

INDIAN JOURNAL OF *ECOLOGY*

Volume 43

Issue-1

June 2016



THE INDIAN ECOLOGICAL SOCIETY

INDIAN ECOLOGICAL SOCIETY

(www.indianecologicalsociety.com)

Past resident: A.S. Atwal and G.S.Dhaliwal
(Founded 1974, Registration No.: 30588-74)

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Impact of Ecological and Climatic Changes on Vectors of Malaria in Four North-Eastern States of India

Akhtar Nasreen, B.N. Nagpal*, Neera Kapoor¹, Aruna Srivastava, H.P. Gupta², Rekha Saxena, A. Shamim, Vikram Kumar, S.K. Gupta, V.P. Singh, Vas Dev², Nutan Nanda and Neena Valecha

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Abstract: A study was conducted during 2010-2013 to assess the impact of climatic and ecological changes on the abundance of the Anopheline species in four endemic North-Eastern states of India namely Assam, Meghalaya, Manipur and Sikkim. Districts and villages were selected on the basis of change in land use pattern using remote sensing classification of images with gap of atleast 6 years and availability of Anophelines species surveys carried out before 2000. Seven entomological and epidemiological surveys in pre and post monsoon seasons were conducted in 287 villages of 16 districts. Ecological and climatic changes like deforestation, development of irrigation channels, change in crop pattern, use of pesticides in tea gardens and increase in annual mean temperature was observed. A total of 29,059 specimens of 29 Anopheline species were collected from entire study area. Pair-wise comparison of *An. culicifacies* and *An. minimus* using Chi square with Yates correction ($\chi^2 = 11443.451$, $p < 0.001$) was statistically significant indicating that *An. culicifacies* is replacing *An. minimus*. Among non vector species *An. ahomi*, *An. aitkenii*, *An. bengalensis*, *An. insulaeflorum*, *An. lindsayi* and *An. umbrosus* were not recorded in the current surveys whereas species namely *An. turkhudi*, *An. karwari*, *An. theobaldi*, *An. dthali*, *An. jamesii*, *An. kochi*, *An. nivipes*, *An. subpictus* *An. nilgircus*, *An. pseudowillmori* reported for the first time in these areas. There is an urgent need to establish the role of *An. culicifacies* as malaria vector to facilitate the policymakers to update malaria control strategies in NE states of India.

Key Words: Anophelines, Deforestation, Ecological changes, Malaria, Remote sensing

In past decades, vast ecological changes have occurred in India due to development activities. Major changes like the loss in forest cover, increase in irrigation and food production, major dams, use of pesticides and migratory population, etc. (Nagpal and Kalra, 1997; Subba Rao, 1999; Forest survey of India 2011). The major ecological and climatic changes were observed in N-E Region. The North-Eastern states namely Assam, Meghalaya, Manipur, Sikkim, Arunachal Pradesh, Mizoram, Nagaland and Tripura together contribute about 4% (44.8 million) of the country's population (Census India, 2011) however, in 2012 these states contributed 7.8% malaria cases with 12.0% Pf and 21.8% deaths from total malaria cases reported in India. Malaria cases reported in NE States were 2.3 lakh about in 2005, which declined to 72 thousand about in 2013 (NVBDCP 2014-15). Vector-borne disease such as malaria is strongly affected by environment and climatic factors, which in turn influence the abundance and survival of the vectors (Patz *et al.*, 2000). The present study was conducted with the objectives to assess the impact of these ecological changes on Anopheline mosquitoes especially on the malaria vectors in the four North-Eastern states of India namely Assam, Meghalaya, Manipur and Sikkim during (2010-2013). Present paper envisage the impact of these

major environmental changes on alteration of mosquito faunal composition, which in turn has impact on the incidences of malaria in these Northeastern States of India

Climatic and Ecological Changes in North-Eastern Region (NER) of India

Rainfall and temperature: The northeast region (NER) of India covers an area of 0.26 million km². This region is one of the highest rainfall receiving regions in India. Trend analysis of rainfall data from 1871-2008 did not show any clear trend for the region as a whole, although there are some seasonal trends and for some hydro-meteorological subdivisions (Khan, 2014) However, rainfalls have increased by 1679mm in Assam in past 50 years. Trend analysis of temperature data of approx 50 years showed that trend fluctuation in selected 4 states. In Assam state maximum and average temperature have increased by 0.44°C and 0.14°C, respectively over 94 years, while there is decrease in minimum temperature of about 0.08°C (IMD 1953-2013).

Forest cover: Over the years due to rapid and uncontrolled urbanization and developmental activities, forest cover is gradually declining. The forest cover in the north eastern region is about 64% of its geographical area and during the years 1993-95 maximum reduction was recorded. Forest cover in Assam was recorded 1419 thousand hectare in

2011-12 compared to 2114 thousand hectare in 1974, a decrease by 32.87%. Similarly in Meghalaya it was recorded 111 thousand hectare in 2003 as compared to 740 thousand hectare in 1973-74 thereafter it gradually increased to 948 thousand hectare in 2008-09. In Manipur forest cover reduced to 1699 thousand hectare in 2013 as compared to 1741 thousand hectares in 1997, *i.e.* decrease by 2.46%. In Sikkim state, forest cover was reported 336 thousand hectare in 2013 as compared to 709 thousand hectare in 1988, *i.e.* decrease by 52.66% (Table 1) (Indian Agriculture in Brief 1977-1981, Statistical handbook of Assam, Meghalaya, Manipur & Sikkim 1977-2013, State of forest report 1999-2007).

Irrigation channels: Apart from forest cover, irrigation channels also increased/decreased in Assam, Meghalaya, Manipur and Sikkim. As a result, the net irrigated area in Assam state decreased by 20.1% in 2010-11 compared with 1975-76, while in Meghalaya state it increased by 20.15% in 2000-01 compared with 1973-74 and in Manipur it increased by 869.14% in 2006-07 compared with 1980-81 (Indian Agriculture in Brief 1977-1981, Statistical handbook of Assam, Meghalaya, Manipur & Sikkim 1977-2013, State of forest report 1999-2007). Data are not available on irrigated

area of Sikkim state (Table 1).

Dams: Many dams got constructed on Brahmaputra river like "Pagladia dam", while one major dam is under construction on the river Subansiri at the border of Arunachal Pradesh and Dhemaji districts of Assam, another 'Hydel power project' dam has been constructed on Umiam lake in Meghalaya. The Khuga dam is a multi-purpose project located to the south of Churachandpur town, Manipur. Rangit dam (Rangit Hydroelectric Power Project Stage III) and Teesta-V-dam on Teesta river have been constructed in Sikkim states (Central Water Commission 2015). Many hydropower plants have also been constructed in these states.

Other changes: Other parameters like net sown area, mining/industry, constructed road length, number of tea gardens, production of rice, and number of fish farms have also increased (Table 1). The impact of these ecological changes on the anopheline fauna and malaria transmission are described in this paper to help in revising control strategy.

MATERIAL AND METHODS

Study site: On the basis of availability of historical data of occurrence of Anopheline (before 2000) and changed ecology, four North-Eastern States of India namely Assam,

Table 1. Ecological and demographical population changes (%) occurred in Assam, Meghalaya, Manipur and Sikkim state

Ecological changes	Assam state	Meghalaya state	Manipur state	Sikkim state
Population growth	16.90 (2001-2011)	27.80 (2001-2011)	18.70 (2001-2011)	12.40 (2001-2011)
Forest cover	-32.87 (1973-2012)	-84.97 (1973-2003)	-2.46 (1997-2013)	-52.66 (1988-2013)
Rainfall pattern	277.5 (1976-2012)	22.9 (1976-85)	NA	NA
Net sown area	14.32 (1973-2011)	73.17 (1973-2009)	68.57 (1978-2011)	75.41 (1978-2011)
Net irrigated area	-20.1 (1975-2011)	20.16 (1973-2001)	869.14 (1980-2007)	NA
Population	847.38 (1901-2011)	192.89 (1973-2011)	856.8 (1973-2011)	190.75 (1971-2011)
No. of villages	43.88 (1973-2011)	20.96 (1973-2001)	34.2 (1971-2011)	114.88 (1974-2011)
Settlement of Mining/Industry	1865.84 (1993-2009)	116.46 (1997-2008)	3512.5 (1980-2006)	83.88 (2003-06)
Construction of roads/highways	43.5 (1997-2012)	308.2 (1970-2008)	42.52 (1995-2005)	NA
Tea gardens	2513.15 (1996-2008)	NA	NA	NA
Production of rice	NA	91 (1974-2009)	NA	4.76 (1997-2005)
Fish farm	NA	NA	100 (1980-2008)	NA
Total Migrant & its reason	NA	NA	NA	2081.85 (1951-2001)

Values in parentheses represent comparison years

*Sources: Indian Agriculture in Brief (1977 to 1981), Statistical handbook of Assam, Meghalaya, Manipur & Sikkim (1999 to 2012), State of forest report (1999-2007)

NA: Not available

Meghalaya, Manipur and Sikkim were selected for study (Fig. 1).

Assam: The state lies at an latitude of 24° 8' N-28° 2' N and longitude of 89° 42' E-96° E covering an area of 78438 km². A total of eight districts of Assam were covered viz. Sonitpur, Nagaon, Lakhimpur, Dibrugarh, Golaghat, Goalpara, Kamrup and Cachar (Table 1).

Meghalaya: The state lies at a latitude of 20.1°N-26.5°N and a longitude of 85.49°E-92.52°E covering an area of 22,429 km². Two districts East Khasi hill and East Garo hill districts were covered under the study (Table 1).

Manipur: The state lies at a latitude of 23°83'N – 25°68'N and a longitude of 93°03'E – 94°78'E covering an area of 22347 km². Two districts of Manipur namely Imphal east and Chandel were included in the study (Table 1).

Sikkim: Sikkim state lies at a latitude of 27°33'N and a longitude of 88°62'E covering an area of 7096 km². All four districts namely East, West, North and South were covered under this study (Table 1).

Selection of villages using RS and GIS: Landsat sensors have a moderate spatial-resolution, individual houses on a Landsat image, are not visible but large man-made activities such as urbanization, settlements, agricultural fields, irrigation channels etc are visible. For Remote sensing data Landsat imagery were downloaded from NASA's 'Global Land Cover Facility' site using Earth Science Data Interface (ESDI). The year of images for different districts was chosen based on availability of images. The images of districts of

Assam e.g., Sonitpur was taken for the years 1999 and 2008; Nagaon for 1999 and 2008; Lakhimpur for 1999, 2002 and 2009; Goalpara for 2002 and 2010; Dibrugarh for 1990, 2003 and 2009; Golaghat for 1989, 2006 and 2011 and Kamrup for 2003 and 2010. In Meghalaya images for districts East Khasi hill were taken for 2003 and 2009, whereas for East Garo hill images were extracted for 1991, 2003 and 2006. For Manipur images for Chandel were extracted for 2006 and 2011 whereas for Imphal East images for 1988, 2006 and 2010 were taken. In Sikkim images for districts East (2002, 2011), West (2005, 2011) and South (2006, 2011) were downloaded and RS images were classified and the ecological changes were identified and quantified, most importantly deforestation, urbanization, settlement, agricultural and irrigation fields.

Laboratory based RS and GIS study on change of ecology was field validated and changes in ecological succession of malaria vectors were studied. For example, many studies carried out in four states of N-E India used NDVI analysis at different time periods to monitor the change in forest cover of districts of each state, experiencing massive reduction in forest cover. In this study also, the deforested areas of each state were identified using RS imageries, for example in Bengenajuli PHC of Sonitpur district, Assam analyzing RS imageries of 1999 and 2008 (Fig. 2a and 2b) found large deforestation and villages of this PHC after recording latitude and longitude were selected for the study (Saxena *et al.*, 2014).

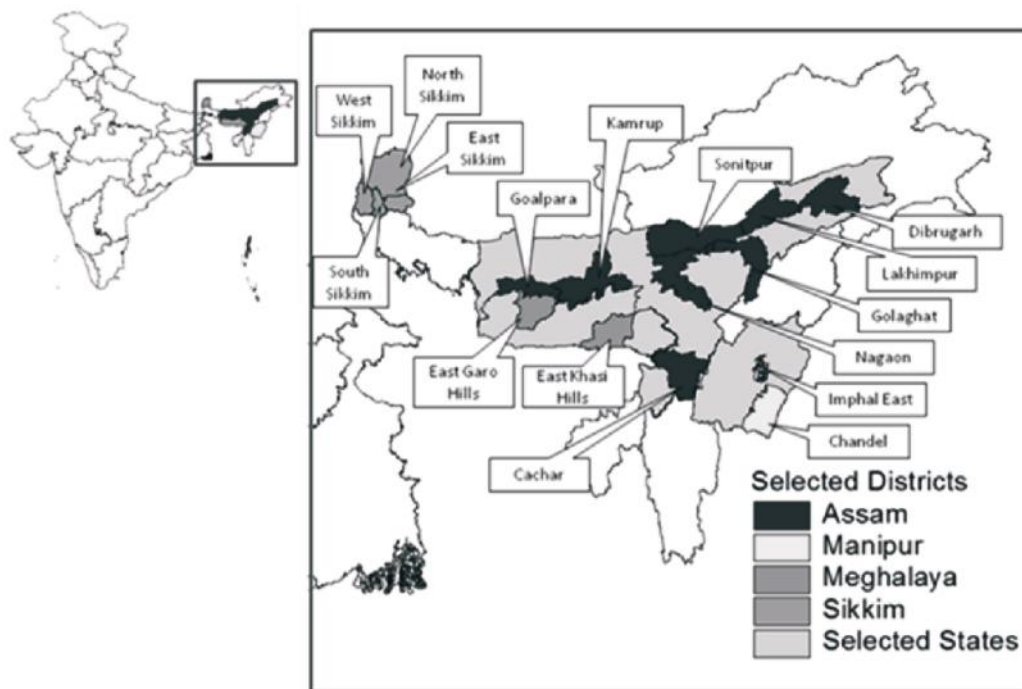


Fig. 1. Study site: 16 selected districts of N-E States of India

Frequency of survey: The field surveys were carried two times in a year *i.e.* pre-monsoon (March-April) and post-monsoon (September-October) seasons. In each survey, entomological and epidemiological data with the help of local health agencies were collected. During 2010 and 2011, two surveys were carried out in each pre-monsoon (March-April) and post-monsoon (September-October) seasons. In 2012 only one survey was done in the month of March-April, while during 2013, two surveys were carried out in the months of February-March and April.

Total seven surveys were done in selected eight districts of Assam (Lakhimpur, Nagaon, Sonitpur, Dibrugarh, Golaghat, Goalpara, Kamrup and Cachar), two districts of Meghalaya (East khasi hill & East garo hill), two districts of Manipur (Chandel and Imphal east) and four districts of Sikkim (East, West, North & South). During this study a total of 287 villages were covered. From Assam, 178 villages (39 with ecological change, 30 villages with available historical data, 49 villages with ecological change and available historical data and 60 new villages), Meghalaya, 25 villages (6 villages with ecological change, 4 villages with available historical data, 2 villages with ecological change & available historical data and 13 new villages), Manipur, 43 villages (8 ecological change villages, 5 villages with available historical data, 7 villages with ecological change and available historical data and 23 new villages) while from Sikkim, 41 villages (4 villages with ecological change, 2 villages with available historical data, 2 villages with ecological change and available historical data and 33 new villages) were selected for surveys.

Entomological Data Collection

Adult collection: Adult mosquitoes were collected in morning and night from both human and cattle sheds with the help of an aspirator and torch light and specimens were transferred to the test tubes in batches of 5-8 mosquitoes per test tube. Soon after the collection, test tubes were marked indicating date, place and time of collection. The following types of adult mosquito collections were made during the present study:

Indoor resting (morning collection): Mosquitoes were collected immediately after day break throughout the period of the study with the help of an aspirator and torch light using WHO technique (1975). The collections were made from cattle sheds, human dwellings and mixed dwelling in each of 15-20 villages representing different ecotypes in each district. In each village six fixed station and two randomly selected houses and cattle sheds were surveyed.

Indoor resting (evening collection): Evening collection was made between 6 PM. and 10 PM in the same villages at the same sites where morning collections were made with the help of an aspirator and torch light using WHO technique (1975). The collections were made from cattle sheds and human dwellings in each selected district of the state.

Space spray collection: Total catch using Aerosol (Hit spray) / pyrethrum spray were carried out in one cattle shed and one human dwelling in selected villages. The structures were sprayed with aerosol. Before spraying care was taken to close all the entry and exit points of the structures and the entire floor space was covered by white bed sheets. After 10-15 minutes of spraying mosquitoes were collected from the

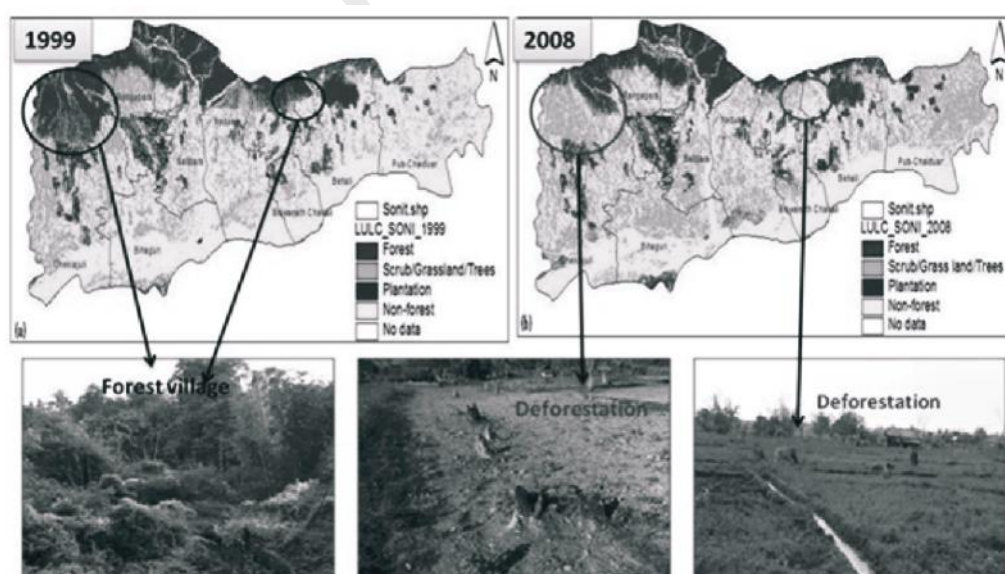


Fig. 2. Forest cover during the year: (a) 1999 and (b) 2008 from satellite imageries of Bengenajuli PHC of Sonitpur district, (Assam) Area encircled by black line indicates dense forest, while purple line indicates deforestation and through GPS validation was done and villages were selected

bed sheets with the help of a pair of fine forceps and transferred to petri-dishes.

Indoor and outdoor resting collection (CDC light trap):

Indoor and outdoor resting mosquitoes were collected from all selected villages where morning collection was made. Mosquitoes were collected with the help of suction tubes and sweep nets in the forenoon for about two hours from tree holes, shrubs, culverts etc. The mosquitoes were also collected using the CDC light trap.

Larval collection for adult emergence: Immature were collected from different habitats such as wells, paddy/rice fields, ponds, streams, sub-stream channels, irrigation channels, rivers, river bed pools, burrow pits, rain water collections, tree holes, containers, flower pots drains etc. from each village. In addition, domestic breeding sites were also checked. A standard WHO dipper of 500 ml vol. was used to sample the ponds, pools, river margins etc. An aluminum spoon of 60 ml. vol. was used to sample the paddy/rice fields, river bed pools etc., well breeding was checked with the help of a well net and also by a bucket (2 lit. capacity). All the adult mosquitoes collected by different methods and adult emerged from the larvae collected were brought to the laboratory and identified to species level by using the keys of Christophers (1933); Barraud (1934); Puri (1954); Nagpal and Sharma (1995); Nagpal *et al.* (2005) and The catalogue of Knight and Stone (1977).

RESULTS AND DISCUSSION

Anopheline fauna of N-E states: During 2010-13 surveys, a total of 29059 specimens belonging to 29 Anophelines species were collected from 287 villages in 16 districts of four selected states. Out of these 29 species, *An. vagus* (53%) was found to be the most dominating species followed by *An. culicifacies* (13%) and *An. annularis* (8%) (Table 2). From total specimens 29059 collected, 3960 specimens (13.6%) belongs to four established malaria vector species viz. *An. culicifacies*, *An. minimus*, *An. fluviatilis* and *An. dirus* from four N-E States. *An. culicifacies* (91.9%) was the most dominating vector species followed by *An. minimus* (5.2%), *An. dirus* (1.99%) and *An. fluviatilis* (0.9%). State-wise results are included in Table 2 and Fig 3a-d.

The results of present study showed that out of 29 *Anopheline* species collected from all four selected states and *An. vagus* was observed to be the most dominating species (53%) followed by *An. culicifacies* (13%), whereas, the surveys carried out by earlier investigators reported 33 *Anopheline* species with *An. vagus* was most dominating species (34%) followed by *An. minimus* (18%) (Table 2). Among the vector species, *An. culicifacies* (91.9%) was most dominating followed by *An. minimus* (5.2%), *An. dirus*

(1.99%) and *An. fluviatilis* (0.9%) in this study, which is in contrast with earlier studies i.e., *An. minimus* (98.5%) as the most dominating species followed by *An. fluviatilis* (0.6%), *An. culicifacies* (0.6%) and *An. dirus* (0.3%) (Rao *et al.*, 1973; Rajgopal *et al.*, 1976; Malhotra *et al.*, 1983; Das *et al.*, 1984; Nagpal and Sharma, 1987; Sarkar *et al.*, 1981; Dutta *et al.*, 1996; Khan, 1998;).

In Assam, out of 24 Anopheline species collected *An. vagus* (56%) was most abundant species followed by *An. culicifacies* (11%) and *An. nigerrimus* (8%), whereas, the surveys carried out by earlier investigators (before 2000) reported 31 *Anopheline* species with *An. vagus* (31%) as the most abundant species followed by *An. minimus* (27%) and *An. annularis* (10%) (Table 2). Our findings showed that *An. culicifacies* (90.2%) was the most dominating malaria vector species followed by *An. minimus* (5.9%), *An. dirus* (3.1%) and *An. fluviatilis* (0.8%) which is inconsistent with the findings reported in other studies that *An. minimus* (99.2%) was the most dominating species followed by *An. fluviatilis* (0.5%), *An. culicifacies* (0.3%) and *An. dirus* (0.04%) (Fig. 3a) (Baruah *et al.*, 2004 and 2007; Challam, 1923; Dev, 2004; Das *et al.*, 1984; Nagpal and Sharma, 1987; Sarkar *et al.*, 1981; Dutta *et al.*, 1996; Khan, 1998).

In Meghalaya, *An. vagus* (35%) was found to be the leading Anopheline species followed by *An. annularis* (20%) and *An. culicifacies* (14%) among 20 Anopheline species collected from this study whereas the surveys carried out before 2000 by other researchers reported that *An. vagus* (40%) was observed to be in high density followed by *An. philippinensis* (21%) and *An. nivipes* (19%), out of 24 Anopheline species collected (Table 2). *An. culicifacies* (92.4%) was also found to be the major malaria vector species followed by *An. minimus* (6.2%) and *An. fluviatilis* (1.4%) in the present study (2010-13), which differ remarkably with other findings (before 2000) reporting *An. minimus* (73.3%) as the most dominating malaria vector species followed by *An. culicifacies* (15.8%), *An. fluviatilis* (9.2%) and *An. dirus* (1.7%) (Fig. 3b) (Nagpal *et al.*, 1987; Das *et al.*, 1984; Rajgopal *et al.*, 1976).

In Manipur state, a total 15 Anopheline species were collected during current study and out of which *An. vagus* (69%) was in profusion followed by *An. subpictus* (12%), *An. nivipes* (4%) and *An. culicifacies* (4%), whereas the field surveys conducted by other researcher (before 2000) stated that *An. vagus* (38%) was most abundant species followed by *An. nigerrimus* (23%) and *An. philippinensis* (20%), out of 20 Anopheline species collected (Table 2). Our findings showed that *A. culicifacies* (88.4%) was also observed to be the most dominating malaria vector species followed by *A. minimus* (10.5%), *A. fluviatilis* (1.1%)

Table 2. Anopheline fauna of N-E States collected by different methods of collection (2010-13) and compared with surveys carried out before 2000

Anopheline species	Assam						Meghalaya						Manipur						Sikkim						Total Specimens					
	2010-13			Before 2000			2010-13			Before 2000			2010-13			Before 2000			2010-13			Before 2000			2010-13			Before 2000		
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
An (Cellia) fuliginosus Lee. 1946/ anularis Vanderwulp. 1884	1254	6%	3185	10%	1010	20%	134	2%	35	2%	1	0%	0	0%	0	0%	0	0%	2299	8%	3320	7%								
An (Cellia) aconitus Doenitz. 1902	39	0%	2067	7%	5	0%	33	0%	1	0%	1	0%	0	0%	0	0%	0	0%	45	0%	2101	4%								
An (An) ahomi Chawdhury. 1924	0	0%	7	0%	0	0%	0	0%	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	8	0%								
An (An) aitkenii James. 1903	0	0%	1	0%	0	0%	1	0%	0	0%	2	0%	0	0%	1	0%	0	0%	0	0%	5	0%								
An (An) barbrostris Vanderwulp. 1884	890	4%	412	1%	107	2%	10	0%	33	1%	2	0%	0	0%	0	0%	0	0%	1030	4%	424	1%								
An (An) bengalensis Puri. 1950	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	1	0%								
An (Cellia) culicifacies Giles. 1901	2305	11%	24	0%	682	14%	19	0%	84	4%	4	0%	568	71%	3	1%	3639	13%	50	0%										
An (Cellia) leucosphyrus Doenitz. 1901/ balanacensis Baisas. 1936/ dirus Peyton & Harrison, 1979 baimaii Sallum & Peyton 2005	79	0%	4	0%	0	0%	2	0%	0	0%	17	0%	0	0%	0	0%	79	0%	23	0%										
An (Cellia) dthali Patton. 1905	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%	0	0%	0	0%	1	0%	0	0%										
An (Cellia) fluviatilis/histonii James. 1902	21	0%	42	0%	10	0%	11	0%	1	0%	1	0%	3	0%	1	0%	35	0%	55	0%										
An (An) gigas Giles. 1901/ baileyi Edwards, 1929	0	0%	1	0%	50	1%	0	0%	0	0%	1	0%	1	0%	0	0%	51	0%	2	0%										
An(An) insulaeflorum Swellengrebel & Swellengrebel de Graaf, 1919	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%										
An (Cellia) jeyporiensis James. 1902	4	0%	10	0%	327	7%	4	0%	0	0%	2	0%	0	0%	0	0%	331	1%	16	0%										
An (Cellia) jamesii Theobald. 1901	52	0%	71	0%	2	0%	5	0%	7	0%	0	0%	0	0%	0	0%	61	0%	76	0%										
An (Cellia) karwari James. 1902	40	0%	8	0%	9	0%	71	1%	0	0%	0	0%	0	0%	0	0%	49	0%	79	0%										
An (Cellia) kochi Doenitz. 1902	642	3%	273	1%	8	0%	206	3%	36	2%	0	0%	0	0%	0	0%	686	2%	479	1%										
An (Topomyia) lindsayi Giles. 1900	0	0%	1	0%	0	0%	0	0%	0	0%	1	0%	0	0%	1	0%	0	0%	3	0%										
An (Cellia) maculatus Theobald. 1901	315	1%	155	0%	85	2%	552	7%	0	0%	1	0%	105	13%	201	89%	505	2%	909	2%										
An (Cellia) minimus Theobald. 1901	151	1%	8632	27%	46	1%	88	1%	10	0%	20	0%	0	0%	0	0%	207	1%	8740	18%										
An (Cellia) majidi Yong and Majidi. 1928	0	0%	10	0%	0	0%	3	0%	0	0%	0	0%	0	0%	0	0%	0	0%	13	0%										
An (An) nigerrimus/hyrcanus Giles. 1900	1736	8%	2649	8%	224	5%	479	6%	83	4%	1914	23%	1	0%	0	0%	2044	7%	5042	10%										
An (Cellia) nives Theobald. 1903	1186	6%	1574	5%	105	2%	1574	19%	96	4%	1574	19%	0	0%	0	0%	1387	5%	4722	10%										
An(An) nilgircus Christophers, 1924	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%	1	0%	0	0%										
An (Cellia) pseudojamei Strickland and Chowdhury 1927/ An. ramsayi Covell 1927	3	0%	161	1%	0	0%	8	0%	0	0%	0	0%	0	0%	0	0%	3	0%	169	0%										
An (Cellia) pallidus Theobald. 1901	6	0%	19	0%	0	0%	19	0%	2	0%	19	0%	0	0%	0	0%	8	0%	57	0%										
An (Cellia) philippinensis Ludlow. 1902	72	0%	2371	7%	2	0%	1669	21%	31	1%	1655	20%	0	0%	0	0%	105	0%	5695	12%										
An(Cellia) pseudowillmori Theobald, 1910	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	56	7%	0	0%	56	0%	0	0%										
An (Cellia) pulcherrimus Theobald. 1902	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%	1	0%										

Cont....

Table 2. Cont....

Anopheline species	Assam						Meghalaya						Manipur						Sikkim						Total Specimens					
	2010-13			Before 2000			2010-13			Before 2000			2010-13			Before 2000			2010-13			Before 2000			2010-13			Before 2000		
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%		
An (<i>Cellia</i>) <i>subpictus</i> Grassi. 1899	173	1%	72	0%	51	1%	4	0%	265	12%	0	0%	0	0%	0	0%	0	0%	0	0%	489	2%	76	0%						
An (<i>An</i>) <i>sinensis</i> Wiedemann. 1828	114	1%	14	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	114	0%	14	0%						
An (<i>Cellia</i>) <i>splendidus</i> Koidzumi. 1920	0	0%	12	0%	1	0%	2	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%	14	0%						
An (<i>Cellia</i>) <i>tessellatus</i> Theobald. 1901	15	0%	4	0%	0	0%	2	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	15	0%	6	0%						
An (<i>Cellia</i>) <i>theobaldi</i> Giles. 1901	17	0%	24	0%	484	10%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	501	2%	24	0%						
An (<i>Cellia</i>) <i>turkhudi</i> Liston, 1901	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%						
An (<i>Cellia</i>) <i>umbrosus</i> Theobald. 1903	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%						
An (<i>Cellia</i>) <i>varuna</i> Iyenger. 1924	55	0%	19	0%	1	0%	1	0%	0	0%	0	0%	1	0%	0	0%	0	0%	0	0%	56	0%	21	0%						
An (<i>Cellia</i>) <i>vagus</i> Doenitz. 1902	11871	56%	9839	31%	1761	35%	3240	40%	1558	69%	3152	38%	70	9%	19	8%	15260	53%	16250	34%	29059	53%	48397							
Total	21041		31663		4970		8137		2243		8370		805		227		29059		48397											

in this state, which is in contrast with the findings of other previous studies (before 2000) reported *An. minimus* (47.6%) as the most dominating malaria vector species followed by *An. dirus* (40.4%), *An. culicifacies* (9.5%) and *An. fluviatilis* (2.4%) (Fig. 3c) (Malhotra *et al.*, 1983; Nagpal and Sharma, 1987).

In Sikkim, *A. culicifacies* (71%) was observed to be the most abundant species followed by *An. maculatus* (13%) and *An. vagus* (9%) from 8 *Anopheline* species collected during the study period, which is very much inconsistent with the findings of the other studies (before 2000) reported *An. maculatus* (89%) as the most dominating followed by *An. vagus* (8%) and *An. culicifacies* (1%) among seven *Anopheline* species collected (Table 2). The results of present study demonstrates that *An. culicifacies* (99.5%) was the most abundant malaria vector species followed by *An. fluviatilis* (0.52%) which corroborate with the findings of previous surveys stated that *An. culicifacies* (75%) was the dominant vector species followed by *An. fluviatilis* (25%) but the proportions of both vector species (*An. culicifacies* and *An. fluviatilis*) differ astonishingly in the present and previous studies. While *An. dirus* and *An. minimus* were absent in present surveys (2010-13) and surveys carried out before 2000 (Fig. 3d) (Rao *et al.*, 1973).

Appearance and disappearance of Anopheline in N-E:

State wise Anophelines collected in the present surveys clearly indicate that during the successive years as there have been ecological changes in north eastern states mostly due to deforestation and change in temperature and humidity influencing habitats of Anopheline mosquitoes. As shown in Table 3 (a, b and c) below, some of the species which were not recorded in earlier surveys, were observed during the current surveys, while some species recorded earlier were absent in the present surveys. This change may be attributed to change in ecological conditions. Similar observations were also recorded by other investigators (Subbarao, 1999; Saxena, 2014).

In Assam *A. karwari*, *An. aitenkii*, *An. gigas*/*An. baileyi* were recorded by Sarkar (1981); *An. splendidus* and *An. ahomi* by Nagpal and Sharma (1987); Dev *et al.* (1996); *An. umbrosus* by Rice *et al.* (1936) were not recorded in the present survey, whereas *An. turkhudi* was recorded for the first time in 2010-2013 surveys (Table 3a).

From Meghalaya *An. aitenkii* by Shroff, 1924; *An. dirus*/*An. leucosphyrus*, *An. pallidus* and *An. tessellatus* recorded by Das *et al.* (1984); Rajgopal *et al.* (1976); *An. majidi*, *An. ramsayi*/*An. pseudojemesii* by Nagpal and Sharma (1987); *An. pallidus* by Rajgopal *et al.* (1976), which were not recorded in the present survey. Whereas *An. karwari* and *An. theobaldi* were not recorded earlier from this

area but surprisingly they were found in the present surveys (2010-13) (Table 3b). During this study, it was observed that *An. ahomii*, *An. aikenii*, *An. bengalensis*, *An. lindsayi*, *An. dirus*, *An. gigas*, *An. jeyporiensis* and *An. maculatus* were absent which were earlier recorded by Malhotra et al. (1983) and Nagpal and Sharma (1987).

From Manipur state, *An. culicifacies*, *An. fluviatilis*, *An. jamesii*, *An. kochi*, *An. minimus*, *An. nigerrimus*, *An. subpictus*, *An. vagus* were recorded during the present

surveys which were absent in the previous surveys conducted (Nagpal and Sharma, 1987; Mortimer, 1946) (Table 3c).

From Sikkim, *An. Aitkenii* and *An. Lindsayi* were reported by Rao et al. (1973); *An. pulcherrimus*, *An. splendidus*, *An. sinensis*, *An. subpictus* and *An. tessellatus* were also recorded by Rao et al. (1973), which were not found in the present surveys and on contrary other Anopheline species like *An. nilgircus* and *An. pseudowullmori* were

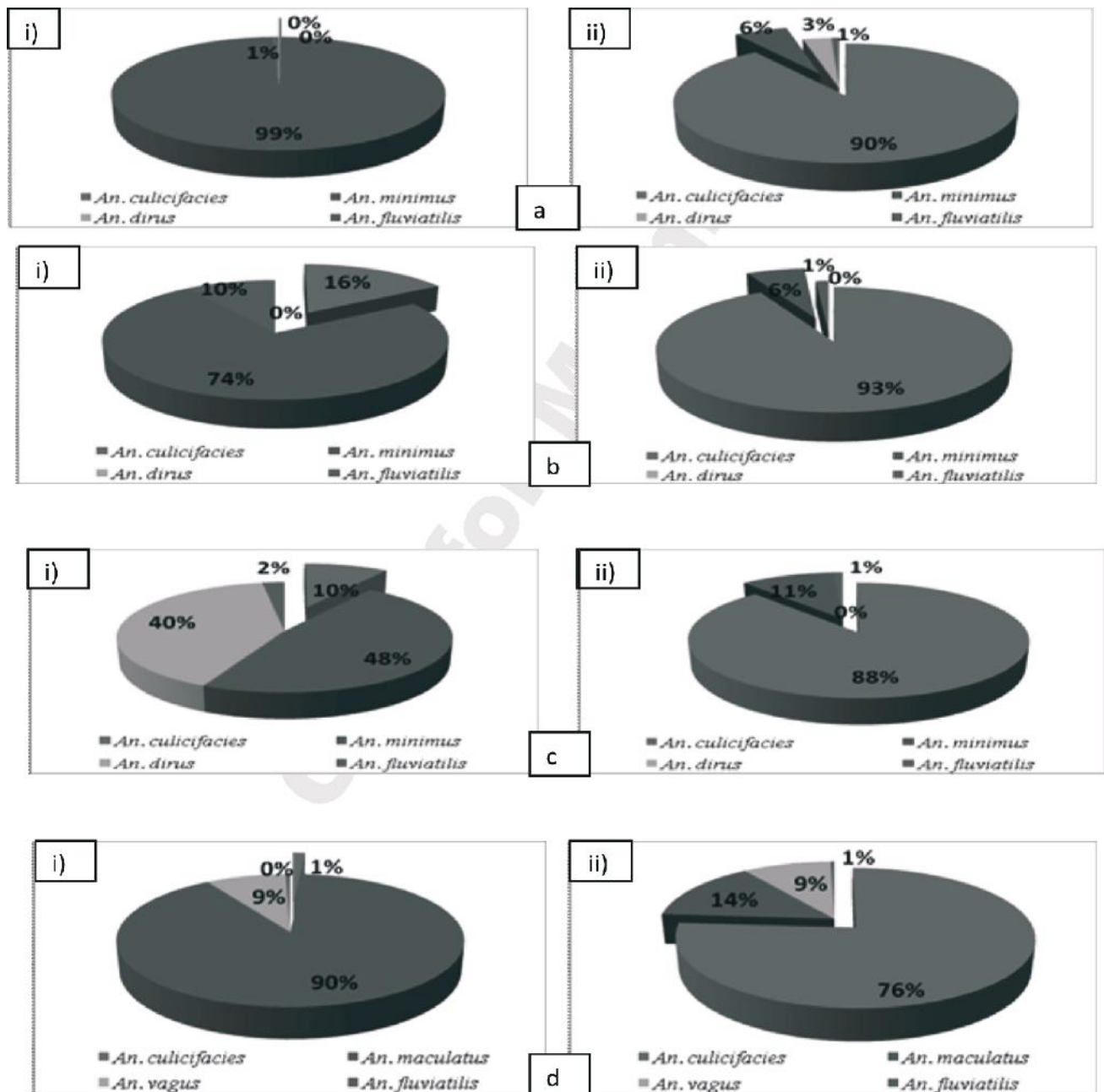


Fig. 3. Comparison of malaria vectors in a) Assam b) Meghalaya c) Manipur d) Sikkim collected i) before 2000 ii) Present survey (2010-2013) which clearly indicates that *An. culicifacies* replacing *An. minimus* in Assam, Meghalaya and Manipur, whereas in Sikkim *An. culicifacies* is replacing *An. maculatus*

Table 3a. Anopheline species prevalence: comparison between current survey and previous surveys (1930-2013) in Assam

Species	Present survey	Dev et al. (1996)	Nagpal et al. (1987)	Sarkar (1981)	Viswanathan et al. (1941)	Rice et al. (1936)	Manson et al. (1931)	Gupta et al. (1932)	Ramsay et al. (1930)
<i>An (Cellia) fuliginosus</i> Lee. 1946/ <i>annularis</i> Vanderwulp. 1884	✓	✓	✓	✓	✓	✓	✓	✓	-
<i>An (Cellia) jaconitus</i> Doenitz. 1902	✓	✓	✓	✓	✓	✓	✓	✓	-
<i>An (An) ahomi</i> Chawdhury. 1924	-	-	-	-	-	-	-	-	-
<i>An (An) aikhenii</i> James. 1903	-	-	-	-	-	-	-	-	-
<i>An (An) barbrostris</i> Vanderwulp. 1884	✓	-	✓	✓	✓	✓	✓	✓	-
<i>An (Cellia) culicifacies</i> Giles. 1901	✓	✓	✓	-	✓	✓	✓	✓	-
<i>An (Cellia) leucosphyrus</i> Doenitz. 1901/ <i>balanacensis</i> Baisas. 1936/ <i>dirus</i> Peyton & Harrison, 1979/ <i>baimaii</i> Sallum & Peyton 2005	✓	✓	✓	-	-	-	✓	-	-
<i>An (Cellia) fluviatilis/istonii</i> James. 1902	✓	✓	✓	-	-	✓	-	✓	-
<i>An (An) gigas</i> Giles. 1901/ <i>baileyi</i> Edwards, 1929	-	-	✓	✓	-	✓	✓	-	-
<i>An (An) insulaefflorum</i> Swellengrebel & Swellengrebel de Graaf, 1919	-	-	-	-	-	✓	-	-	-
<i>An (Cellia) jamesii</i> Theobald. 1901	✓	-	✓	-	-	-	-	-	-
<i>An (Cellia) jeyportensis</i> James. 1902	✓	✓	✓	-	-	-	-	-	-
<i>An (Cellia) kochi</i> Doenitz. 1902	✓	✓	✓	-	✓	✓	✓	✓	-
<i>An (Cellia) karwari</i> James. 1902	✓	-	✓	-	-	-	✓	-	-
<i>An (An) lindsayi</i> Giles. 1900	-	-	-	-	-	✓	-	-	-
<i>An (Cellia) maculatus</i> Theobald. 1901	✓	✓	✓	-	✓	✓	✓	✓	-
<i>An (Cellia) majidi</i> Yong and Majidi. 1928	-	-	✓	-	-	-	-	-	-
<i>An (Cellia) minimus</i> Theobald. 1901	✓	✓	✓	✓	✓	✓	✓	✓	✓
<i>An (An) nigerrimus/hyrcanus</i> Giles. 1900	✓	✓	✓	✓	✓	✓	✓	✓	-
<i>An (Cellia) nivipes</i> Theobald. 1903	✓	-	✓	-	-	-	-	-	-
<i>An (Cellia) philippinensis</i> Ludlow. 1902	✓	✓	✓	✓	-	✓	✓	✓	-
<i>An (Cellia) pallidus</i> Theobald. 1901	✓	-	✓	-	-	✓	-	-	-
<i>An (Cellia) pseudojameii</i> Strickland and Chowdhury 1927/ <i>An. ramsayi</i> Covell 1927	✓	-	✓	✓	-	-	✓	-	✓
<i>An (Cellia) subpictus</i> Grassi. 1899	✓	-	✓	✓	-	-	-	✓	-
<i>An (An) sinensis</i> Wiedemann. 1828	✓	-	✓	-	-	-	-	-	-

Cont.....

Table 3a. Cont...

Species	Present survey	Dev <i>et al.</i> (1996)	Nagpal <i>et al.</i> (1987)	Sarkar (1981)	Viswanathan <i>et al.</i> (1941)	Rice <i>et al.</i> (1936)	Manson <i>et al.</i> (1931)	Gupta <i>et al.</i> (1932)	Ramsay <i>et al.</i> (1930)
<i>An (Cellia) splendidus</i> Koidzumi. 1920	-	✓	✓	-	-	-	-	-	-
<i>An (Cellia) tessellatus</i> Theobald. 1901	✓	-	✓	-	-	-	✓	-	-
<i>An (Cellia) theobaldi</i> Giles. 1901	✓	-	✓	-	-	-	-	-	✓
<i>An (Cellia) turkhudi</i> Liston, 1901	✓	-	-	-	-	-	-	-	-
<i>An (Cellia) umbrosus</i> Theobald. 1903	-	-	-	-	-	✓	-	-	-
<i>An (Cellia) varuna</i> Iyenger. 1924	✓	✓	✓	-	-	✓	-	-	-
<i>An (Cellia) vagus</i> Doenitz. 1902	✓	✓	✓	✓	✓	✓	✓	✓	-

collected and reported in this study (Table 3d).

On comparing fauna of Anopheline in the present surveys (2010-13) with surveys carried out earlier (before 2000) in the same villages of selected districts in each state with addition of few new villages, it was observed that the main factor for succession of Anopheline species was deforestation (shifting cultivation) i.e. clearing of forest land for crop cultivation, which has been a regular phenomenon in north-eastern states resulting in disappearance of native species and influx by other new species into an area (Ranjan *et al.*, 1999). The replacement of vector after deforestation has been reported from other parts of the world as well. Deforestation for rice cultivation and irrigation development in Sri Lanka resulted in change of vector species involved in malaria transmission (Amerasinghe *et al.*, 1991). Species replacement took place in Thailand also where land was converted from forest to cassava/sugarcane cultivations (Prothero *et al.*, 1999). Forest area is important in the context of climate change due to three reasons namely; i) deforestation and land degradation contributes to about 20% of global CO₂ emissions, ii) forest sector provides a large opportunity to mitigate climate change, particularly the REDD (Reducing Emissions from Deforestation and Degradation) mechanism, and iii) forest ecosystems are projected to be adversely impacted by climate change, affecting biodiversity, biomass growth and forest regeneration (Ravindranath *et al.*, 2005).

Land transformation due to deforestation alters every element of local ecosystem, which has profound impact on breeding places, prevalence, density and composition of human disease vectors which in turn modifies the transmission pattern of the diseases (Karla, 1991; Grillet, 2000). *Anopheles minimus* s.l. and *An. dirus* (now known as *An. baimai*) were the major anthropophilic vectors of malaria in North-Eastern region of India and supported perennial transmission of malaria in this region (Dutta *et al.*, 1989; Nagpal and Kalra 1997; Prakash *et al.*, 2005; Dev, 2006).

Establishment of *An. culicifacies* in North-Eastern States of India

The study revealed that *An. culicifacies* is replacing *An. minimus* ($\chi^2 = 11443.451$, $p < 0.001$). This may be mainly attributed to the following reasons:

Change in land use pattern: Deforestation occurred in all the four NE states and about 3565 thousand hectare areas has been deforested (Walsh *et al.*, 1993). This land use was converted into agricultural land to take the crop cultivation. Channels from streams were brought to irrigate the fields. These irrigation channels were the favorable breeding sites for *An. culicifacies* which increased its density.

Increase in temperature: Analysis of temperature data showed that in Assam state maximum and average

Table 3b. Anopheline species prevalence: comparison between current survey and previous surveys (1924-2013) in Meghalaya

Species	Present survey	Rajgopal <i>et al.</i> (1976)	Nagpal <i>et al.</i> (1987)	Das <i>et al.</i> (1984)	Shorrt (1924)
<i>An (Cellia) aconitus</i> Doenitz. 1902	√	√	√	√	-
<i>An (Cellia) fuliginosus</i> Lee. 1946/ <i>annularis</i> Vanderwulp. 1884	√	-	√	√	√
<i>An (An) aitkenii</i> James. 1903	-	-	-	-	√
<i>An (An) barbirostris</i> Vanderwulp. 1884	√	√	√	√	-
<i>An (Cellia) culicifacies</i> Giles. 1901	√	-	√	-	-
<i>An (Cellia) leucosphyrus</i> Doenitz. 1901/ <i>balanacensis</i> Baisas. 1936/ <i>dirus</i> Peyton & Harrison, 1979/ <i>baimaii</i> Sallum & Peyton 2005	-	√	-	√	-
<i>An (Cellia) fluvialis/listonii</i> James. 1902	√	-	√	√	-
<i>An (An) gigas</i> Giles. 1901/ <i>baileyi</i> Edwards, 1929	√	-	-	√	√
<i>An (Cellia) jeyporiensis</i> James. 1902	√	√	-	√	-
<i>An (Cellia) jamesii</i> Theobald. 1901	√	-	√	√	-
<i>An (Cellia) kochi</i> Doenitz. 1902	√	√	√	-	-
<i>An (Cellia) karwari</i> James. 1902	√	-	-	-	-
<i>An (Cellia) maculatus</i> Theobald. 1901	√	√	√	√	√
<i>An (Cellia) minimus</i> Theobald. 1901	√	√	√	√	√
<i>An (Cellia) majidi</i> Yong and Majidi. 1928	-	-	√	-	-
<i>An (Cellia) nivipes</i> Theobald. 1903	√	-	√	-	-
<i>An (An) nigerrimus/hyrcanus</i> Giles. 1900	√	√	√	√	-
<i>An (Cellia) philippinensis</i> Ludlow. 1902	√	√	√	√	-
<i>An (Cellia) pallidus</i> Theobald. 1901	-	√	-	√	-
<i>An (Cellia) pseudojameii</i> Strickland and Chowdhury 1927/ <i>An. ramsayi</i> Covell 1927	-	-	√	-	-
<i>An (Cellia) subpictus</i> Grassi. 1899	√	-	√	√	√
<i>An (Cellia) splendidus</i> Koidzumi. 1920	√	-	√	√	-
<i>An (An) sinensis</i> Wiedemann. 1828	-	-	-	-	√
<i>An (Cellia) theobaldi</i> Giles. 1901	√	-	-	-	-
<i>An (Cellia) tessellatus</i> Theobald. 1901	-	√	-	√	-
<i>An (Cellia) varuna</i> Iyenger. 1924	√	-	√	-	-
<i>An (Cellia) vagus</i> Doenitz. 1902	√	√	√	√	√

* (√): Species found *(-): Species not found

temperature have increased by 0.44°C and 0.14°C, respectively while minimum temperature has decreased by 0.08°C. In Meghalaya state, maximum, minimum and average temperatures have increased by 0.40°C, 0.55°C and 0.23°C, respectively. In Manipur state, maximum, minimum and average temperatures have increased by 2.98°C, 5.92°C and 4.45°C, respectively, whereas in Sikkim state, maximum temperature, average temperature have decreased by 0.34°C and 0.33°C and minimum temperature has increased by 0.97°C, respectively (IMD 1903-2013). As per the study carried out by (Vinogradaskaya *et al.*, 1969) and NIMR in three climatic zones of India (NIMR Annual Report 2004-05)

found that *An. culicifacies* is a thermophilic species i.e. they prefer to established their population in high temperature areas i.e. in Northern India (Tyagi *et al.*, 2001; Joshi *et al.*, 2005) secondly *An. culicifacies* belongs to Indo-Iranian climatic zones and originated from Khandwa district, Madhya Pradesh of India has capability to established their population and transmit malaria in new ecological changed areas in Orissa and Uttar Pradesh Terai (Nagpal and Sharma, 1986; Choudhury *et al.*, 1983).

Disappearance of *An. minimus*, *An. dirus* the vectors of malaria and other non-vector species of study area

a. *An. minimus* is a stream breeder in forested area and

prefers to breed in shady margin (Rao, 1984). As the deforestation occurred and land was used for agriculture cultivation, these streams have been converted into irrigation channels (Saxena *et al.*, 2014).

- b. About 5.5 millions long lasting bed nets were distributed in last 5 years to control malaria in NE States (NVBDCP Annual Report 2015). *An. minimus*, *An. dirus* both the species are susceptible to pyrethroid/DDT insecticides (Dev, 2009; Baruah *et al.*, 2004).
- c. *An. minimus* and *An. dirus* both are a hygrophilic species which prefer high humidity and low temperature but due to deforestation the humidity is decreased and temperature increased in the region (Vinogradaskaya *et al.*, 1969; NIMR Annual Report 2004-05).

- d. Transient disappearance of *An. minimus* & *An. dirus* after 1950 because of DDT spraying and ecological changes (Chakrabarti and Singh, 1957; Nagpal *et al.*, 1983; Sharma *et al.*, 1984).

Non-vector species in India

- a. *An. vagus* was found to be most robust and bio-diverse species as it adapts itself in different ecological and climatic conditions as it was found most dominant species in present survey (54%) as well as in earlier surveys (34%) among all collected Anopheline species.
- b. *An. ahomi*, *An. aitkenii*, *An. bengalensis*, *An. insulaeflorum*, *An. lindsayi* and *An. umbrosus* were not recorded in the present surveys as they prefer to breed in streams, shady pits and due to deforestation these are converted into irrigation channels for agriculture use. Therefore this may be one of the

Table 3c. Anopheline species prevalence: comparison between current survey and previous surveys (1946-2013) in Manipur

Species	Present survey	Nagpal <i>et al.</i> (1987)	Malhotra <i>et al.</i> (1983)	Mortimer (1946)
<i>An (Cellia) fuliginosus</i> Lee. 1946/annularis Vanderwulp. 1884	√	√	√	√
<i>An (Cellia)aconitus</i> Doenitz. 1902	√	-	-	√
<i>An (An) ahomi</i> Chawdhury. 1924	-	-	-	√
<i>An (An) aitkenii</i> James. 1903	-	-	√	√
<i>An (An) barbirostris</i> Vanderwulp. 1884	√	-	-	√
<i>An (An) bengalensis</i> Puri. 1950	-	-	-	√
<i>An (Cellia) culicifacies</i> Giles. 1901	√	√	-	-
<i>An(Cellia)leucosphyrus</i> Doenitz. 1901/ <i>balanacensis</i> Baisas. 1936/ <i>dirus</i> Peyton & Harrison, 1979/ <i>baimaii</i> Sallum & Peyton 2005	-	√	-	√
<i>An (Cellia) dthali</i> Patton. 1905	√	-	-	-
<i>An (Cellia) fluviatilis/listonii</i> James. 1902	√	√	-	-
<i>An (An) gigas</i> Giles. 1901/ <i>baileyi</i> Edwards, 1929	-	-	√	√
<i>An (Cellia) jeyporiensis</i> James. 1902	-	-	-	√
<i>An (Cellia) jamesii</i> Theobald. 1901	√	-	-	-
<i>An (Cellia) kochi</i> Doenitz. 1902	√	-	-	-
<i>An (An) lindsayi</i> Giles. 1900	-	-	-	√
<i>An (Cellia) minimus</i> Theobald. 1901	√	√	-	-
<i>An (Cellia)maculatus</i> Theobald. 1901	-	-	√	√
<i>An (An) nigerimus/hyrcanus</i> Giles. 1900	√	-	√	√
<i>An (Cellia)nivipes</i> Theobald. 1903	√	-	-	-
<i>An (Cellia) pallidus</i> Theobald. 1901	√	-	-	√
<i>An (Cellia)philippinensis</i> Ludlow. 1902	√	√	-	√
<i>An (Cellia) subpictus</i> Grassi. 1899	√	-	-	-
<i>An (Cellia) varuna</i> Iyenger. 1924	-	√	-	-
<i>An (Cellia) vagus</i> Doenitz. 1902	√	-	√	√

* (√): Species found * (-): Species not found

Table 3d. Anopheline species prevalence: comparison between current survey and previous surveys (1933-2013) in Sikkim

Species	Present survey	Rao <i>et al.</i> (1973)	Sur <i>et al.</i> (1933)
<i>An (An) aitkenii</i> James. 1903	-	✓	✓
<i>An (Cellia) fuliginosus</i> Lee. 1946/ <i>annularis</i> Vanderwulp. 1884	-	✓	✓
<i>An (Cellia) culicifacies</i> Giles. 1901	✓	✓	✓
<i>An (Cellia) fluviatilis</i> James. 1902	✓	✓	✓
<i>An (An) gigas</i> Giles. 1901/ <i>baileyi</i> Edwards, 1929	✓	✓	✓
<i>An (Cellia) jeyporiensis</i> James. 1902	-	✓	-
<i>An (An) lindsayi</i> Giles. 1900	-	✓	✓
<i>An (Cellia) maculatus</i> Theobald. 1901	✓	✓	✓
<i>An (An) nigerrimus/ hyrcanus</i> Giles. 1900	✓	✓	✓
<i>An (An) nilgircus</i> Christophers, 1924	✓	-	-
<i>An (Cellia) pulcherrimus</i> Theobald. 1902	-	✓	-
<i>An (Cellia) pseudowillmori</i> Theobald, 1910	✓	-	-
<i>An (Cellia) splendidus</i> Koidzumi. 1920	-	✓	-
<i>An (An) sinensis</i> Wiedemann. 1828	-	✓	-
<i>An (Cellia) subpictus</i> Grassi. 1899	-	✓	-
<i>An (Cellia) tessellatus</i> Theobald. 1901	-	✓	-
<i>An (Cellia) vagus</i> Doenitz. 1902	✓	✓	✓

* (✓): Species found, (-): Species not found

reasons of not recording these species from the areas surveyed.

- c. Species namely *An. turkhudi* in Assam, *An. karwari*, *An. theobaldi* in Meghalaya, *An. dthali*, *An. jamesii*, *An. kochi*, *An. nivipes*, *An. subpictus* in Manipur and *An. nilgircus*, *An. pseudowillmori* in Sikkim recorded in the present surveys but were absent in the past surveys. These species prefer to breed in rice field, irrigation channels and pools with vegetation but due to change in landuse these breeding sites increased.

CONCLUSION

Due to change in landuse and other climatic conditions the population of *An. culicifacies* was found to increase in all four N-E States (Assam, Meghalaya, Manipur

and Sikkim), which should prompt the policymaker of malaria control to develop new control strategies for this region in light of new species composition. Since last five decades these areas were under the influence of *An. minimus* and *An. dirus* so there is an urgent need to establish the role of *An. culicifacies* as a malaria vector in N-E States.

ACKNOWLEDGMENTS

Authors are thankful to the Indian Council of Medical Research, New Delhi for providing funds for this study under N-E Task Force. Thanks are also due to all field staff of NIMR for conducting the surveys and State Health Department for providing malaria epidemiological data of the district.

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Received 14 October, 2015; Accepted 11 April, 2016



Design and General Characteristics of Five Marine Gillnets of Mumbai Coast

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Abstract: Nets of different mesh sizes ranging from 14 to 150 mm operated to target different groups of fishes were prevalent in Mumbai coast. Synthetic materials like polyamide had almost completely replaced the natural fibre in gillnets Mumbai coast. Polyamide (PA) monofilament netting were used to target oil sardine, mackerel and while as PA multifilament were usually used to target seerfish, whitesardine and shrimp.

Key Words: Design, Gillnets, Mumbai coast

Maharashtra is one of the major marine states in India having 720 km long coastal line. With annual marine fish production of 1,43,157 tonnes during 2010-11 (Anon, 2010) There are 12,154 numbers of mechanised and 2,292 non-mechanised fishing units are in operation in the state with gillnet contributes 7.1% (Anon, 2013). Of the total traditional fishing gear in India 40% comprise gillnets. Among the traditional gear of Maharashtra, gillnets mostly surface drift nets of varying mesh sizes are predominant followed by bag nets and hooks and lines (Pillai and Rohit, 2003). Over a period of time, many need based changes have taken place in the netting material, design and operational aspects of gillnets. These are material substitutions, changes in method of operation, introduction of resource specific gear, motorisation and other sequent to this. Design and general characteristics of different types of gillnets operated from different maristates of India are well documented. Thomas and Hridayanathan (2002; 2006) and Kazi *et al.* (2011). Detailed information on gear systems is essential for their improvement in terms of efficiency and cost effectiveness, and for management of fisheries. The present paper deals with the design and operational details of various types of gillnets prevalent from Mumbai coast are communicated here.

MATERIAL AND METHODS

The study was conducted in three landing centres of Mumbai coast viz. Versova, Cuff Parade and Mahim during the period of December 2010 to November 2011. The data pertaining to the technical specifications, design characteristics, rigging and the mode of operation of important gillnets used by the fisherman was collected at weekly interval from minimum ten per cent of the operating

boats centre as per Thomas and Hridayanathan (2006). The design of the gear was documented following Nedelec (1975).

RESULTS AND DISCUSSION

A variety of different nets of mesh sizes starting from 14 to 150 mm were in operation. PA monofilament of diameter 0.16 and 0.23 mm were commonly used for oil sardine fishery locally known as *Taraliche jali* in Mumbai coast (Table 1 and Fig 1). The mesh sizes 32 and 34 mm were common. The hanging coefficient was between 0.53 to 0.65 with an average of 0.63. It was recorded that blue to white coloured gillnets were commonly used for sardine gillnets. Hung depth varied between 3.10 and 18.37 m. Non-motorised gillnetter units used nets of depth 3.10 to 5.25 m, in OBM gillnetter units depth of net ranged from 3.81 to 7.58, while as in the case of IBM gillnetter it ranged between 8.45-18.37 m. The total fleet length of the net ranged between 420 m and 1800 m with a mean of 720 m. Depth of operation of non-motorised vessels ranged between 3.0 and 12 m and that of OBM and IBM vessels ranged between 8 and 40 m.

The white sardine gillnets locally know as *Bhiljeche jali* operated by IBM and OBM crafts from Cuff Parade and Versova with mesh sizes ranging from 12 to 16 mm. (Table 1 and Fig 2). Variations were noticed in certain aspects such as dimensions of the net and meshes in depth from fishing fleet to fleet. Material extensively used for the main webbing of gillnet of the white sardine was PA multifilament of 210×1×2 with mesh size ranges between 12 to 16 mm. The hanging coefficient were between 0.53 – 0.56. It was observed that hung depth varied from 3.80 to 9.20 m. Generally nets of 450-1200 m length are taken for operation. Much variation was not observed with regard to the depth of nets operated from

OBM and IBM fishing vessels. Seerfish gillnets were operated as drifting gear at the surface or in the column waters are restricted to IBM gillnetters (Table 1 and Fig. 3). These nets were operated in column or surface depending on the swimming layer of the fish during different seasons. Seerfish gillnets were operated as surface drift gillnet during September to December and column drift gillnet during October to May. It was observed that seerfish gillnets were used as combined gillnets in which webbings of different mesh sizes were used in combination so that different size groups of same species could be captured. The polyamide (PA) multifilament twines were used in all the centres of study area for seerfish. Variations were seen with respect to the specifications of yarn generally depending on the mesh size. Multifilament yarn having specification of 210d×2×3 to 210d×10×3 were most commonly used according to the mesh size. The mesh size of seerfish gillnet generally ranged between 70 to 150 mm for main webbing. The hanging coefficient of the seerfish gillnet varied in between 0.43 to

0.56. Foot rope was totally absent in this type of gear. The green or brown coloured gillnets were most commonly used for seerfish gillnet operated from Mumbai coast. Total hung depth of the seerfish gillnets ranged from 7.70 to 14.75 m. The mean fleet length of the seerfish gillnet was 1550 m. Seerfish gillnet was mostly operated from IBM fishing vessels and depth of operation of gillnets ranged from 24 to 70 m.

Mackerel gillnets locally known as *Bangada jali* were commonly operated as drift gillnet from IBM, OBM and non-motorised crafts (Table 2 and Fig. 4). In all the selected landing centers where mackerel drift gillnets were operated, gillnets were made up of PA monofilament of diameter 0.16 to 0.23 mm. Much variation was not observed with regard to the mesh size of nets operated from different landing centres of Mumbai. The mesh size for the mackerel drift gillnets ranged from 48 to 56 mm. The hanging ratio of the drift type mackerel gillnet varies between 0.44 to 0.66. The hung length of the mackerel gillnets ranged from between 60 to 85 m with mean 72 m. The total hung depth of the drifting type of mackerel

Table 1. Technical specifications of oil sardine, white sardine and Seer fish gillnets in Mumbai coast

Station	Mumbai	Mumbai	Mumbai
Local name	<i>Taraliche jali</i>	<i>Bhiljeche jali</i>	<i>Surmaichi jali</i>
Main webbing mesh size (mm)	34 (32-36)	(12-16)	150
Twine type	PA mono.	PA multi	PA multi.
Twine specification/diameter (mm)	0.16-0.23	210×1×2	150
No. of meshes in depth	65	250	210×12×3
Hanging ratio (E)	0.52	0.58	75
No. of meshes per unit	2370	12000	0.41 – 0.54
Selvedge twine type	PA multi.	PE	860 - 1520
Selvedge specification / diameter (mm)	210×2×3	2	
Selvedge mesh size	65	80	PE
No. of selvedge meshes in depth	5	2	1.5
Selvedge hung depth (m)	0.20	0.41	150
Total hung depth (m)	15.5-18	9.20	1
Head rope material	pp	PP	0.13
Head rope diameter (mm)	4	3	10.36 – 14.56
Float material	PVC	PVC	pp
Float dimensions (mm)	60×20	45×25	65× 20
No. of floats per unit	55	118	15
Foot rope material	pp	PP	Nil
Foot rope diameter (mm)	4	3	Nil
Sinker material	lead	lead	Nil
Sinker weight (g)	30	35	Nil
No. of sinkers per unit	85	178	1515-2460
Total fleet length (m)	550--1100	800-1200	2460
Depth of operation (m)	18 to 40	9-18	55-70
Fishing craft	Wooden canoe, FRP	Wooden canoe	Wooden canoe
Horse power of engine (hp)	20- 24	20-50	20- 50

Table 2. Technical specifications of mackerel gillnet

Station	Versova			
Local name	<i>Bangadachi jali</i>			
Main webbing mesh size (mm)	48-56			
Twine type	PA mono.			
Twine specification/ diameter (mm)	0.23			
No. of meshes in depth	70-120			
Hanging coefficient (E)	0.44-0.60			
No. of meshes per unit	1240-1887			
Hung length (m)	60-85			
Hung depth (m)	4.5-6			
Colour webbing	Colourless			
Selvedge				
Twine type	Top		Bottom	
Selvedge specification-diameter (mm)	PE	PA multi	PE	PA multi
Selvedge mesh size (mm)	1-1.5	210×2×3	1-1.5	210×2×3
No. of selvedge meshes in depth	45-60	45-60	45-60	45-60
Selvedge hung depth (m)	6-11	3-5	6-11	2-5
Head rope material	0.19-0.60	0.14-0.24	0.19-0.60	0.07-0.24
Head rope diameter (mm)	PP			
Float material	4-6			
Float dimensions (mm)	PVC			
No. of floats per unit	55×15/95×25			
Foot rope material	30-60			
Foot rope diameter (mm)	PP			
Sinker material	3-5			
Sinker weight (g)	Lead			
Total fleet length (m)	200-250			
Depth of operation (m)	410-960			
Fishing craft	5-18			
Horse power of the engine (hp)	Wooden canoe and FRP coated.			
	9.9			

gillnet ranged between 4.50 to 6.0 m. The fleet length of drift mackerel gillnet ranged from 350 to 840 m with mean of 648. It was observed that, mackerel drift gillnets were mostly operated at the surface, mid column and close to the bottom according to the season and behaviour of the target species. The depth of operation for drift gillnet for mackerel ranged from 5 to 22 m.

Trammel nets are three layered nets and are locally known as *Shahehshah Jali* in Mumbai (Table 3 and Fig. 5). These nets are mainly used to catch the shrimps. The materials for inner small panel was exclusively of PA monofilament of 0.16 mm diameter and for outer large mesh panel was of PA multifilament of 210d×2×3. The mesh size used ranged between 45 to 48 mm with mean of 46.16 mm for inner small mesh panel and mesh size of 250 to 280 mm with mean of 268 mm for the two outer panels. Hanging coefficient of the outer panels, which were tightly hung ranged between 0.61 and 0.72. The average hanging coefficient of loosely

hung small meshed inner wall was 0.46. The hung length for each unit of shrimp trammel net varied from 3.16 to 61.82 m with mean of 47.24 m. Total hung depth of shrimp trammel nets ranged from 5.40 to 6.61 m with mean of 5.97. The mean fleet length for shrimp trammel net was 349.12 m. The size of the fleet was determined by the size and capacity of gillnetter and the financial condition of the fisherman. Trammel nets were generally operated as bottom set nets mostly near the shoreline. The depth of operation ranged between 6 and 16 m. PP rope of 3-4 mm diameter was most commonly used for head rope, foot rope, which was present at both upper and lower side of the main webbing of shrimp trammel net. Marker buoys were attached at either end, for identifying location of the gear. It was observed that 4 to 14 numbers of units were joined end to end by making knots at head rope, foot rope and seaming at regular interval throughout the depth of net to form a netting fleet.

The fishing operation was done for targeting a

particular species. Polyamide monofilament gillnets of diameter 0.16 – 0.23 mm with mesh size ranges between 32-36 and 48-56 mm were especially used to catch sardines and mackerel, respectively. Polyamide multifilament gillnets with twine specification of 210d×2×3 to 210d×12×3 mesh size ranges between 70-150 mm were operated for seerfish while as Polyamide multifilament of 210×1×2 with mesh size 14 – 16 mm were especially operated for white sardine. Mesh size varies by type of net because different mesh sizes can catch different species according to their seasonal variability. Reduction in mesh size was been observed in monofilament gillnets mackerel and multifilament seerfish gillnets adversely affect the juvenile and sub-adults of target species.

Among the numerous types of gillnets the sardine gillnets has retained its efficiency operations along Indian

coast and accounts 90% of the sardine catch along east coast (Pillai and Rohit, 2003). Ramarao *et al.* (2002) observed that the use of polyamide multifilament gillnets of small mesh sizes (25-30 mm) in commercial fisheries resulted large quantities of juvenile sardines.

Trammel net or the three walled gillnet, locally known as Shahenshah jali in Mumbai has got wide acceptance as an efficient gear along Indian coast in recent years. This net designed for setting at the bottom is fabricated in such a way that it has a fine net of smaller mesh hung loosely between vertical walls of coarser net of much larger meshes. Mesh sizes of the trammel nets operated along Vishakhapatnam coast have drastically reduced from 50 mm to 20 mm for the inner netting and from 250 mm to 100 mm for the outer netting (Ramarao *et al.*, 2002). Despite the fact that

Table 3. Technical specifications of shrimp bottom set gillnet (trammel net) in different centres of Mumbai coast

Station	Mahim		Versova	
Local name	Shahenshah		Shahenshah	
Webbing	Inner	Outer	Inner	Outer
Mesh size (mm)	45	250-270	45	260-270
Hanging ratio	0.46	0.65	0.46-0.48	0.62-0.65
Twine type	PA mono	PA multi	PA mono	PA multi
Twine specification /diameter (mm)	0.16	210×2×3	0.16	210×2×3
No. of meshes in depth	125	18	125-150	18-20
No. of meshes per unit	1500-1880	156-212	1600-2800	200-280
Hung length (m)	31.64- 48.13		43.81-57.96	
Hung depth (m)	4.00-6		4.5-7.5	
Colour of webbing	Colourless		Colourless	
Selvedge twine type	PA multi.		PA multi.	
Selvedge specification / diameter (mm)	210×2×3		210×2×3	
Selvedge mesh size (mm)	46		46	
No. of selvedge meshes in depth	5		5	
Selvedge hung depth (m)	0.20		0.20	
Total hung depth (m)	5.40-5.57		5.40-6.54	
Head rope material	PP		PP	
Head rope material (mm)	3		3	
Float material	PVC		PVC	
Float dimensions (mm)	45×10		45×10	
No. of floats per unit	60-70		50-70	
Foot rope material (mm)	PP		PP	
Foot rope diameter (mm)	3		3	
Sinker material	Lead		Lead	
Sinker weight (g)	20-23		23-25	
No. of sinker per unit	70-80		60-80	
Total fleet length (m)	240-360		260-750	
Depth of operation (m)	Upto 12		12-18	
Fishing craft	Dug-out canoe		Wooden boat without rigger, Wooden and FRP.	
Horse power of the engine (hp)	Nil		6.6-9.9	

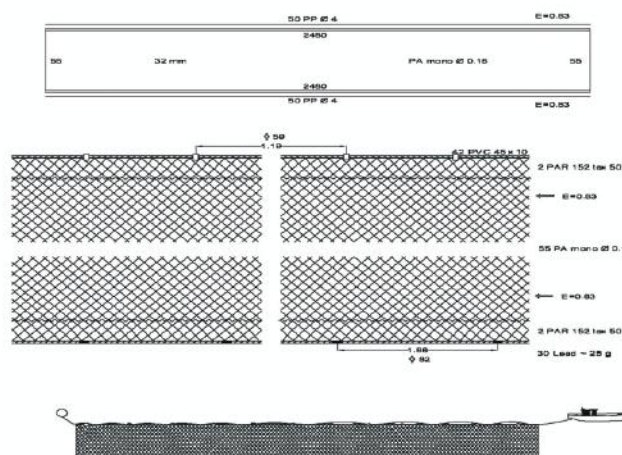
Gillnet
Sardine
Versova (Mumbai)

Fig. 1. Design of gillnet - Sardine

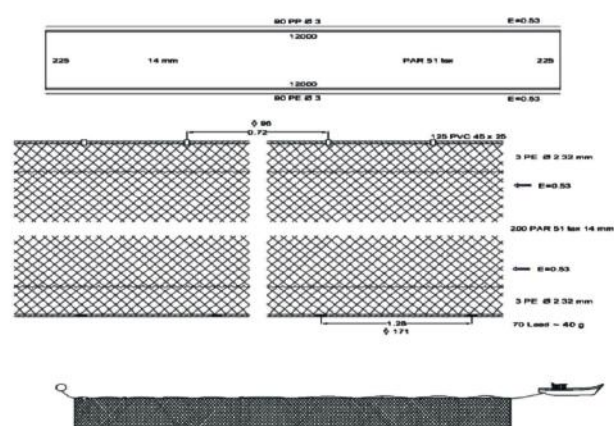
Gillnet
White sardine
Versova (Mumbai)

Fig. 2. Design of gillnet - White sardine

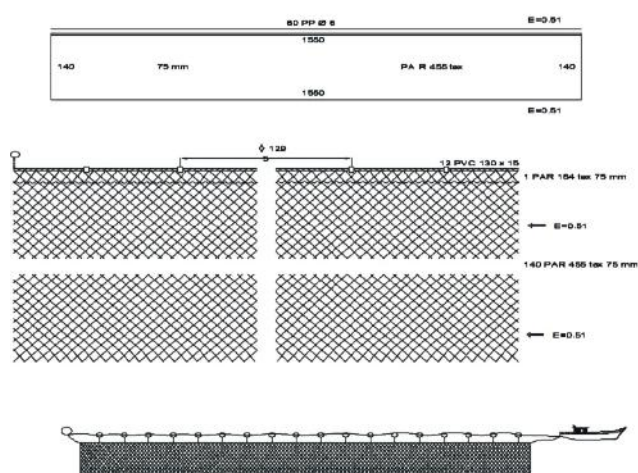
Gillnet
Seerfish
Cuff Parade (Mumbai)

Fig. 3. Design of gillnet – Seerfish

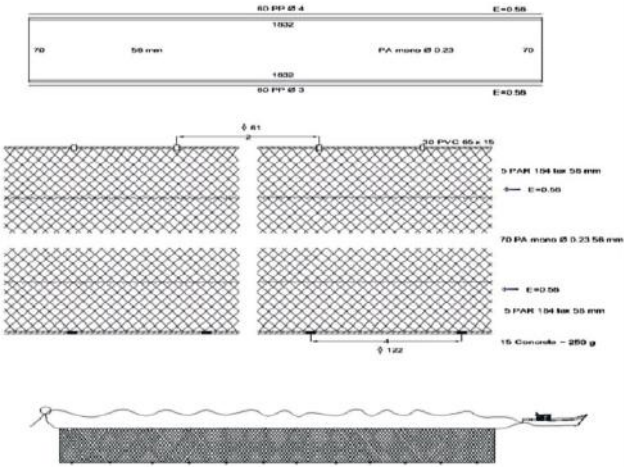
Gillnet
Mackerel
Versova (Mumbai)

Fig. 4. Design of gillnet - Mackerel

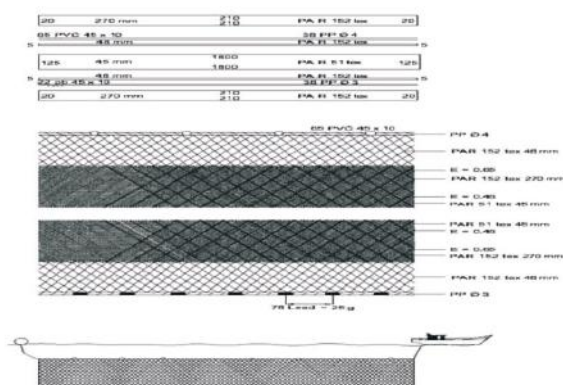
Trawl net
Shrimp
Versova (Mumbai)

Fig. 5. Design of gillnet - Mackerel

there are currently no minimum size regulations for commercial fisheries in Mumbai coast. The present study indicated that PA monofilament was extensively used for oil sardine and mackerel gillnets, because of having very less weight and better catching efficiency compared to multifilament, which will minimize the energy required for operation and ease of lifting the net and minimising number of people required for the operations. However, the use of multifilament gillnets is still in practice for seer and white sardine fishery. Similar results were recorded by (Pravin and Remesan, 2000; Thomas and Hridayanathan, 2002; Ramarao *et al.*, 2002; Thomas *et al.*, 2005). Recommendations on minimum or maximum mesh size could thus be effective tool to enhance juvenile and sub adults stocks.

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Temporal Variation of Phytoplankton in Idukki Reservoir, Kerala

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Abstract: Phytoplankton diversity of Idukki reservoir during there seasons (pre-monsoon, monsoon and post-monsoon) was studied for a period of three years (2007-2010). Samples were collected from the surface area of reservoir on bimonthly basis from 16 stations. Five groups such as Cyanophyceae (Blue-green algae), Chlorophyceae (Green algae), Bacillariophyceae (Diatoms), Dinophyceae (Dinoflagellates) and Desmids represented the phytoplankton community in Idukki reservoir. A total of 37 species, belonging to 31 genera were found in the phytoplankton groups. Phytoplankton was more prevalent during the pre-monsoon season (747.3 l^{-1}) followed by post-monsoon season (662.56 l^{-1}) and monsoon season (217.51 l^{-1}), respectively. Significant variations in the total phytoplankton obtained in different seasons and stations studied. During pre-monsoon season, Chlorophyceae was found to be the dominant group (275.30 l^{-1}) which constituted 36.82% of the total phytoplankton. A total of 30 phytoplankton species were recorded in this season and the most dominant species was *Ankistrodesmus falcatus* (18.57%). During monsoon season, except Dinophyceae all other groups were present under 8 species. In post-monsoon season, all the 5 groups were recorded in the reservoir and Chlorophyceae, was found to be the dominant group (234.77 l^{-1}) accounted for about 35.43% of the total phytoplankton.

Key Words: Phytoplankton composition, Seasonal changes, Fresh water reservoir, Kerala

Phytoplankton are tiny, free-floating, photosynthetic organisms in aquatic systems. These microscopic organisms inhabit the upper sunlit layer of almost all oceans and fresh water bodies. They are agents for 'primary production', which results in accumulation of organic compounds from carbon dioxide dissolved in the water, a process that sustains the aquatic food web (Ghosal *et al.*, 2011). Reservoirs are considered favourable environments to the development of plankton communities, which may establish diverse assemblages in relatively short periods of time after impoundment (Rocha *et al.*, 1999). Several factors usually contribute to the establishment of plankton communities in a reservoir, among which good water quality, presence of nutrients, physico-chemical factors of water, hydrological characteristics of the reservoirs and reservoir ageing are important ones.

Phytoplankton are usually at the base of aquatic food chain and are the most important factor for production of organic matter in an aquatic ecosystem. Most reservoirs will require significant amount of plankton to have productive and sustainable fisheries. The importance of plankton in tropical reservoir ecosystems include its use in estimating potential fish yield (Hecky and Kling, 1981), productivity (Park *et al.*, 2003), water quality (Walsh *et al.*, 2001), energy flow (Simcic, 2005), trophic status (Reynolds, 1999) and management (Beyruth, 2000). Weiss and Stockner (1993) observed that energy in pelagic ecosystems flows from phytoplankton, the primary producers to zooplankton, the consumers through the classical food chains and microbial food webs.

A pre-impoundment survey of the fauna and flora of any water body is necessary because it provides a checklist of organisms present in the water for subsequent exploitation, conservation and sustainable management of the resources. In India, the study on phytoplankton especially their density and diversity and their association as biological indicators in the assessment of water quality or trophic status has been made by several workers (Ingole *et al.*, 2010; Gayathri *et al.*, 2011; Nagabhushan and Hosetti, 2012). The present study was undertaken to investigate the phytoplankton variation in three seasons ie, pre-monsoon, monsoon and post-monsoon in Idukki reservoir.

MATERIAL AND METHODS

The Idukki reservoir is roughly 'V' shaped, situated at an altitude of 693 m above sea level (Fig 1). When full it have a water spread area of 23.1 sq. miles (59.83 sq.km) with depth varying from 50 to 80 m and this storage regulates the yield of over 250.7 sq.miles (1649.3 sq.km) of Periyar and Cheruthoni catchments. The Idukki hydro-electric project encompasses 3 dams. They are Idukki arch dam ($09^{\circ} 50' 30'' \text{N}$ latitude and $76^{\circ} 58' 40'' \text{E}$ longitude) across Periyar, the largest river in Kerala, at Idukki gorge; Cheruthoni a straight concrete gravity dam ($09^{\circ} 50' 48'' \text{N}$ latitude and $76^{\circ} 58' 00'' \text{E}$ longitude) across the tributary River Cheruthoni and Kulamavu dam ($09^{\circ} 47' 15'' \text{N}$ latitude and $76^{\circ} 51' 20'' \text{E}$ longitude) across Kilivallithodu.

For the analysis of seasonal variation of phytoplankton, 40 litre of the surface water (< 0.5m) samples

were taken from each stations, bimonthly over a period of 3 years from February 2007 to January 2010. Phytoplankton was collected by filtering water through plankton net made up of bolting silk (No: 25, Mesh size 40 μ m). The final volume of the filtered sample was 100 ml which was transferred to plastic bottle and labelled mentioning the time, date and place of sampling. The samples were preserved on the field with 2 ml of 4% formalin and added Lugol's iodine solution @1ml/100 ml of sample to arrest cell activity, for sedimentation and better staining. The sample bottles were then transported to the laboratory for plankton analysis and the preserved sample was reduced to 10 ml. 1ml was pipetted out from the 10 ml (after it has been shaken) and qualitative and quantitative estimation of phytoplankton was carried out with the help of 'Sedgwick Rafter' counting cell under an optical microscope (100x magnifications) (Trivedi and Goel, 1984). The systematic identification of the phytoplankton up to the level of species was done adopting the standard keys of Desikachary (1959), Edmondson (1959), Whiteford and Schumacher (1973), Palmer (1980) and Anand (1998). Average of 10 replicates for each sample was taken into account and the density of phytoplankton was expressed in number of organisms per litre.

RESULTS AND DISCUSSION

A total phytoplankton number of 1627.37 l⁻¹ was recorded in the reservoir (Table 1). Five groups of phytoplankton namely, Cyanophyceae (Blue-green algae), Chlorophyceae (Green algae), Bacillariophyceae (Diatoms), Dinophyceae (Dinoflagellates) and Desmids were recorded in the reservoir. A total of 37 species, belonging to 31 genera were found in the phytoplankton groups with Chlorophyceae and Bacillariophyceae with highest population in 11 species each, while Cyanophyceae, Dinophyceae and Desmids had 5, 2 and 8 species each respectively (Table 1). Chlorophyceae (590.15 l⁻¹) dominated the phytoplankton population constituting 36.26% of the total phytoplankton. The next dominant group was Bacillariophyceae (489.03 l⁻¹) which made up 30.05% and this was distantly followed by the Desmids (322.03 l⁻¹) that comprised of 19.79%, Cyanophyceae (165.25 l⁻¹) with 10.15% and Dinophyceae (60.91 l⁻¹) constituted only 3.74% of the phytoplankton population (Table 1), respectively.

The results of three years study showed that phytoplankton was abundant in the reservoir during pre-monsoon season followed by post-monsoon and monsoon season respectively (Fig. 2). ANOVA showed significant variations in the total phytoplankton obtained in different seasons and stations ($P < 0.05$). During pre-monsoon season, Chlorophyceae showed total number of 275.30 l⁻¹ followed by

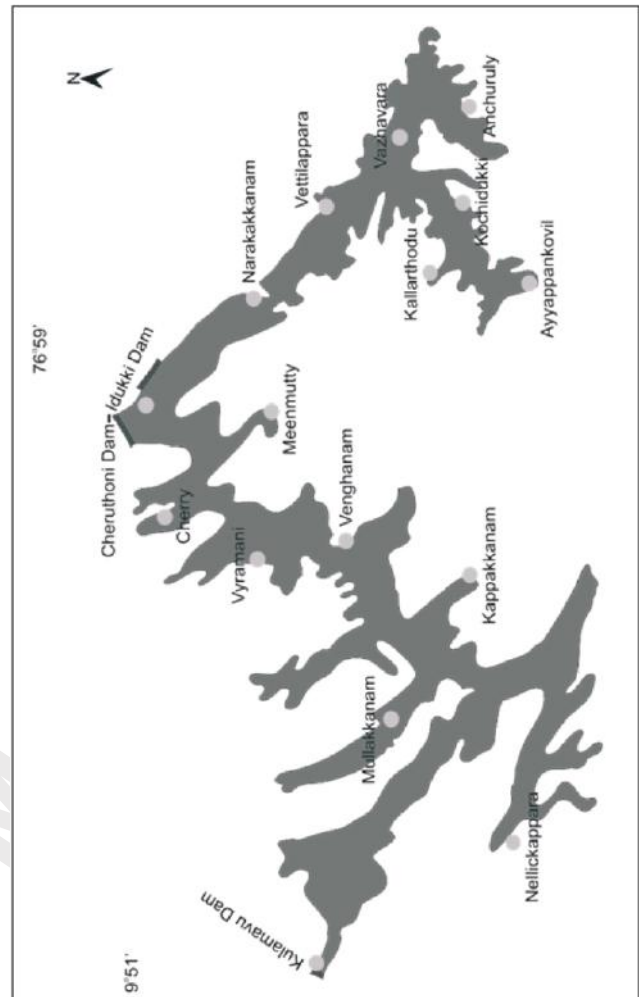


Fig. 1. Map showing different sampling stations in Idukki reservoir

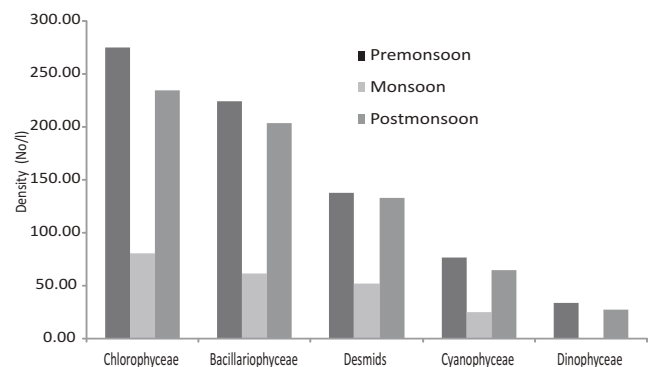


Fig. 2. Seasonal distribution (mean) of phytoplankton groups in Idukki reservoir

Bacillariophyceae (224.47 l⁻¹), Desmids (137.7 l⁻¹) Cyanophyceae (76.05 l⁻¹) and Dinophyceae (33.78 l⁻¹) respectively (Fig.2). Monsoon season also showed the similar pattern in the variation of phytoplankton groups, where Chlorophyceae recorded the highest number (80.08 l⁻¹) followed by Bacillariophyceae (61.15 l⁻¹), Desmids (51.7 l⁻¹) and

Table 1. Groupwise composition of phytoplankton (mean) in Idukki reservoir (No. l⁻¹)

Group/species	Total (no/l)	% (total)	% (Groupwise)
I. Cyanophyceae			
<i>Aphanocapsa</i> sp.	35.71	2.19	21.61
<i>Croococcusturgidus</i>	9.77	0.60	5.91
<i>Oscillatoria</i> sp.	97.86	6.01	59.22
<i>Nostoclinckia</i>	16.82	1.03	10.18
<i>Anabaena sphaerica</i>	5.09	0.31	3.08
Total	165.25	10.15	100.00
II. Chlorophyceae			
<i>Desmidiumquadratum</i>	24.98	1.53	4.23
<i>Pediastrum duplex</i>	7.41	0.46	1.26
<i>Arthrodesmusmenoides</i>	9.08	0.56	1.54
<i>Ankistrodesmusfalcatus</i>	285.44	17.54	48.37
<i>Spirogyra</i> sp.	6.27	0.39	1.06
<i>Coelastrummicroporum</i>	64.64	3.97	10.95
<i>Chlorella vulgaris</i>	114.58	7.04	19.42
<i>Tetraedronproteiforme</i>	4.16	0.26	0.70
<i>Clamydomonas</i> sp.	8.32	0.51	1.41
<i>Ulothrixzonata</i>	36.01	2.21	6.10
<i>Rhizoglonium</i> sp.	29.28	1.80	4.96
Total	590.15	36.26	100.00
III. Bacillariophyceae			
<i>Fragilariavirescens</i>	11.09	0.68	2.27
<i>Naviculacuspadata</i>	86.78	5.33	17.75
<i>Navicula</i> sp.	10.16	0.62	2.08
<i>Melosiragranulata</i>	95.15	5.85	19.46
<i>Cyclotellabodanica</i>	139.29	8.56	28.48
<i>Cyclotellameneghiniana</i>	12.15	0.75	2.48
<i>Pinnulariaacrosphaeria</i>	38.07	2.34	7.78
<i>Amphora ovalis</i>	44.06	2.71	9.01
<i>Diatomavulgare</i>	12.13	0.75	2.48
<i>Stauroneisphoenicenteron</i>	34.31	2.11	7.02
<i>Asterionellaformosa</i>	5.84	0.36	1.19
Total	489.03	30.05	100.00
IV. Dinophyceae			
<i>Ceratiumhirudinella</i>	42.22	2.59	69.32
<i>Peridiniumcinctum</i>	18.69	1.15	30.68
Total	60.91	3.74	100.00
V. Desmids			
<i>Euastrumansatum</i>	9.43	0.58	2.93
<i>Closterium</i> sp.	16.63	1.02	5.16
<i>Staurastrumdentatum</i>	80.81	4.97	25.09
<i>Staurastrumtohopekaligen</i>	157.01	9.65	48.76
<i>Staurastrum</i> sp.	11.60	0.71	3.60
<i>Cosmariumsupergranatum</i>	27.60	1.70	8.57
<i>Cosmariumviridis</i>	9.46	0.58	2.94
<i>Cosmariumsubtumidum</i>	9.49	0.58	2.95
Total	322.03	19.79	100.00
Total Phytoplankton	1627.37	100.00	

Cyanophyceae (24.58 l⁻¹), respectively. But Dinophyceae were completely absent (Fig. 2). During post-monsoon season too, Chlorophyceae registered the highest number (234.77 l⁻¹) followed by Bacillariophyceae (203.41 l⁻¹), Desmids 132.63 l⁻¹, Cyanophyceae 64.62 l⁻¹ and Dinophyceae 27.13 l⁻¹ (Fig.2).

In post-monsoon season, all the 5 groups were recorded in the reservoir, with 11 species of Chlorophyceae. *Ankistrodesmus falcatus* was found to be the most abundant species during three seasons with highest population in pre-monsoon season (138.78 l⁻¹). Seven Chlorophyceae species were recorded during pre-monsoon season, whereas only two species i.e. *Ankistrodesmus falcatus* (57.03 l⁻¹) and *Chlorella vulgaris* (23.05 l⁻¹) were present during monsoon season. Bacillariophyceae was represented by eleven species during post-monsoon season followed by ten species in pre-monsoon and three species during monsoon season. *Cyclotella bodanica*, *Melosira granulate* and *Navicula cuspidate* are the three major species recorded in all season in the Bacillariophyceae group where *Cyclotella bodanica* was present in highest number (65.66 l⁻¹-contributing 8.79% of the total phytoplankton) during pre-monsoon season (Table 2). Among Desmids, two species i.e. *Staurastrum dentatum* and *Staurastrum tohopekaligense* were observed during three seasons with highest quantities during pre-monsoon season (Table 2). *Oscillatoria* sp. showed its presence in three seasons among Cyanophyceae with highest population (41.83 l⁻¹) during pre-monsoon season. Dinophyceae showed the presence of two species with *Ceratium hirudinella* recorded the highest during pre-monsoon season.

Tropical reservoirs are generally characterised by rich population of phytoplankton (Sreenivasan, 1964) with, peaks of plankton abundance observed at different periods in different years. Ei-Gindy and Dorghan (1992) too stated that phytoplankton is variable in different seasons and regions. The phytoplankton population in Idukki reservoir was considerably low during monsoon season and this could be due to the heavy rain fall, excessive flooding, increased water volume, cloudy weather, low transparency, low temperature and low pH. The factors such as cloudy weather, low transparency, heavy flood (Pundhir and Rana, 2002; Rajagopal *et al.*, 2010), increase in water volume, high turbidity, high rain fall dilution, over flooding (Bohra and Kumar, 2004; Verma and Singh, 2010), cool conditions, decreased temperature and low pH (Rajkumar *et al.*, 2009) resulted in decline in phytoplankton population during monsoon season.

The phytoplankton abundance during summer (pre-monsoon) season in Idukki reservoir could be attributed to the prolonged day length, high intensity of light penetration,

Table 2. Seasonal variation (mean) and percentage composition of phytoplankton (No l⁻¹) in Idukki reservoir

Group/Species	Premonsoon		Monsoon		Postmonsoon	
	Average	%	Average	%	Average	%
I. Cyanophyceae						
1. <i>Aphanocapsa</i> sp.	22.79	3.05	0.00	0.00	12.92	1.95
2. <i>Croococcusturgidus</i>	0.00	0.00	0.00	0.00	9.77	1.47
3. <i>Oscillatoria</i> sp.	41.83	5.60	24.58	11.30	31.45	4.75
4. <i>Nostoclinckia</i>	6.33	0.85	0.00	0.00	10.49	1.58
5. <i>Anabaena sphaerica</i>	5.09	0.68	0.00	0.00	0.00	0.00
II. Chlorophyceae						
1. <i>Desmidiumquadratum</i>	11.17	1.49	0.00	0.00	13.8	2.08
2. <i>Pediastrum duplex</i>	0.00	0.00	0.00	0.00	7.41	1.12
3. <i>Arthrodesmusmenoides</i>	0.00	0.00	0.00	0.00	9.08	1.37
4. <i>Ankistrodesmusfalcatus</i>	138.78	18.57	57.03	26.22	89.63	13.53
5. <i>Spirogyra</i> sp.	1.05	0.14	0.00	0.00	5.22	0.79
6. <i>Coelastrummicroporum</i>	35.64	4.77	0.00	0.00	29.00	4.38
7. <i>Chlorella vulgaris</i>	53.99	7.22	23.05	10.60	37.54	5.67
8. <i>Tetraedronproteiforme</i>	0.00	0.00	0.00	0.00	4.16	0.63
9. <i>Clamydomonas</i> sp.	0.00	0.00	0.00	0.00	8.32	1.26
10. <i>Ulothrixzonata</i>	19.78	2.65	0.00	0.00	16.23	2.45
11. <i>Rhizoglonium</i> sp.	14.88	1.99	0.00	0.00	14.39	2.17
III. Bacillariophyceae						
1. <i>Fragilariavirescens</i>	3.82	0.51	0.00	0.00	7.27	1.10
2. <i>Naviculacuspadata</i>	38.36	5.13	16.14	7.42	32.28	4.87
3. <i>Navicula</i> sp.	5.93	0.79	0.00	0.00	4.23	0.64
4. <i>Melosiragranulata</i>	42.06	5.63	16.71	7.68	36.38	5.49
5. <i>Cyclotellabodanica</i>	65.66	8.79	28.3	13.01	45.33	6.84
6. <i>Cyclotellameneghiniana</i>	1.3	0.17	0.00	0.00	10.85	1.64
7. <i>Pinnulariaacrosphaeria</i>	20.18	2.70	0.00	0.00	17.88	2.70
8. <i>Amphora ovalis</i>	23.12	3.09	0.00	0.00	20.95	3.16
9. <i>Diatomavulgare</i>	4.98	0.67	0.00	0.00	7.16	1.08
10. <i>Stauroneisphoenicenteron</i>	19.06	2.55	0.00	0.00	15.25	2.30
11. <i>Asterionellaformosa</i>	0.00	0.00	0.00	0.00	5.84	0.88
IV. Dinophyceae						
1. <i>Ceratiumhirudinella</i>	22.56	3.02	0.00	0.00	19.66	2.97
2. <i>Peridiniumcinctum</i>	11.22	1.50	0.00	0.00	7.47	1.13
V. Desmids						
1. <i>Euastrumansatum</i>	1.62	0.22	0.00	0.00	7.8	1.18
2. <i>Closterium</i> sp.	6.34	0.85	0.00	0.00	10.29	1.55
3. <i>Staurastrumdentatum</i>	34.62	4.63	18.81	8.65	27.38	4.13
4. <i>Staurastrumtohopekaligense</i>	72.5	9.70	32.89	15.12	51.62	7.79
5. <i>Staurastrum</i> sp.	7.32	0.98	0.00	0.00	4.28	0.65
6. <i>Cosmariumsupergranatum</i>	12.7	1.70	0.00	0.00	14.9	2.25
7. <i>Cosmariumviridis</i>	2.59	0.35	0.00	0.00	6.86	1.04
8. <i>Cosmariumsubtumidum</i>	0.00	0.00	0.00	0.00	9.49	1.43
Total Phytoplankton	747.3	100.00	217.51	100.00	662.56	100.00

increased pH, high temperature, low water level and low rainfall. Hujare (2005) and Mohan *et al.* (2009) also made similar findings in Hatkanangale Tahsil (Maharashtra) and Pechiparai reservoirs (Tamil Nadu) respectively. According to Bais and Agarwal (1990), Pulle and Khan (2003); Sivakumar and Karuppasamy (2008), high pH, CO₂, alkalinity, and the dissolved nutrients are important factors responsible for the higher population of phytoplankton. High phytoplankton production during summer season in reservoirs and lakes were also reported by many workers like Wojciechowska *et al.* (2007) Senthilkumar and Sivakumar (2008) and Laskar and Gupta (2009). Khatri (1987) studied the standing crop of phytoplankton in Idukki reservoir and reported extremely poor phytoplankton density (maximum density of $175 \times 10^2 \text{ l}^{-1}$) and diversity, which can be attributed to poor concentration of nutrients in the reservoir.

ACKNOWLEDGEMENTS

Authors extend sincere gratitude towards the Principal St. Berchmans College, Changanassery, Kotayam, Kerala, India for providing all facilities to carry out this work. Thanks are also due to Kerala State electricity Board (KSEB) and faculties of Research and Dam safety subdivision KSEB Cheruthoni, Idukki for providing necessary infrastructure and facilities at the reservoir site for this study.

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Received 2 January, 2016; Accepted 17 May, 2016



Plant Biodiversity of Major Forest Communities in Chail Wildlife Sanctuary of Himachal Pradesh

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Abstract: The study was carried out in Solan district of Himachal Pradesh to assess plant diversity of major forest communities (Chir forest, Oak forest, Deodar + Oak forest, Chir + Oak forest, Deodar forest, Deodar + Chir forest and Mixed forest) of Chail Wildlife Sanctuary. There existed six tree species, 25 shrub species and 67 herb species of 42 families. Oak tree species had higher IVI in all the associated forest. *Rhododendron arboreum*, *Pinus wallichiana* and *Pyrus pashia* had lowest IVI in their community types, hence needs attention. Chir + oak and mixed forest communities were rich in shrub diversity, dominance and richness as compared to other forest communities. Herbaceous diversity, dominance and richness were higher in Chir, mixed and Chir + oak forest communities compared to others, whereas, species richness was maximum in Deodar community.

Key Words: Biodiversity, Coniferous forest, Species richness, Wildlife sanctuary

The Indian Himalayan Region is known for its diverse, unique, natural, and socio-economically important flora and fauna. This rich biodiversity is being utilized by the inhabitants of the region for medicine, food (wild edible), fodder, fuel, timber, agriculture tools, religious and various other purposes. With the increasing human population, the demand for the economically important biodiversity is increasing. Collection of fodder and fuel species from the forests has been identified as one of the chronic problems in the Indian Himalayan region (Samant *et al.*, 2000, 2006). The anthropogenic pressures including heavy grazing coupled with the natural calamities have led to degradation of natural habitats of many species. Such practices are discouraging the moisture loving species and promoting the hardy and spiny species having little value for the society (Samant *et al.*, 2000). This loss of biodiversity and changing pattern of vegetation has necessitated assessing the vegetation composition of tree species of this region.

The State of Himachal Pradesh (geographical area 55,673 km²) comprises the part of Trans and northwest Himalaya that supports a unique biodiversity. Of the total geographical area, 66.45 % is under forests. Protected forests comprise 59.3 % and reserve forests 3.41 %. The state supports 32 Wildlife Sanctuaries, two National Parks and one Biosphere Reserve. The Chail Wildlife Sanctuary lies in Kandaghat Sub-Division of Solan district and part of this also falls under the jurisdiction of Shimla, Sub Division of Shimla District. Total area of the sanctuary is 10,853.16 ha. The whole area of sanctuary was earlier a territorial forest range and, the government declared the intention to convert it into wildlife sanctuary on 21st March 1976. The sanctuary

has been finally notified vide Government of Himachal Pradesh Notification No. FFE-B-F (96)-25/99 dated 03/10/1999. The study area although a reserved area is liable to different kind of biotic as well as abiotic interferences, which include grazing, human encroachment and other anthropogenic pressures. It becomes very imperative to collect knowledge on the biodiversity and its distribution within the ecologically important and sensitive area for proper conservation and better management of the natural resources. Therefore, the study is proposed with objective to account plant biodiversity of different forest communities of Chail Wildlife Sanctuary.

MATERIAL AND METHODS

The major forest species that represent the Chail Wildlife Sanctuary are Chir (*Pinus roxburghii*), Deodar (*Cedrus deodara*), Kail (*Pinus wallichiana*), Oak (*Quercus leucotricophora*) and Burash (*Rhododendron arboreum*) and Kainth (*Pyrus pashia*). These species occurs either pure or mixed with other important or associated species. Based on species dominance, seven forest communities were selected viz. Chir forest, Chir + Oak forest, pure Deodar forest, Deodar + Chir forest, Deodar + Oak forest, pure Oak forest and mixed forest. In each forest community, 10 quadrats of 10m x 10m (100 sq m) size were randomly laid to study tree species. The tree species includes all the saplings, poles and trees present in the study area. The shrub and herbaceous species were studied by laying ten quadrats randomly in each forest community. The relative basal area, relative density and relative frequency were computed and then Importance Value Index (IVI) for each forest community type was

calculated. The species richness (Magurran, 1988), species diversity (Shannon-weaver, 1963) and species dominance Simpson (1949) were determined. In each quadrat, a sub-quadrat of 5m x 5m (25 sq m) size for shrubs and a sub-quadrat of 1m x 1m (1sq m) size for herbaceous vegetation were selected.

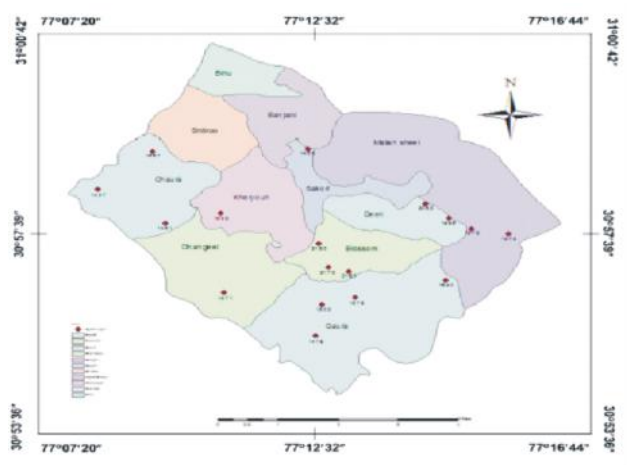


Fig.1. Map of Chail Wildlife Sanctuary

RESULTS AND DISCUSSION

Floristic composition of tree layer: The density ranged from 710 to 1470 ha^{-1} and basal area from 28.22 to 85.23 $\text{m}^2 \text{ha}^{-1}$ among different forest community types of Chail Wildlife Sanctuary (Table 1). In several temperate forests, the value of total basal area and density ranged from 0.15-0.60 $\text{m}^2 100 \text{m}^{-2}$ and 3.2-20.80 $\text{tree} 100 \text{m}^{-2}$, respectively (Pande *et al.*, 1996; Bhandari and Tewari, 1997) which was also found at par with tree density recorded by Lal *et al.* (2015) i.e. 520 ha^{-1} to 990 ha^{-1} in the dry deciduous forests of central India. The value for density and basal area of different forest community in the present study are well within reported range of temperate forest.

Among seven major forest communities, Oak forest community showed highest density (1470 ha^{-1}) as compared to Mixed (1070 ha^{-1}), Deodar + Oak (980 ha^{-1}), Chir + Oak (940 ha^{-1}), Deodar (760 ha^{-1}), Chir (720 ha^{-1}), Deodar + Chir (710 ha^{-1}) forest community (Table 1). The higher density in former four forest communities may be explained in the light of higher proportion of young crop of the oak component i.e. 1470 ha^{-1} in Oak, 590 ha^{-1} in Mixed forest community, 640 ha^{-1} in Deodar + Oak and 670 ha^{-1} in Chir + Oak forest community types. The results are in agreement with the findings of Singh and Gupta (2009) and Devlal and Sharma (2010), who has reported highest density for oak in various sites among all species. The rest of the forest communities i.e., Chir forest (720 ha^{-1}) and Deodar + Chir forest (710 ha^{-1}) had older trees

(Table 1). Mir *et al.* (2011) has reported 240 Individuals ha^{-1} , 11.11-36.37 ha^{-1} and 236.37-333.33 ha^{-1} at different sites for *Pinus roxburghii*, *Quercus leucotrichophora* and *Cedrus deodara*, respectively in Chaupal Forest Division of Himachal Pradesh. The results are in agreement with Devlal and Sharma (2008) that had shown dominance of oak in among other species along altitudinal gradient in the temperate forest of Narayanbagar of Chamoli district of Uttarakhand.

However, basal area was highest in Deodar forest community (85.23 $\text{m}^2 \text{ha}^{-1}$) as compared to Mixed (55.96 $\text{m}^2 \text{ha}^{-1}$), Oak (46.87 $\text{m}^2 \text{ha}^{-1}$), Chir (44.65 $\text{m}^2 \text{ha}^{-1}$), Deodar + Chir (35.09 $\text{m}^2 \text{ha}^{-1}$) and Chir + Oak (30.13 $\text{m}^2 \text{ha}^{-1}$) and Deodar + Oak (28.22 $\text{m}^2 \text{ha}^{-1}$) forest community. The results are in agreement with the findings of Ahmed *et al.* (2011) who had reported highest density and basal area for Deodar forest compared to other forest communities in all the sites in Hind Kush and Himalayan ranges of Pakistan. On the same line Singhal *et al.* (1986) reported dominance of Deodar in regards to density and basal area in Kanasar Range of Chakrata Forest Division of Uttarakhand. Singh and Gupta (2009) also recorded higher density and basal area in all the periodic blocks of Koti Forest Range in Shimla Forest Division of Himachal Pradesh. However, Mir *et al.* (2011) reported a basal area of 34.07, 0.84-8.74 and 41.56-240, for *Pinus roxburghii*, *Quercus leucotrichophora* and *Cedrus deodara*, respectively in different sites of Chaupal Forest Division in Himachal Pradesh. This distribution pattern of basal area in different forest communities may be attributed to the dominance of one species over other species in their respective habitat. In general, Deodar and Chir had older crop in their habitat, whereas, their performance in the transition zone was not as usual as seen in their habitat. Percent frequency ranged between 100 to 300 with maximum in mixed forest community and minimum in both pure Oak and pure Deodar forest community. However, IVI (300) was common in all the forest communities (Table 1).

Among trees, Chir showed maximum abundance (7.20) and IVI (271.45) under Chir forest community. In Chir + Oak forest community, Oak tree showed dominance having maximum abundance (12.89) and IVI (164.07). In pure Deodar forest community, Deodar tree had abundance (7.6) and IVI (300). In Deodar + Oak forest community, Oak tree showed dominance with maximum abundance (6.4) and IVI (173.19). In Deodar + Chir forest community, Chir tree showed dominance having maximum abundance (4.80) and IVI (168.23). In pure Oak forest community, Oak tree had maximum abundance (14.7) and IVI (300). In Mixed forest community, Oak showed maximum abundance (5.90) and IVI (133.85). The abundance was 14.7, 13.07, 10.6, 9.4,

Table 1. Floristic composition of tree layer in different forest community types

Chir forest community					
Trees	Density (No. ha ⁻¹)	Abundance (AB)	Basal area (m ² ha ⁻¹)	Per cent frequency	IVI
Chir forest					
Chir	720	7.20	44.56	100	271.45
<i>Kainth</i>	40	1.33	0.09	30	28.55
Total	760	8.53	44.65	130	300
Chir + Oak forest community					
Chir	270	2.70	17.24	100	135.93
Oak	670	6.70	12.89	100	164.07
Total	940	9.40	30.13	200	300.00
Deodar forest community					
Deodar	760	7.60	85.23	100	300.00
Deodar + Chir forest community					
Deodar	230	2.30	17.58	100	131.77
Chir	480	4.80	18.51	100	168.23
Total	710	7.10	35.09	200	300.00
Deodar + Oak forest community					
Oak	640	6.40	17.61	100	173.19
Deodar	320	3.20	10.29	100	114.57
Burash	20	1.00	0.31	20	12.24
Total	980	10.60	28.22	220	300.00
Oak forest community					
Oak	1470	14.70	46.87	100	300.00
Mixed forest community					
Deodar	210	2.33	19.03	90	83.62
Kail	70	2.33	3.98	30	23.65
Oak	590	5.90	25.39	100	133.85
Burash	200	2.50	7.56	80	58.88
Total	1070	13.07	55.96	300	300.0

8.53, 7.6 and 7.1 in Oak, Mixed, Deodar + Oak, Chir + Oak, Chir, Deodar and Deodar + Chir, respectively in descending order, whereas, Mir *et al.* (2011) reported abundance of 2.96, 1.0 and 2.37-6.10 in *Pinus roxburghii*, *Quercus leucotrichophora* and *Cedrus deodara*, respectively in different sites of Chaupal Forest Division in Himachal Pradesh.

Floristic composition of shrub layer: High density of shrubs was recorded in Mixed forest (6840ha⁻¹) followed by

Oak (6280 ha⁻¹), Deodar (4840 ha⁻¹), Chir + Oak (4720 ha⁻¹), Deodar + Oak (3440 ha⁻¹), Deodar + Chir (2400 ha⁻¹), Chir (2080 ha⁻¹) forest community (Table 2). The lower density of shrubs in Chir forest community was reported by Kumar and Thakur (2008) and Dangwal *et al.* (2012) in Kashmir forests. The basal area ranged from 912.13 - 5,892.34 with maximum in Chir + Oak forest community (5,892.34 cm² ha⁻¹) followed by Deodar (2,975.31cm² ha⁻¹), Mixed (2,101.4 cm² ha⁻¹), Deodar + Oak (1,554.11 cm² ha⁻¹), Chir (1,306.82 cm² ha⁻¹),

Oak ($1049.83 \text{ cm}^2 \text{ ha}^{-1}$) and Deodar + Chir ($912.13 \text{ cm}^2 \text{ ha}^{-1}$) forest community. Whereas, per cent frequency ranged between 180 to 370 with maximum in Chir + Oak forest community followed by Mixed, Deodar + Oak, Oak, Deodar, Deodar + Chir and Chir forest community in descending order. However, IVI (300) was common in all the forest communities.

In shrubs, *Cassia floribunda* showed maximum abundance (12) and IVI (101.30) in Chir forest. *Indigofera pulchella* had maximum abundance (4.25) and IVI (90.84) in Chir + oak forest. *Sarcococca prunifera* had maximum abundance (7.86) and IVI (98.26) in Deodar forest. *Daphne cannabina* had maximum abundance (1.30) and IVI (109.08) in Deodar + Oak forest community. *Rubus ellipticus* had maximum abundance (5.20) and IVI (118.07) in Deodar + Chir forest. *Myrsine africana* had maximum abundance (7.43) and IVI (91.84) in oak forest. *Berberis aristata* had maximum abundance (2.50) and IVI (52.9) in mixed forest community (Table 2).

Floristic composition of herbaceous layer: High density for

herbs was recorded in Deodar forest ($6.73 \text{ lakhs ha}^{-1}$) followed by Oak ($4.78 \text{ lakhs ha}^{-1}$), Chir ($3.97 \text{ lakhs ha}^{-1}$), Chir + Oak ($3.62 \text{ lakhs ha}^{-1}$), Deodar + Chir ($3.02 \text{ lakhs ha}^{-1}$), Mixed ($2.86 \text{ lakhs ha}^{-1}$) and Deodar + Oak ($2.49 \text{ lakhs ha}^{-1}$) forest community (Table 3). The basal area however, was recorded maximum in Deodar forest ($3,45,657.88 \text{ cm}^2 \text{ ha}^{-1}$) followed by Chir ($2,69,613.05 \text{ cm}^2 \text{ ha}^{-1}$), Chir + Oak ($2,06,934.72 \text{ cm}^2 \text{ ha}^{-1}$), Deodar + Chir ($86,983 \text{ cm}^2 \text{ ha}^{-1}$), Deodar + Oak ($58,824.25 \text{ cm}^2 \text{ ha}^{-1}$), Oak ($52,554.15 \text{ cm}^2 \text{ ha}^{-1}$) and Mixed ($42,288.22 \text{ cm}^2 \text{ ha}^{-1}$) forest community. The per cent frequency ranged between 370-520 with maximum in Deodar and minimum in Deodar + Chir forest community. However, IVI (300) was common in all the forest communities (Table 3).

The total basal area and density value were reported in the range of $205 - 2,027 \text{ cm}^2 100 \text{ m}^2$ and $9,050-39,398$ individuals per 100 m , respectively for the herbaceous layer and the same for the shrubs layer in range of $7.24-74.33$ and $9.86-306$, respectively in temperate Himalayan forests (Pande *et al.*, 1996). The value of density and basal area of shrubs in the present study are well within the reported range

Table 2. Floristic composition of shrub layer in different forest community types

Chir forest community					
Shrubs	Density (No. ha^{-1})	Abundance (AB)	Basal area ($\text{cm}^2 \text{ ha}^{-1}$)	Per cent frequency	IVI
Chir forest community					
<i>Berberis aristata</i>	200	1.00	125.05	50	46.96
<i>Woodfordia fruticosa</i>	240	3.00	181.24	20	36.52
<i>Osyris arborea</i>	40	1.00	31.59	10	9.90
<i>Rubus ellipticus</i>	360	2.25	190.15	40	54.08
<i>Cassia floribunda</i>	960	12.00	575.48	20	101.30
<i>Coriaria nepalensis</i>	200	2.50	118.84	20	29.82
<i>Ziziphus mauritiana</i>	80	1.00	84.47	20	21.42
Total	2,080	22.75	1,306.82	180	300.0
Chir + Oak forest community					
<i>Berberis lycium</i>	840	2.63	405.6	80	46.30
<i>Berberis aristata</i>	120	1.50	58.19	20	8.94
<i>Sarcococca prunifera</i>	80	2.00	23.63	10	4.80
<i>Indigofera pulchella</i>	680	4.25	3,866.66	40	90.84
<i>Myrsine africana</i>	720	4.50	221.15	40	29.82
<i>Rubus niveus</i>	400	1.67	192.33	60	27.95
<i>Desmodium floribunda</i>	560	4.67	82.65	30	21.38
<i>Rosa brunonii</i>	200	1.67	119.40	30	14.37
<i>Boenninghausenia albiflora</i>	880	7.33	570.68	30	36.44
<i>Lyonia ovalifolia</i>	160	2.00	282.77	20	13.59
<i>Viburnum coriaceum</i>	80	2.00	69.27	10	5.57
Total	4,720	32.21	5,892.34	370	300.0

Deodar forest community					
<i>Sarcococca pruniiforme</i>	2,200	7.86	852.96	70	98.26
<i>Daphne cannabina</i>	680	2.43	662.78	70	60.46
<i>Boenninghausenia albiflora</i>	200	1.67	488.33	30	30.89
<i>Berberis aristata</i>	400	2.50	291.08	40	31.84
<i>Indigofera pulchella</i>	80	1.00	51.87	20	10.29
<i>Lyonia ovalifolia</i>	40	1.00	40.24	10	5.63
<i>Solanum pseudocapsicum</i>	1,160	9.67	491.65	30	50.84
<i>Lonicera quinquelocularis</i>	40	1.00	49.06	10	5.92
<i>Rubus ellipticus</i>	40	1.00	47.35	10	5.87
Total	4,840	28.12	2,975.31	290	300.0
Deodar + Chir forest community					
<i>Berberis aristata</i>	680	2.43	259.52	70	93.63
<i>Indigofera pulchella</i>	280	3.50	123.46	20	35.73
<i>Rubus ellipticus</i>	1,04,000	5.20	441.67	50	118.07
<i>Lonicera quinquelocularis</i>	8000	1.00	25.99	20	16.71
<i>Meriandra strobilifera</i>	120	3.00	15.38	10	11.95
<i>Daphne cannabina</i>	120	3.00	10.03	10	11.36
<i>Rosa brunonii</i>	80	2.00	36.08	10	12.55
Total	2,40,000	20.13	912.13	190	300.0
Deodar + Oak forest community					
<i>Daphne cannabina</i>	520	1.30	974.68	100	109.08
<i>Rosa brunonii</i>	40	1.00	4.21	10	4.56
<i>Myrsine africana</i>	600	2.50	159.50	60	46.45
<i>Boenninghausenia albiflora</i>	1,200	5.00	102.80	60	60.25
<i>Berberis aristata</i>	4000	1.00	1.93	10	4.41
<i>Randia tetrasperma</i>	80	1.00	19.21	20	9.81
<i>Berberis lycium</i>	40	1.00	3.63	10	4.52
<i>Sarcococca pruniiforme</i>	600	7.50	194.39	20	36.21
<i>Rubus ellipticus</i>	80	1.00	46.67	20	11.58
<i>Hypericum species</i>	240	6.00	47.1	10	13.13
Total	3,44,000	27.30	1,554.11	320	300.0
Oak forest community					
<i>Daphne cannabina</i>	1,400	5.00	312.56	70	74.65
<i>Sarcococca pruniiforme</i>	480	4.00	67.90	30	23.79
<i>Boenninghausenia albiflora</i>	1,920	6.00	166.57	80	72.25
<i>Myrsine africana</i>	2,080	7.43	379.35	70	91.84
<i>Berberis aristata</i>	360	1.80	114.92	50	32.8
<i>Rubus ellipticus</i>	40	1.00	8.52	10	4.67
Total	6,280	25.23	1,049.83	310	300.0

Mixed forest community					
<i>Boenninghauseniaalbiflora</i>	1,360	5.67	142.04	60	43.31
<i>Berberis aristata</i>	800	2.50	398.84	80	52.9
<i>Daphne cannabina</i>	1,000	5.00	389.36	50	47.04
<i>Myrsine africana</i>	2,72000	8.50	841.74	80	102.04
<i>Lyonia ovalifolia</i>	40	1.00	59.37	10	6.19
<i>Indigofera pulchella</i>	240	3.00	59.96	20	11.92
<i>Inula cappa</i>	80	2.00	15.13	10	4.67
<i>Rosa brunonii</i>	40	1.00	34.62	10	5.01
<i>Sarcococca prunifera</i>	120	3.00	46.37	10	6.74
<i>Randia tetrasperma</i>	80	2.00	21.11	10	4.95
<i>Berberis lyceum</i>	120	3.00	70.29	10	7.88
<i>Desmodium triflorum</i>	240	6.00	22.56	10	7.36
Total	6,840	42.67	2,101.40	360	300.0

for temperate forest. While in case of herbaceous vegetation present study density of Chir forest and Deodar forest community showed high value than that of reported range. The high values may be attributed to protected area status of the study site (Singh, 1999).

Dicanthium annulatum showed highest Abundance (17.33) and IVI (86.62) in Chir forest community. *Fimbristylis rigidula* had maximum abundance (8.00) and IVI (64.62) in Chir + oak forest. *Woodsia elongata* had maximum abundance (11.40) and IVI (111.58) in Deodar forest. *Cyperus niveus* had maximum abundance (8.00) and IVI (54.88) in Deodar + Oak forest community. *Senecio nudicaulis* had maximum abundance (15.67) and IVI (47.40) in Deodar + Chir forest. *Fimbristylis rigidula* had maximum abundance (13.60) and IVI (63.21) in Oak forest. *Cyperus niveus* had maximum abundance (14.20) and IVI (57.26) in Mixed forest (Table 3). Importance value index of trees, shrubs, herbs in different forest community is proportional to the amount of resources that they utilized. Where the few species dominating the site and occupy larger space and left the remaining space for the large number of species for inter-specific competition (Whittaker, 1975).

Vegetation Index

Species richness: Tree species richness was 0.64 (Mixed forest), 0.44 (Deodar + Oak forest) and 0.23 (Chir forest, Chir + Oak forest and Deodar + Chir forest). Due to pure nature of Deodar and Oak species, they failed to show any richness in their community types. Species richness for shrub ranged from 0.99 to 2.14 with maximum in Mixed forest and minimum in Oak forest community. However, richness of herbaceous vegetation ranged from 2.10 to 3.69 with maximum in Deodar forest and minimum in Deodar + Oak and pure Oak forest

community, respectively (Table 4). Kharkwal *et al.* (2009) and Pandey *et al.* (2010) contended that it increases/decreases with amount of rainfall and temperature. This is generally because of secondary succession when environmental and edaphic conditions are favorable with low fluctuations. Similar change along the altitude on species richness have also been reported by Kessler (2000); Grytnes and Vetaas (2002); Grytnes (2003) and Kharkwal *et al.* (2009).

Species diversity: Tree species diversity in different forest communities ranged from 0 to 1.23 (Table 4), which is well supported by species diversity index between 0-1.92 (Rahman *et al.*, 1982) for temperate forests of Kumaon Himalaya, between 1.27-2.21 for moist temperate forests in Garhwal (Semwal *et al.*, 2008) and between 0.78-3.45 for forest communities of Garhwal Himalaya (Raturi, 2012). In addition, while comparing diversity of tree species to the reported values elsewhere, 0-3.40 for temperate forest reported by various workers (Rahman *et al.*, 1982; Pande *et al.*, 1996; 2000). The values of the present study falls within the reported range for the temperate forests. The diversity index was 1.23 (Mixed forest), 0.81 (Deodar + Oak), 0.69 (Deodar + Chir and Chir + Oak), 0.31 (Chir) in descending order. Deodar and Oak species were pure in their respective forest community, hence no diversity was observed.

The shrub species diversity ranged from 1.53 to 2.08 and it was maximum in Chir + Oak and minimum in Deodar + Chir forest community. Maximum diversity of the shrubs in Chir + Oak forest (2.08) may be attributed to the lower density and less developed canopy, which might have resulted in sufficient light to the ground favouring growth of shrub species that is well supported by Bhandari and Tiwari

Table 3. Floristic composition of herbaceous layer under different forest community types

	Herbs/Grass/Ferns/Climber	Density	Abundance (AB)	Basal area (cm ² ha ⁻¹)	Per cent frequency	IVI
Chir forest community						
Herbs	<i>Commelina obliqua</i>	9,000	9.00	287.42	10	4.70
	<i>Lespedeza gerardiana</i>	23,000	4.60	8,598.73	50	20.61
	<i>Viola canescens</i>	16,000	8.00	1,035.83	20	9.07
	<i>Cassia mimosoides</i>	8,000	4.00	1,214.17	20	7.12
	<i>Salvia glutinosa</i>	26,000	8.67	23,378.98	30	22.20
	<i>Cnicus argyranthus</i>	7,000	7.00	497.61	10	4.27
	<i>Anaphalis busua</i>	4,000	4.00	497.61	10	3.52
	<i>Bidens pilosa</i>	12,000	6.00	1,990.45	20	8.41
	<i>Verbascum thapsus</i>	3,000	1.50	816.08	20	5.71
	<i>Justicia simplex</i>	21,000	10.50	3,980.89	20	11.42
	<i>Argemone mexicana</i>	5,000	5.00	1,273.89	10	4.06
	<i>Asclepias curassavica</i>	17,000	8.50	7,085.99	20	11.56
	<i>Apluda mutica</i>	31,000	15.50	26,910.82	20	22.44
Grasses	<i>Chrysopogon montanus</i>	15,000	15.00	28,742.04	10	16.76
	<i>Avena fatua</i>	20,000	10.00	12,440.29	20	14.30
	<i>Cenchrus ciliaris</i>	17,000	8.50	5,195.06	20	10.86
	<i>Heteropogon contortus</i>	21,000	21.00	15,605.1	10	13.4
	<i>Setaria glauca</i>	18,000	9.00	3,602.71	20	10.52
	<i>Dicanthium annulatum</i>	1,04,000	17.33	1,25,289.8	60	86.62
	<i>Carex nubigena</i>	4,000	4.00	134.55	10	3.38
Sedge	<i>Woodsiaelongata</i>	16,000	8.00	1035.03	20	9.07
Fern	Total	3,97,000	185.10	2,69,613.05	430	300.0
Chir + oak forest community						
Herbs	<i>Anaphalis busua</i>	18,000	3.60	3,390.12	50	16.81
	<i>Epipactis latifolia</i>	5,000	1.67	85.99	30	7.55
	<i>Rhynchosia sericea</i>	12,000	4.00	1,176.75	30	10.01
	<i>Flemingia fruticulosa</i>	9,000	3.00	701.43	30	8.95
	<i>Viola canescens</i>	6,000	6.00	7.17	10	3.70
	<i>Anaphalis fruticulosa</i>	9,00000	3.00	447.45	30	8.82
	<i>Commelina obliqua</i>	40,000	20.00	10,707.01	20	20.31
	<i>Plectranthus striatus</i>	9,000	4.50	2,488.06	20	7.77
	<i>Carpesium cernuum</i>	3,000	3.00	318.47	10	3.02
	<i>Artemisia roxburghiana</i>	2,000	2.00	50.96	10	2.62
	<i>Galium triflorum</i>	11,000	11.00	79.62	10	5.12
	<i>Urochloa panicoides</i>	6,000	6.00	114.65	10	3.75
Grasses	<i>Fimbristylis rigidula</i>	64,000	8.00	63,343.16	80	64.62
	<i>Themeda anathera</i>	47,000	23.50	61,226.12	20	46.65
	<i>Heteropogon contortus</i>	26,000	13.00	8,777.07	20	15.51
	<i>Apluda mutica</i>	16,000	8.00	35,369.42	20	25.59
	<i>Cyperus rotundus</i>	51,000	10.20	9,902.07	50	29.08
Sedges	<i>Cyperus nivens</i>	7,000	7.00	6,738.85	10	7.23
	<i>Carex nubigena</i>	15,000	15.00	1,612.26	10	6.96

Fern	<i>Polystichum squarrosus</i>	4,000	4.00	318.47	10	3.30
Climber	<i>Vitis himalayana</i>	2,000	2.00	79.62	10	2.63
	Total	3,62,000	158.47	20,6934.72	490	300.0
Deodar forest community						
Herbs	<i>Roscoea purpurea</i>	9,000	9.00	1,273.89	10	3.27
	<i>Arisaema spp</i>	7,000	1.75	2,510.26	40	8.78
	<i>Viola canescens</i>	25,000	12.50	899.02	20	7.58
	<i>Viola serpens</i>	47,000	15.67	3,021.50	30	12.79
	<i>Bistorta spp</i>	6,000	6.00	495.96	10	3.02
	<i>Ainsliaea aptera</i>	6,000	6.00	421.18	10	2.82
	<i>Anemone rivularis</i>	10,000	10.00	8,945.86	10	3.51
	<i>Rumex nepalensis</i>	8,000	4.00	23,4983.00	20	7.63
	<i>Galium triflorum</i>	7,000	7.00	179.14	10	2.97
	<i>Anaphalis busua</i>	3,000	1.50	308.92	20	4.30
	<i>Strobilanthes alatus</i>	26,000	8.67	5,460.99	30	9.69
	<i>Girardinia diversifolia</i>	6,000	3.00	10,971.34	20	4.86
	<i>Taraxacum officinale</i>	3,000	3.00	1,612.26	10	2.39
	<i>Fragaria indica</i>	77,000	38.50	3,875.80	20	15.33
	<i>Salvia spp.</i>	2,000	2.00	257.96	10	2.22
Grasses	<i>Urochloa panicoides</i>	1,41,000	20.14	12,583.20	70	36.20
	<i>Themeda anathera</i>	25,000	12.50	5,748.41	20	7.62
Sedge	<i>Cyperus rotundus</i>	53,000	35.00	17,714.17	30	13.84
	<i>Eriophorum comosum</i>	35,000	17.67	4,843.95	10	7.18
	<i>Carex nubigena</i>	7,000	7.00	1,149.68	10	2.98
Ferns	<i>Woodsia elongata</i>	57,000	11.40	16,615.00	50	111.58
	<i>Thymus serpyllum</i>	20,00000	10.00	130.57	20	6.82
	<i>Onychium contiguum</i>	42,000	42.00	6,165.61	10	8.23
	<i>Dryopteris nigropaleacea</i>	37,000	37.00	4,013.54	10	7.47
Climber	<i>Hedera helix</i>	14,000	7.00	1,476.69	20	6.94
	Total	6,73,000	328.29	3,45,657.88	520	300.0
Deodar + chir forest community						
Herbs	<i>Erigeron annuus</i>	15,000	15.00	13,455	20	10.70
	<i>Senecio nudicaulis</i>	47,000	15.67	20,640	30	47.40
	<i>Anaphalis busua</i>	26,000	6.50	165	10	12.27
	<i>Cnicusargyracanthus</i>	6,000	3.00	2,880	40	13.94
	<i>Gerbera lanuginosa</i>	46,000	15.33	11,520	40	15.67
	<i>Dicliptera bupleuroides</i>	7,000	1.75	710	30	22.13
	<i>Viola canescens</i>	12,000	3.00	770	50	26.67
	<i>Potentilla katherine</i>	30,000	10.00	3,558	20	23.50
	<i>Rubia cordifolia</i>	37,000	7.40	785	40	19.61
Grasses	<i>Dicanthium annulatum</i>	18,000	4.50	13,980	10	23.14
	<i>Themeda anathera</i>	26,000	13.00	8,250	30	36.58
Sedge	<i>Eriophorum comosum</i>	26,000	26.00	3,680	10	15.54
	<i>Cyperus rotundus</i>	6,000	6.00	6,590	40	32.84
	Total	3,02,000	127.15	86,983	370	300.0

Deodar + oak forest community						
Herbs	<i>Plectranthus striatus</i>	50,000	7.14	6,820.06	70	49.62
	<i>Strobilanthes alatus</i>	1,000	1.00	2,866.24	10	7.84
	<i>Rumex nepalensis</i>	5,000	5.00	2,236.47	10	8.37
	<i>Viola canescens</i>	13,000	13.00	114.65	10	7.98
	<i>Viola serpens</i>	27,000	6.75	2,331.21	40	25.06
	<i>Artemisia roxburghiana</i>	4,000	2.00	372.61	20	7.37
Grasses	<i>Fimbristylis rigidula</i>	25,000	6.25	6,723.73	40	31.73
	<i>Echinochloa colona</i>	26,000	6.50	6,134.75	40	31.13
	<i>Oplismenus burmannii</i>	33,000	11.00	7,484.08	30	33.67
Sedge	<i>Cyperus niveus</i>	32,000	8.00	18,688.70	40	54.88
Ferns	<i>Polystichum squarrosum</i>	7,000	1.40	3,761.94	50	22.03
	<i>Pteris cretica</i>	8,000	8.00	458.60	10	6.56
	<i>Woodsia elongata</i>	14,000	14.00	716.56	10	9.40
Climber	<i>Hedera helix</i>	4,000	4.00	114.65	10	4.37
	Total	2,49,000	94.04	58,824.25	390	300.0
Oak forest community						
Herbs	<i>Viola canescens</i>	12,000	6.00	369.43	20	7.86
	<i>Achyranthes bidentata</i>	24,000	6.00	1,261.94	40	16.72
	<i>Plectranthus striatus</i>	53,000	17.67	5,079.62	30	27.73
	<i>Anaphalis fruticulosa</i>	13,000	3.25	1,430.73	40	14.74
	<i>Plectranthus incanus</i>	26,000	13.00	392.52	20	10.84
	<i>Adenocaulon bicolor</i>	14,000	7.00	1,433.12	20	10.31
	<i>Cnicus argyranthus</i>	5,000	1.25	554.94	40	11.40
	<i>Rhynchosiasericea</i>	16,000	8.00	322.45	20	8.61
Grasses	<i>Fimbristylis rigidula</i>	68,000	13.60	19,632.17	50	63.21
	<i>Avena fatua</i>	39,000	13.00	1,479.30	30	17.95
	<i>Oplismenus burmannii</i>	28,000	7.00	1,908.44	40	18.79
Sedge	<i>Cyperus niveus</i>	1,77,000	29.50	16,679.14	60	82.72
Fern	<i>Polystichum squarrosum</i>	1,000	1.00	1,990.45	10	6.32
Climber	<i>Hedera helix</i>	2,000	2.00	19.90	10	2.78
	Total	4,78,000	128.27	52,554.15	430	300.0
Mix forest community						
Herbs	<i>Carpesium cernuum</i>	1,000	1.00	458.60	10	3.97
	<i>Viola canescens</i>	13,000	6.50	1,248.41	20	12.51
	<i>Viola patrinii</i>	10,000	10.00	1,404.46	10	9.16
	<i>Viola serpens</i>	27,000	6.75	1,605.89	40	23.44
	<i>Anaphalis busua</i>	12,000	4.00	9,326.43	30	32.24
	<i>Erigeron annuus</i>	3000	3.00	1,149.68	10	6.16
	<i>Adenocaulon bicolor</i>	10,000	10.00	458.60	10	7.12
	<i>Plectranthus striatus</i>	12,000	12.00	3,790.61	10	15.02
	<i>Cnicusargyracanthus</i>	2,000	1.00	293.79	20	6.60
Grasses	<i>Oplismenus burmannii</i>	39,000	13.00	5,649.68	30	33.74
	<i>Themeda anathera</i>	19,000	9.50	4305.73	20	21.21
Sedge	<i>Cyperus niveus</i>	71,000	14.20	8,921.98	50	57.26

Ferns	<i>Woodsia elongate</i>	29,000	7.25	3,551.75	40	28.34
	<i>Dryopteris nigropaleacea</i>	1,000	1.00	2,152.87	10	7.63
	<i>Thymus serpyllum</i>	22,000	7.33	985.67	30	17.72
	<i>Onychium contiguum</i>	5,000	5.00	716.56	10	5.93
Climber	<i>Hedera helix</i>	10,000	3.33	267.52	30	11.97
	Total	2,86,000	114.87	46,288.22	380	300.0

Table 4. Vegetation indices of trees, shrubs and herbs under different forest community types

Major forest community type	Plant category	Vegetation index		
		Shannon weaver	Simpson's dominance	Species richness
Chir forest	Trees	0.31	0.83	0.23
Chir + Oak forest		0.69	0.50	0.23
Deodar forest		0.00	1.00	0.00
Deodar + Chir		0.69	0.51	0.23
Deodar + Oak forest		0.81	0.48	0.44
Oak forest		0.00	1.00	0.00
Mixed forest		1.23	0.32	0.64
Chir forest	Shrubs	1.75	0.8	1.27
Chir + Oak forest		2.08	0.84	2.10
Deodar forest		1.81	0.8	1.67
Deodar + Chir		1.53	0.73	1.47
Deodar + Oak forest		1.81	0.78	2.02
Oak forest		1.55	0.77	0.99
Mixed forest		1.92	0.8	2.14
Chir forest	Herbs	2.63	0.88	3.34
Chir + Oak forest		2.58	0.9	3.40
Deodar forest		2.49	0.83	3.69
Deodar + Chir		2.47	0.91	2.10
Deodar + Oak forest		2.37	0.89	2.36
Oak forest		2.23	0.85	2.10
Mixed forest		2.59	0.91	2.83

(1997) and Semwal *et al.* (2007) whereas, low diversity in Deodar + Chir forest community is due to well developed dense canopy allowing less sunlight to support the shrub vegetation.

The herbaceous vegetation diversity ranged from 2.23 to 2.63 with minimum in Oak forest community and maximum in Chir forest community. The recorded index value (2.23-2.63) for herbaceous vegetation in the forest community of present study is within the range (1.44-3.00) reported by Pande (2001) and Sharma *et al.* (2009) for forests in Himalayan region. The high diversity index of herbs in Chir forest (2.63) may be due to less density and open canopy. Both Oak and Deodar forests exhibited nil diversity. The lowest diversity index values in Oak forest (2.23) may be attributed to the climax nature of the species with dense cover that might not have restricted herbaceous species to enter and establish.

Species richness: The lower concentration of tree dominance indicates the sharing of dominance by many species. In present study, Simpson's concentration of dominance ranged between 0.32 to 1.00 with maximum in Deodar and minimum in mixed forest community (Table 4). This is in similarity with the findings of other workers, who found concentration of dominance for certain temperate forests ranging between 0.10 to 0.99 (Whittaker, 1965; Risser and Rice, 1971) and between 0.31 to 1.0 (Rahman *et al.*, 1982). The index value of present study falls within the reported range for temperate forest. The concentration of dominance for shrub vegetation ranged between 0.73-0.84 and for herbaceous vegetation between 0.83-0.91.

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Evaluation of Site Suitability and Storage Capacity of Constructed Rainwater Harvesting Structure in Vindhyan Region, India

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Abstract: The study was under taken to evaluate the suitability of site of constructed rainwater harvesting structures. Volume of stored rainwater and area of submergence of check dam was used for the understanding the suitability of constructed site. The collection runoff water starts form July and remains up to February/March. Maximum volume of the harvested runoff rainwater and area of submergence was 24954 m³ and 30985 m², at 1.11 m level of stored water at the check dam. There was no water during the month of April to June in the reservoir.

Key Words: Rainwater harvesting, Check dam cum reservoir, Storage capacity, Site suitability, Vindhyan plateau

Among several alternative water resources available for reuse, rainwater is the most preferred by the general public, especially when compared to recycled wastewater. Reduction of surface runoff can be achieved by constructing suitable structures, which automatically helps to manage the other natural resources like soil and vegetation. Rainwater harvesting and reuse is a widely used practice, which deals with collection, storage, treatment and distribution through various engineering approaches (Hatt *et al.*, 2006; Mitchell *et al.*, 2007; Goonrey *et al.*, 2009). Continuous failure of monsoon, increasing demand and over exploitation leads to depletion of ground-water level, which in turn tends to increase both the investment and the operational costs.

This problem could be sorted out to certain extent by artificially recharging the potential aquifers. In hard rock terrains, availability of groundwater is of limited extent. Occurrence of groundwater in such rocks is essentially confined to fractured and weathered horizons. Rainwater harvesting into check dam type reservoir for supplemental irrigation during intra-seasonal dry spells for the crop growing seasons of plateau regions could therefore prove to be a possible solution. Rainwater harvesting may also be used for restoration of the productivity of land, which suffers from insufficient rainfall, increasing productivity of rainfed farming (Oweis and Hachum, 2006), minimizing risk of drought in areas prone to it and decrease the threat of desertification through decreasing runoff and increasing infiltration. In the present study, potential sites for construction of rainwater harvesting structures in the Barkachha farm, Mirzapur district part of central Vindhyan plateau have been identified by using Golden Software SURFER 7.0.

MATERIAL AND METHODS

The site selected for present study is Barkachha farm in Rajiv Gandhi South Campus (Banaras Hindu University), located in the Mirzapur district (central Vindhyan plateau) and situated between 25°00' to 25°15' North latitude and 82° 45' East longitude with an altitude of 180.59 m above mean sea level. The topography of the study area is undulating and surface is rough. The area of Barkachha farm is 1010 ha, it is positioned in Mirzapur - Rabertsganj highway and only 8 km far from Mirzapur. According to ICAR survey report (NBSSLUP, 1971), nearly 40% land of this farm is rocky, i.e. surface with bed rock exposure (1-25% slope). Approximately 60% farm land is having low to moderate deep soil (1-5% slope with slight to moderate surface erosion). The red soil, generally red to reddish brown, lighter texture with friable structure is commonly observed at the farm, but, slightly alkaline, calcareous soil is also found (6.68%) in some patches. This farm has both types of land, i.e. cultivable and non-cultivable. Variety of crops such as cereals, pulses, oilseed crops, horticultural crops (fruits and vegetables), bio-fuel crop as well as medicinal and aromatic plants are grown in cultivable land of Barkachha. Overall climate of the area is semi-arid and sub-humid.

Rainwater harvesting structure: Rainwater harvesting check dam cum reservoirs was constructed by the Irrigation Department, Government of Uttar Pradesh in collaboration with Krishi Vigyan Kendra, Barkachha during 2001, on the back side of the girl's hostel in Barkachha farm. The specification of rainwater harvesting structure (Table 1) is consisted of 400 m length, 6.0 m top width of check dam, 6.0 m maximum height, 2:1 side slope up to 3 m and 2.5:1 in between 3m to 5m and 2.59 km² catchment areas.

Data collection and analysis: Monthly average

meteorological data of the study year (2009) was collected from the Meteorological Division, Krishi Vigyan Kendra, Mirzapur. The climate of Brakachha is typically semi-arid, characterized by extremes of temperature both in summer and winter with low rainfall and moderate humidity. Maximum temperature in summer is as high as 41°C and minimum temperature in winter falls down below 10°C. The annual rainfall of locality was 773.9 mm in 2009, of which nearly 90% is contributed by South-West monsoon between July to September. The relative humidity fluctuated between 58 to 79 % and average evaporation between 1.5 to 8.7 mm day⁻¹.

Table 1. Construction design of check dam reservoirs in Barkachha, Mirzapur

Design parameter	Check dam cum reservoirs
Length (m)	400.0
Top width (m) of check dam	6.0
Maximum height/depth (m)	6.0
Side slope	2:1(up to 3 m) & 2.5:1(>3m<5m)
Catchment area (km ²)	2.59

Depth of the check dam reservoirs at different slopes were measured randomly by setting the graduated wooden poles and conformed by measuring tape. The depth of the water level of the harvested rainwater at different locations of the reservoirs was measured by graduated wooden poles in a grid way. The monthly changes of level of the harvested rainwater was used for generating the contour map and analysis of volume of harvested rainwater and submerged area up-side of check dam by using Golden Software SURFER 7.0.

RESULTS AND DISCUSSION

Rainfall distribution of study site: The period of onset of monsoon in this region was last week of May. More than 95 % of the annual rainfall was received during monsoon season, but it was erratic and unpredictable (Fig.1). Only 31 days rained in 2009 and maximum rainfall (354.2 mm) was received during the month of July. There was a light shower during the month of May and June. The period of November to April did not receive any rain. This trend of monsoon rains coupled with high temperature and the existing toposquence of the central Vindhyan plateau of Barkachha, Mirzapur district leads to imbalance between rain received and vegetation water demand.

Storage capacity and suitability of site constructed rainwater harvesting: The check dams were constructed by scrapping the soil from upstream. The stone pitching was done on downstream face of the check dams by using locally available stone spalls and boulder. The surface runoff was started in the month of July and it is harvested upside of check dam. Maximum depth of stored rainwater was 1.11 m near the check dam in the month of September. The maximum length of submerge area was 140m when the width of harvested rainwater was 195m at the check dam. Maximum volume of the harvested runoff rainwater in the check dam was 24954 m³ and the area of submergence 30985 m² at 1.11 m depth of stored rainwater (Table 2; Fig. 2). The stored water was retained in the check dam up to February month and dam remained dry in the month of April to June. The rate area of submergence was lower ($R^2 =$

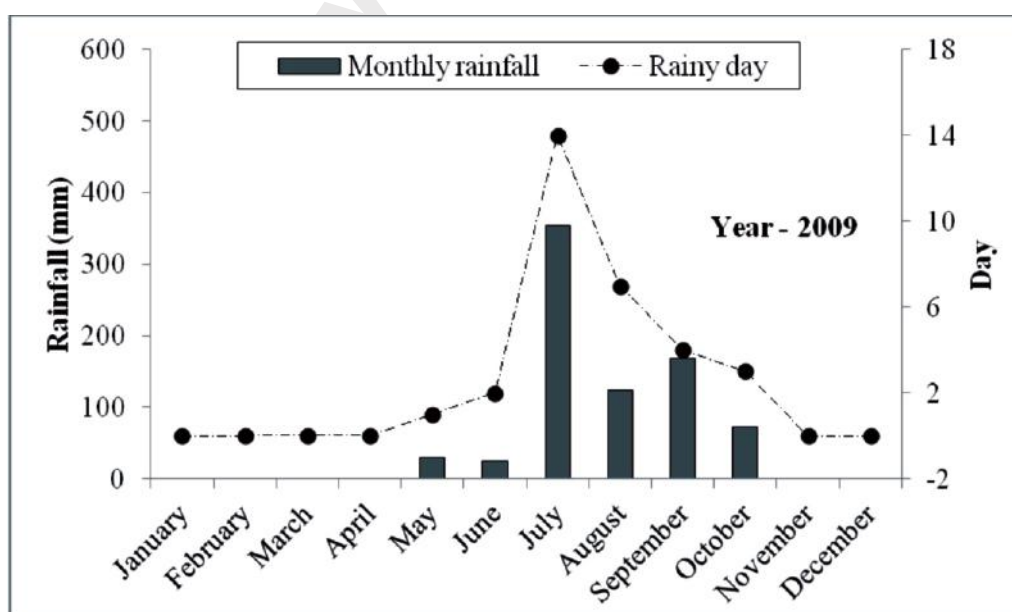


Fig. 1. Monthly distribution of rainfall and rainy days of Barkachha

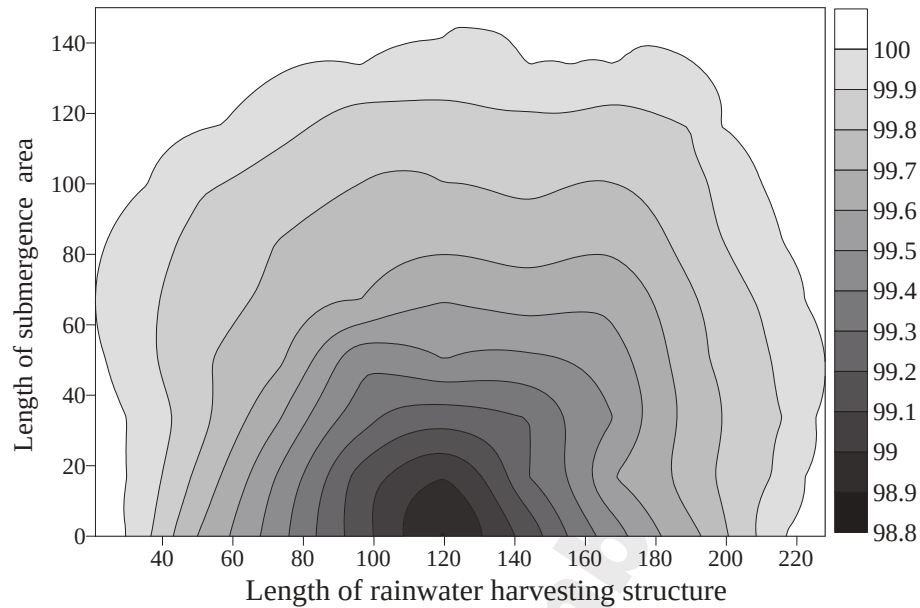


Fig. 2. Contour map of rainwater harvesting structure of Barkachha drwan by Golden Software SURFER 7.0

Table 2. Seasonal average (Av.) water depth (2009-10) and volume of water in check dam reservoirs in Barkachha, Mirzapur

Month	Checkdam cum reservoir	
	Av. depth of water (m)	Volume of water ('000 m ³)
July,2009	0.81	15.38
August,2009	0.65	10.84
September,2009	1.11	24.96
October,2009	1.00	23.073
November,2009	0.80	15.13
December,2009	0.61	9.87
January,2010	0.52	7.01
February,2010	0.29	2.54

0.986) than volume of stored water ($R^2 = 0.997$) when the depth of stored rainwater is increased (Fig. 3). It means, increased per unit of harvested rainwater had covered low unit area of submerged land, and also term as per unit cost of submerged land upward of check dam was decreased due to higher storage of runoff, so the suitability of construction site.

Rainwater harvesting has emerged a sustainable alternative water resource to cope with growing urban water demands. The selection of suitable rainwater harvesting sites is essential and equally challenging for the urban water infrastructure planners. This technique is greatly useful for the selection of construction sites for new rainwater

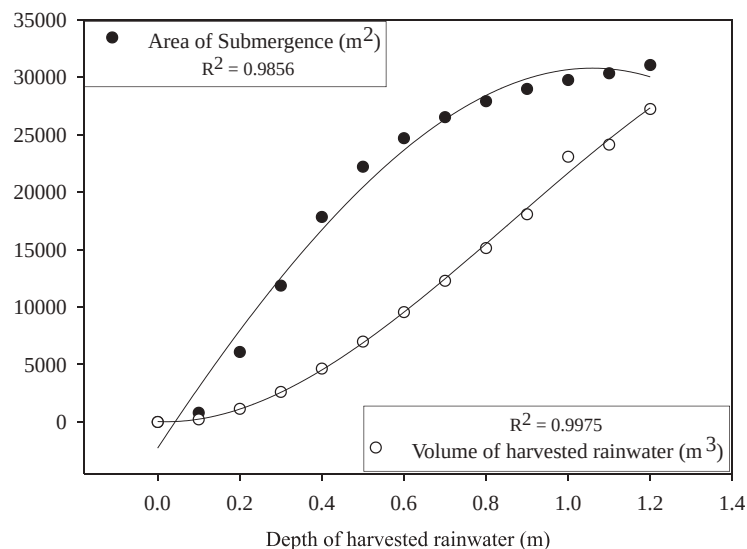


Fig. 3. Volume harvested rainwater and area of submergence rainwater in check dam of Barkachha.

harvesting structures and also renewal of already constructed structure for enhancing the storage capacity of per unit area of land.

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Received 13 October, 2015; Accepted 15 December, 2015



Kinetics of Adsorption and Desorption of Boron in Major Soils of Lesser Himalayas

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Abstract: All the forms of boron were positively and significantly correlated with pH, EC, CEC, clay and silt content, and negatively with organic carbon and sand content. About 50 % of the soils were deficient in available boron. The parabolic diffusion kinetic model was best in describing the rate of boron adsorption, followed by Elovich and zero order model, whereas, the first order, second order and power function equations failed in describing the boron adsorption kinetics. The desorption kinetics showed that boron was desorbed from two separate sites. The total desorbable boron was higher and faster during initial stages and then became slower. The power function kinetic model best described the boron desorption, followed by Elovich kinetic model.

Key Words: Adsorption/desorption, Boron, Lesser Himalayas, Modelling

Boron is a vital micronutrient for plants and range between deficient and toxic concentration. Both deficient and toxic levels of B in the soil solution can occur during a single growing season. Different kinetic models can be used to evaluate the adsorption and desorption characteristics of soils. The best fit kinetic model with high R^2 and low standard error value can infer the dynamics of boron in soils and suggest the adsorption and desorption pattern of boron from various mineral surfaces in the soil systems. Goldberg (1997) studied various reactions of B in soils and concluded that B adsorption reactions can be described empirically using the Langmuir adsorption isotherm equation, the Freundlich adsorption isotherm equation, and the phenomenological Keren model.

Sakal *et al.* (1994) used five kinetic equations (second approximation or pseudo-first order, two constant rate, first order, parabolic diffusion and Elovich) for their suitability in describing B desorption in four calcareous soils. The comparison of coefficients of determination (R^2) and standard errors of estimate (SE) showed that the second approximation, two constant rate and first order equations followed by parabolic diffusion adequately described the boron release, whereas Elovich equation failed to describe it. Chaudhary and Shukla (2005) also studied the kinetics of boron desorption from arid soils of India. Eight kinetic models were tested to describe boron desorption in arid soils of Rajasthan varying in physical and chemical characteristics. The comparison of coefficient of determination (R^2) and standard error of estimate (SE) revealed that the order of application to describe boron desorption kinetics in these soils was as follow: second approximation>two-constant rate equation>Elovich rate equation>parabolic diffusion rate equation.

Understanding the mechanism of adsorption-desorption of boron on/from soil materials is vital to understand as plants respond primarily to the B-activity in soil solution. Boron adsorption-desorption is one of the most important phenomenon in soil that regulates its supply from soils, determining the release and fixation of applied boron and thus deciding on the efficiency of boron fertilization (Arora and Chahal, 2009) for plant growth. The studies of B kinetics may be helpful in evolving empirical relationship for predicting boron availability to plants. Very little is known about boron reaction rates and mechanisms in Kashmir soils. Considering these aspects, the study was conducted with the objective of kinetics of Boron adsorption-desorption studies in soils of Kashmir.

MATERIAL AND METHODS

Ten bulk soil samples representing low, medium and high altitude temperate zones of Kashmir state, which come under the lesser Himalayas belt were selected varying in physical and chemical properties for adsorption-desorption studies. All the soil samples taken were geo-referenced with the available GPS equipment. Anantnag (S3), Shalimar (S6), Bandipora (S7) and Sagipora (S9) soils are from low altitude zone, Khag (S2), Kulgam (S4) and Tral (S5) are from mid altitude zone and Tangmarg (S1), Kangan (S8) and Shopian (S10) represent high altitude zone of Kashmir. The physical and chemical characteristics of the soils were determined by adopting standard procedures. Mechanical analysis of the soil was carried out by the Hydrometer method described by Moodie *et al.* (1959). Organic carbon was determined by achromic acid rapid titration method as described by Walkley and Black (1934).

Calcium carbonate content of soils was determined by the procedure given by Puri (1930) using bromothymol blue and bromocresol green as indicators. The cation exchange capacity (CEC) was estimated by using neutral normal sodium acetate according to the procedure described by Rhoades (1982). Adsorption-desorption kinetics of boron was studied on the surface soil samples representing ten soils of Kashmir, varying in physical and chemical properties.

Adsorption kinetics: Adsorption reactions of boron in soils are important for retention and release of boron for plant nutrition. For adsorption kinetic study, 10 gm of soil sample in triplicate was taken in 50 ml polyethylene centrifuge tubes, and 10 ml of 0.01 M CaCl_2 solution containing $40 \mu\text{g B mL}^{-1}$ was added to each tube. The samples were equilibrated at $25 \pm 1^\circ\text{C}$ for 0, 1, 2, 4, 8, 16, 24, 48 and 72 hours on a reciprocating shaker. At the end of reaction time, the suspension was centrifuged for 20 minutes. The samples were filtered using Whatman's No. 42 filter paper. The boron concentration was determined using Azomethane-H method as per the procedure outlined by Wolf (1974). The amount of boron adsorbed was calculated from the difference between the amount initially added and the quantity remaining in solution after equilibration.

Desorption kinetics: The soil samples used for adsorption kinetics after 48 hours were equilibrated with 0.05M mannitol in the same centrifuge tube. The mixture was equilibrated at $25 \pm 1^\circ\text{C}$ for 0, 1, 2, 4, 8, 18, 24, 48 and 72 hours on end to end shaker, followed by centrifugation for 10 minutes. The samples were filtered using No. 42 Whatman's filter paper and kept in refrigerator for B analysis. The boron concentration in the filtrate was determined using Azomethane-H method (Wolf, 1974).

The various kinetic models used to describe Boron adsorption / desorption from soils were tested for goodness of fit by least square regression analysis. These are;

1) Zero order

$$qt = q_0 + K_0 t$$

where K_0 is the zero order rate constant $[(\text{mg B kg}^{-1}) \text{ S}^{-1}]$.

2) First order

$$\ln qt = \ln q_0 + K_1 t$$

where K_1 is the first order rate constant $[\text{S}^{-1}]$.

3) Second order

$$1/qt = 1/q_0 - K_2 t$$

where K_2 is the second order rate constant $[(\text{mg B Kg}^{-1}) \text{ S}^{-1}]$.

4) Parabolic Diffusion

$$q = + K_d t^{1/2}$$

where K_d is the Diffusion rate constant $[(\text{mg B Kg}^{-1}) \text{ S}^{-1/2}]$.

5) Elovich

$$q_t = q_0 + (1/B) \ln - (B) + (1/B) \ln t$$

where is the initial B adsorption rate $(\text{mg B Kg}^{-1} \text{ h}^{-1})$ and is B desorption constant $(\text{mg B Kg}^{-1} \text{ h}^{-1})$.

6) Power function

$$\ln y = \ln a + b \ln t$$

where y is the quantity of boron adsorbed/desorbed at time 't' and a & b are constants.

In all equations, q_0 and q_t are the amount of boron adsorbed (mg B Kg^{-1}) at time zero and t, respectively. To determine the equation that best described the adsorption of boron in soils, a standard error of estimate was calculated for each equation. A relatively high value of the coefficient of determination (R^2) and low standard error (SE) was used as criteria for the best fit (Chien and Clayton, 1980). The standard error was calculated as follows;

$$SE = [(q - q')^2 / (N - 2)]^{0.5}$$

where q & q' are the measured and calculated amounts of B in soil, respectively at time 't' and N is the number of measurements (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The results showed that majority of the soils were slightly acidic to neutral reaction with good amount of organic matter content. The clay percentage of soils varies from 18.5-36.5%. The data indicates that pH value ranged from 5.6 to 7.8. The pH influences oxidation reactions, solubility and ionic forms of several metallic constituents. The soil conductivity varies from 0.11 to 0.36 dSm^{-1} . The organic carbon content varied from 0.98 to 3.93 per cent. The cation exchange capacity varied between 10.48 and 18.17 $\text{cmol}_c \text{ kg}^{-1}$ with an average value of $14.93 \text{cmol}_c \text{ kg}^{-1}$, highest in S_3 ($18.17 \text{cmol}_c \text{ kg}^{-1}$) and lowest in S_{10} soils ($10.48 \text{cmol}_c \text{ kg}^{-1}$). Calcium carbonate varied from 0.01 to 1.28 per cent.

The concentration of water soluble boron in the soil samples ranged from 0.07 to 0.95 mg kg^{-1} with a mean value of 0.35 mg kg^{-1} and there was no specific distribution pattern. Results showed that hot water soluble varied from 0.10 to 1.27 mg kg^{-1} soil, with a mean value of 0.46 mg kg^{-1} soil. The content of boron extracted in 0.05 N HCl varied from 0.36 to 1.24 mg kg^{-1} soil with an average value of 0.64 mg kg^{-1} soil. Acid extractable boron averaged of 0.6 mg kg^{-1} in the soils of North East hills of India (Dwivedi *et al.*, 1993). Leachable boron consists of "adsorbed + soluble B". It ranged from 0.57 to 1.74 mg kg^{-1} soil with a mean value of 0.93 mg kg^{-1} soil. This shows that most of the boron is prone to leach from the soils. The recently developed AB-DTPA extractant has proven to be effective for boron on alkaline soils (Gupta, 1993). The concentration of AB-DTPA extractable boron ranged from 0.77 to 1.94 mg kg^{-1} soil with a mean value of 1.13 mg kg^{-1} soil. Similar results were reported by Dwivedi *et al.* (1993) for

Table 1. Physico-chemical Characteristics of different soils of Kashmir

Parameters	Locations									
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀
pH (1:2.5)	6.1	6.5	7.8	5.9	6.4	5.6	6.2	6.2	7.2	5.8
EC (dSm ⁻¹)	0.12	0.21	0.36	0.15	0.19	0.13	0.11	0.19	0.22	0.19
OC (%)	3.93	1.17	0.98	2.80	1.33	2.57	1.72	1.76	1.01	2.69
CaCO (%)	1.28	0.68	0.03	0.12	0.85	0.01	0.58	0.66	0.72	1.20
CEC [cmol _e kg ⁻¹]	15.32	16.20	18.17	11.85	17.30	16.11	14.63	11.63	17.64	10.48
Coarse sand (%)	1.12	0.96	1.23	0.98	1.18	1.15	1.10	0.95	1.47	1.36
Fine sand (%)	19.08	26.64	15.77	28.02	18.62	34.05	32.20	27.65	14.53	40.64
Silt (%)	45.20	42.45	46.64	52.55	44.30	30.20	39.20	51.40	47.50	27.00
Clay (%)	34.60	29.95	36.36	18.45	35.90	34.60	27.50	20.00	36.50	31.00
Textural class	Silty clay loam	Clay loam	Silty clay loam	Silt loam	Silty clay loam	Clay loam	Clay loam	Silt loam	Silty clay loam	Clay loam

Meghalaya soils. Although total boron does not affect the plant growth and its contribution is low as compared to other forms of boron, but it helps in knowing its potential reserve in soil. Total boron content ranged from 40.72 to 50.55 mg kg⁻¹ soil with an average value of 45.46 mg kg⁻¹ soil. Das (2000) stated that total boron in Indian soils varied from 7 to 630 ppm.

Clay and silt were positively and significantly correlated with water soluble boron ($r = 0.572^{**}$ and 0.374^{**}). Water soluble boron and silt/clay content was correlated significantly ($r = 0.84^{**}$) in both non-saline and low saline soils of Delhi (Gajbhiye *et al.*, 1980), indicating that water soluble boron increases with increasing content of silt and clay. Water soluble boron also showed significant relation with pH ($r = 0.872^{**}$). Significant positive correlation of water soluble boron with EC of soils ($r = 0.609^{**}$) suggested that boron concentration increases during the process of salt accumulation. Water soluble boron showed negatively significant relationship with organic carbon content ($r = -0.451^{*}$). It may be due to low organic matter status of some soils and might not have contributed to a considerable extent in releasing water soluble boron. Diana and Beni (2006) also observed negative correlation of different forms of boron with organic carbon content of soils. Calcium carbonate content showed positive and non-significant relationship with water soluble boron.

The clay content was positively and significantly correlated ($r = 0.477^{*}$) with hot water soluble boron content of soil but negative relationship between available boron and organic matter of the soils (Table 3). Calcium carbonate content of soil showed non-significant negative correlation ($r = -0.095$) with hot water soluble boron. This may be due to the affinity of CaCO₃ towards boron adsorption. Cation exchange capacity, pH and EC also showed positive and significant correlation ($r = 0.492^{*}$, $r = 0.773^{**}$ and $r = 0.589^{**}$, respectively). Positive significant correlation was noted between hot water soluble and CEC by Sharma and Katyal (2006). Calcium chloride extractable boron was positively and significantly correlated with pH ($r = 0.742^{**}$) and EC ($r = 0.593^{**}$). The clay content was positively and significantly correlated ($r = 0.576^{**}$) with calcium chloride soluble boron while as silt content was non-significantly correlated ($r = 0.163$). Clay content contributed positively to boron extracted by CaCl₂ (Datta *et al.*, 1998). The results are also in accordance with the findings of Saha and Singh, 1998. Significant negative correlation ($r = -0.432^{*}$) was observed between CaCl₂ extractable boron and organic carbon content of soils. Calcium Carbonate showed negative but non-significant relationship ($r = 0.110$) with CaCl₂ extractable boron. Similar results were obtained by Datta *et al.* (1998).

Positive and significant correlation ($r=0.510^*$) between cation exchange capacity and CaCl_2 extractable boron was observed.

There was a significant positive curvilinear correlation between 0.05 N HCl extractable boron and clay ($r=0.564^{**}$), pH ($r=0.637^{**}$) and EC ($r=0.520^{**}$) of the soils. Organic carbon content was negatively and non-significantly correlated ($r=-0.148$) with acid extractable boron. Significant and positive correlation of acid soluble boron with pH, EC and negative and non-significant correlation with organic carbon suggested that in these soils, high in pH and EC, the dilute acid could not extract appreciable amount of boron that is associated with organic matter. These results clearly revealed that the available boron (water soluble, hot water soluble, CaCl_2 and acid extractable) were mainly associated with silt and clay fractions followed by pH and EC. There was positive and non-significant correlation ($r=0.359$) between cation exchange capacity and acid soluble boron. A negative but significant correlation ($r=-0.677^{**}$) was observed between acid soluble boron and sand content. The water soluble, hot water soluble, CaCl_2 and 0.05 N HCl extractable

forms of boron were found to be highly and significantly correlated with each other. This suggests that all the forms of boron can be considered to be almost equally efficient indices of boron availability in the soils under study.

Leachable boron was significantly and positively correlated with silt, clay, pH and EC following curvilinear relationship with these parameters. Leachable boron content was strikingly higher in fine textured soils as compared to coarse textured alluvial and lateritic soils of Assam (Borkakati, 1988). pH and EC were significantly correlated with AB-DTPA extracted boron with coefficient values of $r=0.874^{**}$ and $r=0.684^{**}$, respectively. Dwivedi *et al.* (1993) reported positive but non-significant correlation between pH and AB-DTPA extractable boron. It is interesting to note that this form of boron was significantly correlated with other forms of boron. Thus, it can also be used as an index of boron availability in these soils. Total boron content showed significant correlation with pH ($r=0.686^{**}$), EC ($r=0.421^*$), CEC ($r=0.766^{**}$), silt ($r=0.498^*$) and clay content ($r=0.563^*$) of the soil. Total boron was associated with finer fractions of soil. Negative but significant correlation was seen for total

Table 2. Different forms of boron in different soils of Kashmir

Soils	Water soluble	Hot Water soluble	CaCl_2 extractable	Acid extractable	Leachable	AB-DTPA extractable
mg kg ⁻¹						
S ₁	0.34	0.41	0.58	0.63	0.88	1.08
S ₂	0.22	0.47	0.64	0.51	0.94	1.14
S ₃	0.86	0.78	0.95	1.15	1.25	1.45
S ₄	0.25	0.29	0.46	0.54	0.76	0.96
S ₅	0.34	0.48	0.65	0.63	0.95	1.15
S ₆	0.10	0.18	0.35	0.39	0.65	0.85
S ₇	0.07	0.10	0.27	0.36	0.57	0.77
S ₈	0.15	0.13	0.30	0.44	0.60	0.80
S ₉	0.95	1.27	1.44	1.24	1.74	1.94
S ₁₀	0.22	0.47	0.69	0.51	0.94	1.19
Mean	0.35	0.46	0.63	0.64	0.93	1.13

Table 3. Coefficient of Correlation between different forms of Boron and physico-chemical characteristics of soils

Forms of Boron	pH	EC	CEC	Organic carbon	CaCO_3	sand	Silt	Clay
Water soluble	0.860 ^{**}	0.609 ^{**}	0.620 [*]	-0.451 [*]	0.030	-0.813 ^{**}	0.374 [*]	0.572 ^{**}
Hot water soluble	0.773 ^{**}	0.589 ^{**}	0.492 [*]	-0.362 [*]	-0.095	-0.783 ^{**}	0.419 [*]	0.477 [*]
CaCl_2 extractable	0.742 ^{**}	0.593 ^{**}	0.510 [*]	-0.432 [*]	0.110	-0.608 ^{**}	0.163	0.576 ^{**}
Acid extractable	0.637 ^{**}	0.520 ^{**}	0.359	-0.148	0.460 [*]	-0.677 ^{**}	0.242	0.564 ^{**}
Leachable-B	0.576 ^{**}	0.610 ^{**}	0.140	-0.140	0.327	-0.486 [*]	0.203	0.362
AB-DTPA extractable	0.874 ^{**}	0.684 ^{**}	0.663 ^{**}	-0.436 [*]	0.043	-0.463 ^{**}	0.399 [*]	0.580 ^{**}
Total	0.686 ^{**}	0.421 [*]	0.766 ^{**}	-0.219	-0.080	-0.928 ^{**}	0.498 [*]	0.563 [*]

^{*}, ^{**} Significant at 5%, 1 % level of significance, respectively

boron with sand ($r=-0.928^{**}$) and non-significant with organic carbon ($r=-0.219$).

Kinetics of boron adsorption by soils: Batch studies were conducted to investigate the kinetics of boron adsorption process. CaCl_2 (0.01 M) solution containing $40 \mu\text{g B ml}^{-1}$ was added to each 10 g soil in 50 ml polyethylene centrifuge tubes. In all the soils, the boron adsorption was characterized by an initial fast reaction followed by a slow process. Adsorption of boron was almost complete after 48 hr in all the soils, though complete equilibrium was not attained until 72 hr (Table 4). The concentration of boron in the equilibrium solution ranged from 27.5 to $36.1 \mu\text{g ml}^{-1}$ at the completion of the adsorption reaction. Six different kinetic models viz. zero order, first order, second order, Elovich, power function and parabolic diffusion were tested by least square regression analysis for describing boron adsorption in representative soils of Kashmir. The model, which had the highest coefficient of determination (R^2) and the lowest standard error of estimates (S.E) values was considered to be best fit. The zero order, first order, second order and power function kinetic equations could not describe the adsorption of boron by soils, as coefficient of determination (R^2) values were very low and S.E values were large (Table 5).

The amount of boron adsorbed by eight soils of Tamil Nadu was poorly described by zero order, first order and third order reaction equations (Krishnaswamy *et al.*, 1997). The Parabolic diffusion was best of the other kinetic equations studied to describe the rate of boron adsorption as evidenced by the overall highest values of coefficient of determination (R^2) and lowest standard error of estimates (SE) over the entire time range. Such conformity has also been reported by Krishnaswamy *et al.* (1997), while studying boron adsorption kinetics of Tamil Nadu soils. Elovich kinetic model was as good as parabolic diffusion equation in describing boron adsorption kinetics in all the soils.

Table 5. Parameters of Elovich and Zero order Kinetic equation for B-adsorption

Soils	Elovich			Zero order		
	β	R^2	SE	β	R^2	SE
S1	4.831	0.956	0.478	5.906	0.871	0.816
S2	5.773	0.962	0.441	6.863	0.848	0.876
S3	6.731	0.974	0.346	7.802	0.841	0.861
S4	4.328	0.928	0.539	5.198	0.932	0.575
S5	6.051	0.974	0.362	7.166	0.827	0.925
S6	4.713	0.965	0.465	5.931	0.844	0.985
S7	4.555	0.958	0.422	5.509	0.892	0.672
S8	4.239	0.937	0.453	5.048	0.907	0.551
S9	6.272	0.972	1.305	7.377	0.818	0.935
S10	3.970	0.948	0.421	4.815	0.913	0.543

Kinetics of boron desorption by soils: All the soils used for boron adsorption kinetics were also subjected to desorption study after 48 hours of adsorption (Table 6). The soils were pre-treated with $40 \mu\text{g B g}^{-1}$ soil. The amount of desorbable boron was strikingly higher in the coarse textured soils as compared to the fine textured soils. The increase in desorbable boron with time can be attributed to the less number of exchange sites due to the low clay content to retain boron more tightly. Also desorbed boron when expressed in percentage of adsorbed content was lower in fine textured soils. This again suggests that boron was held more tightly and thereby more time for its desorption was taken from fine textured soils.

The desorption reaction was initially fast and thereafter became slow. The desorption reactions seemed to have completed in 48 hours in most of the soils and only in few soils the reaction was not complete even after 72 hr and therefore, the values of total desorbable boron could not be ascertained. For the kinetic treatment of the data, the concentration of solution boron at the plateau of the curves

Table 4. Amount of boron adsorbed ($\mu\text{g g}^{-1}$) by the soils under investigation with respect to time

Soils	Time (h)								
	0	1	2	4	8	16	24	48	72
S1	35.6	34.5	34.0	33.5	32.7	32.1	31.6	30.0	29.0
S2	34.6	33.8	33.2	32.4	31.5	31.0	30.8	29.4	28.1
S3	33.6	32.8	32.3	31.8	30.7	30.0	29.8	28.4	27.5
S4	35.7	35.0	34.9	34.2	33.8	33.1	32.7	31.1	30.0
S5	34.4	33.4	33.0	32.2	31.1	30.8	30.2	29.1	28.0
S6	35.5	34.7	34.6	33.4	32.0	31.7	31.5	29.7	28.7
S7	35.6	34.9	34.6	34.0	33.2	32.8	32.1	30.8	29.9
S8	36.0	35.1	35.0	34.4	34.0	33.3	33.0	31.8	30.7
S9	34.2	33.1	32.9	32.0	31.0	30.5	30.0	28.9	28.0
S10	36.1	35.7	35.1	34.8	34.1	33.8	33.1	31.8	31.0

Table 6. Amount of boron desorbed ($\mu\text{g g}^{-1}$) from the soils in 0.05 M mannitol solution with respect to time

Soils	Time (h)								
	0	1	2	4	8	16	24	48	72
S1	7.65	7.94	8.35	8.56	8.85	9.25	9.79	10.25	10.25
S2	7.11	7.44	7.78	8.56	8.89	9.22	9.46	9.87	10.22
S3	6.82	7.28	7.78	8.55	8.69	9.42	9.84	10.23	11.28
S4	9.33	9.92	10.56	10.98	11.94	12.29	12.63	12.95	12.95
S5	6.89	7.73	8.89	9.04	9.72	10.67	11.15	11.41	11.54
S6	7.33	7.84	9.47	9.63	10.45	11.09	11.29	11.59	11.59
S7	8.88	9.35	9.45	9.78	9.98	10.22	10.49	11.16	11.62
S8	8.76	9.35	10.13	10.55	11.02	11.78	12.18	13.58	13.58
S9	6.35	6.80	7.52	7.76	8.34	9.03	9.14	9.55	9.55
S10	9.82	10.27	10.89	11.65	11.88	12.88	13.45	13.86	14.58

Table 7. Parameters of boron desorption kinetics

Soils	Zero order		First order		Second order		Parabolic diffusion		Elovich		Power function	
	R ²	SE	R ²	SE	R ²	SE	R ²	SE	R ²	SE	R ²	SE
S1	0.779	0.4821	0.752	0.0569	0.723	0.0068	0.948	0.2347	0.976	0.1595	0.977	0.0174
S2	0.721	0.6172	0.678	0.0775	0.635	0.0098	0.910	0.3494	0.982	0.1557	0.971	0.0233
S3	0.818	0.6648	0.761	0.0864	0.699	0.0113	0.959	0.3171	0.971	0.2649	0.972	0.0297
S4	0.607	0.9072	0.577	0.0842	0.546	0.0079	0.842	0.5759	0.954	0.3092	0.939	0.0321
S5	0.627	1.0895	0.574	0.1268	0.518	0.0151	0.856	0.6773	0.946	0.4154	0.915	0.0566
S6	0.533	1.1646	0.489	0.1297	0.446	0.0146	0.775	0.8081	0.908	0.5165	0.874	0.0644
S7	0.899	10.4215	0.873	0.0328	0.844	0.0036	0.987	3.0561	0.936	0.2378	0.945	0.0217
S8	0.803	0.8147	0.757	0.0813	0.705	0.0082	0.960	0.3684	0.974	0.2985	0.973	0.0269
S9	0.640	0.7614	0.600	0.1010	0.558	0.0136	0.866	0.4648	0.964	0.2417	0.945	0.0375
S10	0.781	0.8278	0.739	0.0751	0.695	0.0069	0.948	0.4042	0.986	0.2075	0.983	0.0190

was considered as the total desorbable boron (B) with the assumption that the desorption reaction at that stage was complete in these soils. The perusal of the data showed that Elovich kinetic equation was best among different models as evident by the overall highest values of coefficient of determination (R^2) and lowest standard error of estimates (SE) values over the entire range of time. The requirement of short reaction time for fitting data to the Elovich equation may be one of the reasons for higher SE values observed in the present study. The Power function model was found to be equally good in describing boron desorption kinetics in all the soils. Sharma *et al.* (1989) also indicated that power function, pseudo-first order and Elovich kinetic models adequately described boron release from salt affected soils of Punjab.

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Received 15 December, 2015; Accepted 10 February, 2016



Calculation and Analysis of the Urban Ecological Footprint: A case Study of Ajmer City, India

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Abstract: The ecological impact of urbanization in terms of built up land footprint of Ajmer City of Rajasthan state in northwest India indicate that residential areas have highest footprint among other developmental activities in Ajmer city. Results show that urban footprint of Ajmer city is twice the area of the city itself, which concludes that Ajmer city cannot meet its domestic needs alone and has to depend on outside sources to meet its sustenance.

Key Words: Urbanization, built footprint, infrastructural development, sustainability

Urbanization is an index of transformation from traditional rural economies to modern industrial economies. The process of urbanization in developed countries has been very slow but steady accompanied by agricultural and industrial revolution, higher per capita income and high standard of living, whereas in developing countries the rate of urbanization is very fast however not accompanied by industrialization but with rapid growth of service sector in the economies (Macbeth *et al.*, 2002). In the search of economic opportunities and various facilities of urban area, more and more people migrate from rural to urban areas. Increasing growth of human population in urban areas has resulted in physical growth, be it horizontal or vertical. It has been predicted that by the end of 2050, 64.01% of developing and 85.9% of developed world would be urbanized. India is witnessing increasing levels of urban population. As per census 2011, nearly 31% of the country's population lives in cities and urban areas. Moreover, the level of urbanization is expected to reach 50% mark in the next 2-3 decades. While cities are regarded as engines of growth, they continue to face enormous challenges. Increasing urbanization has led to tremendous pressure on land, civic infrastructure, transport, open spaces, etc. (Kundu, 2006). Census 2011 has provided total, rural and urban population of India and Rajasthan along with respective percent decadal growth in population (Table 1).

According to the census report of 2011, the share of urban population in Rajasthan has enhanced up to 24.87% as compared to 23.38%. Number of towns in the Rajasthan has also increased to 216 as per census 2011 against 196 in the 2001. A shift from rural to urban society represents major and irreversible changes in production and consumption and the way people interact with environment. Urban environment is a composite of natural environment, built

environment and socio-economic environment. Urban sustainability primarily means that the needs of city dwellers are met without imposing unsustainable demand on natural resources. The aim of urban sustainability is to create the smallest ecological footprint, to produce the lowest quantity of pollution, to efficiently use land and other natural resources, to recycle or convert waste-to-energy and to overall contribute to minimal climate change (Lenzen *et al.*, 2003). Basically, ecological footprint is defined as the land area that would be needed to meet the consumption of a population and to absorb all their waste (Wackernagel *et al.*, 1995). To assess whether an urban society is moving towards or against sustainability, it is necessary to consider five dimensions of urban sustainability - Economic Sustainability, Social Sustainability, Ecological Sustainability, Political Sustainability and Physical Sustainability.

Table 1. Census 2011 study of India and Rajasthan

Particulars	India	Rajasthan
Total population	1,210,193,422	68,621,012
Rural population	833,087,662	51,540,236
Urban population	377,105,760	17,080,776
Percent decadal growth	17.64	21.44
Percent decadal rural growth	12.18	19.05
Percent decadal urban growth	31.80	29.26

MATERIAL AND METHODS

Ajmer is situated in the cradle of the Aravalli mountain range and is the fourth largest city of Rajasthan covering an area of 241.50km². As per 2011 census, the population of Ajmer is estimated at around 5.4 lakhs (at the rate of 2% annual growth). According to Ajmer Master plan 2001-03, the total urbanized area is 13,444 acres (53.8 km²) of which 11482 acres (45.9 km²) is the developed area. 85%

of the total urban area is developed so far. 5% of the total area is government reserved land under CRPF and Defense force. The remaining 10% comprises of open spaces and water bodies. Out of the total developed area the largest land is under residential use (45%) followed by transport (18%) and public and semi-public (12%). The high portion of land use under transport is because of National Highway 8 (NH-8) passing through the city and the newly built NH-8 by pass.

In this paper, ecological footprint is calculated in terms of land area of built environment of Ajmer City by using component methodology. The study of footprint may help in making choices that will have less impact on the environment.

Built-up land footprint is calculated based on the area of land covered by human infrastructures. Ecological footprint calculations are usually presented in units of global hectares. Historically, ecological footprint analyses have utilized a yield factor (YF) for each land use type to capture the difference between local and global productivity. Yield factor (YFs) is area specific and vary by land use type and year. They may reflect natural factors such as differences. The 2011 Edition of the National Footprint Accounts assumes that built-up land occupies what would previously have been cropland. This assumption is based on the observation that human settlements are generally situated in fertile areas with the potential for supporting high yielding cropland (Imhoff *et al.*, 1997; Wackernagel *et al.*, 2002); in precipitation or soil quality, as well as anthropogenic differences such as management practices. The YF is the ratio of national average to world average yields. It is calculated in terms of the annual availability of usable products. In order to combine the ecological footprint of different land-use types, equivalence factors (EQFs) is necessary (Galli *et al.*, 2007). Equivalence factors (EQFs) convert the areas of different land use types, at their respective world average productivities, into their equivalent areas at global average bio-productivity across all land use types. EQFs vary by land use type as well as by year. For the ecological footprint, proper information related to land use pattern of the specific area is required. The total bio-capacity is expressed in global hectares, equals the total number of biologically productive physical hectares on Earth (Kitzes *et al.*, 2007).

To calculate each built land ecological footprint, a yield factor is applied to the raw data to convert it into hectares of global average crop area. A crop area equivalence factor is then applied to convert the data into global hectares.

RESULTS AND DISCUSSION

Land use pattern of Ajmer City may be categorized

mainly into 06 types -residential, commercial, industrial, governmental, public/semi-public, recreation and transport. Ajmer have total area (km²) 8,481.00 which contain 8,091.33 rural and 389.67 urban area. The residential area is 24km² followed by 9.9km² of transport area. Public/semi-public area contains 6.3km² whereas, commercial and industrial areas are same i.e. 2.3 Km². Land use for government use and recreation purpose are also same but least among the land use pattern (1.3%). Calculation of ecological footprint of city show that residential area have maximum ecological footprint (4997.05) followed by transport and public/ semi public area.

Table 2. Ecological footprint of different land pattern of Ajmer city

Land use	Acres	Hectares	Ecological footprint
Residential	6000	2428.11	4997.05
Commercial	564	228.24	469.71
Industrial	586	237.14	488.03
Governmental	140	56.65	116.58
Public/Semi Public	1571	635.76	1308.39
Recreation	138	55.84	114.91
Transport	2483	1004.83	2067.94

The resultant built up land, footprint for Ajmer city is 9562.61gha and out of the total built up land footprint, residential area contributes to maximum and recreational land has minimum built land footprint.(Fig. 1 and 2)

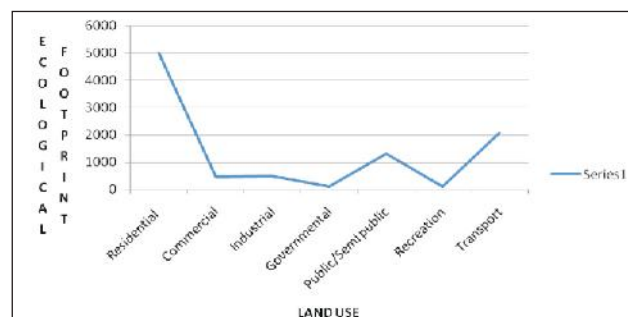


Fig. 1. Line chart of ecological footprint of different area of Ajmer

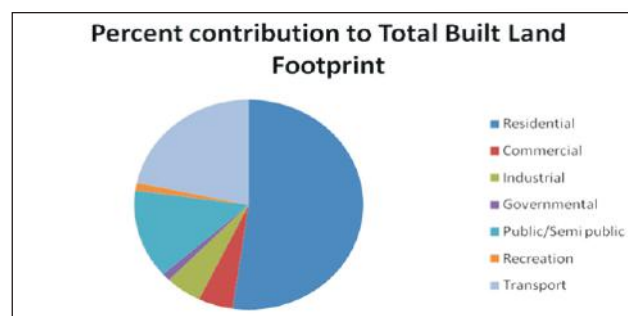


Fig. 2. Pie Chart of ecological footprint of different area of Ajmer

More than 50% of total built footprint is due to residential dwellings in Ajmer city (Table 2). According to the

census 2001, population of Ajmer city was 4, 85, 575 and thus, built up footprint per capita comes to be 0.0196 gha capita⁻¹.

Results show that urban footprint of Ajmer city is twice the area of the city itself, which concludes that Ajmer city cannot meet its domestic needs alone. Similar results have been drawn from the study by Cole (2002) wherein ecological footprint was analysed to quantify sustainability of Manali, a rapidly growing tourist center. Ecological footprint of Manali was found to be 25 times greater than its size and thus Manali relies on outside ecosystem for its sustenance. Since footprint of Ajmer city is greater than its bio-capacity, a scientific study is required to line up the growth of city towards sustainable development. The present study indicates that land areas for recreation and commercial facilities have been poorly developed as compared to residential land. An approach to develop recreational and commercial facilities in areas with residential dwellings may probably balance the inequity of footprint of different land areas and future development of residential areas should be in peri-urban interfaces, connecting cities to the rural spaces. A study conducted by Holden (2004) shows that housing related consumption of two Norwegian towns and concluded that decentralized concentration could lead to smaller ecological footprints. The research favors formation of polycentric cities which implies dense and concentrated centers within larger cities, containing variety of housing and workplaces as well as private and public transport services. Our study is in accordance with study of Holden referring with development of peri-urban areas with effective and environmentally sound transport infrastructure that connects Ajmer city to all such nearby areas.

Ecological footprint of transportation for Ajmer city is second among the other footprint areas and thus demands a new transportation policy so as to reduce impacts of increasing means of transport. A transportation policy does not only deal with travel flows but can also foster effects of land use policies that are dealing with excessive urban sprawl. Vishal (2013) observed that with urbanization, ecological footprint of city grows and creates a need of interaction among urban dwellers and authorities with peri-urban residents to evolve a transparent collaborative effort, especially in terms of improving transportation and mobility. Likewise, peri-urban areas surrounding Ajmer city should be developed in such a way that problem of heavy and

congested transportation is resolved and transport footprints are minimized. Ajmer city is one among the rapidly growing cities and holds a major concern due to its location near to capital city, Jaipur and also because, Ajmer as a pilgrimage center and tourist place is visited during both Pushkar and Urs Fair. Even though, city of Ajmer have lower ecological footprint than the national average; yet depends on outside sources to meet its sustenance. In order to achieve self supportive ecosystem, sustainable future plans have to be formulated taking into consideration the urban form, public transport system, lowering fuel dependence, eco-friendly tourism, systematic land use plans and public awareness towards environmental concerns of the city.

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Waste Water: A Source of Multidrug Resistant *Escherichia coli* and Faecal streptococci Contamination

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Abstract: Ten isolates each of *Escherichia coli* and faecal streptococci purified from different samples of waste water showed high resistance to 24 antibiotics tested by disc diffusion method. All the *Escherichia coli* strains were sensitive only to three antibiotics viz. cotrimoxazole, levofloxacin and impenem and were sensitive or partially sensitive to norfloxacin. For nalidixic acid, amikacin, sparflaxacin and vancomycin 60, 40, 20 and 10% *Escherichia coli* strains respectively showed resistance. Similarly for faecal streptococci, high degree of drug resistance was observed and all isolates were resistant to as many as 21 antibiotics and for cotrimoxazole, vancomycin and impenem 70, 60 and 40% isolates. The high multiple antibiotic resistance index (MAR) indicates high risk to public health and calls for proper monitoring of waste water treatment and disposal.

Key Words: Antibiotic resistance, *Escherichia coli* and faecal streptococci (FS), Multidrug resistant, Wastewater

Emergence and prevalence of antibiotic resistant microorganisms in environment poses a serious public health problem. As food and water are known for transmission of microbial diseases, the prevalence of antibiotic resistance has added another dimension to food safety. *Escherichia coli* (Gram negative rods) and faecal streptococci (Gram positive cocci) are normal flora of gastrointestinal flora of human beings and warm blooded animals (Harwood *et al.*, 2000). They are the indicators of contamination by fecal matter (Cray *et al.*, 1998). The proportion of faecal streptococci in fecal matter of animals is higher than *Escherichia coli*, while in human beings the reverse is true (Sinton *et al.*, 1993). Coliforms pollution of surface water is attributed to livestock industry, open defecation and improper treatment of sewage and waste water (Ahmed *et al.*, 2010; Baquero *et al.*, 2008; Carlet *et al.*, 2012). Antibiotics are routinely used in treatment of microbial diseases as well as in rearing of animals to prevent and treat illness. Thus through prolonged exposure, microorganisms develop resistance to antibiotics and furthermore spread fast among other population as the genetic markers for antibiotic resistance are largely carried on plasmids and transposons (Aminov and Mackie, 2007; Zbigniew *et al.*, 2010). The risk of exposure of food and water to such pathogens result in gastrointestinal diseases such as diarrhoea and enteric fever. Lack of environmental sanitation and poor personal hygiene particularly among food handlers accounts for contamination of foods and improper storage of foods leads to multiplication of pathogens to infective doses. Therefore, the present study was undertaken to examine waste waters for prevalence of antibiotic resistant coliforms-*Escherichia coli* and faecal streptococci.

MATERIAL AND METHODS

Ten wastewater samples were collected from five different sources, the sewage treatment plants (WS-1, WS-2), food industries (WS-3, WS-4), milk and milk product industries (WS-5, WS-6), cattle feed industries (WS-7, WS-8) and Dhaba kitchens (WS-9, WS-10). Samples measuring 200 ml were collected in clean sterile plastic bottles. All samples were stored at 4°C and analyzed within 24 hrs.

Isolation and identification of bacterial isolates: The number of *Escherichia coli* and Faecal streptococci was determined by using MPN method as per guide lines of BIS (Oblinger and Koburger, 1975). The media used for MPN of *Escherichia coli* was Mac Conky broth and for MPN of faecal streptococci was ethyl violet azide dextrose broth (EVDB). Test for thermo-tolerant coliforms was performed by modified Eijkman test at a temperature of 44.5°C. Pure cultures of *Escherichia coli* and faecal streptococci were obtained by repeated sub culturing on specific medium plates from the tubes showing positive results (Ibe and Okpluye, 2005). The selective media used for *Escherichia coli* was eosin methylene blue lactose Agar (EMB) and for faecal streptococci was ethyl violet azide dextrose agar (EVDA). The pure cultures were identified on the basis of pigmentation of the colonies, cell morphology, motility and Gram's reaction followed by standard biochemical characterization viz. MR-VP test, urease, TSI (triple sugar iron), indole production, citrate utilization, glucose fermentation, catalase and the oxidase tests. Pure cultures were maintained on slants at 4°C (IS: 5887(I), 5887(II):1976). Ten isolates of each of *Escherichia coli* and faecal streptococci selected and purified on selective medium were used for determining antimicrobial

susceptibility to 24 different antibiotics by using disc diffusion antibiotic discs.

Antimicrobial susceptibility: Antimicrobial susceptibility of the bacterial isolates was checked by Kirby-Bauer disc diffusion method (Bauer *et al.*, 1996) on Muller Hinton Agar medium using Hi-media antibiotic impregnated discs (National Committee for Clinical Laboratory Standards). Twenty four different antibiotics (Hi-media) selected for diffusion test were Amikacin (30µg), Ampicillin (10µg), Amoxicillin (20µg), Azithromycin (15µg), Ciprofloxacin (15µg), Erythromycin (15µg), Clindamycin (2µg), Tetracycline (30µg), Gentamycin (10µg), Oxycillin (1µg), Vancomycin (30µg), Ofloxacin (5µg), Sparfloxacin (5µg), Penicillin-G (10U), Cotrimoxazole (25µg), Kanamycin (30µg), Levofloxacin (5µg), Cephalothin (30µg), Norfloxacin (10µg), Nalidixic acid (30µg), Neomycin (30µg), Sulphafurazole (30µg), Trimethoprim (5µg), Imipenem (10µg). The results were interpreted as per CLSI guidelines as S-sensitive, I-intermediate resistant and R-resistant (CLSI 2005).

$$\text{MAR index of isolate} = \frac{\text{No. of antibiotics to which an isolate is resistant}}{\text{Total no. of antibiotics to which the isolate was exposed}}$$

RESULTS AND DISCUSSION

Results presented in tables shows that water from different sources differed in their characteristics and all the samples were positive for *Escherichia coli* and faecal streptococci. The MPN count for *Escherichia coli* and faecal streptococci were in the range of 34-175 MPN and 16-110 MPN/100ml of water sample, respectively. The pH was close to neutral as it ranged between 6.8 and 7.4 for different samples. Physical appearance indicated that waste was not clear and contained organic matter, dirt and grit. The presence of *Escherichia coli* and faecal streptococci indicated fecal matter as a source of contamination and was not suited for any type of use including irrigation.

Antibiotic sensitivity: Ten isolates of each of *Escherichia coli* and faecal streptococci were purified by repeated streaking on selective media plates, identified and maintained as pure cultures. These isolates were subjected to antibiotic sensitivity test to 24 antibiotics using disc diffusion method (Table 2 and 3). All the isolates of *Escherichia coli* were resistant to Ampicillin, Amoxicillin, Azithromycin, Ciprofloxacin, Erythromycin, Clindamycin, Tetracycline, Gentamycin, Oxycillin, Ofloxacin, Penicillin-G, Kanamycin, Cephalothin, Neomycin, Sulphafurazole and Trimethoprim (Table 2). Only 20% of the isolates were resistant to Sparfloxacin and all isolates were sensitive to

Cotrimoxazole, Levofloxacin and Imipenem. For Norfloxacin two isolates, EcW3 and EcW9 were sensitive, while all others showed intermediate reaction. As well as none of the isolate was sensitive to amikacin while four strains were resistant and remaining six showed intermediate reaction. Strain EcW5 was resistant to Vancomycin and one strain EcW3 was sensitive to Vancomycin while other showed intermediate reaction. Two strains EcW5 and EcW7 were resistant to Sparfloxacin and one strain EcW8 was sensitive and all other showed partial resistance as indicated in intermediate reaction. None of the isolates was resistant to Norfloxacin, EcW3 and EcW9 were sensitive and all others were partially resistant. Sixty per cent of the isolates were resistant to Nalidixic acid and rest were partially resistant. Sensitivity towards these antibiotics can be attributed to their restricted use. Resistance to antibiotics is attributed to mechanisms such as reduced uptake of antibiotics, decreased cell permeability, alteration at the ribosomal binding sites or by the production of modifying enzymes (Maira-Litran *et al.*, 2000).

Faecal streptococci were resistant to all selected antibiotics except Imipenem (40%), Vancomycin and Cotrimoxazole for which 40, 60 and 70% isolates were resistant (Table 3). High percentage of isolates resistant to fluoroquinolone group of antibiotics such as Levofloxacin and Ofloxacin can be credited either to alteration in drug target enzymes or to amendments that limit the permeation of drug to target, both resulting from chromosomal mutations (Dang *et al.*, 2008). Resistance to Cotrimoxazole can be conferred by mutational loss modifying the bacterial ability to methylate deoxyuridylic acid to thymidylic acid. This makes them dependent on the external supply of thymine.

Multiple antibiotic resistance (MAR) index: Results show a high degree of resistance to antibiotics in both types of organisms isolated from waste waters (Fig. 1.). MAR index of 0.2 or higher indicates high risk sources of contamination, MAR index of 0.4 or higher is associated with human fecal contamination (Krumperman, 1983). MAR indices of all the

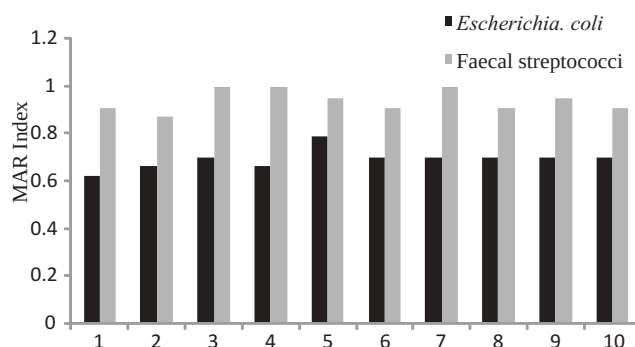


Fig. 1. Multiple Antibiotic resistance indices of *Escherichia coli* and Faecal Streptococci isolates

isolates of *Escherichia coli* and faecal streptococci imply that they originate from high risk sources of contamination.

Wastewaters of milk product industries are heavily loaded with multidrug resistant bacteria. The sources of multidrug resistance may be used as antibiotics in preservation of milk products to increase their shelf life (Zubir *et al.*, 2009). Continuous exposure of bacteria to these antibiotics enables them to survive as antibiotic resistance bacteria and when these come in contact with other resistant bacteria, these become multidrug resistant by sharing their resistance genes.

Highest MAR index in case of faecal streptococci is 1.0 for isolates FSW3 and FSW4 isolated from wastewaters of food industries and strain FSW-7 isolated from waste water of cattle feed industries. Rest of the isolates showed higher value of MAR index that is between 0.87-1.0, which is much higher than that of *Escherichia coli* isolates. Raw material used, improper handling, improper cleaning of equipments and utensils used in production, unhygienic conditions prevailing in these industries contribute to large scale contamination of waste water of these industries. Waste waters of food industries and cattle feed industries are heavily loaded with multidrug resistant faecal streptococci. The waste waters may further be contaminated with surface runoff water laden with debris and street garbage besides fecal matter.

Five different sources of waste waters characterized in this study were investigated for isolation of *Escherichia coli* and *faecal streptococci* because they differ in terms of the origin of bacterial strain in the environment and the expected exposure of these bacteria to antimicrobial selective pressure. Both strains of all bacterial isolates were

resistant to all selected antibiotics except *Escherichiacoli* isolates were sensitive to Cotrimoxazole, Levofloxacin, Norfloxacin and Imipenem and *faecal streptococci* isolates were not sensitive to any of the selected antibiotics but some isolates showed intermediate reaction. The percentage of resistance in the *faecal streptococci* isolates was observed to be 40% for Imipenem and 60% Vancomycin, while all other isolates were found to be resistant to all the antibiotics tested. This study support the idea that β -lactam resistance is now widespread in pathogenic bacteria isolated from variety of location and sources (Maciedo *et al.*, 2010).

Highest MAR in case of *Escherichia coli* isolate EcW5 was observed, with Sample ID WS-5 of milk and milk products industries indicate that high percentage of *Escherichiacoli* in food product industries have become multidrug resistant, which could be the cause of morbidity. Highest MAR index in case of faecal streptococci isolates FSW3 and FSW4 isolated from waste waters of food industries and strain FSW-7 isolated from waste water of cattle feed industries was found to be 1.0. This indicates that infected raw material with multidrug resistant bacteria could be the reason of heavily contaminated wastewater. Throwing of contaminated wastewater without treatment and stagnation of water before releasing might be the reason for heavy load of microbes which become multidrug resistance under adverse conditions.

High resistance to antibiotics may be attributed to several factors: first It may be that commensal bacteria excreted into wastewaters had become resistant due to their higher exposure, secondly introduction of antibiotic supplemented foods, cattle feed might have initiated the resistance. Higher resistance may also be attributed to the

Table 1. Total count of *Escherichiacoli* and faecal streptococci as MPN

Sample ID	Location	Appearance	pH	<i>Escherichia.coli</i> count MPN/100ml	Faecal streptococci count MPN/100ml
WS-1	Sewage treatment plant	Blackish Muddy water with some particles	7.2	94	21
WS-2	Sewage treatment plants	Blackish Muddy water with some particles	7.4	120	24
WS-3	Food industries	Grayish black water	6.8	70	46
WS-4	Food industries	Grayish black water	7.0	175	110
WS-5	Milk product industries	Straw colored clear water	7.2	46	29
WS-6	Waste waters of milk product industries	Straw colored clear water	7.3	109	16
WS-7	Waste water of cattle feed industries	Brownish clear water	7.2	34	24
WS-8	Waste water of cattle feed industries	Brownish clear water	7.2	70	110
WS-9	Waste waters of house kitchens	Brownish black clear water	7.0	141	110
WS-10	Waste waters of house kitchens	Brownish black clear water	7.1	79	46

Table 2. Antimicrobial susceptibility of the *Escherichia coli* isolates

Antibiotics	EcW 1	EcW 2	EcW 3	EcW 4	EcW 5	EcW 6	EcW 7	EcW 8	EcW 9	EcW 10	% Resistance
Amikacin	I	I	R	I	R	R	I	R	I	I	40
Vancomycin	I	I	S	I	R	I	I	I	I	I	10
Sparfloxacin	I	I	I	I	R	I	R	S	I	I	20
Cotrimoxazole	S	S	S	S	S	S	S	S	S	S	0
Levofloxacin	S	S	S	S	S	S	S	S	S	S	0
Norfloxacin	I	I	S	I	I	I	I	I	S	I	0
Nalidixic acid	I	R	R	R	I	I	R	I	R	R	60
Imipenem	S	S	S	S	S	S	S	S	S	S	0
*Other antibiotics tested	R	R	R	R	R	R	R	R	R	R	100

*Ampicillin, Amoxycillin, Azithromycin, Ciprofloxacin, Erythromycin, Clindamycin, Tetracycline, Gentamycin, Oxycillin, Ofloxacin, Penicillin-G, Kanamycin, Cephalothin, Neomycin, Sulfafurazole, Trimethoprim

Table 3. Antimicrobial susceptibility of the faecal streptococci isolates

Antibiotics	FSW 1	FSW 2	FSW 3	FSW 4	FSW 5	FSW 6	FSW 7	FSW 8	FSW 9	FSW 10	% Resistance
Vancomycin	R	I	R	R	I	R	R	I	R	I	60
Cotrimoxazole	I	I	R	R	R	I	R	R	R	R	70
Imipenem	S	I	R	R	R	I	R	S	S	S	40
**Other antibiotics tested	R	R	R	R	R	R	R	R	R	R	100

**Amikacin, Ampicillin, Amoxycillin, Azithromycin, Ciprofloxacin, Erythromycin, Clindamycin, Tetracycline, Gentamycin, Oxycillin, Ofloxacin, Sparfloxacin, Penicillin G, Kanamycin, Levofloxacin, Cephalothin, Norfloxacin, Nalidixic acid, Neomycin, Sulfafurazole, Trimethoprim

Note: R=Resistant, I= Intermediate, S=Sensitive

history, frequency and high dose of antibiotic utilization by self medication or their prolonged use which is excreted in sewage water (Aminov and Mackie, 2007). High MAR index found in this study has also been reported in other aquatic environments (Matyar *et al.*, 2007). MAR index beyond 0.20 in isolates of both types indicates that these pose a high risk and exposure can be cause of major public health concern (Mc Donald *et al.*, 1997). Resistance to one antibiotic could contribute to selection of resistance to antibiotics for other groups. This situation calls for strict public health regulation regarding the disposal of wastewaters from mentioned sources. Enforceable legislation regarding the regular check on environmental sanitation may improve the situation. There is also need of public awareness about proper wastewater treatment before disposal and regulation should be set so that occurrence of multidrug resistant bacteria through waste water can be avoided.

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Surface Water Quality and Associated Aquatic Insect Fauna under Different Land-Uses in Solan (District Solan), Himachal Pradesh

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Abstract: The common land uses of mid hills have resulted in significant variations in the physico- chemical properties of the surface water. The pH and EC of surface water ranged from 6.01 to 7.53 and 455.00 to 618.50 $\mu\text{S cm}^{-1}$, respectively. The TDS, BOD and COD ranged from 45.75 to 248.25 mg l^{-1} , 0.35 to 3.43 mg l^{-1} and 0.25 to 6.25 mg l^{-1} , respectively. The land use wise trend of these properties was Agriculture>Urban >Forest. The Cl^{-} and NO_3^{-} content of surface water ranged from 10.33 to 132.50 mg l^{-1} and 1.50 to 29.50 mg l^{-1} . The land use wise trend of these parameters was agriculture>Urban > Forest. The contents of Ca^{2+} and Mg^{2+} were ranged from 7.50 to 56.50 and 7.50 to 29.88 mg l^{-1} respectively. The study indicated that the common land uses in the mid hills of Solan district did not influence the surface water quality adversely and was within the acceptable limit as prescribed by WHO. The study further indicated that the agriculture land use tended to increase TDS, BOD, COD, Cl^{-} and NO_3^{-} content in surface water. The seasons of the year also influenced the physico-chemical properties of surface water. During rainy season all the parameters exhibited relatively higher values as compared to winter and summer months. The identification of aquatic insects in surface water up to family level indicated that in the region water is composed of 173 individuals m^{-2} falling under 10 families and six orders. The Land use wise aquatic insect recorded were 78, 62 and 33 individuals m^{-2} under forest, agriculture, and urban land uses, respectively. Forest land use registered maximum diversity of aquatic insect fauna as compared to other land use.

Key Words: Agriculture, BOD, COD, Forest, Seasons, Urban

The availability of safe water, which has been described as water that meets the standard for drinking water quality (Federal Ministry of Water Resources 2004) is decreasing rapidly. Since last few decades, rapid increase in human population, increasing anthropogenic activities, land use changes, discharge of sewage and municipal waste into water bodies have deteriorated water quality (Meitei *et al.*, 2004). Water resources have been rendered unsafe for human consumption as well as for other activities like irrigation and industrial needs. The severe contamination near urban areas indicate that industrial and domestic sectors contribute significantly to water pollution in our country. Besides, growing trend of high input based commercial farming is also contributing to this problem. The deteriorating water quality of surface and ground water sources contribute to water scarcity as it limits its availability for both human use and for the ecosystem. Therefore, there is urgent need for monitoring quality parameter of water like Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids, pH, Electrical Conductivity, hardness, toxic metals pesticides, oil and grease, indicators of pathogenic bacteria like total coliform and faecal coliform. Moreover, fast changes in the ecosystem due to various anthropogenic activities also demand for monitoring of aquatic vertebrates and invertebrates which act as natural bio indicators of water quality.

In Solan district of Himachal Pradesh has also come

up as a hub for various types of industries. Presently it has about 38 small scale and 6 large scale industries. State is having 56 urban/ sub urban habitation, which are further growing rapidly and releasing about hundreds tonnes of solid waste to the environment. Consequently, various anthropogenic activities like construction of roads, establishment of industries and educational institutes, deforestation, change in cropping pattern and excessive use of chemical fertilizers and pesticides in the field for better crop yield has led to change in the land use pattern, which has degraded the water quality. Moreover, the waste water effluents are discharged directly to the water bodies without any treatment. The physical and chemical parameters of portable water are permitted in Table 1. Consequently, problem of drinking water contamination, water conservation and water quality management has assumed a very complex shape. Attention on water contamination and its management has become need of the hour because of far reaching impact on human health. Keeping this in view present study was undertaken with objective to assess the effect of different land uses, seasonal variation and aquatic insect fauna on surface water quality.

MATERIAL AND METHODS

In order to assess surface water quality under dominant landuses of mid hills in Himachal Pradesh Solan block of the district Solan was selected. The study site fall

Table 1. Permissible limit of water quality parameters for drinking and domestic purpose set by WHO, CPCB & ICMR

Parameters	CPCB	WHO	ICMR
pH	6.5 to 8.5	6.5 to 8.5	7.5-8.5
EC ($\mu\text{S cm}^{-1}$)	750	Not-mentioned	Not-mentioned
TDS (mg l^{-1})	500	1000	Not -mentioned
BOD (mg l^{-1})	5	5	
COD (mg l^{-1})	Not- mentioned	Not-mentioned	20
Ca (mg l^{-1})	75	75	75
Mg (mg l^{-1})	30	30	50
NO_3 (mg l^{-1})	45	50	20
Cl (mg l^{-1})	250	250	200
Fe (mg l^{-1})	0.3	Not-mentioned	0.1
Zn (mg l^{-1})	5	3	0.1
Cd ($\mu\text{g l}^{-1}$)	10	3	Not -mentioned
Ar ($\mu\text{g l}^{-1}$)	50	10	Not -mentioned
Pb ($\mu\text{g l}^{-1}$)	100	10	Not -mentioned

between 30°50'30" to 30°52.00 N latitude and 77°08'30" to 77°11'30 E longitude with rivers and springs under different land use systems and geographical area of 21,053 ha with 15.98% cultivated area. The major seasons of this region are rainy, winter and summer commences from November to March and summer season from March to June and the rainy period, from July to September. The study area experience average annual rainfall of about 1200 mm in 64 rainy days. In the region the major part of the rains (70 %) is received during rainy months. During the study period (2011-2012) the region experienced 483.4, 234.00 and 123.10 mm rainfall during rainy, winter and summer seasons, respectively, (Fig. 1).

**Fig. 1.** Rainfall distribution during the study period

To assess the effect of common landuses on surface water quality in the region, the system like agriculture, Forest and Sub-Urban were selected to study the effect of season on the surface water quality. In total there were nine treatments, which were replicated thrice under Randomized Block Design Factorial.

Surface water samples from each selected land use system were collected during rainy, winter and summer seasons as per standard procedure prescribed by APHA (1998). The samples were immediately analyzed for pH, EC, TDS, BOD, COD, NO_3^- , Cl^- and preserved at -4°C for Ca^{2+} and Mg^{2+} .

The pH of the water samples was determined using microprocessor based pH meter (Model 510 of EIA make). Electrical conductivity and total dissolved solids were measured using microprocessor based conductivity/ TDS meter (Model- 1601 of EIA make), and expressed as $\mu\text{S cm}^{-1}$. BOD was determined by using BOD system Oxi-direct (Aqualytic make) and chemical oxygen demand (COD) with TR320 Spectroquant. Chloride (Cl^-), nitrate (NO_3^-) Magnesium (Mg) of surface water samples was determined photometrically by using spectroquant pharo 300 (Merck make) and expressed as mg l^{-1} . The calcium (Ca^{2+}) was estimated by using flame-photometer and expressed as mg l^{-1} .

Collection, preservation and clearing of aquatic insects:

The aquatic insects were collected by using the method suggested by Subramanian and Sivarama Krishnan (2005) from surface water sources under different land use systems by using aquatic insect net (Mesh size 40-80/ cm^2). The insects were identified up to family level by using the keys of Brues *et al.* (1954). EPT (Ephemeroptera Plecoptera Trichoptera) diversity were calculated (Kirsch, 1999; Mandaville, 1999; NCDEHNR, 1997).

RESULTS AND DISCUSSION

In mid hills of Himachal Pradesh the dominant landuses was found to influenced the surface water quality. The pH of surface water under different land uses ranged from 6.59 -7.13, which was found within the acceptable limit as prescribed by WHO. The highest value of pH (7.13) was noticed under agriculture land use, whereas, lowest value of pH (6.59) was observed under forest land use system. The maximum value of pH under agriculture land use was probably due to accumulation of more salts in agriculture fields because of intensive cultivation and application of fertilizers and other chemicals in the fields. Significantly, different seasons of the year also exert significant influence on water quality in the selected land use system. Significantly, highest pH value (7.33) was observed during summer season and lowest (6.48) was in rainy season. The maximum pH during summer season may be due to decreased volume of water by evaporation and minimum in winter season may be due to short day length and decrease in evaporation rate. Sharma and Capoor (2010) also reported similar results.

The EC of surface water of this region was found to be influenced by different land uses. The EC of water samples ranged from 483.25 – 589.75 $\mu\text{S}/\text{cm}$, which, was found within the safe limit as prescribed by WHO. Maximum value of EC (589.75 $\mu\text{S}/\text{cm}$) was recorded under agriculture land use, whereas, minimum (483.25 $\mu\text{S}/\text{cm}$) was noticed under forest land use systems. The highest content of EC under agriculture land use system might be due to accumulation of more salts and intensive farming practices with excessive fertilizer application in this land use. The results are in conformity with the findings of Taiwo *et al.* (2011) and Akujieze *et al.* (2003). Further, decomposition and mineralization processes were also considered responsible for high EC under agriculture land use (Abida and Hari Krishna, 2008). Similarly, different season of the year were also found to have significant influence on EC of surface water in the selected land uses. Among all the seasons significantly, highest EC value (563.75 $\mu\text{S}/\text{cm}$) of surface water was recorded during rainy season, whereas, lowest (516.42 $\mu\text{S}/\text{cm}$) was noticed during summer season.

Total dissolved solids of surface water under different land use systems varied from 66.43- 207.43 mg l^{-1} , which was found within the permissible limit prescribed by WHO. The maximum value of TDS (207.43 mg l^{-1}) was recorded under suburban land use, whereas, minimum TDS (66.43 mg l^{-1}) was observed under forest land use systems. This might be due to increased anthropogenic activities in urban land uses, which might have increased the dumping of waste materials and other household waste water effluents and ultimately increased TDS of surface water sources. The results are in conformity with the findings of Khound *et al.* (2012). Similarly, different season of the year were also showed significant influence on surface water of Solan block in the selected land uses. Significantly, highest mean TDS value (191.95 mg l^{-1}) was noticed during rainy season, whereas, lowest value of TDS was observed during summer season (116.92 mg l^{-1}). The results are in conformity with the findings of Shaikh and Mandre (2009) who also reported maximum TDS in rainy season, which could be due to addition of solids from surface run off.

The dominant land uses of this region also exert significant influence on both BOD and COD of water samples. The BOD and COD of water samples under different land uses was found to range from 0.55 to 3.07 mg l^{-1} , 1.00 to 4.42 mg l^{-1} , respectively. Which, was found within the acceptable limit prescribed by WHO. Agriculture land use system showed the maximum values of both BOD (3.07 mg l^{-1}) and COD (4.42 mg l^{-1}), whereas, minimum values of BOD (0.55 mg l^{-1}) and COD (1.00 mg l^{-1}) was recorded under forest land use system. Which might be due to enrichment of

water sources with organic matter as a reason for high BOD. The results are in conformity with the findings of Trivedi and Goel (1986). Further, the presence of higher amount of oxidizable matter under these land use systems lead to high content of COD under agriculture land use. Whereas, different seasons of the year was found to be non-significant with respect to the BOD of surface water. Similarly, different season of the year exert significant influence on water sources. Significantly, highest COD value (4.08 mg l^{-1}) was observed during winter season, whereas, lowest COD value (1.75 mg l^{-1}) was during summer season. Similarly, Kaushik and Saksena (1999) reported heavy load of organic, inorganic pollution that require more oxygen to oxidize under increased thermal conditions.

The Cl^{-} and NO_3^{-} content ranged from 15.53 to 130.00 mg l^{-1} , 3.99 to 27.17 mg l^{-1} , respectively, which was found within the acceptable limit as prescribed by WHO (Table 2). Significantly, maximum content of Cl^{-} (130.00 mg l^{-1}) and NO_3^{-} (27.17 mg l^{-1}) was observed under agriculture land use system, whereas, forest land use reported minimum content of Cl^{-} (15.53 mg l^{-1}) and NO_3^{-} (3.99 mg l^{-1}). This might be due to intensive farming practices wherein input of chemical fertilizer and manure are applied at higher rates by the farmers of Solan block. Jeyarubu and Thushyanthy (2009) also indicated that excess application of inorganic fertilizers contribute to high Cl^{-} and NO_3^{-} content in surface water. Khaund *et al.* (2012) also reported maximum chloride and nitrate content under agriculture land use system. However, different seasons of the year showed a non-significant effect with respect to chloride and nitrate concentration of surface water.

The dominant land uses of this region was found to exert significant influence on Mg^{2+} and Ca^{2+} content of surface water samples. The Mg^{2+} and Ca^{2+} concentration was found within the permissible limit as prescribed by WHO. Under different land uses Mg^{2+} and Ca^{2+} content varied from 9.75 to 24.46 mg l^{-1} , 8.42 to 38.08 mg l^{-1} , respectively. The highest content of Mg^{2+} (24.46 mg l^{-1}) and Ca^{2+} (38.08 mg l^{-1}) was recorded under agriculture land use system, whereas, lowest value of Mg^{2+} (9.75 mg l^{-1}) and Ca^{2+} (8.42 mg l^{-1}) was observed under forest land use system. The increase in Mg^{2+} and Ca^{2+} content of surface water under agriculture land use might be attributed to application of manures, fertilizers and pesticides to the agricultural fields. The results are in conformity with the findings of Shaikh and Mandre (2009). The present results are in agreement with the findings of Pallisamy and Geetha (2007) who have also reported that 98% of world water sources are dominated by calcium and bicarbonate due to limestone weathering. Similarly, different seasons of the

Table 2. Effect of different land use on surface water quality parameters in mid hills of Solan district in H.P.

Season (s)	Land use (L)			Mean
	Agriculture	Forest	Sub-Urban	
pH				
Rainy	7.17	6.25	6.01	6.48
Winter	6.70	6.31	6.75	6.58
Summer	7.53	7.22	7.25	7.33
Mean	7.13	6.59	6.67	
CD (p=0.05) L= 0.20, S = 0.20, L × S= 0.35				
EC (μS cm ⁻¹)				
Rainy	606.50	538.25	546.50	563.75
Winter	618.50	455.00	526.75	533.42
Summer	544.25	456.50	548.50	516.42
Mean	589.75	483.25	540.58	
CD (p=0.05) L= 33.12, S = 33.12, L × S= 57.37				
TDS (mg l ⁻¹)				
Rainy	248.25	85.05	242.55	191.95
Winter	177.00	45.75	240.25	154.33
Summer	142.75	68.70	139.50	116.92
Mean	189.33	66.43	207.43	
CD (p=0.05) L= 21.69, S = 21.69, L × S= 37.56				
BOD (mg l ⁻¹)				
Rainy	3.43	0.45	1.10	1.66
Winter	2.53	0.35	1.68	1.52
Summer	3.26	0.85	1.08	1.73
Mean	3.07	0.55	1.28	
CD (p=0.05) L= 0.82, S =NS, L × S= NS				
COD (mg l ⁻¹)				
Rainy	6.25	1.00	3.75	3.67
Winter	4.25	1.75	6.25	4.08
Summer	2.75	0.25	2.25	1.75
Mean	4.42	1.00	4.08	
CD (p=0.05) L=1.65, S = 1.65 , L × S= NS				
Cl (mg l ⁻¹)				
Rainy	Rainy	Rainy	Rainy	Rainy
Winter	Winter	Winter	Winter	Winter
Summer	Summer	Summer	Summer	Summer
Mean	Mean	Mean	Mean	Mean
CD (p=0.05) L=16.70, S = NS , L × S= NS				
NO ₃ (mg l ⁻¹)				
Rainy	29.00	2.98	19.00	16.99
Winter	29.50	7.50	17.75	18.25
Summer	23.00	1.50	21.75	15.42
Mean	27.17	3.99	19.50	
CD (p=0.05) L= 2.96, S = NS , L × S= 5.12				
Mg ²⁺				
Rainy	29.88	12.00	28.25	23.38
Winter	22.00	7.50	10.50	13.33
Summer	21.50	9.75	16.25	15.83
Mean	24.46	9.75	18.33	
CD (p=0.05) L= 2.67, S = 2.67, L × S=4.62				
Ca ²⁺				
Rainy	56.50	11.75	32.75	33.67
Winter	36.25	7.50	18.00	20.58
Summer	21.50	6.00	24.75	17.42
Mean	38.08	8.42	25.17	
CD (p=0.05) L= 3.50, S = 3.50, L × S=6.05				

Table 3. Effect of land use systems and seasons on biodiversity of aquatic insects of surface water sources in mid hills of Solan district in H.P.

Order	Family	Seasons			Total	Percentage (%)
		Rainy	Winter	Summer		
Agriculture						
Ephemeroptera	<i>Batidae</i>	2	4	3	62	35.8%
	<i>Haptagenidae</i>	1	0	0		
	<i>Ephemerellidae</i>	0	0	4		
	<i>Ephemeridae</i>	0	0	3		
Plecoptera	<i>Leuctridae</i>	1	0	0		
Trichoptera	<i>Hydropsychidae</i>	2	5	13		
Odonata	<i>Gomphidae</i>	1	2	0		
	<i>Libellulidae</i>	1	0	0		
Hemiptera	<i>Girridae</i>	5	10	2		
Coleoptera	<i>Hygrobiidae</i>	1	1	1		
Forest						
Ephemeroptera	<i>Batidae</i>	2	2	3	78	45.08%
	<i>Haptagenidae</i>	0	1	1		
	<i>Ephemerellidae</i>	0	0	5		
	<i>Ephemeridae</i>	0	0	4		
Plecoptera	<i>Leuctridae</i>	0	0	0		
Trichoptera	<i>Hydropsychidae</i>	0	0	17		
Odonata	<i>Gomphidae</i>	3	3	1		
	<i>Libellulidae</i>	1	2	3		
Hemiptera	<i>Girridae</i>	3	11	5		
Coleoptera	<i>Hygrobiidae</i>	5	4	2		
Urban/Sub -Urban						
Ephemeroptera	<i>Batidae</i>	0	0	1	33	19.07%
	<i>Haptagenidae</i>	0	0	1		
	<i>Ephemerellidae</i>	0	0	2		
	<i>Ephemeridae</i>	0	0	3		
Plecoptera	<i>Leuctridae</i>	0	0	0		
Trichoptera	<i>Hydropsychidae</i>	1	5	4		
Odonata	<i>Gomphidae</i>	1	1	1		
	<i>Libellulidae</i>	1	2	2		
Hemiptera	<i>Girridae</i>	1	4	3		
Coleoptera	<i>Hygrobiidae</i>	0	0	0		
		32	57	84	173	

year also exert significant influence on water in the selected land uses. The maximum values of Mg^{2+} (23.38 mg l⁻¹) and Ca^{2+} (33.67 mg l⁻¹) was observed during rainy season which might be due to increased dissolution and its further leaching to the water sources. The results are in conformity with the findings of Trivedi *et al.* (2010), who have reported, increased concentration of calcium due to carbonates and bicarbonates

ions and their runoff during rainy season. However, minimum content of Mg^{2+} (13.33 mg l⁻¹) and Ca^{2+} (17.42 mg l⁻¹) was noticed during winter and summer season, respectively.

A total number of 173 individuals m⁻² of aquatic insects under 10 families and 6 orders were recorded (Table 3). Among different land use systems maximum number of insects i.e 78 individuals was collected from forest land use

system followed by agriculture land use (62 individuals) and urban/suburban (33 individuals). Among seasons, maximum number of aquatic insects (84 individuals) were during summer season, followed by winter and rainy season. Most of the individuals belonged to the order Trichoptera family Hydropsychidae (13 individuals) followed by order Ephemeroptera family Ephemerellidae (4 individuals). Maximum abundance of aquatic insects under agriculture land use during summer season may be due to availability of food, consistent flow rate of water and temperature of the water body. In present findings, the temperature of water body was in the range of 17.70°C to 29.10°C. The present findings are in confirmation with the findings of Mishra and Nautiyal (2011) who reported Hydropsychidae of Trichoptera, Baetidae of Ephemeroptera and Gomphidae of Odonata under agriculture land use at the temperature range between 16.5 to 21.50°C. During winter season, out of the total aquatic insects encountered, maximum belonged to the order Hemiptera family Girridae, 5 to order Trichoptera family Hydropsychidae, 4 to order Ephemeroptera family Batidae and 2 individuals to order Odonata family Gomphidae. The order Coleoptera was represented by only one insect belonging to the family Hydrobiidae. During rainy season, maximum number of aquatic insects encountered belonged to the order Hemiptera family Girridae, followed by order Ephemeroptera family Batidae, order Trichoptera family Hydropsychidae. The families Haptagenidae of order Ephemeroptera, family Leuctridae of order Plecoptera and family Gomphidae and Libellulidae of order Odonata was represented by one each. Similarly, in forest land use systems also maximum number of aquatic insects were collected during summer season. Out of the total collection, maximum individuals, belonged to the order Trichoptera, family Hydropsychidae followed by the families Ephemerellidae of order Ephemeroptera and Girridae of order Hemiptera. One insect represented the Odonata family Gomphidae and Ephemeroptera family Haptagenidae. During winter season, maximum numbers belonged to the order Hemiptera family Girridae followed by Coleoptera family Hydrobiidae. During rainy season, out of the total individuals collected from forest land use system, maximum belonged to the order Coleoptera family Hydrobiidae (5 No's) followed by order Odonata family Gomphidae and order Hemiptera family Girridae. During summer season, under forest land use maximum abundance of aquatic insects may be due to presence of food mainly algae, constant flow of water body and availability of oxygen for the survival of insects. These findings are in confirmation with the findings of Bahuguna et al. (2004) who reported Ephemeroptera, Trichoptera, Coleoptera in the Veerbhadra barrage during the month of

January when the temperature of water body was in the range of 20 to 22°C under forest land use system. In the present investigations on an average temperature of water body at the time of sampling ranged between 17.70°C to 29.10°C. This low number of aquatic insects in urban/suburban land use system may be attributed to discharge of household waste and other toxic effluents in the rapid growing areas including Solan city of this block. Wahizatul *et al.* (2006) and Foreman *et al.* (2008) also reported that human activities add nitrogen and phosphorous to the water sources which lead to eutrophication, low BOD and ultimately reduce the population of aquatic insects.

The study indicated that the common land uses in the mid hills of Solan district did not influence the surface water quality adversely and was within the acceptable limit as prescribed by WHO. The study further indicated that the agriculture land use tended to increase TDS, BOD, COD, Cl⁻ and NO₃⁻ content in surface water. The seasons of the year also influenced the physico-chemical properties of surface water. During rainy season all the parameters exhibited relatively higher values as compared to winter and summer months. The Land use wise aquatic insect recorded were 78, 62 and 33 individuals m⁻² under forest, agriculture, and urban land uses, respectively. Forest land use registered maximum diversity of aquatic insect fauna as compared to other land use, which indicated that aquatic biodiversity distribution varied from excellent for forest and agriculture land use to good for urban land use system.

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Quantification of Water Loss Due to Uneveled Surface of Conventionally Puddled Wet Rice Fields

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Abstract: The amount of water wasted due to uneveled conventionally puddled fields was quantified by measuring elevations of the wet rice fields with dumpy level at 2.5 m grid points in six conventionally puddled wet rice fields at Sangareddy, Telangana. The surface SD's of the six fields from the mean level were observed as ± 0.019 , ± 0.034 , ± 0.029 , ± 0.039 , ± 0.044 , and 0.038 m and the amount of water to be filled to have a leveled surface for six fields were quantified as 78,205, 1,42,828, 1,33,950, 1,43,968, 1,70,830 + or -0.038 m and 1,47,323 lit ha⁻¹ respectively.

Key Words: Cut, Dumpy level, Fill, Uneveled surface, Wet rice fields

Rice (*Oryza sativa*) is one of the most important cereal crops which feed millions of mouths and playing an important role in the world food security. Rice is known for its high water requirement for its cultivation. It is reckoned that approximately 5000 liters of water is required for production of 1 kg of rice (Bouman and Hengsdisk, 2002, The Hindu Nov 10, 2011). The alarming statistic spectacularly explaining the need for increasing water productivity in the cultivation of rice, to feed ever increasing population and to save water for future generations. Puddling is the seed bed preparation technique followed in the cultivation of rice. Puddling reduces the percolation losses by 75% (Kukul and Aggarwal, 2003) and provides soft seed bed for transplanted rice seedlings. Puddling refers to the tillage system in which soil is repeatedly ploughed and harrowed under submerged condition with standing water of 5 to 10 cm depth (Bhagath, 2003). Conventionally puddled seed bed is mostly being prepared by using rotavator, cultivator with cage wheels arrangements. As these are mechanical implements, there is no control over the depth of puddling and surface levels of the puddled field in the preparation of very soft seed bed, which should be scrupulously studied to design precision implements for preparation of puddled field and planting of rice seeds in the field.

Surface of the rice field seems leveled when it is seen from the top. In fact, the leveled view is not due to the leveled soil surface but due to the water covered on the uneveled surface of the puddled field. It is revealed from research that, there is a variation from one end to the other end of the field. Hence to quantify the amount of water that is used to cover the surface of the wet rice fields to have a leveled surface view is quantified in the study.

MATERIAL AND METHODS

Six conventionally puddled wet rice fields of Sangareddy were studied for their variation in surface levels. The sizes of the fields were 337.5, 393.7, 243.7, 250.0, 175.0 and 225.0 m² respectively for 1, 2, 3, 4, 5 and 6 fields. Dumpy level was used to measure the elevations of surface of the puddled fields. The field was divided into 2.5 X 2.5 m grids. The surface elevations were taken just one day before the transplanting by keeping the leveling staff at the surface of the soil which is just below the ponding water depth (Fig. 1). The surface elevations of the wet rice field were depicted as wireframes and contour maps using surfer 7 software. Σ cut and Σ fill volumes were calculated by using Eq.1 and 2, to quantify the amount of water used to fill the bumps of the field as well as to cover the continuous slope of the field from one end to the other end.

Cut = (Mean reduced level – Reduced level at particular grid point) * Area of the Experimental plot

= A / Area of the plot A values are all positive values (1)

Fill = (Mean reduced level – Reduced level at particular grid point) * Area of the Experimental plot

= B / Area of the plot B values are all negative values (2)

The benchmarks were taken separately for each field and the datum was taken as 100m for all the fields. The Reduced levels of the field elevations were calculated (Kanetkar and Kulkarni, 2011). The corresponding mean and standard deviations (SD) for reduced levels of the six fields were calculated by using MS Excel.

RESULTS AND DISCUSSION

Reduced levels of the six fields which were measured by using dumpy level and leveling staff are

Table 1. Reduced levels of the six experimental plots

Field No. 1 Area: 337.5 m ² Datum: 100 m Bench mark: 1.65 m				Field No. 2 Area: 393.75 m ² Datum: 100 m Bench mark: 1.695 m				Field No. 3 Area: 243.75 m ² Datum: 100 m Bench mark: 1.695m			
99.850	99.860	99.860	99.885	99.820	99.885	99.820	99.885	99.850	99.855	99.855	99.825
99.830	99.870	99.850	99.842	99.855	99.875			99.840	99.895	99.895	99.875
99.840	99.835	99.845	99.840	99.860	99.880			99.840	99.895	99.895	99.895
99.830	99.890	99.855	99.865	99.855	99.835			99.835	99.845	99.845	99.825
99.830	99.840	99.850	99.850	99.860	99.845			99.870	99.895	99.895	99.845
99.830	99.840	99.870	99.850	99.810	99.840			99.875	99.920	99.920	99.835
99.820	99.840	99.895	99.885	99.870	99.875			99.855	99.875	99.875	99.850
99.845	99.850	99.870	99.850	99.865	99.870			99.860	99.850	99.850	99.790
99.845	99.840	99.830	99.840	99.830	99.870			99.860	99.830	99.830	99.845

furnished in Table 1. The amount of water loss due to unlevelled surface was quantified by calculating Cut and fill of the six fields as mentioned in art 3. The Mean reduced levels and its standard deviations, Cut and fill volumes were given in Table 2. The depictions drawn using surfer software was also given in Fig. 1, 2, 3, 4, 5, and 6 for 1, 2, 3, 4, 5 and 6 fields, respectively.

The SD is varying from 1.9 cm to 4.4 cm. but for ideal leveled surface should have a SD of 0 cm. It is also obtained that the Cut and Fill values for six fields are varying from minimum 2.678 m³ in 337.5 m² area and maximum of 5.670 m³ in 393.75 m² area. Hence, the amount of water to be

filled to have a leveled surface for six fields were quantified as 78,205, 1,42,828, 1,33,950, 1,43,968, 1,70,830 and 1,47,323 lit ha⁻¹ respectively for 1,2,3,4,5 and 6 fields.

Substantial amount of water is lost through conventional puddling practices which is approximately as high as 1, 36,000 lit ha⁻¹ due to unlevelled surface of the puddled fields, which constitutes to 5894 GL of total water loss for 44M ha of rice cultivable land in India. These alarming statistics clear-cutted the need for leveled surface of wet rice fields. Hence, precision controlled machinery has to be developed to eradicate issues of unlevelled puddled fields.

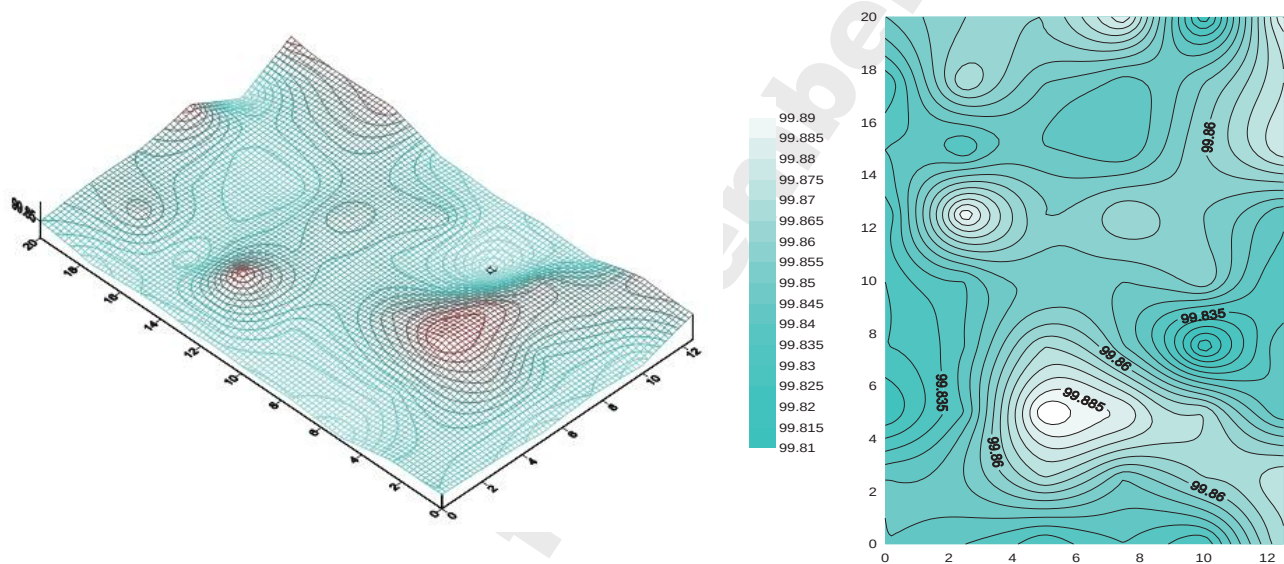


Fig. 1. Wireframe and contour map of surface of the field 1

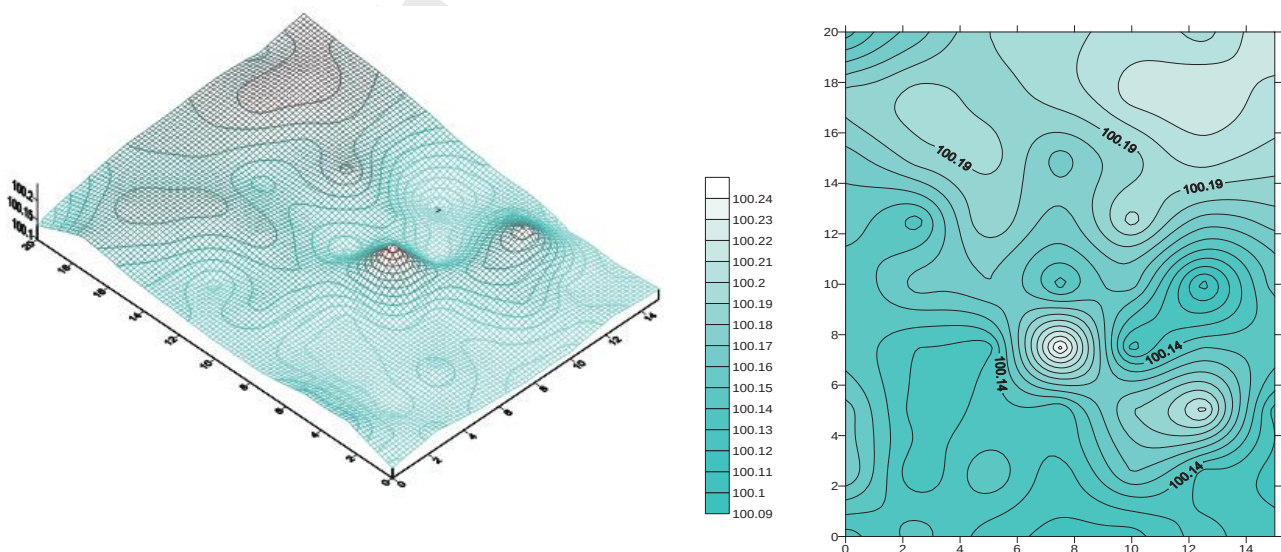


Fig. 2. Wireframe and contour map of surface of the field 2

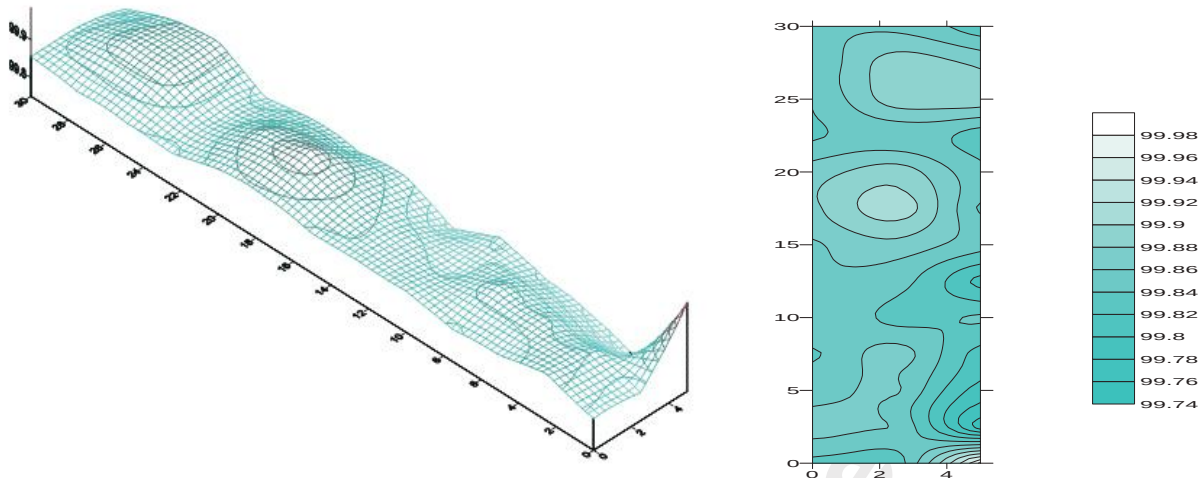


Fig. 3. Wireframe and contour map of surface of the field 3

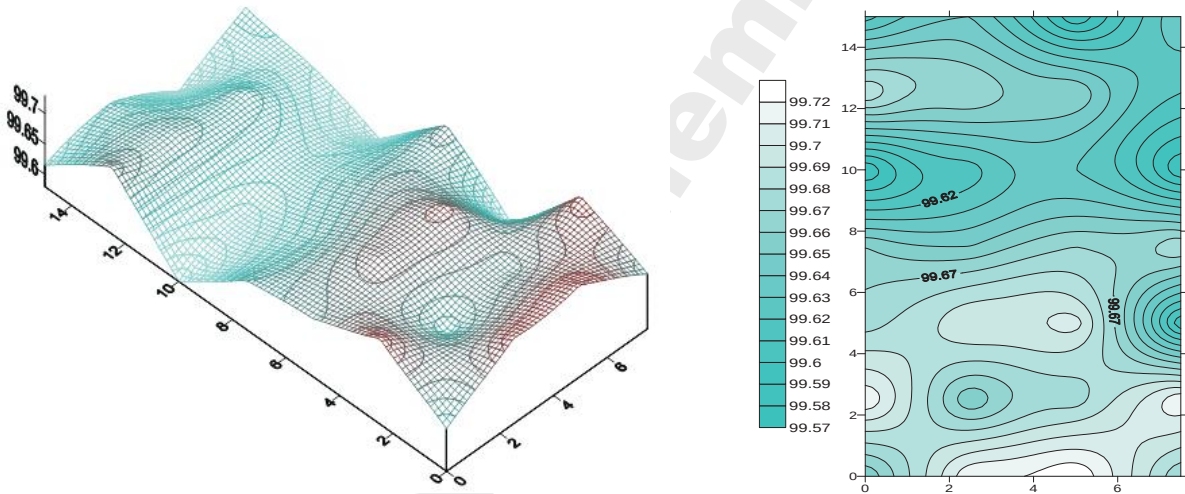


Fig. 5. Wireframe and contour map of surface of the field 5

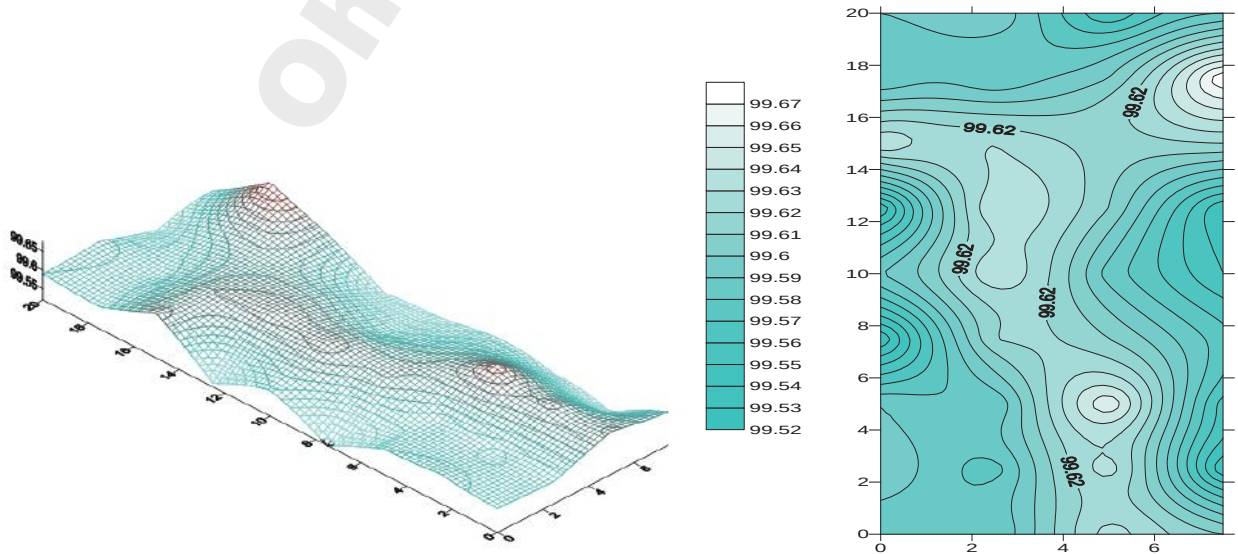


Fig. 6. Wireframe and contour map of surface of the field 6

Table 2. Mean and SD of the Surface levels of the wet rice fields

Field No.	Area (m ²)	Surface level		
		Mean (m)	SD (m)	Σ fill = Σ cut (m ³)
1	337.5	99.852	0.019	2.658
2	393.75	100.161	0.034	5.670
3	243.75	99.853	0.039	3.292
4	250	99.673	0.039	3.941
5	175	99.657	0.044	3.099
6	225	99.595	0.038	3.342

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Received 14 December, 2015; Accepted 7 April, 2016



Performance of Direct-Seeded Rice in Relation to Water and Weed Management Scenarios in a Sandy Loam Soil

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Abstract: A study was conducted to monitor the performance of direct seeded rice under differential water and weed management scenarios. The treatments included four water management scenarios as main plots viz. continuous shallow flooding, irrigation of 5 cm depth at 20 kPa, irrigation of 5 cm depth at 20 kPa + kept flooded for 5 days after application of herbicide and shallow flooding during crop establishment + 40 kPa during mid-season + subsequent irrigation at 20 kPa. The treatments included four weed management scenarios as sub plots viz. weedy, weed free, pre-emergence (pendimethalin @ 1 kg a.i. ha⁻¹) + post-emergence (bispyribac @ 20-25 gm a.i. ha⁻¹) at 20-25 DAS and early post emergence (propanil @ 4 kg a.i. ha⁻¹ + pendimethalin 1 kg a.i. ha⁻¹) at 12 DAS. Among the yield attributes, thousand grain weight was statistically significant among the two scenarios. Among the two management practices, the highest average yields under water and weed management scenarios were 3.07 t ha⁻¹ (flooded scenario) and 4.93 t ha⁻¹ (weed free scenario) respectively. However, the highest grain yield was 6.10 t ha⁻¹ for continuous shallow flooded irrigation under the weed free management scenario. The grain yield was lowered significantly with water stress and the weed management practices.

Key Words: Direct seeded rice, Grain yield, Soil matric potential, Water management, Weed management

Rice (*Oryza sativa* L.) is an important target for irrigation water use reductions, because of its relatively large water requirements compared with other crops (Humphreys *et al.*, 2010). The high water requirement of conventionally flooded puddled transplanted rice (PTR) has become a major threat to the sustainability of rice production due to over exploitation of ground water resources in Indo-Gangetic Plains (IGP), more so in the north-west IGP of India. Direct seeded rice (DSR) with intermittent irrigation could offer potential water savings at the field level due to reduced evaporation losses, intermittent irrigation and avoidance of puddling. However, it needs to develop optimum thresholds for irrigation scheduling to DSR. The effect of DSR on water use and water productivity is not well understood in medium textured soils. Yadav *et al.* (2011c) while simulating the irrigation effects on DSR, observed that DSR if irrigated with lower soil matric potential at sensitive growth stages could help in increasing crop water productivity.

Weeds are the main biological constraint to the production of DSR and are floristically diverse and higher in abundance than transplanted rice system (Rao *et al.*, 2007). Timing of application of herbicide needs to be more precisely identified in DSR than transplanted rice (Rao *et al.*, 2007). Aerobic soil conditions and dry-tillage practices, besides alternate wetting and drying conditions are conducive for germination and growth of highly competitive weeds, resulting in grain yield losses of 50–91% (Fujisaka *et al.*, 1993). Several pre-emergence herbicides alone or supplemented with hand weeding may provide a fair degree

of weed control. The use of any single approach, however, would not provide season-long and sustainable weed control because of the variation in dormancy and growth habits of weeds. There is a need to study weed biology and ecology in DSR systems in different rice ecosystems. The present study was thus undertaken in a sandy loam soil to monitor the performance of DSR in relation to differential water and weed management scenarios.

MATERIAL AND METHODS

A field experiment was carried out during 2013 at Punjab Agricultural University on sandy loam soil to monitor the performance of conventional direct seeded rice (DSR) under different irrigation water and weed management scenarios. The study soil was slightly alkaline and low in soil organic carbon (Table 1). The site was under a rice-wheat cropping system for at least 2-3 years prior to establishment of the experiment.

The site was given stall bed treatment to kill the weeds, cultivated and laser leveled prior to establishment of the experiment. Before sowing DSR, the weed seed (*Amaranthus spinosus* L., *Commelina diffusa* Burm. F. and *Trianthema portulacastrum* L.) was broadcasted. The DSR was sown on 26th June during 2013 following recommended agronomic practices from package of practices for kharif crops of Punjab Agricultural University for year 2012.

Treatments: The experiment was laid out in 3 replicates in a split plot design with four main plot treatments viz. M1-Continuous shallow flooding, M2-Irrigation of 5 cm depth at

20 kPa at 15 cm soil depth, M3-M2+kept flooded for 5 days after application of herbicide and M4-Shallow flooding during crop establishment+40 kPa (mid-season)+subsequent irrigation at 20 kPa at 15 cm soil depth. The study included four subplot treatments viz. S1-Weedy, S2-Weed free (Pre fb post and hand weeding as and when require), S3-Pre-emergence (Pendimethalin @ 1kg a.i. ha⁻¹) followed by post-emergence (Bispyribac @ 20-25 gm a.i ha⁻¹) at 20-25 DAS and S4-Early post emergence (Segment @ 40g a.i. ha⁻¹ followed by rice star at 12 DAS).

Water productivity (WP_i and WP_{i+R}): The quantity of irrigation water applied was measured using area velocity method. The first irrigation given at the time of sowing was common to all the treatments and the rest of the irrigations were scheduled using tensiometers installed. Irrigation water productivity (WP_i) was computed as the ratio of grain yield (12% moisture) to the irrigation water applied and the total input water productivity (WP_{i+R}) was computed as the ratio of grain yield (14% moisture) to the total input water (irrigation + rainfall) and expressed as g kg⁻¹.

Rainfall: Effective rainfall of 422 mm was recorded during crop seasons (Fig. 1). The rainfall was below average (14 rainy days) during crop season having ≥ 10 mm rain.

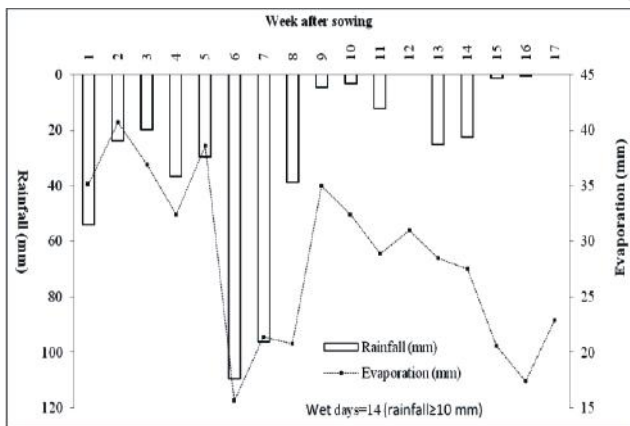


Fig 1. Rainfall and evaporation distribution

RESULTS AND DISCUSSION

The thousand grain weight was statistically significant under water and weed management scenarios

having a significant interaction. Fertility percent, harvest index and filled grains per panicle were statistically similar under differential irrigation water and weed management scenarios with no significant interaction (Table 2). Among weed management scenarios, the average thousand grain weight was maximum (19 g) under weed free and minimum (0 g) under weedy scenarios respectively. The average thousand grain weight was maximum (14.7 g) under flooded scenario (M1) and minimum under M2 scenario i.e. irrigation of 5cm depth at 20 kPa at 15 cm soil depth. The trend among the fertility and filled grains per panicle showed the same trend. The highest fertility percents under water and weed management scenarios were 57.6 (flooded scenario) and 80.5 (weed free scenario) respectively. Similar was the trend for filled grains per panicle..

The periodic biomass and tiller density remained statistically similar under different water and weed management scenarios throughout the crop season having non-significant interaction (Fig. 2 and 3).

Rice grain yield: The grain yield differed significantly under both weed and water management scenarios with a significant interaction between the two (Table 3). The grain yield was highest (6.10 t ha⁻¹) for flooded water management scenario followed by M3 scenario (4.68 t ha⁻¹) under the same weed management scenario (weed free). The average grain yield was maximum (3.07 t ha⁻¹) under M1 followed by M4 and minimum under M2 scenario.

Water productivity: The water productivities (WP_i and WP_{i+R}) were significantly different under different water and weed management scenarios having a significant interaction between the two (Fig. 4a, b). Among weed management scenarios, the irrigation water productivity (WP_i) was maximum under weed free scenario followed by pre-emergence+ post-emergence at 20-25 DAS scenario and minimum under weedy scenario. Similar was the trend for total input water productivity (WP_{i+R}).

Among water management scenarios, the irrigation water productivity (WP_i) was maximum under M4 followed by M2 and minimum under flooded scenario. Similar was the trend for total input water productivity (WP_{i+R}). The irrigation was scheduled using tensiometer installed in the main plots

Table 1. Soil properties at the experimental site

Depth	pH	EC	SOC	Bulk density	Soil water content (%)		Sand	Clay
					FC	PWP		
0-15	8.27±0.08	0.29±0.10	0.29±0.07	1.43±0.06	17.1	7.6	78	17
15-30	8.44±0.16	0.21±0.04	0.26±0.02	1.53±0.04	19.3	8.8	65	20
30-45	8.36±0.05	0.14±0.01	0.17±0.02	1.50±0.02	22.1	10.8	58	25
45-60	8.31±0.05	0.13±0.01	0.21±0.11	1.47±0.03	25.6	12.7	52	28

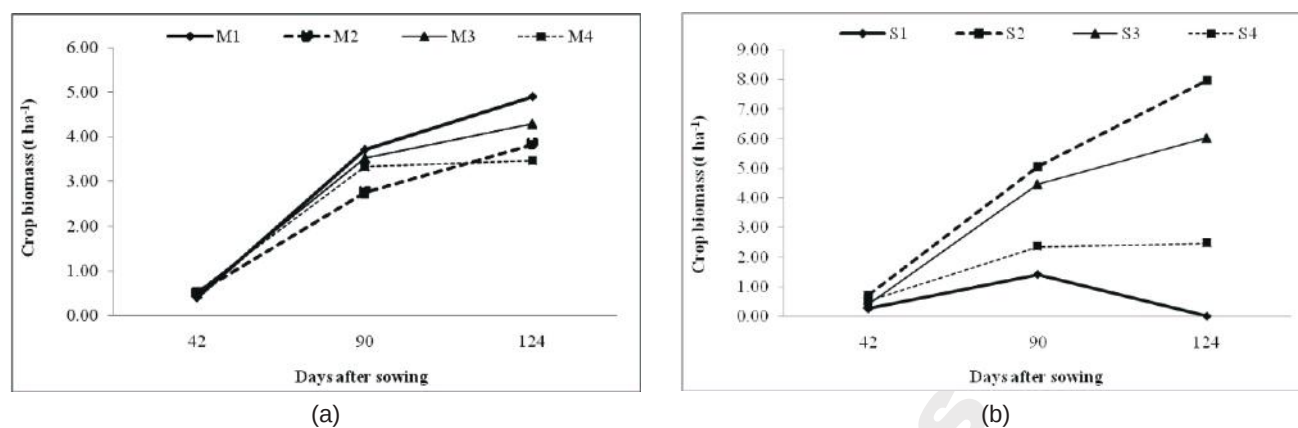


Fig. 2. Crop biomass in relation with (a) water and (b) weed management scenarios

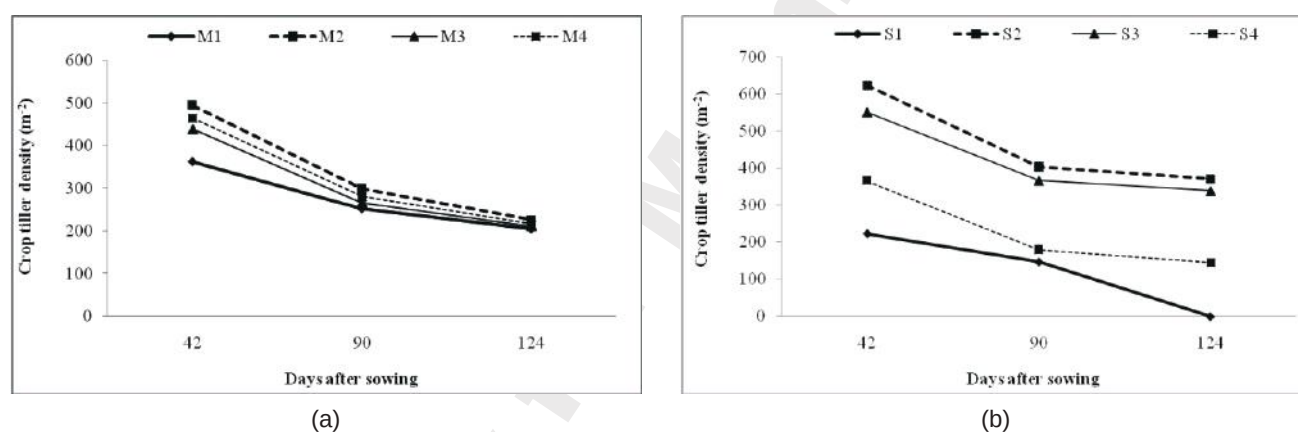


Fig. 3. Crop tiller density in relation with (a) water and (b) weed management scenarios

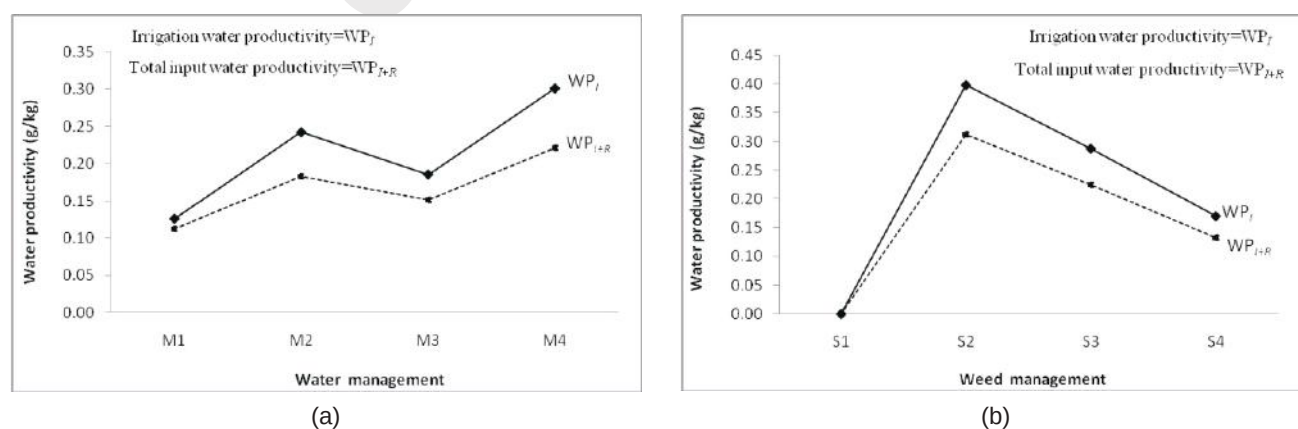


Fig.4. Water productivities in relation with (a) water management and (b) weed management scenarios

Table 2. Grain yield components

Weed Management					
Water Management	Thousand grain weight (at 14% moisture content)				
	S1	S2	S3	S4	Mean
M1	0	20.4	19.4	18.9	14.7
M2	0	18.1	17.9	17.4	13.4
M3	0	19.1	18.8	18.8	14.2
M4	0	18.5	16.6	20.7	14.0
Mean	0	19.0	18.2	18.9	
LSD (0.05)		M=0.76, S=0.65, MxS=1.29			
	Fertility (%)				
	S1	S2	S3	S4	
M1	0	83.8	78.8	67.7	57.6
M2	0	78.0	74.3	66.4	54.7
M3	0	80.1	72.0	73.6	56.4
M4	0	80.2	75.6	63.2	54.7
Mean	0	80.5	75.1	67.7	
LSD (0.05)		M=NS, S=7.16, MxS=NS			
	Filled grains per panicle				
	S1	S2	S3	S4	
M1	0	128	122	90	85
M2	0	98	108	89	74
M3	0	117	104	106	82
M4	0	102	100	76	69
Mean	0	111	109	90	
LSD (0.05)		M=NS, S=11.35, MxS=NS			

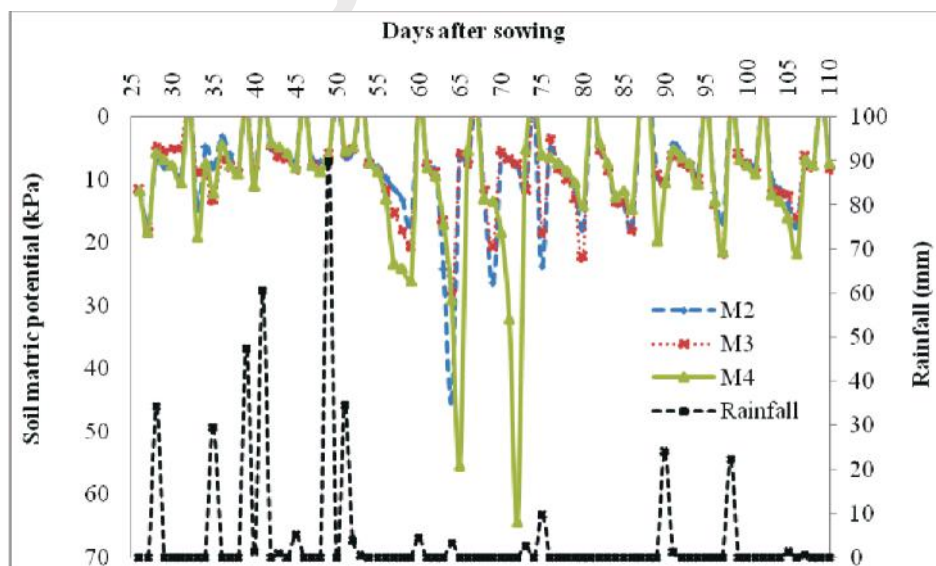
**Fig. 5.** Soil matrix potential based irrigation scheduling

Table 3. Grain yield of DSR

Yield (t ha ⁻¹) at 14% m. c.					
Weed management					
Water management	S1	S2	S3	S4	Mean
M1	0	6.10	4.17	2.01	3.07
M2	0	4.51	2.44	2.27	2.31
M3	0	4.68	3.47	1.89	2.51
M4	0	4.43	3.96	1.86	2.56
Mean	0	4.93	3.51	2.01	
CD (p=0.05)	M=0.30, S=0.21, MxS=0.42				

at 15 cm depth. Among the main treatments, the most frequently irrigated plot was kept flooded partially throughout the crop season maintaining soil matric potential (SMP) value of <10 kPa. The Figure 5 shows the irrigation frequencies of other three main plot treatments (M2, M3 and M4).

The results indicate that the direct seeded rice under differential water and weed management scenarios did not perform well in sandy loam soil as the yield was not at

par with the puddle transplanted rice under the same soil conditions. However, the crop yield was affected significantly by interaction of water and weed management scenarios. The grain yield was highest under weed free scenario among all the water management scenarios. The grain yield was lowered significantly with water stress and the weed management practices.

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Received 10 October, 2015; Accepted 15 May, 2016



Energy Requirement under Different Planting Methods of Sugarcane with and without Preparatory Tillage

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Abstract: The output energy was higher by 1.73 % in no pre-planting tillage than conventional due to 1.4 t ha^{-1} higher cane yield. Energy use efficiency and energy productivity were also higher by 3.7 and 4.1 % in the absence of pre-planting tillage over the conventional tillage treatment. In contrast, energy intensity was higher by 3.7 % in conventional than no pre-planting tillage indicating more energy requirement to produce per unit cane yield. Trench method recorded the maximum energy use efficiency, energy productivity and net energy gain and was the lowest energy intensive.

Key Words: Energy, Planting method, Productivity, Sugarcane, Tillage

Late planting of sugarcane after harvest of wheat is a common practice in North-West India. Sugarcane is grown in tilled prepared soil conditions and despite enormous benefits, wheat residue is removed and/or burnt primarily to clean the field resulting in loss of food for microbes and wastage of energy. In addition, burning of wheat residue after harvest in field takes a toll of soil biota, which is a principle component of soil ecology dictating varying bio-geochemical cycles and soil properties. Frequent tillage operations for land preparation consume huge amount of energy apart delay in sowing, escalate the cost of cultivation and enhance the mineralization of soil organic carbon contributing to atmospheric CO_2 content. Escaping preparatory tillage operations and residue retention is an emerging concept for transition to sustainability of intensive production system besides conserving energy (Srivastava, 2003) and generating net social gains to the society (Bhale and Wanjari, 2009). Presence of residue in no- tillage system can conserve energy, maintain moisture, suppress weeds, reduce soil loss and lower the cost of cultivation (Singh *et al.*, 2005). Besides this, no tillage system also saves the fuel and soil organic carbon, which in turn reduces CO_2 emission protecting the environment.

Adoption of a suitable planting method is also of utmost significance as it alters the cane growth and productivity thereby influencing energy output from the system. Presently, in north India, flat planting is the common method, which results in lower cane productivity (Singh *et al.*, 2009) and demands greater use of energy in primary tillage operations. Alternate eco -friendly planting methods such as trench, ridge and furrow, dual row trench and pit may be of potential value for energy production and saving in sugarcane. Considering the above facts, the study targeted

to work out the energy use parameters in different planting methods with and without preparatory tillage operations for sugarcane cultivation.

MATERIAL AND METHODS

The field experiment was carried out at the Norman E. Borlaug Crop Research Centre, GBPUA&T, Pantnagar during the summer season of 2012-13. The site is located at 29°N latitude, 79.5°E longitude at an elevation of 243.8 meters above the mean sea level under the foot hills of *Shivalik* range of Himalayas, representing the *tarai* region of Uttarakhand. The soil was silty clay loam in texture with neutral reaction (pH 7.1). The bulk density of top 0-15cm soil layer was 1.46 Mg / m^3 . The soil moisture content at field capacity and permanent wilting point in the upper 0-30 cm surface was 22.9 and 7.4%, respectively. The experiment comprising 10 treatments with 2 pre- planting tillage practices (with and without pre-planting tillage) and 5 planting methods (flat, ridge and furrow, trench, dual row trench and pit planting) was set in a factorial randomized block design replicated thrice. Sugarcane variety 'Co Pant 90223' was planted on 26 April, 2012 and harvested on 16 February, 2013. Input energy was worked out by considering the practices used for raising sugarcane and their energy equivalents. For land preparation a common pre-sowing irrigation depth of 6 cm was given. In conventional system, pre-planting tillage operations consisted of four cross harrowings and two plankings and fuel use in harrowing and planking was 20 and 5 litres ha^{-1} , respectively. After land preparation, for flat and trench planting; 15 cm deep furrows were opened at 60 cm distance with the help of tractor mounted furrow opener. In trench planting, soil from the first furrow was removed with the help of a small spade to increase its depth up to 20- 25 cm. Then

cane setts were placed and covered with the soil removed from the next furrow, and so on. In flat and trench methods, 3 budded 4 setts per meter furrow length were placed in bud to bud fashion. In flat method, the furrows were completely filled with soil while in trench and pit plantings, initially the furrows and pit were half filled. In pit method, the distance between centers of one to another pit was 90 cm having actual pit diameter of 60 cm. In all, 12346 pits ha⁻¹ were prepared. Pits were prepared manually and after fertilizer application, 2 budded 10 setts were placed in a circular fashion. Pits were connected to each other by small channels to facilitate irrigation. At the time of last intercultural operation at the end of June, the trenches and pits were completely filled with soil making the surface flat. Dual row trenches were made at a spacing of 40/80 cm. In ridge and furrow method, crop was sown in flat bed and at 60 days after planting, earthing was done to make the ridges. In no pre-planting tillage treatment plots, the preceding wheat crop was harvested manually at 25 cm above the soil surface and planting under different methods was done as that of in conventional method. The seed rate was 7.0 t ha⁻¹ in all the planting methods except in pit (11.1 t ha⁻¹). For weed management, two manual weeding were done in the month of May and June. The crop was

uniformly fertilized with 150 - 26.2 – 33.3 kg N-P-K ha⁻¹. One third dose of nitrogen and the entire amount of phosphorus and potassium was applied at the time of planting. The remaining N was top dressed in two equal splits in the last week of May and June, respectively. During the crop period, a total rainfall of 1007.3 mm was received in 46 rainy days. After the crop establishment, 4 irrigations were applied till the commencement of monsoon. The depth of irrigation in flat, ridge and furrow, trench, dual row trench and pit planting methods was 6, 6, 4.8, 3.4 and 4.2 cm, respectively. For pest control, Furadon was applied @ 30 kg ha⁻¹. For energy calculations, the harvesting capacity of a labour was taken as 800 kg labour⁻¹ (8 working hours). Variable yield under different treatments was taken in to account for computing the energy requirement. For other inputs and operations carried out, the energy equivalents are presented in Table 1. The labors used to raise sugarcane under conventional and no preparatory tillage system are presented operation/activity wise in Table 2.

Energy use efficiency, energy productivity, net energy gain and energy intensity for sugarcane production were calculated by using the following formula of Canakci *et al.* (2005).

Table 1. Energy equivalent of inputs and outputs used in sugarcane cultivation

Sl. No.	Particulars	Unit	Energy equivalent	References
A. Inputs				
1.	Human labor	hr	1.96	(Erdal <i>et al.</i> , 2007)
2.	Machinery	hr	62.7	(Erdal <i>et al.</i> , 2007)
3.	Diesel fuel	L	56.31	(Erdal <i>et al.</i> , 2007)
4.	Chemical fertilizers	kg	66.14	(Esengun <i>et al.</i> , 2007)
5.	Chemicals	kg	120	(Canakci <i>et al.</i> , 2005)
6.	Water for irrigation	m ³	1.02	(Acaroglu and Aksoy, 2005)
7.	Sugarcane	kg	5.3	Dey (2000)
B. Outputs				
1.	Cane stalk	kg	5.3	Dey (2000)

Table 2. Labor requirement for different operations

Operation /Activity	Labors hrs (mean over the planting methods)	
	Conventional tillage	No preparatory tillage
Pre-sowing irrigation	16	16
Harrowing and planking	5.5	-
Fertilizer application	12	12
Irrigation	64	64
Sowing(for planting+ covering+ tractor operator)	266.8	290.8
Weeding	320	320
Pest control	8	8
Harvesting	821.2	871.4

Energy equivalents given in Table 2 were used for the computation of energy use indicators

$$\text{Energy use efficiency} = \frac{\text{Energy Output (MJ ha}^{-1}\text{)}}{\text{Energy Input (MJ ha}^{-1}\text{)}}$$

$$\text{Energy productivity} = \frac{\text{Cane yield (kg ha}^{-1}\text{)}}{\text{Energy Input (MJ ha}^{-1}\text{)}}$$

$$\text{Net energy} = \text{Energy output (MJ/ ha)} - \text{Energy Input (MJ/ ha)}$$

$$\text{Energy intensity} = \frac{\text{Energy Input (MJ ha}^{-1}\text{)}}{\text{Cane yield (kg ha}^{-1}\text{)}}$$

RESULTS AND DISCUSSION

No pre-planting tillage produced 1.9 per cent more number of millable canes than conventional tillage (101875 ha⁻¹) but differences were not enough to be significant (Table 3). Trench planting recorded the maximum number of millable canes which remained at par with pit and ridge & furrow method but was significantly superior to the flat and dual row trench methods. The minimum number of millable canes (92181 ha⁻¹) was counted in dual row trench method. Weight of individual cane did not show significant variations due to the tillage practices and crop establishment methods. Trench planting produced the heaviest cane (904 g) followed by pit planting (891 g).

Pre-planting tillage treatments did not affect the cane yield significantly. Crop grown in the absence of pre-planting tillage operation produced 1.4 t ha⁻¹ higher cane yield than of the conventional tillage (Table 3). Non-significant difference in number of millable canes and individual cane weight of conventional tillage and no pre-planting tillage method led to comparable cane yields. Trench planting produced at par cane yield with pit and ridge & furrow methods, but gave significantly higher cane yield (90.8 t ha⁻¹) over the flat and dual row trench plantings. The lowest cane yield (70.7 t ha⁻¹) was noticed in dual row trench method. Ridge & furrow and flat planting methods also showed significantly higher cane yield than that of the dual row trench method, but both the former treatments did not differ significantly with the pit planting. Cane yield is a function of number of millable canes and weight of individual cane. The higher cane yield under trench planting method could be attributed to more number of millable canes and relatively more individual cane weight. Pit method recorded second highest cane yield next to the trench planting. Increased cane yield in pit planting over ridge & furrow, flat and dual row trench planting could be assigned to more number of millable canes and individual cane weight as a result of more contribution of the proportion of mother shoots in cane population under pit planting than secondary and tertiary tillers in flat planting. Moreover, these secondary and tertiary tillers continue to depend on primary shoots until they form their own roots, thus restricting development of the primary shoot in flat planting. The localized placement of

fertilizers in pit method has been reported to increase nutrient use efficiency *vis-a-vis* to enhance production of healthy canes (Gupta *et al.*, 2004).

Input energy: Results revealed that total energy used in various farm operations for sugarcane cultivation under no preparatory tillage system fluctuated from 62269 to 79475 MJ ha⁻¹ in ridge & furrow and pit planting, respectively (Table 3). Energy consumed in land preparation, fertilizer application, weeding and pest control remained constant amongst all the planting methods. Among different farm operations, energy used in seed and planting shared the maximum percentage of total energy and ranged from 68 to 75.4 % in ridge & furrow and pit planting, respectively. The higher energy consumption for seed and planting in pit method was due to use of 72 % higher seed rate and also more energy requirement for the pit preparation. Fertilizer input was next to seed and planting in order from energy consumption point of view. The share of fertilizer input was the maximum (17.9 %) in dual row trench method and the minimum (14.0 %) in pit planting. The energy used for irrigation also varied being the highest in both flat and ridge & furrow planting methods and the lowest in dual row trench method owing to variation in irrigation depth (6.0 and 3.4 cm, respectively). Percentage wise, energy consumed for irrigation was the lowest in pit planting due to less irrigation depth (4.2 cm) and overall total high energy requirement. Energy involved in harvesting also showed variations among the planting methods. Crop produced by trench planting consumed the maximum energy for harvesting was higher by 9.9, 7.8, 5.9 and 4.8 % over flat, ridge & furrow, dual row trench and pit methods of planting, respectively (Table 3). Trench planting produced the maximum cane yield thus required more number of labors for the harvesting and resulted in the maximum energy consumption. Dual row trench method accounted for the lowest energy consumption for harvesting due to production of the lowest cane yield. Among various farm operations, energy requirement for weeding purpose was found to be the lowest as only 2 manual weedings were performed by labors (20 labors/weeding).

The total energy used in conventional tillage system was higher than no- preparatory tillage system irrespective of the planting method. The higher energy consumption in conventional tillage system was due to more energy consumed in fuels and labors for land preparation consist of 4 harrowings and 2 plankings. Energy required for land preparation in conventional system was higher by 220.4 % over the no- preparatory tillage system (Table 3). Energy requirement for harvesting was also different between conventional and zero tillage system due to the variations in cane yield. Crop planted by trench method consumed the maximum energy for harvesting while the minimum by dual

Table 3. Energy use parameters of sugarcane as influenced by different treatments

Treatment	Number of millable canes (ha ⁻¹)	Individual cane weight (g)	Cane yield (t ha ⁻¹)	Input energy (MJ)	Output energy (MJ)	Energy use efficiency	Energy productivity (kg MJ ⁻¹)	Net energy gain (MJ)	Energy intensity (MJ kg ⁻¹)
Tillage practice									
Conventional tillage	101875	884	82.1	67621	435243	6.47	1.22	367622	0.832
No-pre planting tillage	103801	890	83.5	66302	442814	6.72	1.27	376512	0.800
SEm±	1241	114	1.8	-	9387	0.14	.03	9387	.019
CD (p=0.05)	NS	NS	NS	-	27890	0.43	.08	27890	.055
Planting method									
Flat	102660	875	80.9	64129	428580	6.69	1.26	364451	0.799
Ridge & furrow	103260	889	84.4	64042	447513	6.99	1.32	383471	0.763
Trench	108448	904	90.8	63624	481078	7.56	1.43	417453	0.702
Dual row trench	92181	878	70.7	62837	374865	5.97	1.13	312028	0.896
Pit	107640	891	87.4	80175	463105	5.78	1.09	382930	0.919
SEm±	1963	180	2.8	-	14842	0.23	0.04	14843	.029
CD (p=0.05)	5831	NS	8.3	-	44097	0.68	0.13	44098	.087

row trench method. In conventional tillage system, energy consumed in fertilizer application, seed and planting, weeding, and pest control was similar to no preparatory tillage system. In conventional tillage system, crop raised by pit planting accounted for the maximum energy consumption while the minimum energy was consumed in dual row trench planting. Among different farm operations, energy used in seed and planting contributed towards the maximum percentage of total energy used in sugarcane cultivation and it ranged from 66.6 to 74.1 % in ridge & furrow and pit planting, respectively (Table 4). Conventional tillage consumed 2.0 % higher input energy than crop grown in the absence of pre -planting tillage. The results corroborate with the findings of Jha *et al.* (2011). They observed 6227 MJ ha⁻¹ more input energy consumption under conventional tillage system compared to zero till sowing of wheat owing to more tillage operations. Srivastava (2003) also reported less input energy consumption in zero-tilled sugarcane compared to conventional tillage system. Saving of 6700 MJ ha⁻¹ energy by avoiding pre- planting tillage in sugarcane also have been reported by Singh *et al.* (1995). The lower input energy used in no -preparatory tillage system was due to the saving of 1418.3 MJ ha⁻¹ energy consumed for land preparation in conventional tillage system. Conventional tillage system spent 220% higher energy than no preparatory tillage system for the land preparation.

Among the different planting methods, the total input energy use was the highest in pit planting and the lowest in dual row trench planting. The higher total input

energy use in pit planting was associated with the more input energy incurred in seed and planting being higher by 25.0, 25.2, 26.0 and 27.6% over flat, ridge & furrow, trench and dual row trench planting, respectively. The lowest total input energy was recorded by dual row trench method as it saved 10.4, 10.4, 5.6 and 3.5 cm irrigation water compared to flat, ridge and furrow, trench and pit planting, respectively.

Output energy: The output energy which is the function of cane yield and their energy equivalent was higher by 1.74 % in no pre-planting tillage treatment over the conventional tillage with a significant margin due to 1.4 t ha⁻¹ higher cane yield. The variations in cane yield caused differences in output energy therefore, trench method recorded the highest and dual row trench attained the lowest output energy. Trench method recorded at par output energy with pit and ridge & furrow method but was significantly superior to flat and dual trenching methods (Table 3). It gave 12.2 % higher output energy than flat planting due to higher cane yield.

Energy use efficiency: Energy use efficiency, which is the ratio of energy output to energy input, was also significantly higher in the absence of pre-planting tillage due to relatively higher cane yield, which in turn reflected in higher output energy and 1.95 % less energy consumption in the aforesaid treatment against the conventional tillage (Table 3). Jha *et al.* (2011) also observed 17.3% higher energy use efficiency in zero tilled system of wheat than the conventional. Among different planting methods, trench method recorded the maximum energy use efficiency. It gave 13.0 % higher output energy than flat planting due to higher cane yield.

Table 4. Energy (MJ/ha) used by different planting methods under no preparatory and conventional tillage systems

Practice/operation	No- preparatory tillage					Conventional tillage				
	Flat	Ridge & furrow	Trench	Dual row trench	Pit	Flat	Ridge & furrow	Trench	Dual row trench	Pit
Land preparation	643.4	643.4	643.4	643.4	643.4	2061.7	2061.7	2061.7	2061.7	2061.7
Fertilizer application	11136.9	11136.9	11136.9	11136.9	11136.9	11136.9	11136.9	11136.9	11136.9	11136.9
Irrigation	2573.4	2573.4	2083.8	1512.6	1839	2573.4	2573.4	2083.8	1512.6	1839.0
Seed and planting	43238.2	43082.3	43029.5	43029.5	59892	43238.2	43082.3	43029.5	43029.5	59892
Weeding	627.2	627.2	627.2	627.2	627.2	627.2	627.2	627.2	627.2	627.2
Pest control	3615.7	3615.7	3615.7	3615.7	3615.7	3615.7	3615.7	3615.7	3615.7	3615.7
Harvesting	1640.5	1671.9	1803.2	1703.2	1720.8	1528.8	1636.6	1754.2	1423	1703.2
Total	63475.3	63350.1	62939.7	62268.5	79475	64781.9	64733.8	64309	63406.6	80875.3

Energy productivity and net gain: Energy productivity i.e., the production of cane yield per unit energy used was significantly higher in no pre-planting tillage condition than conventional tillage treatment having difference of 4.1% energy productivity. Among different planting methods, trench method recorded the maximum energy productivity followed by ridge & furrow planting system. Although, cane yield of pit planting was higher than ridge & furrow planting but due to higher input energy incurred in pit method, ridge& furrow planting method recorded higher energy productivity.

The net grain of energy was also significantly higher in no-pre planting tillage than conventional tillage treatment (Table 3). It was higher by 8890 MJ ha⁻¹ than conventional tillage method. Among different planting methods, trench method recorded the maximum net energy gain due to higher cane yield. Dual row trench proved the poorest owing to the lowest cane yield.

Energy intensity: Energy intensity shows the amount of energy spent to produce a unit of marketable product. It was higher by 3.7 % in conventional than no pre- planting tillage indicating more energy requirement to produce per unit cane yield (Table 3). The findings revealed less energy requirement and efficient utilization of energy in no pre-planting tillage practice with more gain of energy. Among different planting methods, pit method required the maximum energy to produce per unit cane yield and showed maximum energy intensity while trench the minimum.

Based on the findings of present study, it can be inferred that crop grown without pre-planting tillage operations is more energy effective as it spent 220% less energy for land preparation and also registered higher energy use efficiency and energy productivity compared to conventional tillage method. Among the planting methods, trench method gave the maximum energy use efficiency, energy productivity and net energy gain and was the lowest energy intensive.

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Integration of Organic and Chemical Fertilizers as Affected by Growth and Yield of Wheat (*Triticum aestivum*)

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Abstract: A field experiment was conducted at Students' Agriculture Farm of Khalsa College Amritsar, to study the effect of different levels of organic and chemical fertilizers as affected by growth and yield of wheat in Punjab region. Among all the treatments highest plant height, leaf area index, dry matter accumulation, effective tillers per plant, ear length and number of grains per ear were recorded in 75% of Recommended NPK + Biofertilizers + CDS and this treatment was higher over all other treatments but statistically at par with Recommended NPK (125, 62.5, 30 kg ha⁻¹). Maximum grain and straw yield were recorded (40.5 and 60.2 q ha⁻¹) with application of 75% of Recommended NPK + Biofertilizers + CDS and which was statistically at par with Recommended NPK (125, 62.5, 30 kg ha⁻¹).

Key Words: Biofertilizers, Cow dung slurry, Farm yard manure, Grain yield, Wheat

Wheat (*Triticum aestivum*) is one of the most important cereal crop of India. It is usually placed at premier position among cereals on account of vast acreage under it. The protein found in wheat, known as *gluten*, makes wheat very versatile in the culinary arts, and is a primary protein source for much of the world's population. In India during 2008-09, the area under wheat was 28.46 million hectare with production of 74.9 million tonnes and average yield 26.70 q ha⁻¹ (Anonymous, 2009a) In Punjab, during 2008-2009 wheat was grown on an area of 35.20 lac hectare with production of 157.33 lac tonnes and average yield of 44.62q ha⁻¹ (Anonymous, 2009b). No doubt chemical fertilizers will continue to be the main component for meeting the increased crop nutrient need of the country. However, due to energy crisis, their use has become the costliest input, which constitutes about 30-40 per cent of farmer's budget for wheat production. Tremendous quantities of nutrients are required to produce necessary food for the country and continuous use of high level of fertilizers over a long period is posing a great threat to soil as well as environmental quality. The role of fertilizers in boosting crop production has already been proved and those become so essential that the cultivation of present day wheat without them is rather a dream. The crop removal of nutrients is presently more than 32 million tonnes and will continue, resulting in depletion of soil fertility. Such a scenario will lead to more widespread and acute nutrient deficiencies. The gap between demand and supply of the fertilizers is expected to reach 9-10 million tonnes (Gupta *et al.*, 2006). Therefore, it is an urgent need to harness and put to use all the available sources of plant nutrients to reduce the extent of soil mining and maintaining the soil health.

The basic concept underlying the principal of integrated nutrient supply system is to improved soil fertility

for sustainable crop production on long-term basis. It may be achieved through integrated use of all the available sources of plant nutrient and their scientific managements in different cropping systems. Potential alternatives to chemical fertilizers are available in nature, which do not pollute the environment and maintain soil health such biological and natural inputs include: Biofertilizers like *phosphorus solubilizing bacteria* (PSB), *Azotobacter*, *Azospirillum*, *Vascular arbuscular mycorrhiza* (VAM), *farm yard manure* (FYM) and *cow dung slurry* (CDS) etc. Moreover, specific application of these organic sources would change the environment of rhizosphere by positively affecting the porosity, aeration, temperature, water holding capacity and soil microflora etc. Biofertilizers are the preparation of efficient strains of micro-organisms capable of converting atmospheric nitrogen to available form or solubilize phosphorus from its fixed form in the soil. Non-symbiotic nitrogen fixer viz *Azotobacter*, *Azospirillum* can add 20-30 kg N ha⁻¹ to the soil (Kizilkaya 2009). Moreover VAM and PSB help in solubilizing the fixed form of phosphorus in the soil improve water transport and also protect the roots from soil borne pathogens (Boparai *et al.*, 2001). Integration of various sources of organic and inorganic fertilizers is more suitable, as they reduce the cost of cultivation and improve physical condition of the soil. Use of chemical fertilizers in combinations with organic manure is essential to improve the soil health (Bajpal *et al.*, 2006). The objective of this study was to evaluate the various combinations of organic sources and inorganic fertilizers for maximum wheat production.

MATERIAL AND METHODS

The field experiment was conducted at students' agriculture farm Khalsa college Amritsar during Rabi season

2009-2010. The variety of wheat crop PBW 550 was sown in 2nd week of November with recommended PAU practices except fertilizer applications. There were twelve treatment combination (Table 1) in Randomized block design with four replications. The soil of the experiment field was sandy loam texture, low in available nitrogen, medium in phosphorus and high in potash. The farm yard manure contains 0.63% N, 0.22% P₂O₅ and 0.79% K₂O. Organic manure was incorporated in the soil as per the treatments about three weeks before sowing of the crop. Seed of wheat was inoculated with biofertilizers viz. Azospirillum, Azotobacter @ 250g inoculants ha⁻¹ and for inoculation, 10% sugar solution was prepared by dissolving 100 g sugar in 1 liter water and heated for 20-25 minutes. The solution was cooled at room temperature and mixed with cultures treatment-wise. The wheat seed were inoculated (1 kg + 10 g culture) with the solution and then dried under shade before sowing. Whereas, VAM and PSB @ 0.5 and 5 kg ha⁻¹ are mixed in 50 kg sand and broadcasted in field at time of sowing. Wheat crop was fertilized as per treatments Total three irrigation were applied in order to raise a successful crop of wheat. CDS with the ratio 10:10:200 (cow dung, urine and water, respectively) was applied with all irrigations as per the treatments. The data was analyzed with CPCS 1 software

Table1. Effect of Integration of organic and chemical fertilizer on growth of wheat

Treatments	Emergence count per meter row length	Plant height (cm)	Dry matter accumulation (q ha ⁻¹)	Leaf area index
T1	31.2	78.3	55.9	3.00
T2	33.2	87.5	96.7	3.37
T3	32.0	86.4	92.7	3.34
T4	31.7	85.7	90.8	3.16
T5	31.7	81.6	89.8	3.12
T6	31.5	86.9	87.5	3.30
T7	31.6	83.4	89.3	3.24
T8	31.4	81.8	88.4	3.10
T9	33.5	88.1	100.7	3.40
T10	31.7	86.2	98.7	3.27
T11	31.2	82.2	77.5	3.21
T12	31.6	82.6	75.2	3.17
CD (p=0.05)	NS	7.1	7.1	0.26

T1 - Control, T2- Recommended NPK (RDF) (125, 62.5, 30 kg ha⁻¹), T3 - 75% of Recommended NPK + FYM @ 5 t/ha, T4 - 50% of Recommended NPK + FYM @ 10 t/ha, T5 - 25% of Recommended NPK + FYM @ t/ha, T6 - 75% of Recommended NPK + Biofertilizers, T7 - 50% of Recommended NPK + Biofertilizers+ FYM @ 5 t/ha, T8 - 25% of Recommended NPK +Biofertilizers + FYM @ 10 t/ha, T9 - 75% of Recommended NPK + Biofertilizers +CDS, T10 - 50% of Recommended NPK + Biofertilizers +CDS, T11 - 25% of Recommended NPK + CDS+ FYM @ 5 t/ha, T12 - Biofertilizers + FYM @ 15 t/ha + CDS.

developed by Punjab Agricultural University, Ludhiana. Differences among the treatments means were compared using least significant difference (LSD) at 5% probability level (Steel and Torrie, 1984). Data on growth and yield parameters were recorded.

RESULTS AND DISCUSSION

Plant emergence constitutes the basis of optimum plant population which ultimately account for the crop yield. Data presented in table 1 revealed that the emergence count was not significantly affected by various treatments. Plant height is a reliable index of growth and metabolic activities of the crop plant. Highest plant height was recorded with 75% of recommended NPK + Biofertilizers + CDS (T9), however, it was statistically at par with all other treatments except control. This could be attributed to improved soil fertility status through microbial activities and better utilization of plant nutrients by wheat. Singh and Panwar (1997) also reported improved growth attributes of wheat with Azotobacter and Azospirillum inoculation. Maximum leaf area index was recorded with application of 75% of Recommended NPK + Biofertilizers + CDS and this treatment was statistically at par with T2, T3, T4, T6, T7, T11, T12 and T10 respectively and which was significantly better than T1, T5 and T8 respectively. The increase in leaf area index may be due to continuous supply of nutrients to the crop from inorganic fertilizers and organic sources (Solunke *et al.*, 2006). The dry matter accumulation increased progressively with advancement of the age and the maximum values were recorded at harvest. The treatments T9, produced the highest dry matter accumulation which was statistically at par with T2, T10 and significantly better than all other treatments. The increase in the dry matter accumulation of wheat with integrated use of organic sources of nutrients along with inorganic fertilizers could be attributed to its ability to improve soil health and help to increase plant height, leaf area index by virtue of which dry matter accumulation was higher (Rao *et al.*, 2004).

The number of effective tiller is one of the most important yield contributing characters which serve as direct index for determining the actual yield of the crop. Maximum number of effective tiller per meter were recorded in application of 75% of recommended NPK + Biofertilizers + CDS but which was statistically at par with T2, T3, T6, T10 respectively and significantly better than control. (Table 2). The higher value of yield attributing character of wheat due to biofertilizers has been reported by (Tomar *et al.*, 1995). Application of 75% of recommended NPK + Biofertilizers + CDS (T9) showed significant more ear length than T5, T6, T8, T10, T11, T12, respectively. The ear length was statistically

Table 2. Effect of Integration of organic and chemical fertilizer on yield of wheat

Treatments	Effective tillers per plant	Ear length (cm)	Number of grains per ear	Test weight (g)	Grain yield (q ha ⁻¹)	Straw yield (q ha ⁻¹)	Harvest index
T1	1.40	8.70	25.6	36.4	22.5	33.4	40.2
T2	2.80	12.6	39.0	39.6	39.2	57.5	40.5
T3	2.70	12.3	37.1	39.3	38.3	54.4	41.3
T4	2.50	12.2	36.5	38.9	37.8	53.0	41.6
T5	2.50	10.7	36.1	37.5	36.9	52.8	41.1
T6	2.10	10.8	37.0	39.1	35.2	52.3	40.2
T7	2.60	12.1	36.1	38.6	36.8	52.5	41.2
T8	1.80	11.5	30.1	37.3	36.5	51.9	41.3
T9	1.60	13.1	39.0	39.7	40.5	60.2	40.2
T10	2.90	10.5	36.1	38.5	37.7	59.0	40.2
T11	2.60	10.3	30.8	37.1	32.2	45.3	41.5
T12	2.40	10.6	36.9	38.8	31.3	43.9	41.6
CD (p=0.05)	0.36	1.5	2.1	NS	3.2	5.1	NS

comparable under T2, T3, T4 and T7, but differed significantly under control. The increase in ear length of wheat may be attributed to various integrated treatments by continuous supply of photosynthates to the sink due to slow release of nutrient from different organic sources and inorganic fertilizers and there long term availability to plants. The trend of increasing ear length with the combined application of organic and inorganic fertilizers had been also reported by (Sushilla and Giri, 2000). The treatment T9 and T1 recorded maximum (39.0) and minimum (25.6) number of grains per ear respectively. Among all other treatments, T2, T3, T6, T12 recorded significantly higher number of grains per ear as compare to T4, T5, T7, T10, T11, and T8, respectively. (Nehra *et al.*, 2001) also reported that the increase in number of grains per ear may be due to favorable effect of combined application of organic sources and chemical fertilizers on plant growth parameters which might have enhanced number of grains per ear. The data presented in table 2 showed that the test weight was found to be non-significant for all treatments. The results are in close agreement with the finding of (Tulsaram and Mir, 2006).

Significantly higher grain yield was obtained with the application of 75% of recommended NPK + Biofertilizers + CDS (40.5 q ha⁻¹) over T3, T4, T10, T5, T7, T6, T11, T12 and T1 (22.5 q ha⁻¹) but was statistically at par with recommended NPK (125, 62.5,30 kg ha⁻¹) (T2). Application of 75% of recommended NPK + Biofertilizers + CDS was 5.4, 6.6, 6.9, 8.8, 9.1, 10.6, 13.0 ,20.4 ,22.7 ,22.7 and 44.4 % grain yield over T3, T4, T10, T5, T7, T6, T11, T12 and T1, respectively (Table 2). Maximum straw yield was obtained with the application of 75% of Recommended NPK + Biofertilizers + CDS (60.2 q ha⁻¹) and this treatment was significantly higher

straw yield over T3, T4, T5, T7 T6, T8, T11, T12, and T1 (33.4 q ha⁻¹) but statistically at par with recommended NPK (125, 62.5,30 kg ha⁻¹) (T2) and 50% of recommended NPK + Biofertilizers +CDS (T10). Application of 75% of recommended NPK + Biofertilizers + CDS was 9.6, 11.9, 12.2, 12.7, 13.1, 13.7, 24.7 ,27.0 and 44.5 % straw yield over T3, T4, T5, T7 T6, T8, T11, T12, and T1, respectively (Table 2). The combined application of organic and inorganic sources produced higher grain and straw yield than organic alone might be attributed to higher and continuous supply of nutrient and improvement in physical environment of soil which increased significantly the yield characteristic of wheat. This was in agreement with finding of (Kachroo *et al.*, 2006; Walia *et al.*, 2005; Aulakh *et al.*, 2013). Barik and Goswami (2003) and Bhat (2013) also obtained higher grain and straw yield of wheat crop by integration of organic and inorganic sources.

It is recommended that application of 75% of Recommended NPK + Biofertilizers + CDS treatment showed the highest growth and yield attributes among all other treatments. However, Recommended NPK (125, 62.5,30 kg ha⁻¹) was the next best treatment under irrigated ecosystem of Punjab.

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Chemical Partitioning and Distribution of Copper, Zinc and Nickel in Soils of Lesser Himalayas

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Abstract: Distribution and availability of copper (Cu), zinc (Zn) and nickel (Ni) in agricultural soils of nine districts of Lesser Himalayas were studied during the year 2011-12. Sequential extraction was used to fractionate the metals (Zn, Cu and Ni) from soils into six operationally defined groups: water soluble, exchangeable, carbonate, Fe-Mn oxide, organic, and residual. The residual fraction was the most abundant pool for all the three metals examined (65.93, 80.08 and 48.7% respectively for Cu, Zn and Ni). Results of these analysis showed that 4.35, 1.0 and 1.44% of total Cu, Zn and Ni, respectively is associated to water soluble fraction, 5.14, 2.01, 7.62% with exchangeable fraction, 3.58, 2.07, 8.86% with carbonate bound, 8.28, 8.42, 20.25% with oxide and 12.72, 6.42, 13.13% with organic matter. Only a small amount of Cu and Zn (34.07 and 19.92%) was present in the potentially available fraction: nonresidual fraction indicating lithogenic origin and low bioavailability of the metals considered. A significant amount (51.3%) of Ni was present in potentially available fraction. Assuming that mobility and bioavailability of these metals are related to their solubility and geochemical forms, and that they decrease in the order of extraction sequence, the apparent mobility and potential bioavailability for these three metals in the soils were: Ni > Cu > Zn.

Key Words: Chemical Partitioning, Heavy metals, Lesser Himalayas, Potential mobility

Soils are the main source of nutrient elements for plants, and soil conditions play a crucial role in metal ion behavior. Soil properties, metal speciation, plant species, water regime, and especially soil plant interactions determine the bioavailability of soil metal ions (Manouchehri *et al.*, 2006; Kubova *et al.*, 2008). The soil-plant transfer of metal ions is a part of the cycling of chemical elements in nature. It is a very complex process governed by several factors, both natural and human-related. In general, plants readily take up the species of elements that are dissolved in the soil solution in either ionic or chelated or complexed forms (Moralejo and Acebal, 2014).

Metals are diverse in their chemical and physical forms, which change with perturbation of the system. As such, these metals are distributed among the solid and liquid phases of the soils, which determine their bioavailability. Much of the micronutrients associated with the solid phase are not available for plant uptake. In fact, <10% generally are in soluble and exchangeable forms. However, the redistribution among forms is brought about by changes in soil properties due to natural and anthropogenic causes (Shuman, 1991). The forms are classified as include (i) water soluble (ii) exchangeable (iii) linked to organic substances (iv) occluded or co-precipitated with oxides, carbonate and phosphates, or other secondary minerals and (v) ions in the crystalline lattices of the primary minerals (Kashem *et al.*, 2007). The first three chemical forms are considered to be balanced among themselves and to be most available forms for plant nutrition, each successive form representing less

availability (Petrizzelli, 1989). The fate of metals added to a soil is controlled by a number of physical and biological processes acting within the soil (Buba *et al.*, 2013). Their initial mobility after addition to soil will largely depend on the form in which the metals are added, which in turn depends on their sources. Plants depend so much on soil micronutrient metals for the essential function of their organs. Since no speciation studies on micronutrients in soils in this part of the country has been reported, it is expected that the results from this study would form a baseline data for future micronutrient status of soils in the area under study. This research is also aimed at determining metals redistribution among extractable fractions caused by changes in soil properties.

MATERIAL AND METHODS

Study area : The study area is the Lesser Himalayas located in the North-western corner of India, occupies the depression formed by the bifurcation of the Great Himalayan Range whose south-western arm is known as the Pir Panjal Range and the north-eastern arm as the Main Himalayan Range. At the northern limit of the valley is the Qazi-Nag Range. In between the complexly folded and faulted mountain ramparts lies the bowl shaped Kashmir Valley between the parallels of 33°30' and 34°30' N latitude and 74°10' and 75°03' E longitude.

Sampling and sample pre-treatment: Soil samples were collected from nine districts of Lesser Himalayas. A total of forty four composite surface soil samples (0-20 cm) were taken randomly from different sites constituting mostly the

Table 1. Description of sampling locations of Kashmir Himalayas

Village	District	Land use	Latitude	Longitude
Mali bonigam	Pulwama	Apple orchard	33.882	74.958
Rajpora	Pulwama	Apple orchard	33.913	75.045
Keegam	Shoupian	Apple orchard	33.784	74.858
Keegam pashpora	Shoupian	Apple orchard	33.777	74.861
Akipora	Kulgam	Apple orchard	33.686	74.973
Katianwaley	Baramula	Apple orchard	34.186	74.393
Muqam	Baramula	Apple orchard	34.226	74.454
Shangargund	Baramulla	Apple orchard	34.321	74.434
Mohrpur	Anantnag	Forest	33.644	75.24
Duksum	Anantnag	Forest	33.613	75.44
Mugpath	Budgam	Agricultural crop	33.861	74.703
Buzgo	Budgam	Agricultural crop	33.883	74.621
Tangmarg	Baramula	Agricultural crop	34.07	74.435
Sarfara	Ganderbal	Agricultural crop	34.217	75.044
Zganeir	Ganderbal	Agricultural crop	34.286	75.194
Tangdar	Kupwara	Agricultural crop	34.395	73.86
Tral	Pulwama	Agricultural crop	33.922	75.101
Shurant	Kulgam	Agricultural crop	33.667	75.039
Achabal	Anantnag	Agricultural crop	33.689	75.215
Aloosa	Bandipora	Agricultural crop	34.421	74.549
Ajas	Bandipora	Agricultural crop	34.345	74.654
Wangipora	Bandipora	Agricultural crop	34.217	74.645
Mirgund	Baramula	Agricultural crop	34.136	74.677
Pattan	Baramula	Agricultural crop	34.16	74.562
Magam	Baramula	Agricultural crop	34.092	74.601
Salamabad	Baramula	Agricultural crop	34.083	74.036
Gawata	Baramula	Agricultural crop	34.275	74.316
Manigam	Ganderbal	Agricultural crop	34.277	74.803
Kangan	Ganderbal	Agricultural crop	34.274	74.897
Waskoor	Ganderbal	Agricultural crop	34.233	74.663
Solar	Ganderbal	Agricultural crop	34.202	74.755
Tulmula	Ganderbal	Agricultural crop	34.23	74.721
Taratpora	Kupwara	Agricultural crop	34.47	74.113
Mawar	Kupwara	Agricultural crop	34.349	74.205
Duksum	Anantnag	Pasture	33.598	75.466
Cherwan	Bandipora	Agricultural crop	34.65	74.891
Lathipora1	Pulwama	Saffron crop	33.988	74.934
Lathipora 2	Pulwama	Saffron crop	33.988	74.934
Haran	Budgam	Vegetable crop	34.057	74.707
Gambora	Budgam	Vegetable crop	34.043	74.622
Alapora	Budgam	Vegetable crop	34.04	74.537
Doru	Budgam	Vegetable crop	34.043	74.736
Hayathpora	Budgam	Vegetable crop	34.01	74.606
Ismerg	Bandipora	Agricultural crop	34.655	74.689

cultivated areas cropped with paddy, maize, vegetables, wheat, oats, mustard, horticultural crops, pastures and forests using GPS (Table 1). Soil samples were air-dried in the laboratory before being crushed in a ceramic mortar and sieved in a 2mm screen plastic sieve.

Metal speciation: Metals were fractionated using sequential extraction scheme proposed by Tessier *et al.* (1979) and modified by Abu-Kukati, (2001). The metal species were extracted into six fractions i.e., water soluble, exchangeable, bound to carbonates, bound to Fe-Mn oxides (Fe/ Mn ox.), bound to organic matter and residual fraction according to the following methods:

Fraction 1 - Water soluble: Water soluble portion of each micro element (fraction 1) was determined by shaking the soil sample (1g) with 10 ml of deionized water for one hour at 20°C. The solution was then filtered and stored for analysis.

Fraction 2 – Exchangeable fraction: The residue from fraction 1 was extracted at room temperature for 1hour with 16ml of magnesium chloride solution (1M MgCl₂) at pH 7. Soil and extraction solution were thoroughly agitated throughout the extraction. The leachate was then analysed for metals using AAS and the residue reserved for further analysis.

Fraction 3 – Bound to carbonates: The metals bound to carbonate phase are affected by ion exchange and changes of pH. The residue of Fraction 2 was extracted with 16ml of 1M sodium acetate/acetic acid buffer at pH 5 for 5 hours at room temperature. The extracted metal solution was decanted from the residual soil. The residual soil was used for the next extraction.

Fraction 4 – Bound to iron and manganese oxides : The residue from fraction 3 was extracted under mild reducing conditions. 13.9g of hydroxyl amine hydrochloride (NH₂OH.HCl) was dissolved in 500ml of distilled water to prepare 0.4M NH₂OH.HCl. The residue was extracted with 20ml of 0.4M NH₂OH.HCl in 25% (v/v) acetic acid with agitation at 96°C in a water bath for 6 hours. The leachate was then analysed for metals using AAS and the residue reserved for further analysis.

Fraction 5 – Bound to organic matter: The residue from

fraction 4 was oxidized as follows: 3ml of 0.02M HNO₃ and 5ml of 30% (v/v) hydrogen peroxide, which has been adjusted to pH 2, was added to the residue from fraction 4. The mixture was heated to 85°C in a water bath for 2 hours with occasional agitation and allowed to cool down. Another 3ml of 30% hydrogen peroxide, adjusted to pH 2 with HNO₃, was then added. The mixture was heated again at 85°C for 3 hours with occasional agitation and allowed to cool down. Then 5ml of 3.2M ammonium acetate in 20% (v/v) nitric acid was added, followed by dilution to a final volume of 20ml with de-ionized water. The extracted metal solution was decanted from the residual soil, which was used for the next extraction.

Fraction 6 – Residual or inert fraction: Residue from fraction 5 was oven dried at 105°C. Digestion was carried out with a mixture of 5ml conc. HNO₃ (HNO₃, 70% w/w), 10ml of hydrofluoric acid (HF, 40% w/w) and 10ml of perchloric acid (HClO₄, 60% w/w) in Teflon beakers. Fraction 6 largely consists of mineral compounds where metals are firmly bonded within crystal structure of the minerals comprising the soil.

Elemental analysis and instrumentation: The concentration of Cu, Zn, Ni, and Fe was determined from the soil extracts using a BUCK SCIENTIFIC Atomic Absorption Spectrophotometer Model 210 VGP. The spectrophotometer was calibrated by aspirating standard solutions of Cu, Zn and Ni nitrates at their various wavelengths and Acetylene-air mixtures in the ratio of 6:8 was used. Measurements were made after calibration, instrument settings optimized and blue flame obtained.

RESULTS AND DISCUSSION

Results of Cu, Zn and Ni fractions expressed as percentage of the sum of individual fractions for the overall (0-20cm) soils revealed principally the partitioning of these metals among six fractions, F1 – F6, (Water soluble, F1; Exchangeable, F2; Carbonate bound, F3; Iron-Manganese oxides bound, F4; Organic Matter and Sulphide bound, F5; Residual Fractions, F6). The distribution of the metals varies greatly among fractions. On average, the order of partitioning for the metals are shown in Table 2.

Table 2. Percentages of metals in different fractions

Fraction	Description	Cu	Zn	Ni
1	Water soluble	4.35±0.36	1.00±0.64	1.44±0.48
2	Exchangeable	5.14±0.57	2.01±0.59	7.62±0.91
3	Carbonate bound	3.58±0.47	2.07±1.96	8.86±0.93
4	Oxide bound	8.28±1.50	8.42±1.53	20.25±2.63
5	Organic bound	12.72±1.14	6.42±5.42	13.13±2.47
6	Residual	65.93±11.27	80.08±5.44	48.70±5.23

± Standard Deviation

Copper: There is variation in the distribution of copper among the different fractions (Table 2). Organic bound was the dominant fraction for copper (8.28%) while carbonate (3.58%) and water soluble bound (4.35%) was the least dominant fraction among non residual fractions (Fig.1) for the 0 – 20 cm depth soil. The relatively higher content of Cu in organic bound fraction can be attributed to its higher affinity for organic matter (Iwegbue *et al.*, 2006). The preference of Cu for organic matter is supported by high stability constant of Cu complexes with organic matter. The lowest concentration of copper in carbonate fraction might be due to increased manganese oxide and Fe/Mn oxides activity in concentrating Cu, thereby reducing co-precipitation reactions with carbonates (Buba *et al.*, 2013). Nevertheless, the non-residual form accounts for 34% and this indicates the quantity of Cu that could readily be available. The residual fraction constitutes 66%, thus suggesting that the Cu must have, to a great extent, been derived from lithogenic sources. The order of distribution in various chemical fractions is $F_6 > F_5 > F_4 > F_2 > F_3 > F_1$.

Zinc: The difference in distribution patterns of zinc (Table 2) can be inclined towards possible mobilization of the metals. The dominant form was found in the residual fraction (80.0%) likely indicated the greater tendency of Zinc to become unavailable in the soils. This is because the residual fraction represented metals that are largely embedded in sedimentary matrix of the soils and may not be available for remobilization except under very harsh conditions (Ma and Rao, 1997). Similar results were reported by Aydinalp (2009) and Kamali *et al.* (2010). The oxide bound fraction (reducible fraction) amongst the non residual fractions contained the highest concentration of Zn (8.420%), followed by the organic bound (6.42%), exchangeable fraction (2.0%) and acid soluble fraction (2.0%). Ashraf *et al.* (2011) found that most of Zn was strongly retained in the residual phase in soils. These heavy metals are contained in the crystal lattices of minerals with strong bindings and consequently they will not be released into the environment. The major portion of total Zn in residual fraction is also examined by Zauyah *et al.* (2004), Jaradat *et al.* (2006), Han *et al.* (2007), Aydinalp (2009) and Kamali *et al.* (2010). The order of the chemical partitioning of Zn in the soils is $F_6 > F_4 > F_5 > F_2 > F_3 > F_1$.

Nickel: The dominant fraction for nickel in soils was the residual phase (48.7%) (Table 2). The results are in close agreement with the findings of Zauyah *et al.* (2004), Guerra *et al.* (2007), Aydinalp (2009) and Osakwe (2012). This chemical form was closely followed by the Fe/Mn oxide phase (20.25%). The levels of nickel in this fraction depend on how much Mn oxide is adsorbed in a given soil sample because Ni^{2+} could substitute for surface manganese in

mixed valence Mn oxides and may also be due to higher affinity of Ni by goethite and hematite. The partitioning of Ni varies among all the fractions. Almost 51.3% Ni was held in the non-residual form, which indicates the potential bioavailability of Ni. The stability of metals bound to sediments as inorganic compounds (metal oxides, hydroxides, carbonate and sulphides) is controlled primarily by the system pH. The order of the partitioning of Ni in the soils is $F_6 > F_4 > F_5 > F_3 > F_2 > F_1$.

Potential mobility of metals in soil: The average potential mobility of Cu is 34.07%. Under strong oxidizing conditions, due to degradation of organic matter, Cu becomes very available and toxic in the environment (Table 3). The Zn had the least average potential mobility (19.92%) and may be the reason for large scale deficiency of Zn in the soils. Both the Cu and Zn were highly abundant in the residual fraction (65.93% and 80.08%), implying that they were not derived from anthropogenic sources but have lithogenic origin. The average potential mobility of Ni was 51.30%. High potential mobility indicates high reduction in concentration soil. Therefore, compared with other metals that were mostly abundant in the non-bioavailable fraction, Ni was more available and toxic in the environment.

Table 3. Potential mobility of metals in soil (%)

Metal	Available fraction	Non-bioavailable fraction
Cu	34.07	65.93
Zn	19.92	80.08
Ni	51.3	48.7

The chemical pools of the micronutrients/metals shows that they are distributed among six soil fractions. Most of the metals resided in the residual fraction, suggesting their lithogenic origin. This indicates that the metals were immobile and the environment was not likely to be polluted by these metals. The average potential mobility of the metals arranged in decreasing order was as follows: $Ni > Cu > Zn$. Considering that the metals mostly occur in the unavailable form except nickel, it might be assumed that the metals must have been deprived from being taken up by plant. Major portion of Zn and Cu found in potentially unavailable fraction may be the fact for their widespread deficiency. This also buttresses the points that high percentage of these metals found in residual fraction is an important repository of iron in the soil. The results of the investigation will be useful in monitoring deficiencies and toxicities of micro-elements in soils of Kashmir Himalayas.

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Influence of Silicon and Phosphorus on Growth, Yield and Nutrient Uptake by Maize (*Zea Mays L.*) in *Typic Ustochrepts* Soils

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Abstract: Application of Si @ 300 mg Si kg⁻¹ soil along with P @ 40 mg P kg soil⁻¹ recorded significantly highest green and dry shoot yield (93.03 g pot⁻¹, 52.25 g pot⁻¹, respectively) of maize in loamy sandy and silty loam soils, whereas, the highest root yield (13.39 g pot⁻¹) was also recorded under the same treatment in silty loam soil. The highest chlorophyll content in maize leaf (22.05 cci) was noted with the application of Si @ 400 mg Si kg⁻¹ soil along with P @ 40 mg P kg soil⁻¹ in both the soils. Results of investigation indicated that application of silicon @ 300 mg kg⁻¹ and phosphorus at 40 mg P₂O₅ kg⁻¹ gave maximum maize yield under P stress condition and also improved silicon, phosphorus and micronutrients utilization by maize plants.

Key Words: Growth, Maize, Nutrient uptake, Phosphorus, Silicon, Yield

The efficiency of phosphorus fertilizer and crop yields mostly reduces due to high soil sorption of phosphorus in some P deficient soils. Silicon is the second most abundant element after oxygen in soil and its application has been reported to decrease P sorption and increase yields in such soils. It is one of beneficial element for maize growth, which is very important for its morphological and physiological characters. Intensive cropping with a constant application of NPK fertilizers has depleted plant available silicon from the soil. Crops like rice, barley, cucumber, wheat, maize, sorghum and sugarcane absorb silicon in greater amounts than N, P and K (Epstein, 1994). Phosphorus is also an essential nutrient required by plants for normal growth and development of maize. Soils deficient in available P not only produce economically unacceptable yields, but other inputs, particularly N, are also used less effectively. Thus, there is an urgent need to seek strategies by which P fertilizers can be used more effectively in those farming systems where P is deficient and its use is economically feasible.

The solubility of Si in soils is important both for P and Si availability and Si movement, including leaching, in the soil. The beneficial effect of silicon on the plant growth and resistance to biotic stress has been proven in higher plant. Alleviation of Si on P-stressed maize and accumulation of Si and P in maize were well documented by various researchers which provide a theoretical foundation for the scientific application of fertilizers in maize in P deficient area (Yang *et al.*, 2008). Application of soluble Si in acid soil could decrease absorption of phosphorus in soil and soil pH, which improve dry weight and absorption by maize (Owino and Gascho, 2004). Hence, pot study was conducted to study the relationship between silicon and phosphorus under P

stressed soils keeping maize as a test crop.

MATERIAL AND METHODS

The experiment was conducted at Anand Agricultural University, Anand, during summer 2011, which is located at 22°-35' north latitude and 72°-55' east longitude with an elevation of 45 m above MSL. The area is characterized by low and erratic rainfall with mean annual rainfall of 864 to 870 mm with peaks in July to August. The nature of soil was neutral to alkaline, pH ranged from 7.25 to 8.94. The soluble salts (EC) content was low to high and an overall range was 0.17 to 1.06 dS m⁻¹ with a mean value of 0.38 dS m⁻¹. The cation exchangeable capacity (CEC) ranged from 15.0 to 23.0 cmol (p+) kg⁻¹. The available phosphorus, potassium, sulphur and sodium ranged from low to medium.

Earthen pots was taken for the conducting experiment and six kg soil was taken in each pots and calculated quantities of graded levels of Si viz. 0, 100, 200, 300 and 400 mg kg⁻¹ soil and two levels of P (0 and 40 mg P kg soil⁻¹) applied and mixed properly in soils. The source of silicon was calcium silicate and P as potassium di-hydrogen phosphate (KH₂PO₄). After sowing of maize seeds, pots were regularly watered and maintained optimum moisture up to 60 days required for optimum growth and development. A top dressing of nitrogen with urea was done in 15 days after sowing. Maize plants were harvested once they attained the age of 60 days. Soil and plant samples (shoot) were drawn for the further analysis of soil.

RESULTS AND DISCUSSION

Chlorophyll content: Chlorophyll content in the leaf was significantly improved due to different levels of silicon up to

Si₃₀₀ in both the soils (Table 1). The mean chlorophyll content was 15.32 cci and 20.44 cci in S₁ and S₂ soils, respectively. The interaction effect of Si x P on chlorophyll content in maize was non-significant. The appropriate Si could not only alleviate chlorophyll decomposition at a certain extent, but also enhance absorption of light in leaf (Yang *et al.*, 2008). Similar result was also reported by Rashid and Iqbal (2012).

Table 1. Effect of Si and P levels on chlorophyll content (cci) in maize

Si levels	Chlorophyll content (cci)			
	Soil (S ₁)		Soil (S ₂)	
	P ₀	P ₄₀	P ₀	P ₄₀
Si ₀	9.98	11.50	15.10	16.62
Si ₁₀₀	9.95	15.40	15.07	20.52
Si ₂₀₀	11.63	15.95	16.75	21.07
Si ₃₀₀	15.50	16.93	20.62	22.05
Si ₄₀₀	15.50	16.83	20.62	21.95
CD (p=0.05)	0.71	0.71	NS	NS

Plant height: Application of Si @ 300 mg kg⁻¹ soil at different levels significantly increased the plant height of maize at all three growth stages (30 and 60 DAS) in both P deficient S₁ and S₂ soils except at 60 DAS in S₁ soil, where in maximum plant height was noted under Si₃₀₀ level. The plant height was increased significantly under S₃₀₀ over control and Si₁₀₀ and Si₂₀₀ levels in S₂ soil at all growth stages, however, it was at par with S₄₀₀ at 30 DAS, While at 60 DAS it was significantly the higher under Si₃₀₀. In case of S₁ soil, initial two growth stages, Si₃₀₀ registered maximum plant height, while in S₂ soil, later two growth stages, Si₄₀₀ recorded maximum plant height, but it was on par with Si₃₀₀ level. Thus the results indicated that under both the soil condition varying in texture class, application of 300 mg Si kg⁻¹ was good enough to get beneficial effect on growth of maize crop. In both P deficient soils, the application of phosphorus P₄₀ also significantly increased the plant height at 30 and 60 DAS. The increase in

plant height at 60 DAS was 5.8 per cent under S₁ soil and 2.9 per cent under S₂ soil due to P₄₀ over P₀ level. The difference in P response is mainly due to differences in the initial fertility of soil and also difference in texture of soil. The increase in plant height due to P application @ 40 mg kg⁻¹ might be due to the increase in P availability to maize plants. The interaction effect indicate that in absence of P, Si application partly meet the requirement of the crop.

Green and dry shoot yield: The application of Si @ 300 mg kg⁻¹ soil significantly increased green shoot yield and dry shoot yield in S₁ and S₂ soils (Table 3). The increase in dry shoot yield due to Si₃₀₀ level was 24.2 and 22.7 per cent over control in S₁ and S₂ soils, respectively. The higher maize yield obtained in S₂ soil as compared to S₁ soil may be attributed to better fertility status of S₂ soil. The results are in accordance with those of Yang *et al.* (2008), who reported that Si with appropriate concentration could promote the growth of maize seedlings, dry matter accumulation of different organs, which significantly alleviated the ill effects caused by low-P stress. Therefore, Si application under low-P stress could improve not only the weight of different organs in maize seedlings, but also the dry matter distribution in different organs, On the other hand Si improved chlorophyll content and net photosynthesis rate of leaves, which promoted dry matter accumulation of maize seedlings. In both P deficient soils, the application of phosphorus (P₄₀) significantly increased the green and dry shoot yields. The mean dry shoot yield was 49.76 and 58.98 g pot⁻¹ due to P₄₀ level in S₁ and S₂ soils, respectively. The shoot yield was also significantly increased by P supplementation under P stress condition of both soils. This is mainly due to deficiency of available P in both the soils. Ma *et al.* (2001) also noted that higher P dose increased its availability allowing less adsorption of P in soil and so improved plant growth. Owino and Gascho (2004) also observed that under limiting condition of phosphorus in the soil, applied P at different rates increased maize dry

Table 2. Effect of Si and P levels on plant height (cm) at different growth stages of maize

Si levels	Plant height (cm)							
	30 DAS				60 DAS			
	Soil (S ₁)		Soil (S ₂)		Soil (S ₁)		Soil (S ₂)	
	P ₀	P ₄₀	P ₀	P ₄₀	P ₀	P ₄₀	P ₀	P ₄₀
Si ₀	26.62	30.72	33.25	35.84	105.95	110.50	120.40	121.26
Si ₁₀₀	29.56	28.97	31.23	38.09	110.89	112.30	117.65	121.45
Si ₂₀₀	35.92	37.62	37.04	42.74	117.25	122.95	125.58	128.16
Si ₃₀₀	30.42	44.82	38.54	46.94	115.85	129.15	123.96	135.45
Si ₄₀₀	35.62	37.52	42.12	42.64	120.95	128.85	128.65	128.06
CD (p=0.05)	NS	NS	NS	NS	2.05	2.05	NS	NS

weight. The interactions effect of Si x P for green and dry shoot yields of maize was found to be non-significant.

Dry root yield: Dry root yield in S_1 Soils was significantly higher under Si_{400} than rest of the treatment barring Si_{300} level. However, in case of S_2 soil, Si_{300} registered significantly the highest dry root (11.70 g pot⁻¹) yield, thereafter it was significantly decreased at Si_{400} to 10.22 g pot⁻¹ over preceding level (Table 3). The result showed that application of Si increased the root mass. Yang *et al.* (2008) also noted that Si enhanced permeability of the root cell membrane, allowing nutrients to enter more efficiently, as a result the root mass was increased. The dry root yield was also significantly increased by the application of P_{40} phosphorus in both soils as in case of shoot yields.

Nutrients Uptake

Silicon: Silicon uptake by maize straw (Table 4) was significantly increased by Si application at different levels. In S_1 soil, the highest Si uptake (19.40 g pot⁻¹) was observed under Si_{400} level but in (S_2) soil it was under Si_{300} level 24.32 g pot⁻¹. The Si uptake in both P deficient soils was significantly increased by the application of phosphorus ($P: 40$). The mean Si uptake was 15.91 and 23.89 g pot⁻¹ due to $P: 40$ level in (S_1) and (S_2) soils, respectively. Barman *et al.* (1998) showed that in soil addition of Si up to 5 mM/g soil would not increase the P availability, but there is every likelihood of

increasing Si availability due to P fertilization. The interaction effect of Si x P on Si uptake by maize was found significant, it means that P increased Si uptake by increasing the available Si in soil solution. Owino and Gascho (2004) observed that the applied silicate ion was converted to silicic acid (H_3SiO_4). In soils having low pH the negative charge of soluble silicon (H_3SiO_4) was less than negatively charged phosphate anion, therefore, the phosphorus was preferably adsorbed on the soil particle and it replaced the Si into soil solution.

Phosphorus: Uptake was increased significantly by the silicon application in both soils (Table 4). In (S_1) soil, significantly the highest P uptake (1.67g pot⁻¹) was observed under treatment Si_{300} and similar results were found in case of (S_2) soil (S_2). Owino and Gascho (2004) showed that Si concentration in the soil solution improved growth of maize resulting greater P uptake and utilization. The mean P uptake obtained was 1.68 g pot⁻¹ and 1.86 g pot⁻¹ due to P_{40} level in (S_1) and (S_2) soils, respectively. The application of phosphorus ($P: 40$) also significantly increased the P uptake in both P deficient soils. Sahoo and Panda (2001) indicated that soils low in P will adsorb large amounts of P leaving little for plants and higher P dose increased its availability allowing less adsorption and so improved uptake of P. Data on interaction effect of Si x P in (S_1) soil indicated that significantly the highest P uptake (2.26 g pot⁻¹) was under Si_{300}

Table 3. Effect of Si and P levels on green, dry shoot yield and dry root yield (g pot⁻¹) of maize

Si levels	Green shoot yield (g pot ⁻¹)				Dry shoot yield (g pot ⁻¹)				Dry root yield (g pot ⁻¹)			
	Soil (S_1)		Soil (S_2)		Soil (S_1)		Soil (S_2)		Soil (S_1)		Soil (S_2)	
	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}
Si_0	66.23	77.69	95.12	107.23	39.05	45.12	50.24	54.62	2.69	4.31	6.53	9.59
Si_{100}	71.36	83.56	103.45	113.56	39.45	43.30	41.70	60.45	2.91	4.33	6.42	10.89
Si_{200}	76.56	88.36	111.85	120.25	45.12	48.10	47.22	55.12	4.08	5.08	8.04	10.24
Si_{300}	89.36	96.70	117.12	121.52	47.56	56.44	58.45	70.20	4.35	6.25	10.02	13.39
Si_{400}	82.12	96.12	121.45	122.30	41.86	55.86	52.49	54.52	3.85	7.25	9.02	11.43
CD ($p=0.05$)	1.89	1.89	NS	NS	2.14	2.14	NS	NS	0.60	0.60	NS	NS

Table 4. Effect of Si and P levels on nutrient uptake by maize plant in different soils

Si levels	Nutrient uptake (g pot ⁻¹)								Nutrient uptake (mg pot ⁻¹)							
	Si				P				Fe				Mn			
	Soil (S_1)		Soil (S_2)		Soil (S_1)		Soil (S_2)		Soil (S_1)		Soil (S_2)		Soil (S_1)		Soil (S_2)	
	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}
Si_0	7.77	8.80	10.10	16.39	0.66	0.90	0.80	0.98	13.74	16.53	17.40	20.79	1.30	1.63	1.60	1.76
Si_{100}	8.88	12.34	11.47	24.66	0.63	1.52	0.63	1.99	14.47	16.96	17.97	16.73	1.27	1.56	1.47	1.68
Si_{200}	13.58	13.23	11.81	23.43	0.95	1.59	0.94	1.71	12.99	13.95	13.74	16.16	1.15	1.38	1.19	1.77
Si_{300}	9.99	22.29	17.42	31.24	1.09	2.26	1.29	2.67	11.94	11.94	10.01	16.86	1.12	1.26	1.43	1.42
Si_{400}	15.91	22.90	17.95	23.72	0.88	2.12	1.05	1.96	11.23	12.34	11.81	15.12	1.13	1.35	1.34	1.55
CD ($p=0.05$)	1.61	1.61	NS	3.61	0.09	0.09	NS	0.21	0.58	0.58	0.83	NS	0.06	0.06	NS	NS

$\times P_{40}$ and the lowest (0.90 g pot^{-1}) was in $Si_0 \times P_{40}$ combination, whereas in (S_2) soil the same treatment combinations gave the highest (2.67 g pot^{-1}) and the lowest (0.98 g pot^{-1}) P uptake, respectively. Owino and Gascho (2004) showed that uptake of P was increased as a result of Si and P interaction, which could be due to increase in soil pH that made P more available and better plant growth because of improved P uptake and utilization. Similar results were also recorded by Ma *et al.* (2001).

Uptake of Fe and Mn: Application of Si at different levels decreased Fe and Mn uptake by maize (Table 4). The phosphorus ($P: 40$) application significantly increased Fe and Mn uptake by maize. The increase in uptake of Fe and Mn could be attributed to increase in shoot and root yield of maize due to P addition over P_0 level. Owino and Gascho (2004) also showed that better P utilization, enhance the root elongation and increased concentrations of Fe, Mn and Zn therefore increasing in their uptake.

CONCLUSION

From the forgoing results, it could be concluded that the application of Si @ $300 \text{ mg Si kg}^{-1}$ soil along with P @ $40 \text{ mg P kg soil}^{-1}$ significantly increased the plant height,

chlorophyll content, green shoot yield, dry shoot yield, dry root yield and nutrient uptake of maize in loamy sand and silt loam soils.

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Soil organic Carbon Stock under Different Land Use Systems in Mountainous Ecosystem

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Abstract: Soil physico-chemical property revealed maximum bulk density (1.25 g cm^{-3}) in the agriculture land use system and least in the forest land use system (0.97 g cm^{-3}). The bulk density recorded at the upper soil layer (0-20 cm) was significantly lower than the deeper soil layer (20-40 cm). Maximum soil organic carbon (2.50%) was found in the forest land use system and least in agriculture land use system (0.68%). The soil organic carbon also enhanced significantly with the ascending altitudinal ranges. Forest land use system displayed the maximum value of soil organic carbon stock (49.04 t ha^{-1}) and least in agriculture land use system (16.94 t ha^{-1}). In the altitudinal range, the total soil organic carbon stock increased with increasing altitudinal range. Soil organic carbon stock in 0-20 cm in layer was significantly higher than that of lower layer. Maximum soil organic carbon stock (0-40 cm layer) was recorded in forest land use system (98.08 t ha^{-1}), which was followed by agri-horticultural (41.05 t ha^{-1}) and horticultural system (39.16 t ha^{-1}).

Key Words: Altitudinal gradient, Bulk density, Carbon stocks, Land use systems, Soil layer

Emission of greenhouse gases has become a matter of great concern because of the future projection of the global warming and related effects on biological life. While nations struggle to lower the greenhouse gas emissions at source, complimentary efforts are required to enlarge the sinks of these gases. The reduction in concentration of CO_2 in the atmosphere can be achieved by reducing the demand for energy and by altering the way the energy is used, and by increasing the rates of removal of the atmospheric CO_2 through carbon sequestration. Carbon sequestration refers to the provision of long term storage of carbon in the terrestrial biosphere, underground or the ocean so that the buildup of carbon dioxide concentration in the atmosphere will be reduced or slowed in order to improve environmental conditions and check the processes of environmental degradation. The soil organic carbon (SOC) varies with the land-use system (Chauhan *et al.*, 2010; Sharma *et al.*, 2016). Depending on land-use type, vegetation change the soil organic carbon (SOC) accumulation. Tree-based land-use systems have greater potential of soil carbon sequestration (CSP) than agronomic crops (Chauhan *et al.*, 2011; 2015). No systematic study has been undertaken to estimate the soil organic carbon pool in forests, as well as in other land use systems in the north western Himalayas, India. Although some investigations have been carried out and data generated on the soil organic carbon pool in the forests, but it was on the basis of desk review by using some indirect methods or using some assumptions. Therefore, a study was conducted to

estimate SOC pool under different land uses. The results of this study have provided the authentic and comprehensive estimates of the SOC pool under different land use systems. Information generated from this study can be used as a benchmark for future work to estimate the changes in SOC pool in these land uses.

MATERIAL AND METHODS

The study was carried out in Kullu district of Himachal Pradesh. District is bounded by Lahaul -Spiti and Kangra districts, on the East and South East by Kinnaur and Shimla district. The district receives moderate rainfall and bulk of it is received during the months of July, August, December and January. The parent material consists of shale, schist and micaceous schist. The soil types belong to order entisols, inceptisols and mollisol with sandy loam and clay loam texture. The pH varied between 6.3 to 6.6. The forests of study area were having vegetation of temperate nature mainly the forest types 12/C₁ - Low Himalayan temperate forest, 12/C₁₆ - Mohru Oak forest, 12/C₂ - Upper west Himalayan temperate forest, 12/2S₁ - Low-level blue-pine forest and 13/C₄ - West Himalayan high level dry blue pine forest. Soil samples were collected by dividing each main plot area into five areas each 10 x 10 m. Soil samples for each sub area were obtained by digging five profiles of 20 x 50 cm (sub-surface area) by 50 cm deep. Composite samples from all sub area were obtained for each depth. Samples were air dried in shade, ground with wooden pestle,

passed through 2 mm sieve and stored in cloth bags for further laboratory analysis.

Soil analysis and carbon stock: Soil organic carbon was determined by wet digestion method (Walkley and Black, 1934). Bulk density of soil was estimated through specific gravity method (Singh, 1980). Soil organic carbon stock was calculated using equation of Nelson and Sommers (1996), Soil Organic Carbon stock = [(Soil bulk density (g cm^{-3}) x Soil depth (cm) x Carbon (%)] x 100.

RESULTS AND DISCUSSION

Bulk density: The bulk density (g cm^{-3}) varied significantly under different land use system, altitudinal ranges and soil

layer layers (Table 2) and under different land use system followed the decreasing trend i.e. agriculture > horticulture > agri-horticulture > silvi-pasture > forest. It was observed that intensively managed land use system higher bulk density and it declined as the intensity of land management system declined. The high value of bulk density in the soils can also be ascribed to lower soil organic carbon content. Higher bulk density in horticulture based system as reported in present study has also been reported by Kumar (2003). The current studies further revealed that agroforestry system types integrating fruit trees as woody perennial in place of forest/ fodder tree had higher bulk density value. The above findings indicated that fruit tree based agroforestry system has less

Table 1. Tree-crop combinations and their distribution in mountainous ecosystem

Land use systems/ elevation range	Code	Tree-crop combinations	Plot size m^2	Net cropped	Area under tree ha^{-1}	Area under crop/ grass	No of trees ha^{-1}	Average age of tree component
Agriculture	A							
1100-1400 m		Wheat+ maize	50x10	1.00	-	-	-	Annuals
1400-1700 m		Wheat +maize		1.00	-	-	-	Annuals
1700-2000 m		Pea +Maize		1.00	-	-	-	Annuals
2000-2300 m		Garlic+ maize		1.00	-	-	-	Annuals
Agri horticulture	AH		50x10					
1100-1400 m		Plum+ wheat- maize		0.40	0.60	-	278	~20 yr
1400-1700 m		Apple + wheat-		0.40	0.60	-	278	~20 yr
1700-2000 m		Apple + pea -maize		0.40	0.60	-	278	~20 yr
2000-2300 m		Apple + Garlic –		0.30	0.70	-	333	~18 yr
Horticulture	H		50x10					
1100-1400 m		Plum ,apple		-	1.00	-	278	~20 yr
1400-1700 m		Apple		-	1.00	-	278	~20 yr
1700-2000 m		Apple		-	1.00	-	278	~20 yr
2000-2300 m		Apple		-	1.00	-	278	~18 yr
Silvi- pasture	SP		50x10					
1100-1400 m		<i>Pinus roxburghii</i> + grasses		-	0.70	0.3	250 (Pr)	~60 yr(Pr)
1400-1700 m		<i>P wallichiana</i> + grasses		-	0.70	0.3	217 (Pr)	~60 yr(Pw)
1700-2000 m		<i>Cedrus deodara</i> + grasses		-	0.70	0.3	212 (Pw)	~70 yr (Cd)
2000-2300 m		<i>Cedrus deodara</i> + grasses		-	0.70	0.3	218 (Cd)	~90 yr (Cd)
Forest	F		50x10					
1100-1400 m		<i>Pinus roxburghii</i>		-	1.00	-	650	~70 yr(Pr)
1400-1700 m		<i>P. wallichiana</i> , <i>C. deodara</i>		-	1.00	-	580 (Pw), 400 (Cd)	~90 yr(Pw) ~90 yr(Cd)
1700-2000 m		<i>P. Wallichiana</i> , <i>C. deodara</i>		-	1.00	-	370(Cd), 450 (Pw)	120 yr(Pw), 140 yr (Cd)
2000-2300 m		<i>C. deodara</i> , <i>Abies pindrow</i> , <i>Picea smithiana</i>		-	1.00	-	192 (Ap), 202 (Cd), 190 (Ps)	~140 yr (Cd) ~180yr(Ap) ~180yr(Ps)

Table 2. Average soil organic carbon, bulk density and soil organic carbon (SOC) as influenced by average affect of land use system, altitudinal ranges and soil layer

Land use systems	Soil organic carbon (%)	Bulk density (g cm ⁻³)	Soil organic carbon stock (t ha ⁻¹)	Available nitrogen (kg ha ⁻¹)
Agriculture	0.68 e	1.25 a	16.94 e	425.2 bc
Agri-horticulture	0.89 b	1.16 c	20.59 b	510.4 a
Horticulture	0.87 c	1.20 b	20.52 c	510.4 a
Silvi-pasture	0.81 d	1.12 d	17.90 d	392.6 d
Forest	2.50 a	0.97 e	49.04 a	443.2 b
SE±	0.03	0.01	0.56	11.2
CD (p=0.05)				
Altitudinal range				
1100-1400 m	1.03 d	1.20 a	23.06 d	419.4 bc
1400-1700 m	1.09 c	1.19 b	25.20 bc	438.2 b
1700-2000 m	1.15 b	1.14 c	25.96 ab	484.7 a
(2000-2300 m	1.34 a	1.04 d	26.77a	480.0 a
SE±	0.02	0.013	0.51	10.0
CD (p=0.05)				
Soil Layer				
0-20 cm	1.32 a	1.13 b	28.42 a	495.5 a
20-40 cm	0.98 b	1.15 a	21.58 b	415.6 b
SE±	0.02	0.01	0.35	7.1
CD (p=0.05)				

potential to improve upon the physico-chemical properties of the soil e.g. bulk density in the current studies. Bulk density showed a decreasing trend with increasing altitudinal range. This can again be owed to increasing trend of soil organic matter with increasing altitudinal range. The soil organic matter increased with the increasing altitude because of slower decomposition rate with increasing altitude. Bulk density increased with the increasing depth of soil layer. The lower bulk density values in upper layer of soil profile may have resulted from the dilution of soil matrix (mineral matter) with lesser denser material (organic matter) and improvement in soil aggregation. The reduction of bulk density of soil due to increase in soil organic carbon has been reported in literature. (Sharrow and Ismail, 2004; Singh *et al.*, 2016).

Soil organic carbon (%): Soil organic carbon was significantly influenced due to land use system. Maximum soil organic carbon (2.50%) was found in the forest land use system, which was followed by agri-horticulture (0.89%), horticulture (0.87%), silvi-pasture (81%) and agricultural system (0.68%), in the descending order. The increased organic carbon content in soils under tree based system may be ascribed to more leaf litter deposition and root turnover from trees (Dhillon *et al.*, 2012). Lower organic carbon in agriculture land use system than grassland as recorded in our studies has also been reported by Pal *et al.* (2013). The

organic carbon increased with increasing altitudinal range, which can be owed to continuous accumulation of leaf litter and slower decomposition rate at the higher altitude than at lower ones. Slower decomposition means less mineralization and hence losses of organic carbon through erosion will be lower at higher altitude and hence more carbon content conservation. Similar results have been reported earlier (Chisanga, 2012; Bhardwaj *et al.*, 2013). Soil carbon content declined significantly from upper (0-15 cm) to lower soil layer (15-30cm). Similar findings i.e. decrease in organic carbon content with increase in soil depth have also been reported by Chauhan *et al.* (2012) and Singh *et al.* (2016).

Soil organic carbon stocks: The soil organic carbon was significantly influenced by land use system, altitudinal range and soil layer effect. Maximum soil organic carbon stock (SOC stock) was recorded in forest land use system (49.04 t ha⁻¹), which was followed by agri - horticultural (20.59 t ha⁻¹), horticulture (20.52 t ha⁻¹), silvi-pastural (17.90 t ha⁻¹) and agricultural system (16.94 t ha⁻¹), in the descending order. This may be because of enhanced accumulation of leaf litter in the tree and fruit based land use systems. The abundant litter and/or pruned biomass returns to soil, combined with the decay of roots contribute to the improvement of organic matter under complex land use systems (Sharma *et al.*, 2014). Low amounts of soil organic carbons stocks under the

Table 3. Soil carbon inventory (t ha^{-1}) under different land use systems along an altitudinal gradient in the mountain ecosystem of Kullu district (H.P.)

Land use systems	1100-1400 m			1400-1700 m			1700-2000 m			2000-2300 m			Mean
	0-20 cm	20-40 cm	0-40 cm	0-20 cm	20-40 cm	0-40 cm	0-20 cm	20-40 cm	0-40 cm	0-20 cm	20-40 cm	0-40 cm	
Agriculture	18.20	13.48	31.68	19.96	12.46	32.42	18.57	14.66	33.23	20.48	17.71	38.19	33.88
Agri-horticulture	21.69	16.95	38.64	22.56	17.18	39.74	26.81	16.96	43.77	24.55	17.85	42.05	41.05
Horticulture	20.82	16.06	36.88	22.31	15.77	38.08	24.21	17.08	41.29	24.21	16.25	40.37	39.16
Silvi-pasture	19.41	14.34	33.75	22.08	17.31	39.39	19.95	14.26	34.21	21.77	14.05	35.82	35.79
Forest	47.67	41.98	89.65	52.90	49.46	102.36	54.44	42.69	97.13	59.24	43.95	103.19	98.08
Mean	25.66	20.56	46.12	27.96	22.44	50.40	28.80	21.13	49.93	31.35	22.20	53.55	

agriculture land use system can be ascribed to intensive cropping as also reported by Lal *et al.* (1998). In the altitudinal range, soil organic carbon stock increased with increasing altitudinal ranges from 1100-2300 m, however, altitudinal range of 1700-2000 masl remained statistically at par with 2000-2300 m above sea level. It may be due to sharp decline in the bulk density of the A_2 and A_4 soil layers. The soil organic carbon stock declines appreciably from 0-20 cm to 20-40 cm (Table 2). The increase or decrease in the soil organic carbon pool was associated with the bulk density and organic carbon content of the soil of a particular depth. The higher amount of soil organic carbon stock on the surface layer i.e. 0-20 cm may be explained in the sense that there is continuous accumulation of leaf litter on the surface, which keeps on decomposing and thus enriches the upper layer (0-20 cm) continuously.

Inventory

Soil organic carbon pool inventory: The maximum soil organic pool in 0- 40 cm layer was found in forest land use system (98.08 t ha^{-1}) followed by agri-horticulture (41.05 t ha^{-1}), horticulture (39.16 t ha^{-1}), etc. in descending order (Table 3). The total soil organic carbon stock as observed in the forest land use system was 3 times higher over other land use systems as explained earlier also. Higher soil organic carbon pool in the forest land use system can be ascribed to greater incorporation of leaf litter and addition of decayed roots to the upper soil layers than in the other land use systems. In addition to it, the intensity of cropping in other land use is higher than forest land use. The soil carbon stock i.e. 98.08 t ha^{-1} in our system is slightly lower than the world average ($134.9 \text{ t C ha}^{-1}$) for temperate evergreen forest (Houghton, 1995).

This may be due to the nature/composition of the forest. Forests in Kullu region are categorized as warm temperate Himalayan forest, hence the average temperature of our forest is higher than the average temperature of cool temperate forest of the world. Higher temperature may have helped in greater decomposition and loss of the soil organic

carbon from our forest land use system. Moreover, the soil of our study site is sandy loam, which is known for lower soil organic carbon content.

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Received 20 August, 2015; Accepted 17 November, 2015



Effect of Field Conditions and Different Growing Media on Seed Germination in *Rheum australe* D. Don

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Abstract: The experiment was conducted to study the seed germination and growth behaviour of *Rheum australe* under different field conditions and growing media. Maximum germination percentage, early initiation and completion of germination, maximum mean daily germination and maximum peak value was under shade net house conditions and soil + cocopeat + vermicompost (1:1:1) media.

Key Words: Endangered species, Medicinal plants, *Rheum australe*, Seed germination

Current estimates by the Threatened Plants Species Committee of the Survival (TPSSC) of IUCN indicate that 1 in 10 species of vascular plants on earth is endangered or threatened due to commercial exploitation and increasing international trade. It has been pointed out that nearly 60,000 plant species may be in danger of extinction leading to gene erosion during the next 30-40 years. The Himalaya covers 18% of the Indian sub continent, accounts for more than 50% of Indian forest and contains 405 of India's endemic species (Maikhuri *et al.*, 2000). The Indian Himalayan Region (IHR) is a rich reservoir of biological diversity in the world. Over 1748 species of medicinal and aromatic plants reported from IHR are used in different systems of medicines. There is an urgent need to develop regeneration/conservation strategies for over exploited medicinal and aromatic plant species. *Rheum australe* is one of the important high value species, which has huge economic potential, being used in pharmaceutical industries and due to heavy anthropogenic pressure its natural population is threatened, which in turn has affected the natural regeneration. The status of this species has been listed under the endangered plant category (Nautiyal *et al.*, 2003). The genus *Rheum* includes perennial, stout herbs, found in the temperate Himalayas from Kashmir to Sikkim at an elevation of 2200-3800 m, is distributed in sub-tropical regions of the world, chiefly in Asia. Present study was conducted with combination of different growing media with soil as a control under glass house and shade net house conditions for better germination and healthy seedlings that can be transplanted to field at higher altitudes

MATERIAL AND METHODS

The experiment was conducted at department of forest products under glass house and shade net house conditions at Nauni, Solan, Himachal Pradesh at 1250 m altitude. Seeds were collected from natural ecological zones

of *Rheum australe* during October month from different parts of Himachal Pradesh. Freshly collected seeds were sown in pots under two field conditions i.e. glass house conditions and shade net house having different combination of growing media in pots and soil as a control. The experiment was laid out in completely randomized design (CRD factorial) in glass house and under shade net house conditions with nine treatments (Table 1) and three replications. Observations were recorded on germination and growth parameters including germination percentage, initiation of germination, germination period, mean daily germination and peak value.

RESULTS AND DISCUSSION

Among different growing media maximum mean germination was in S_8 (73.75 %), which was statistically higher than all other medium. The minimum value was observed in S_1 (22.50%). In field conditions, maximum mean germination percentage was recorded in F_1 (48.07%) and minimum in F_2 (42.77%). Among interaction effects between soil media and field conditions, maximum germination percentage was recorded in S_8F_1 (82.68%), which was statistically superior to all other treatments. Minimum germination percentage was found in S_1F_2 (21.66%) and was statistically at par with S_1F_1 (23.33%).

Initiation and completion of germination (days): The early initiation and completion of seed germination was recorded in S_8 (10.50 and 17.50 days, respectively) and maximum days taken for initiation and completion of seed germination were in S_1 (16.00 and 25.10 days, respectively). Among the field conditions early initiation and completion of seed germination was in F_1 . The early initiation of seed germination was in S_8F_1 (10 days), whereas, the maximum time taken for initiation of seed germination was recorded in S_1F_2 (17 days). But trend varied for completion of

Table 1. Effect of different growing media on germination percentage, initiation of germination and completion of germination

Field conditions Growing media	Germination %			Initiation of germination (days)			Completion of germination (days)		
	Shade net house (F ₁)	Glass house (F ₂)	Mean (%)	Shade net house (F ₁)	Glass house (F ₂)	Mean	Shade net house (F ₁)	Glass house (F ₂)	Mean
S ₁ : Soil	23.33 (28.76)	21.66 (27.69)	22.50 (28.23)	15.00	17.00	16.00	24.00	26.33	25.16
S ₂ : Soil + FYM (2:1)	36.66 (37.24)	30.00 (33.19)	33.33 (35.22)	14.00	16.00	15.00	21.66	24.66	23.16
S ₃ : Soil+ forest leaf litter (2:1)	41.66 (40.18)	38.33 (38.22)	40.00 (39.20)	13.33	15.33	14.33	21.00	24.33	22.66
S ₄ : Soil + vermicompost (2:1)	46.66 (43.07)	43.33 (41.14)	45.00 (42.10)	13.00	15.00	14.00	20.00	23.00	21.50
S ₅ : Soil + cocopeat (2:1)	31.66 (34.21)	28.34 (32.12)	30.00 (33.17)	14.33	16.33	15.33	22.33	25.00	23.66
S ₆ : Soil+ sand + FYM (1:1:1)	51.66 (45.93)	48.34 (44.02)	50.00 (44.98)	12.66	13.66	13.16	20.00	20.33	20.16
S ₇ : Soil + cocopeat + FYM (1:1:1)	61.67 (51.73)	58.34 (49.78)	60.00 (50.75)	11.00	12.00	11.50	18.66	18.00	18.33
S ₈ : Soil + cocopeat + Vermicompost (1:1:1)	82.68 (64.72)	64.83 (52.72)	73.75 (58.72)	10.00	11.00	10.50	17.33	17.66	17.50
S ₉ : Soil + cocopeat + forest leaf litter (1: 1:1)	56.66 (48.81)	53.33 (46.89)	55.00 (47.85)	12.00	13.00	12.50	19.00	18.33	18.66
Mean	48.07 (43.85)	42.77 (40.64)		42.77 (40.64)	14.37		20.44	21.96	
CD (p=0.05)									
Field conditions (F)		1.68		0.43			0.37		
Soil media (S)		3.57		0.91			0.80		
Field conditions x Soil media (FxS)		5.04		1.30			1.13		

*Values in parenthesis are arcsine transformed values

germination. The maximum value for completion of germination was in S₁F₂ (26.33 days) and minimum in S₈F₁ (17.33 days).

Mean daily germination and peak value: The maximum mean daily germination and peak value was in S₈ (3.76 and 0.74, respectively), which was statistically superior to all other treatments. The minimum value was in S₁. Mean daily germination and peak value under glass house condition was 2.48% and 0.48, respectively, which was statistically superior than the value (2.02%) recorded under shade net house conditions (2.02% and 0.39, respectively). Interaction values followed the trend of germination values.

Growing under organic conditions is highly desired especially in medicinal crops as such crops are free from residual accumulation of harmful agro-chemicals. However, organic conditions can be manipulated by supplementing the soil with various organic components like forest litter, FYM, vermicompost, etc. These enrichments in addition help in

boosting yield which to a large extent determines the commercial success of any cultivation activity in medicinal plants. National and international scenario put a large emphasis on their organic cultivation avoiding any harmful synthetic enrichment. The recent concept of developing good agricultural practices (GAP) in medicinal plants largely incorporates organic cultivation. Enhancing seed germination is surely the first step towards profitable cultivation followed by yield.

In the present studies, soil + cocopeat + vermicompost (1:1:1) was more suitable for seed germination under both glass house and shade net house conditions. However, shade net house conditions favoured better results for all the above parameters. Vermicompost supplements plant nutrient, growth enhancing substances and number of beneficial micro-organisms like nitrogen fixing, phosphorus solubilizing and cellulose decomposing organisms (Sultan, 1997). Also among the sources of available organic manures,

Table 2. Effect of different growing media on mean daily germination and peak value

Field conditions Growing media	Shade net house (F ₁)	Glass house (F ₂)	Mean	Shade net house (F ₁)	Glass house (F ₂)	Mean
	Mean daily germination			Peak value		
S ₁	0.97	0.92	0.94	0.21	0.18	0.19
S ₂	1.88	1.31	1.60	0.35	0.26	0.30
S ₃	2.01	1.80	1.90	0.39	0.32	0.35
S ₄	2.44	1.89	2.17	0.45	0.37	0.41
S ₅	1.61	1.13	1.37	0.29	0.22	0.25
S ₆	2.66	2.12	2.39	0.52	0.43	0.47
S ₇	3.54	2.91	3.22	0.70	0.58	0.64
S ₈	4.12	3.41	3.76	0.81	0.67	0.74
S ₉	3.09	2.74	2.91	0.61	0.52	0.57
Mean	2.48	2.02		0.48	0.39	
CD (p=0.05)						
Field conditions			0.37			0.05
Soil media (S)			0.80			0.12
Field conditions x Soil media			1.13			NS

See table 1 for treatment details

vermicompost is potential source due to the presence of readily available plant nutrient. The cocopeat has high water holding capacity, thus availability of enough moisture favours the high seed germination and plant growth. Appropriate moisture and light intensity facilitates the seeds to germinate and grow early under shade net condition than glass house, where the high temperature plays negative role. These findings are in line with those of Nautiyal and Chauhan (2007) in *Nardostachys jatamansi* where they reported poor seed germination under field conditions compared to polyhouse conditions. Similar results were also reported by Nautiyal *et al.* (2001) in *Picrorhiza kurroa*. Therefore, seeds of *Rheum australe* sown under shade net house conditions with soil + cocopeat + vermicompost (1:1:1) media is best suitable condition for seed germination.

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Diversity, Anti-microbial and Plant Growth Promoting Activity of Endophytic Actinomycetes Isolated from *Azadirachta indica* A. Juss

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Abstract: A total of 35 isolates of endophytic actinomycetes were obtained from 20 trees of *Azadirachta indica* A. Juss and evaluated for their biocontrol potential against an array of pathogenic fungi and bacteria. The dominant genus identified was *Streptomyces* sp. (22) and rest was related to *Micromonospora* sp. (5), *Microbiospora* sp. (5), *Nocardia* sp. (2) and *Rhodococcus* sp. (1). The prevalence of endophytic actinomycetes was more in roots (42.9%), followed by stem (37.1%) and leaves (20.0%). Eighteen (51.42%) of the isolates were showing inhibitory activity against different plant pathogenic fungi and also eighteen isolates were displaying antagonism against human pathogenic bacteria. Nine of the isolates were active against *Fusarium oxysporum* and ten others were displaying acute activity against *Phytophthora dreschlea*. Sixteen showed a broad antibacterial spectrum against *Escherichia coli*, only nine isolates were antagonistic to *Klebsiella* sp. and ten isolates were displaying strong inhibition against *Pseudomonas aeruginosa*. *Micromonospora* sp. AzR12 displayed maximum inhibition against a fungus *F. oxysporum* and bacteria *E. coli*, *Staphylococcus aureus* and *Klebsiella* sp. Four isolates solubilized phosphate ranging from 0.10 to 0.59 mg/ml and 35 isolates were producing IAA in a range of 3.0-107.29 µg/ml. The catechol type of siderophores production by these isolates ranged from 3.04-52.0 µg/ml while hydroxamate type of siderophores was in the range of 1.33-88.6 µg/ml.

Key Words: *Azadirachta indica*, Biocontrol agents, Endophytic actinomycetes, Phytopathogenic fungi, Pathogenic bacteria

Actinomycetes are a diverse group of filamentous Gram-positive bacteria that are characterized as prolific producers of diverse bioactive secondary metabolites. Endophytes enter their host plant primarily through the root zone; however aerial portion of plants such as flowers, stems and cotyledons may also be used for entry. The most commonly encountered endophytic actinomycete in surface-sterilized plant tissues is *Streptomyces* sp. (Coombs and Franco, 2003).

The association of actinomycetes with plants is found to confer many advantages to host plants such as the production of phytohormones and siderophores, nitrogen fixation and protection against plant pathogens by producing antibiotics or extracellular enzymes (Bailey *et al.*, 2006). Endophytic infected plants often grow faster than the non-infected ones. This effect is due to the production of the phytohormone Indole-3-acetic acid (IAA) (Lee *et al.*, 2004), pteridic acid A and B, which has auxin like activity (Igarashi *et al.*, 2002), phosphate solubilizing activity (Wakelin *et al.*, 2004), production of siderophores (Costa and Loper, 1994), cytokinin and other plant growth promoting substances. Some endophytic *Streptomyces* sp. can enhance crop yield through protection of their host against pathogens as in case of 'take all disease' of wheat (Coombs, 2002).

Each part of *Azadirachta indica* is used in medicines for centuries and now commercially exploitable. The bark, seeds, leaves, fruit, extracts and oils of the neem tree contain pharmacological constituents, which offer some impressive

therapeutic qualities, like antimicrobial, anti-pyretic, anti-inflammatory, anti-tumour and anti-helminthic activities (Shenpagam *et al.*, 2012). The aim of the work reported in this paper was to screen the endophytic actinomycetes isolated from parts of *Azadirachta indica* tree, for their potential as biocontrol agents against a range of pathogenic bacteria and fungi and to find effective plant growth promoting agents for enhanced crop yield.

MATERIAL AND METHODS

Twenty healthy trees of *Azadirachta indica* (neem) were randomly selected from different locations in Ludhiana district of northern India and samples of leaves, roots and stems were taken as per standard practices.

Isolation of endophytic actinomycetes: The leaf, root and stem segments were washed in running tap water to remove adhered epiphytes and soil debris and subjected to surface sterilization as described in Taechowisan and Lumyong (2003). After the treatment, the plant samples were soaked in 10% NaHCO₃ solution for 10 min to inhibit fungal growth. Small fragments of each sample were mashed using sterile pestle and mortar and an aliquot of 1ml of suspension was poured on to Petri plates containing starch-casein agar (SCA) and spread with the help of glass spreader. Petri plates were incubated at 28° C for 7-10 days. To verify the surface sterilization procedure, aliquots of rinsed water were spread on SCA medium and incubated at 30° C for 3-5 days.

Dual culture antagonistic bioassay: Actinomycete isolates

were tentatively identified according to traditional morphological criteria (Yan, 1992; Tan *et al.*, 2006). The actinomycete isolates were evaluated for their antagonistic activity against five phyto pathogenic fungi (*Alternaria brassicicola*, *Fusarium oxysporum*, *Phytophthora dreselea*, *Rhizoctonia solani* and *Helminthosporium oryzae*) by dual-culture *in vitro* assay on Potato Dextrose Agar (PDA) plates. Spread plate method was used for detecting antagonism against six pathogenic bacterial cultures i.e., *Escherichia coli*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Klebsiella* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa* and one pathogenic yeast *Candida albicans* on Sabouraud's dextrose agar was also evaluated. A lawn of these pathogenic cultures was prepared on nutrient agar plates by spreading 0.1 ml of these pathogenic cultures. A well was made in the center of each nutrient agar plate. Culture filtrate (50µl) of the actinomycete isolates was put in that well and incubated at 28°C. After 4-7 days of incubation the width of inhibition zones were measured.

Screening of isolates for phosphate solubilization: Screening of isolates for phosphate solubilization was done using National Botanical Research Institute bromophenol blue (NBRI-BPB) medium. Plates containing NBRI-BPB medium were inoculated with actinomycetes cultures and incubated at 28°C for 7 days. The cultures forming yellow zone of halos were grown in NBRI-BPB broth for 10 days. The total phosphorus solubilized in the culture supernatant was estimated by vandatomolybdate Jackson's method (1973).

Siderophore Production: Siderophore production by actinomycete isolates was detected by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye chrome azurol S (CAS). Cultures positive for siderophore production formed an orange halo around the colony where siderophores had chelated iron that had been bound to the dye. These positive isolates were cultured using Yeast malt broth. Catechol-type siderophores were assayed using the method of Arnow (1937), while for hydroxamate siderophores the method of Csaky (1948) was used.

Production of Indole acetic acid (IAA) : Characterization of isolates for the production of IAA was done by method of Gordon and Weber (1951). Standard curve was prepared by taking standard IAA solution in 0-100 g ml⁻¹ concentration by taking absorbance at 530 nm.

Identification of the potent biocontrol agent using 16S rDNA based molecular technique: DNA was isolated from the culture. Its quality was evaluated on 1.2% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of 16S rDNA gene was amplified by PCR from the above isolated DNA. A single discrete PCR

amplicon band of 1500 bp was observed when resolved on Agarose Gel (Gel Image-1). The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 1326bp 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the nr database of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4.

RESULTS AND DISCUSSION

A total of 35 isolates of endophytic actinomycetes were obtained from tissues of roots, leaves and stems, revealing that healthy living tissues of *Azadirachta indica* harbour a variety of the endophytic actinomycetes. The results indicate the predominance of *Streptomyces* sp. from this host, as it was also confirmed by other report (Qin *et al.*, 2009), where 87% of the isolates were *Streptomyces* sp. Besides *Streptomyces* sp. other prevalent genera were *Micromonospora* sp. *Microbispora* sp., *Nocardia* sp and *Rhodococcus* sp. Out of 35 isolates of neem, 15, 13 and 1 were recorded from roots, stems and leaves, respectively (Table 1). The results also revealed that the surface treatment was adequate for the isolation of endophytic actinomycetes, as surface sterilized imprinted Petri plate (control) did not produce any growth. Thus, all the actinomycetes recorded in this experiment must have been endophytic and not the epiphytic. Based on colony and

Table 1. Distribution of endophytic actinomycete isolates from *Azadirachta indica* trees

Types	No. of isolates		
	Root	Stem	Leaf
<i>Streptomyces albosporus</i>	4	2	4
<i>S. griseofuscus</i>	2	5	-
<i>S. grieseorubroviolaceus</i>	2	-	-
<i>S. aureus</i>	1	-	-
<i>S. flavus</i>	-	-	2
<i>Micromonospora</i> sp.	4	1	-
<i>Microbispora</i> sp.	2	3	-
<i>Nocardia</i> sp.	-	1	1
<i>Rhodococcus</i> sp.	-	1	-
Total	15	13	7

Values represent mean of three replicates

cultural characteristics, various subgroups were identified and among *Streptomyces* sp., the subgroup *S. albosporus* (10) was more frequently present followed by *S. griseofuscus* (7) *S. griseorubroviolaceus* (2), *S. flavus* (2) and *S. aureus* (1) (Table 2). *Streptomyces albosporus* was obtained from all the plant parts. Similar results were obtained by Verma *et al.* (2009), who isolated 55 endophytic actinomycetes from *Azadirachta indica* A. Juss. Actinomycetes were most commonly recovered from roots (54.5% of all isolates), followed by stems (23.6%), and leaves (21.8%). The dominant genus was *Streptomyces* (49.09% of all isolates), while *Streptosporangium* (14.5%), *Microbispora* (10.9%), *Streptoverticillium* (5.5%), *Sacchromonospora* sp. (5.5%), and *Nocardia* (3.6%) were also recovered.

Dual culture antagonistic bioassay: Eighteen (51.4%) out of 35 isolates were showing promising activity against different plant pathogenic fungi (Table 3). Most of the isolates displaying antagonistic activity belonged to the *Streptomyces* sp. (13) and rest belonged to *Micromonospora* sp., *Microbispora* sp. and *Nocardia* sp. (3, 1 and 1). About 4% isolates recovered showed a broad antimicrobial spectrum against both pathogenic fungi and bacteria. *Streptomyces* sp. was more active against pathogenic bacteria (Table 4). Isolates belonging to *Streptomyces griseofuscus* were more active, and these were displaying antagonistic activity against *Yersinia enterocolitica*, *Klebsiella* sp., *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Isolates of *Streptomyces albosporus* were observed to have antibacterial activity against *E. coli*, but did not form any clear zone and had only retarded the growth of bacteria. The isolates of *Streptomyces griseorubroviolaceus* showed antibacterial activity against *E. coli* and *Klebsiella*

sp., while *Streptomyces flavus* was active against *Klebsiella* sp. only. Isolates of *Micromonospora* sp. were displaying maximum antibacterial activity against *E. coli*, *Klebsiella* sp. and *Staphylococcus aureus* while *Microbispora* sp. was observed to have inhibitory activity against *Pseudomonas aeruginosa* and *E. coli*. The isolates of *Nocardia* sp. were active against *E. coli* and *Pseudomonas aeruginosa*. None of the isolates were able to inhibit the activity of *Candida albicans* and *Aeromonas hydrophila*. These results are in accordance with Singh and Padmavathy (2015), who reported that out of 11 endophytic actinomycetes from *Ocimum sanctum*, isolate no.5 showed maximum activity against all the human pathogenic microorganisms such as *Staphylococcus aureus* (21mm), *Candida albicans* (18 mm), *Enterococcus faecalis* (19mm), *Vibrio cholera* (18mm). These results are supported by Gangwar *et al.* (2015), who reported that 29 isolates from *Embllica officinalis* were displaying the antibacterial activity against human pathogens.

In the present study, *Streptomyces albosporus* was found to have antifungal activity against *Fusarium oxysporum*, *Alternaria brassicicola*, *Phytophthora dresclea* and *Rhizoctonia solani* while *Streptomyces griseofuscus* was effective against *F. oxysporum*, *P. dresclea*, *Aspergillus niger* and *R. solani* (Fig. 1). The isolates of *Streptomyces griseorubroviolaceus* were observed to have antifungal activity against *F. oxysporum*, *P. dresclea* and *A. niger* and *Streptomyces aureus* showed antifungal activity against *F. oxysporum* and *P. dresclea*. This may be attributed to the hydroxamate type siderophores production ability of *Streptomyces* sp., which inhibit phytopathogen growth by competing for iron in rhizosphere soils (Khamna *et al.*, 2009).

Table 2. Morphological and biochemical characterization of endophytic *Streptomyces* sp.

Property	<i>S. albosporus</i>	<i>S. griseofuscus</i>	<i>S. griseorubroviolaceus</i>	<i>S. cinereus</i>	<i>S. aureus</i>	<i>S. flavus</i>
Colony color	white	grey	purplish-pink	grey	grey	yellow
Pigmentation	-	grey	brown	-	yellow	yellow
Spore arrangement	chain	chain	chain	chain	chain	chain
Hydrolysis of casein	+	-	-	+	+	-
Starch	+	+	+	+	+	+
Decomposition of esculin	-	+	+	-	+	+
Tween-80	+	-	+	+	-	+
Xanthine	+	+	+	+	+	+
Hypoxanthine	+	-	+	+	+	-
Tyrosine	+	+	+	+	+	+
Effect on growth in the	+	+	-	+	-	+
NaCl (5%)	+	+	+	+	+	+
NaCl (10%)	-	+	+	+	+	-

(+) = Growth detected : (-) = Growth not detected

Table 3. Antifungal activity (% inhibition) of the isolates (Mean $\pm$ S.D.)

Genus	Isolate	<i>A. niger</i>	<i>A. brassicicola</i>	<i>F. oxysporum</i>	<i>P. dresclea</i>	<i>R. solani</i>
<i>Streptomyces albosporus</i>	AzS4	-	-	56.25 $\pm$ 0.8	-	-
<i>S. albosporus</i>	AzS13	-	33.50 $\pm$ 0.8	62.50 $\pm$ 0.8	-	30.25 $\pm$ 0.6
<i>Rhodococcus qingshengii</i> strain BJC15-A38	AzS96	-	42.25 $\pm$ 0.8	63.75 $\pm$ 0.8	26.95 $\pm$ 0.7	-
<i>S. albosporus</i>	AzL3	-	-	-	35.75 $\pm$ 0.7	-
<i>S. albosporus</i>	AzL5	-	-	62.50 $\pm$ 0.8	-	-
<i>S. albosporus</i>	AzL6	-	-	63.75 $\pm$ 0.8	-	-
<i>S. albosporus</i>	AzR9	-	-	-	28.25 $\pm$ 0.7	-
<i>S. albosporus</i>	AzR14	-	-	-	37.25 $\pm$ 0.7	-
<i>S. griseofuscus</i>	AzR1	-	-	60.00 $\pm$ 0.3	-	33.25 $\pm$ 0.9
<i>S. griseofuscus</i>	AzS11	-	-	-	46.50 $\pm$ 0.9	29.25 $\pm$ 0.9
<i>S. griseorubroviolaceus</i>	AzR7	75.25 $\pm$ 0.5	-	-	-	-
<i>S. griseorubroviolaceus</i>	AzR13	-	-	68.75 $\pm$ 0.6	30.25 $\pm$ 0.5	-
<i>S. aureus</i>	AzR10	-	-	62.50 $\pm$ 0.1	8.25 $\pm$ 0.4	-
<i>Nocardia</i> sp.	AzL2	-	-	-	50.00 $\pm$ 0.5	-
<i>Microbispora</i> sp.	AzR3	-	-	-	27.00 $\pm$ 0.2	-
<i>Micromonospora</i> sp.	AzR2	-	-	-	-	-
<i>Micromonospora</i> sp.	AzS10	-	-	-	27.25 $\pm$ 0.2	-
<i>Micromonospora</i> sp.	AzR12	-	50.00 $\pm$ 0.7	75.00 $\pm$ 0.2	-	-

Values represent mean of three replicates

Table 4. Antibacterial activity of isolates against human pathogens

Genus	Isolate	<i>Yersinia</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella</i>	<i>Pseudomonas aeruginosa</i>
<i>Streptomyces albosporus</i>	AzS4	-	$\pm$	-	-	-
<i>S. albosporus</i>	AzL6	-	$\pm$	-	-	-
<i>S. griseofuscus</i>	AzR1	++	$\pm$	$\pm$	$\pm$	$\pm$
<i>S. griseofuscus</i>	AzR11	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
<i>S. griseofuscus</i>	AzS2	-	$\pm$	$\pm$	+	+
<i>S. griseofuscus</i>	AzS9	$\pm$	$\pm$	$\pm$	+	+
<i>S. griseorubroviolaceus</i>	AzR7	-	$\pm$	-	$\pm$	-
<i>S. griseorubroviolaceus</i>	AzR13	-	$\pm$	-	$\pm$	-
<i>S. flavus</i>	AzL4	-	-	-	+	-
<i>Microbispora</i> sp.	AzS1	-	$\pm$	-	-	$\pm$
<i>Microbispora</i> sp.	AzS5	-	$\pm$	-	-	$\pm$
<i>Microbispora</i> sp.	AzS12	-	$\pm$	-	-	$\pm$
<i>Microbispora</i> sp.	AzR3	-	$\pm$	-	-	$\pm$
<i>Nocardia</i> sp.	AzL2	-	$\pm$	-	-	$\pm$
<i>Nocardia</i> sp.	AzS3	-	+	-	-	+
<i>Micromonospora</i> sp.	AzR5	-	$\pm$	$\pm$	$\pm$	-
<i>Micromonospora</i> sp.	AzR12	-	+	+	+	-
<i>Micromonospora</i> sp.	AzS10	-	$\pm$	+	$\pm$	-

*(+) zone of inhibition ranged between 4 and 7 mm, (++) zone of inhibition ranged between 8 and 15mm, ($\pm$) inhibition in traces, (-) no inhibition

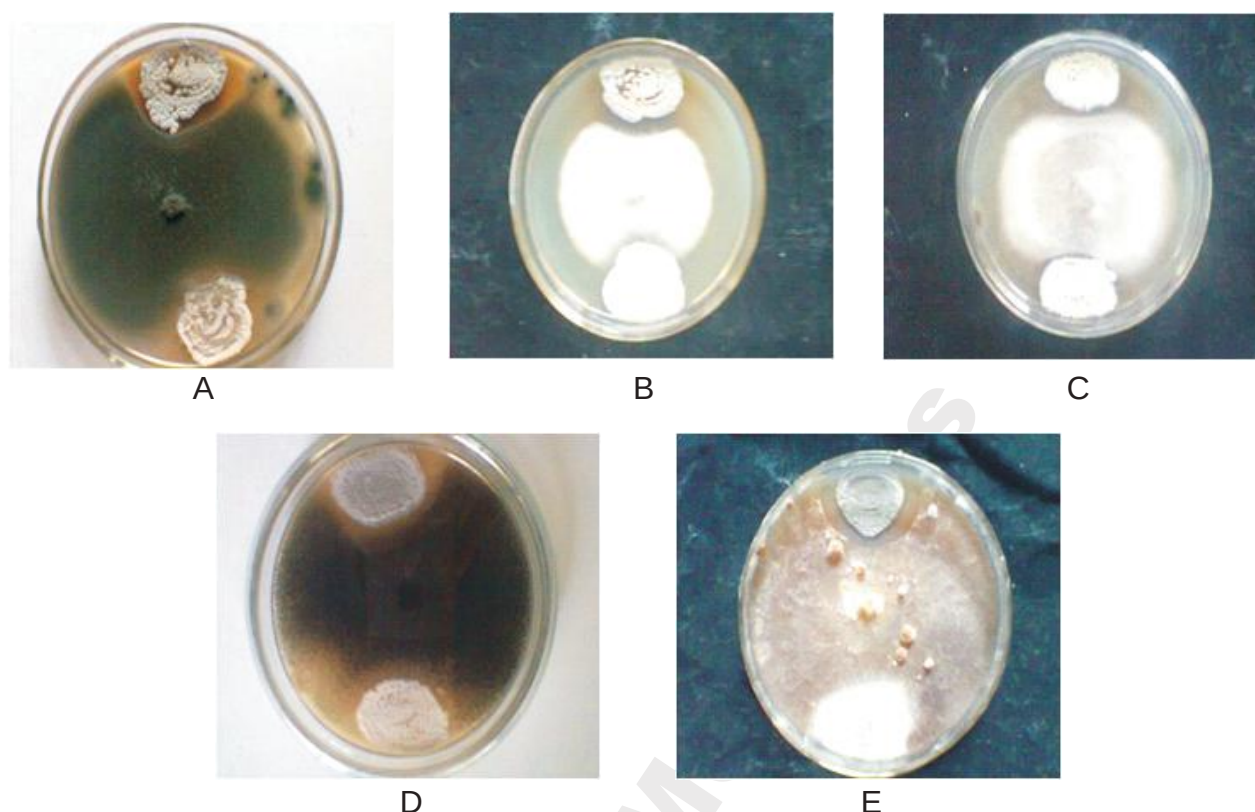


Fig. 1. Inhibition effect of *Streptomyces* sp. isolates against different phytopathogenic fungi (A) *Alternaria brassicicola* (B) *Fusarium oxysporum* (C) *Phytophthora dreselea* (D) *Aspergillus niger* (E) *Rhizoctonia solani*

Three isolates of *Micromonospora* sp. were displaying antagonistic activity against most of the fungi tested and one isolate of *Microbispora* sp. was active against *P. dreselea*. One isolate of *Nocardia* sp. was observed to have antifungal activity against only *P. dreselea*. The results are in accordance with Goudjal *et al.* (2014) who reported that 6 out of the 34 endophytic actinomycetes isolates from native plants of Algerian Sahara were found to have broad spectrum significant antifungal activity against *Rhizoctonia solani* damping-off disease, both *in vivo* and *in vitro*. Actinomycete-fungus antagonism has been demonstrated for a variety of plant pathogens such as, *Alternaria*, *Rhizoctonia*, *Verticillium*, *Fusarium*, *Phytophthora* and *Phytium* sp. (Aghighi *et al.*, 2004). The ability of isolates to inhibit the growth of fungal pathogens is due to the secretion of the secondary metabolites by actinomycetes and this shows that endophytic actinomycetes can be developed as potential biocontrol agents. The antagonistic test revealed that mostly the isolates from roots showed antagonistic activity against phytopathogenic fungi and lesser from stems and leaves. Non-*Streptomyces* isolates such as *Amycolatopsis*, *Micromonospora*, *Nonomuraea* and *Nocardia* also demonstrated promising activities against pathogens (Huang *et al.*, 2012).

Phosphate solubilization: Out of 35 isolates of neem screened for phosphate solubilization, four (11.40%) were observed to solubilize phosphate i.e. formation of yellow colored zone around the colony on NBRI-BPB medium. This test indicated the ability of isolates to solubilize phosphate but not the amount of solubilized phosphate, therefore quantitative method was used. The maximum amount of phosphate solubilization was shown by *Streptomyces griseofuscus* AzS8 (0.59 mg/ml) and minimum amount was shown by *Nocardia* sp. AzL2 (0.10 mg/ml) (Table 5). It is suggested these actinomycetes could be used as plant growth promoters. Biological phosphate solubilization is an

Table 5. Phosphate solubilization by neem isolates after 10 days of incubation

Genus	Isolate	Phosphate solubilization (mg ml ⁻¹)
<i>Nocardia</i> sp.	AzL2	0.10 ± 0.006
<i>Streptomyces griseofuscus</i>	AzS7	0.24 ± 0.005
<i>S. griseofuscus</i>	AzS8	0.59 ± 0.008
<i>S. griseofuscus</i>	AzS9	0.12 ± 0.005
CD (p=0.05)		0.22

*Average ± standard error from three replicates

alternative for improving the efficiency of natural phosphate utilization in agriculture.

Siderophore production: Siderophores are iron chelating compounds which impart antimicrobial ability to the producing microbe. Nineteen (54.2%) isolates of neem out of 35, produced distinct orange halo on chrome-azurol S (CAS) plates after 10 days of incubation, indicating siderophore production. Diameter of halo formed varied from 1.2 to 4.0 cm on CAS agar plates and most of the isolates belong to *Streptomyces* sp., maximum being produced by *Streptomyces albosporus* AzS4. Nineteen isolates of neem were producing orange halo on CAS plates, 13 produced catechol type of siderophore ranging from 3.04-52.00 µg/ml and maximum production was observed by *Streptomyces albosporus* AzL6 and minimum production of catechol was reported from *Micromonospora* sp. AzR5. While 9 isolates produced hydroxamate type siderophores ranging from 1.33 to 88.6 µg/ml, maximum being produced by *Streptomyces*

griseofuscus AzS8 (88.6 µg/ml) and minimum being produced by *Microbispora* sp. AzR15 (1.33 µg/ml) (Fig 3). Our results are in agreement with Nimnoi *et al.* (2010), who reported that eight endophytic actinomycetes isolates from *Aquilaria crassna* Pierre ex Lec produced hydroxamate type of siderophore in the range of 3.21-39.40 µg/ml and one isolate produced catechol type siderophore (4.12 µg/ml). Siderophores are iron chelating metabolites. Microbial siderophores may also be utilized by plants as an iron source (Wang *et al.*, 1993).

Indole acetic acid (IAA) production: All the 35 isolates of neem were observed to produce the phytohormone indole acetic acid. *Nocardia* sp. AzS3 was observed to produce highest amount of IAA viz. 107.29 µg/ml and minimum was produced by *Micromonospora* sp. AzR4 i.e. 3.00 µg/ml (Fig 4). Our findings are in accordance with Gangwar *et al.* (2014) who reported that out of 22 endophytic actinomycetes from Indian medicinal plants, 16 isolates produced IAA ranging

Table 6. Sequence producing significant alignments

Accession	Description	Max score	Total score	Query coverage (%)	Max ident
JX401475.1	<i>Rhodococcus qingshengii</i> strain BJC15-A38	2442	2442	100	99%
AF442523.1	Bromate-reducing bacterium B7	2108	2108	100	95%
DQ366094.1	Uncultured <i>Microbacterium</i> sp. clone FQ3_Pitesti	2054	2054	100	95%
JN981943.1	Uncultured <i>Actinobacterium</i> clone OTU-9_BFBA	1628	1628	99	89%
HQ132397.1	Uncultured <i>Propionibacterium</i> sp. clone H-101	1620	1620	100	89%
EU723140.1	<i>Actinobacterium</i> kmd_152	1618	1618	100	89%
JN836957.1	<i>Streptomyces</i> sp. AQB.SKKU 20	1613	1613	100	89%
EU371586.1	Uncultured <i>Rhodococcus</i> sp. clone CBFOS-04	1596	1596	100	89%
HQ008548.1	Uncultured <i>Acidothermus</i> sp. clone AL_22	1552	1552	99	88%
HQ132789.1	<i>Streptomyces</i> sp. N56	1528	1528	98	88%

E value was zero for all accessions

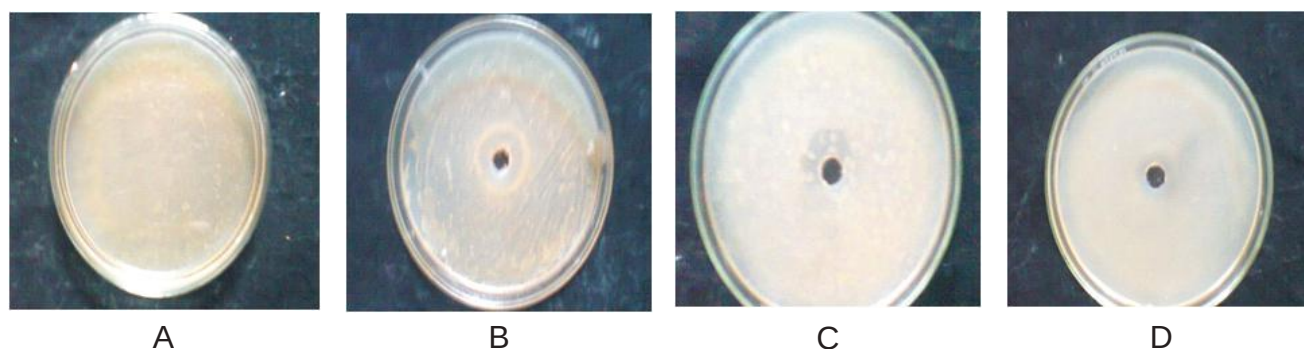


Fig. 2. Inhibition effect of *Micromonospora* sp. against different pathogenic bacteria, (A) Control, (B) *Staphylococcus aureus* (C) *Klebsiella* sp. (D) *E. coli*

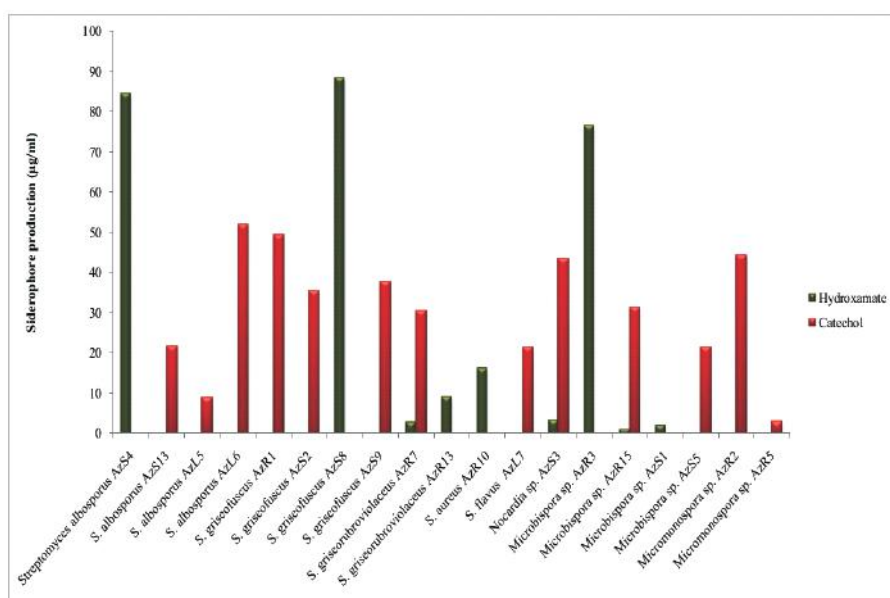


Fig. 3. Siderophore production by different neem isolates

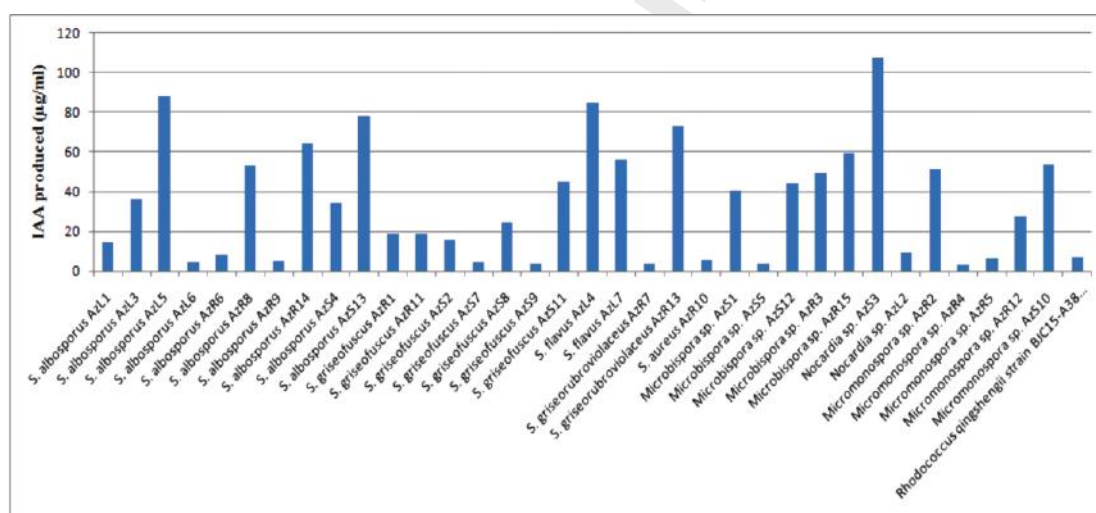


Fig. 4. Indole acetic acid production (IAA) by neem isolates

between 8.3–38.8 µg/ml. The root exudates are natural sources of tryptophan for rhizospheric microorganisms which can enhance auxin biosynthesis in actinomycetes. Singh and Padmavathy (2014) obtained three isolates from neem tree which were having IAA-producing activity along with other plant growth promoting traits.

Optical microscopy : Optical microscopy of the inoculated and uninoculated (control) fungal culture of *Fusarium oxysporum* was performed. The intact hyphae and numerous spores of *F. oxysporum* were observed in the control fungus (Fig. 5 A) as compared to the *F. oxysporum* inoculated with isolate *Rhodococcus* sp. AzS96 (Fig. 5 B). The result indicated that potent antagonists inhibited the growth of the

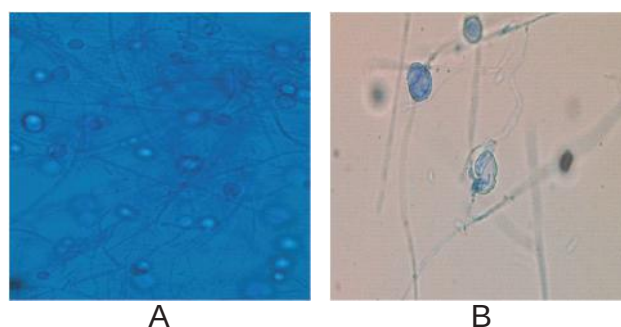


Fig. 5. Control (A) and *Fusarium oxysporum* infected with *Rhodococcus qingshengii* strain BJC15-A38 (AzS96) shows reduction in the spore size, number of spores and thinning of hyphae and bursting of spore in (B)

test fungi by releasing extracellular diffusible metabolites that inhibited the hyphal growth of *F. oxysporum* and reduction in spore number, spore size and bursting of spores was also observed along with thinning of hyphae. Our results are in accordance with Taechowisan *et al.* (2005) who reported that fungal mycelium along the edges of the colonies facing CMUAc130 appeared thickened, with bulbous formations along the ends under a dissecting microscope. On the control plate fungal mycelium was showing regular thin hyphae with normal sized spores.

Scanning electron microscopy: Scanning electron microscopy of the co-culture containing *F. oxysporum* and endophytic actinomycete isolate *Rhodococcus* sp. AzS96 was performed. Results obtained from SEM showed the degradation of cell walls of *F. oxysporum* mycelia growing towards *Rhodococcus* sp. AzS96 as compared to control. The single culture plate of *F. oxysporum* served as control, which showed the regular vegetative cells (Fig. 6 A), whereas fungal colony inoculated with *Rhodococcus* sp. AzS96 (Fig. 6: B) and observed that hyphae got disrupted and reduced in size, whereas control intact cells had a smooth surface with overall intact morphology. The spores were also partially deformed and reduced in size. Many cells were enlarged and elongated, but having extremely low viability. Our results are in corroboration with Tang-um and Niamsup (2012) who reported that breakage of the cell walls of *F. oxysporum* f. sp. *lycopersici* mycelia growing towards *Streptomyces* sp. P4 as compared to control *F. oxysporum*. It is suggested that the actinomycetes isolate produced extracellular secondary metabolites and/or hydrolytic enzymes including chitinase, which play a crucial role in fungal growth inhibition. The effect was investigated and compared with the control.

Identification of Microbial Culture using 16S rDNA based Molecular Technique: The culture, which was labeled as *Rhodococcus* sp. AZS96 was found to be *Rhodococcus qingshengii* strain BJC15-A38 (GenBank Accession Number: JX401475.1) based on nucleotide homology and phylogenetic analysis. The evolutionary history was inferred using the Neighbor-Joining method suggested by Saitou and Nei (1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All

positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1179 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007). The isolate shared 88-95% identity to those of the closely related strains (Table 6). Isolate is the most commonly related to JX401475.1 under a bootstrap supported value of 100% (Fig. 7).

The study revealed that endophytic actinomycetes isolated from neem have broad-spectrum antimicrobial activity and are also the valuable reservoirs of novel bioactive compounds. Not only the *Streptomyces*, but also the other



Fig. 6. Scanning electron microscopic analysis of *Fusarium oxysporum* grown alone showed regular, radial growth (A) and co-cultured with *Rhodococcus qingshengii* strain BJC15-A38 (AzS96) reduction in number and size of spores along with hyphal degradation in (B)

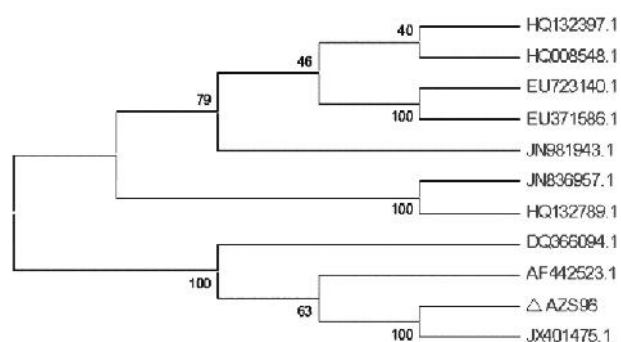


Fig. 7. Evolutionary relationships of 11 taxa

genera of actinomycetes also act as the antagonistic agents displayed antifungal activity as well as antibacterial activity. Our survey also suggested that medicinal plants are a potent source of endophytic actinomycetes with wide plant growth promoting activities for the development of sustainable agriculture.

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Received 30 August, 2015; Accepted 21 January, 2016

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Effect of Superimposed Treatments of Long Term Fertilizer Experiment on Soil Biological Properties

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Abstract: Significant differences were noticed among the treatments in enzymatic activities, soil microbial biomass carbon and total carbon content of soil. Higher enzymatic activities soil microbial biomass carbon and total carbon content of soil after harvest of maize and finger millet was observed in 150% NPK+10 tons FYM ha⁻¹. Significantly lowest enzymatic activities, soil microbial biomass carbon and total carbon content of soil recorded in 100% N. Similar trend was followed with respect to soil microbial populations (bacteria, fungi and actinomycetes) with highest population of bacterial 37.54cfu X 10⁶ g⁻¹, fungi 37.48cfu X 10³ g⁻¹, and actinomycetes 5.96cfu X 10⁴ g⁻¹ was seen in 150% NPK+10 tons FYM ha⁻¹ and lowest population of bacterial 16.67cfu X 10⁶ g⁻¹, fungi 6.33cfu X 10³ g⁻¹, and actinomycetes 1.85cfu X 10⁴ g⁻¹ was seen in 100% N.

Key Words: Biomass carbon, Microbial biomass, Soil enzymatic activities, Total carbon content

Soil biota plays a vital role in the maintenance of soil fertility and productivity and soil microorganisms that drive most soil processes, e.g. nutrient cycling, availability and retention, decomposition of organic materials, soil organic matter build-up and stabilisation of soil aggregates (Coleman *et al.*, 2004). Interactions amongst soil organisms of different trophic levels can have an effect on crop quality, soil-borne plant diseases and beneficial organisms i.e., involved in nutrient cycling processes or antagonistic relationships with pest species. Consequently, soil biota and their interactions indirectly affect production levels and the sustainability of agroecosystems. Studying links between microbial community composition and key soil processes (e.g. N mineralisation) will enable us to better understand the role of soil biota, in general, and biodiversity and soil microbial community structure, in particular, in protecting soil ecosystems against disturbances. This, in turn, is essential to ensuring sustained productivity of agricultural production systems (Coleman *et al.*, 2004; Brussaard *et al.*, 2007). Although the significance of microorganisms in the soil is well documented but little information on the importance of microbial diversity in the functioning of soil systems and most research suggests that the relationships are neither consistent nor direct (Nannipieri *et al.*, 2003; Brussaard *et al.*, 2004).

MATERIAL AND METHODS

Field experiments were carried out in superimposed treatments of long term fertilizer experiment during *Rabi* 2010-11 and *kharif* season of 2011-12 to quantify the soil enzymatic activities, soil microbial biomass carbon and total carbon content of soil after the harvest of maize and

finger millet under the AICRP on Long-Term Fertilizer Experiment plots which was initiated during 1986-87 at GKVK, UAS, Bengaluru. The soil is classified as fine, mixed Isothermic Kandic Paleustalfs of Vijayapura series.

The long term fertilizer experiment consists of permanently laid out plots in randomized block design with eleven fixed treatments replicated four times. In replication three (R₃), four treatments (T₃, T₄, T₆ & T₇) were superimposed in 2002 (T₄, T₆ & T₇) and 2005-06 (T₃), which are further divided into three sub treatments retaining original treatment in one of the sub treatment (S₁). The present investigation consists of four main and three sub-treatments combinations, which were laid out in Nested linear model design with two replications (Table 1).

The crop was sown in 5m x 4.25m sized plots using NAC-6004 of maize and GPU-28 of finger millet variety at a spacing of 60cm x 30cm and 22.5cm x 10cm respectively. Soil samples were collected from rhizosphere of effective crop root zone soon after harvest of both the crops and stored in refrigerator for further analysis. The standard procedures and statistical methods were followed to analyze the soil samples and other parameters.

RESULTS AND DISCUSSION

The trend of available soil enzyme activities like urease, dehydrogenase and phosphatase in the soil remains same after the harvest of maize (Fig. 1) and finger millet (Fig. 2). The enzyme activities were low in imbalanced fertilizer applied plots compared to the balanced fertilizer applied plots. Further, it is evident from the study that in treatments receiving farm yard manure, the enzyme activities were higher compared to the no FYM applied plots and this may be

attributed to more availability of carbon as revealed in the present study. This has been supported by the results obtained in the present study that there is an increased level of soil biomass carbon and also total carbon in the soil.

The microbial biomass carbon was studied in long-term field experiments with continuous cultivation of silage maize and with crop rotation. Positive effect of organic fertilizers on the microbial biomass carbon content in soil was observed, which is higher compared to control without statistical significance (Černý *et al.*, 2008).

Organic manure had a significantly greater

($P < 0.05$) impact on the soil biomass C and the activity compared with mineral fertilizers. The microbial metabolic activity (dehydrogenase activity per microbial biomass C) was significantly higher ($P < 0.05$) under balanced fertilization than under nutrient-deficiency fertilization.

There was increase in bacterial population upon addition of FYM and the increase was significant compared to non FYM added treatments. The highest population of bacteria of $37.5 \times 10^6 \text{ cfu g}^{-1}$ and $86.4 \times 10^6 \text{ cfu g}^{-1}$ in soil after harvest of maize and finger millet was under T_4S_3 (150% NPK + 10 tonnes FYM ha^{-1}), lowest value ($16.7 \times 10^6 \text{ cfu g}^{-1}$ and

Table 1. Treatment details of the experiment

Treatments	NPK dosage (kg ha^{-1})		Fertilizer source
	Finger millet	Hybrid maize	
T_1S_1 : 100%N	100–00–00	100–00–00	Urea
T_1S_2 : 100%N, 100%P, 100%K, FYM	100–22–42	100–33–82	Urea, SSP, MOP
T_1S_3 : 100%N, 100%P, 100%K, FYM, Lime	100–22–42	100–33–82	Urea, SSP, MOP, Lime
T_2S_1 : 100% NP	100–22–00	100–33–00	Urea, SSP
T_2S_2 : 100%N, 50%P, 100%K, FYM	100–11–42	100–16.5–82	Urea, SSP, MOP
T_2S_3 : 100%N, 50%P, 100%K, FYM, Lime	100–11–42	100–16.5–82	Urea, SSP, MOP, Lime
T_3S_1 : NPK+ HW	100–22–42	100–33–82	Urea, SSP, MOP
T_3S_2 : 100%N, 50%P, 100%K, FYM	100–11–42	100–16.5–82	Urea, SSP, MOP
T_3S_3 : 100%N, 50%P, 100%K, FYM, Lime	100–11–42	100–16.5–82	Urea, SSP, MOP, Lime
T_4S_1 : 150% NPK	150–33–63	150–48–123	Urea, SSP, MOP
T_4S_2 : 150% NPK, 5 tons FYM/ha	150–33–63	150–48–123	Urea, SSP, MOP
T_4S_3 : 150% NPK, 10 tons FYM/ha	150–33–63	150–48–123	Urea, SSP, MOP

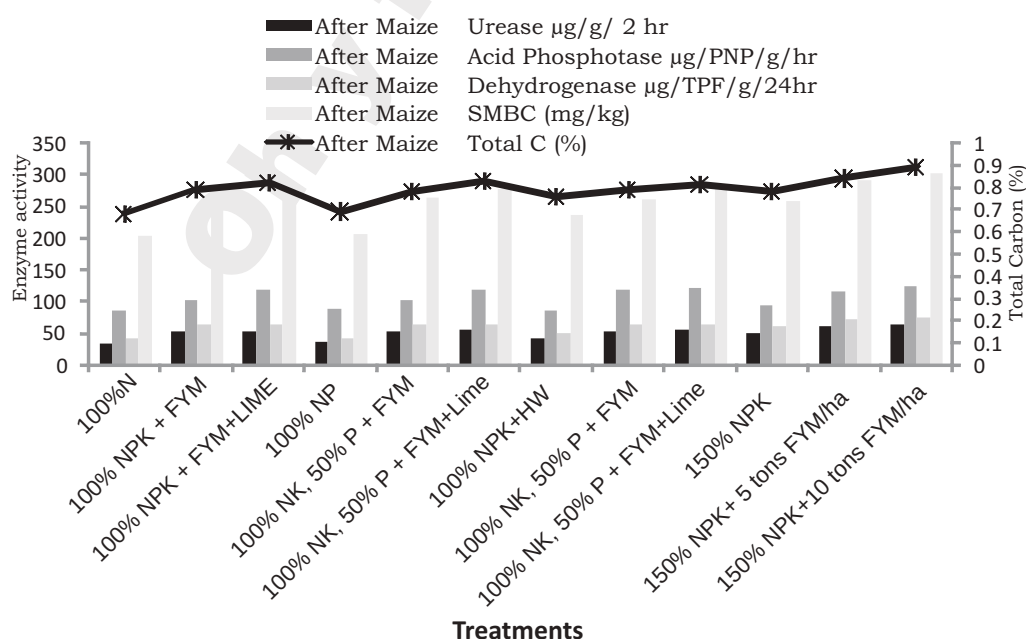


Fig. 1. Effect of superimposed treatments of long term fertilizer experiment on soil enzymatic activities, soil microbial biomass carbon and total carbon content after harvest of maize

45.1X 10⁶cfu g⁻¹) was observed under treatment, which received only nitrogen fertilizer (T₁S₁) (Table 2 & 3).

The FYM and lime amended plots sustained crop productivity and microbial populations at higher levels than rest of the mineral fertilizer treatments. The nitrogenous fertilizers alone had the most deleterious effect on crop

productivity and the biological soil environment (Mahajan *et al.*, 2007).

The population of fungi in soil after harvest of maize followed similar trend as bacteria and showed significant differences among the treatments. The highest population of 37.5 X 10³cfu g⁻¹ and 28.5 X 10³cfu g⁻¹ was noticed in T₄S₃ (150%

Table 2. Effect of superimposed treatments of long term fertilizer experiment on soil microbial populations (cfu g⁻¹) after harvest of maize during *rabi* 2010

Treatments	Bacteria (cfu X 10 ⁶ g ⁻¹)	Fungi (cfu X 10 ³ g ⁻¹)	Actinomycetes (cfu X 10 ⁴ g ⁻¹)
T ₁ S ₁ : 100% N	16.67	6.33	1.85
T ₁ S ₂ : 100% NPK +FYM	26.56	12.64	3.99
T ₁ S ₃ : 100% NPK+FYM+ Lime	28.43	10.47	4.93
T ₂ S ₁ : 100% NP	20.13	9.72	1.86
T ₂ S ₂ : 100% NK, 50% P + FYM	29.24	13.04	3.94
T ₂ S ₃ :100%NK,50%P+FYM+ Lime	32.29	11.13	4.99
T ₃ S ₁ :100% NPK + HW	20.28	12.41	3.19
T ₃ S ₂ :100% NK, 50% P + FYM	27.44	13.49	3.66
T ₃ S ₃ :100%NK,50%P+FYM+ Lime	28.35	11.63	4.13
T ₄ S ₁ :150% NPK	28.56	15.18	2.98
T ₄ S ₂ :150% NPK+ 5 tons FYM/ha	31.35	28.61	5.18
T ₄ S ₃ :150%NPK+10 tons FYM/ha	37.54	37.48	5.96
CD (p=0.05)	7.68	4.90	2.20

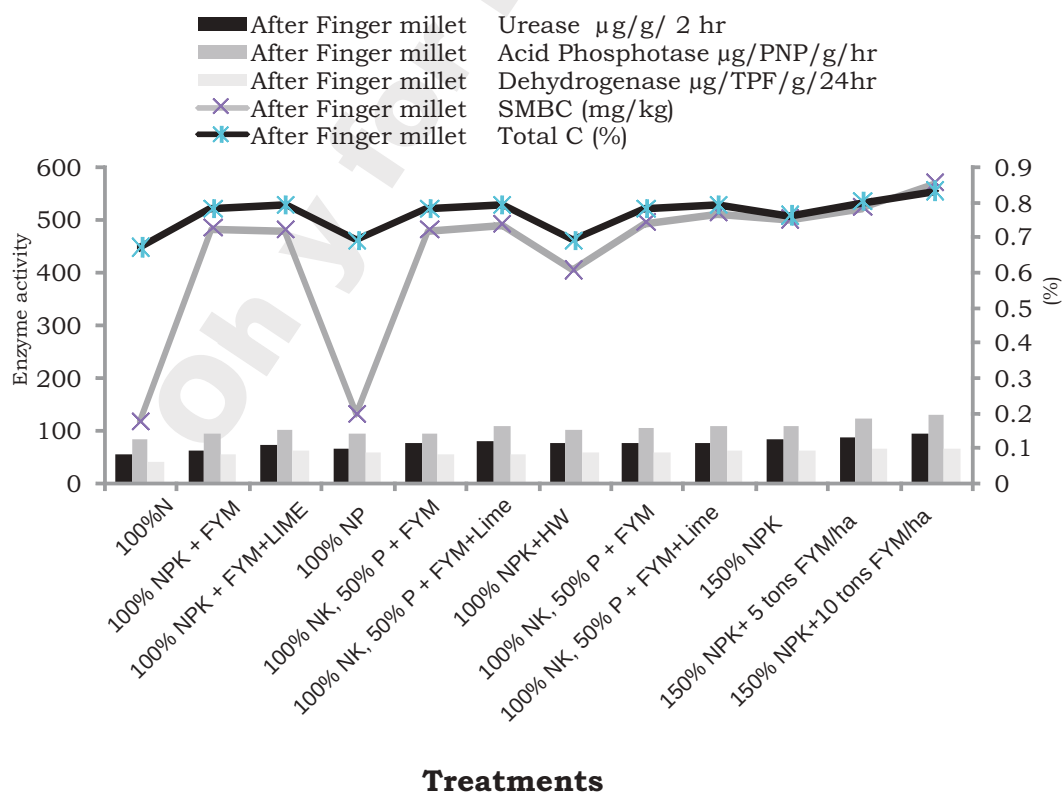


Fig. 2. Effect of superimposed treatments of long term fertilizer experiment on soil enzymatic activities, microbial biomass carbon and total carbon content after harvest of finger millet crop

Table 3. Effect of superimposed treatments of long term fertilizer experiment on soil microbial populations (cfu g⁻¹) after harvest of finger millet crop during *kharif* 2011

Treatments	Bacteria (cfu X 10 ⁶ g ⁻¹)	Fungi (cfu X 10 ³ g ⁻¹)	Actinomycetes (cfu X 10 ⁴ g ⁻¹)
T ₁ S ₁ : 100% N	45.41	15.83	1.52
T ₁ S ₂ : 100% NPK +FYM	72.56	22.64	3.47
T ₁ S ₃ : 100% NPK+FYM+ Lime	71.43	23.47	4.86
T ₂ S ₁ : 100% NP	50.13	18.72	1.54
T ₂ S ₂ : 100% NK, 50% P + FYM	70.24	23.04	3.44
T ₂ S ₃ :100%NK,50%P+FYM+ Lime	71.29	24.13	4.59
T ₃ S ₁ :100% NPK + HW	57.28	20.41	2.79
T ₃ S ₂ :100% NK, 50% P + FYM	71.44	23.49	3.66
T ₃ S ₃ :100%NK,50%P+FYM+ Lime	73.35	24.63	3.82
T ₄ S ₁ :150% NPK	74.56	25.18	2.69
T ₄ S ₂ :150% NPK+ 5 tons FYM/ha	81.35	26.59	4.86
T ₄ S ₃ :150%NPK+10 tons FYM/ha	86.41	28.51	5.51
CD (p=0.05)	13.80	7.11	2.22

NPK + 10 tonnes FYM ha⁻¹) followed by 28.6 X 10³cfu g⁻¹ and 26.6 X 10³cfu g⁻¹ in T₄S₂ (150% NPK + 5 tonnes FYM ha⁻¹) and lowest population 6.3 X 10³cfu g⁻¹ and 15.8 X 10³cfu g⁻¹ was recorded in T₁S₁ (100% N) after harvest of maize and finger millet, respectively.

Use of FYM alone or in combination with chemical fertilizers led to higher numbers of microbes and enhanced microbial respiration than use of chemical fertilizers alone (Catherine *et al.*, 2010).

The population of actinomycetes was found to show significant variation among the treatments. Highest population of 5.96 X 10⁴cfu g⁻¹ and 5.51 X 10⁴cfu g⁻¹ was noticed in T₄S₃ (150% NPK + 10 tonnes FYM ha⁻¹) after harvest of maize and finger millet respectively and lowest of 1.85 X 10⁴cfu/g and 1.52 X 10⁴cfu/g was recorded in T₁S₁ (100% N). There was low population of actinomycetes compared to bacteria and fungi in soil after harvest of both maize and finger millet (Table 2 and 3).

Unbalanced fertilization to rice crop had negative effect on the diversity of the microbial community and total microbial biomass in the soil. The long-term fertilizer experiment (LTFE) also showed that balanced application of N, P and K promoted microbial biomass growth and improvement of community composition. Unbalanced fertilization reduced microbial N and increased C/N ratio of the microbial biomass (Zhang *et al.*, 2005).

The soil enzymes activity like urease, dehydrogenase and phosphatase in the soil remains same after the harvest of maize and finger millet. It is observed that the enzyme activities were low in imbalanced fertilizer applied plots compared to the balanced fertilizer applied plots. Further, it is evident from the study that in treatments

receiving farm yard manure the enzyme activities were higher compared to the no FYM applied plots. Microbial populations were less in imbalanced fertilizer applied plots compared to the balanced fertilizer applied plots and this may be attributed to more availability of carbon as revealed in the present study.

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Influence of Nitrogen, Zinc and Iron Fertilizer on Growth Parameters and Yield of *Parmal* Rice in Transplanted Condition

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Abstract: A field experiment was conducted at Punjab Agricultural University, Ludhiana for two consecutive years (during *kharif* season 2013 and 2014) to study the influence of N, Zn and Fe on growth parameters and yield in *parmal* rice (var. PR-122). All the growth parameters significantly increased with increased level of nitrogen. Soil application of Zn followed by foliar Zn significantly affected dry matter, leaf area and SPAD value. Foliar application of Fe had significant effect only on SPAD value. 125% of RDN (N_3) recorded 10.40 per cent higher grain yield over 75% of RDN (N_1) and soil Zn @ 50 kg $ZnSO_4$ ha⁻¹ + foliar Zn @ 0.5% (Zn_4) recorded 12.20 per cent higher grain yield over no Zn application.

Key Words: Growth parameters, Iron, Nitrogen, *Parmal* rice, Yield, Zinc

Increase in productivity of rice is an important requirement to meet the needs of over increasing population in world. Growing of hybrid rice is an appropriate strategy to increase the yield potentiality of rice to help the world to meet the future projected demand. Growth and development processes associated with higher grain yields of rice hybrids include a more vigorous and extensive root system, increased growth rate during vegetative growth, more efficient sink formation, greater carbohydrate translocation from vegetative plant parts to the spikelets, and larger leaf area index (LAI) during the grain-filling period. The age of seedling had a significant effect on number of grains per panicle. Kim *et al.* (1999) observed that 10-day old seedlings had more vigorous stem elongation and higher tillering ability compared with 15- and 40-day old seedlings. It was observed that the tiller production was higher among 30- day old seedlings transplanted in *kharif* season than other. Modern production agriculture requires efficient, sustainable, and environmentally sound management practices. Nitrogen is normally a key factor in achieving optimum lowland rice grain yields. It is also a prime nutrient for protein and carbohydrate synthesis, growth and development of plant body. The effect of nitrogen on rice growth and grain productivity are derived from several biochemical, physiological and morphological processes in the plant system. Hence, use of N efficiently is important for increasing crop yield, quality, and environmental safety and has economic considerations in crop production. The requirement of N vary from field to field, season to season and year to year due to high variability among fields, seasons and years in soil N supplying capacity and crop growth due to difference in climatic factors.

Ding *et al.* (2006) reported that rice is one of highly sensitive crops to zinc deficiency and zinc is the most important micronutrient limiting rice growth, yield and quality. Zinc and iron is one of the most important micronutrient essential for plant growth especially for rice grown under submerged condition. Combining Zinc fertilizer with SO_4^{2-} can improve the effect of Zn fertilization, reduce adverse impacts of a single-form Zinc fertilizer on crude protein and starch accumulation in rice seeds, and strengthen rice against disease or adversity, thereby improve quality of irrigated rice and increase yield. Zinc is essential for several biochemical processes in the rice plant, such as cytochrome and nucleotide synthesis, auxin metabolism, chlorophyll production, enzyme activation, and membrane integrity reported by IRRI (2006). Fe is also essential for plant growth and development and also an essential component of protein synthesis, which improve the quality of rice. Iron deficiency is therefore a yield limiting factor which also affects the quality of the grain.

MATERIAL AND METHODS

The present study was conducted at PAU, Ludhiana for two consecutive years (during *kharif* season 2013 and 2014). The soil of the experimental field was loamy sand in texture, pH 7.3, EC 0.25 dSm⁻¹ and medium in organic carbon (0.61%), available N (332 kg ha⁻¹), available P (53.2 kg ha⁻¹) but low in available K (120 kg ha⁻¹). With respect to availability of Zn and Fe, DTPA Zn was normal (0.62 mg kg⁻¹) and adequate Fe (4.48 mg kg⁻¹). The experiment was laid out in split-split plot design with three levels of nitrogen in main plot (N_1 : 75% of RDN, N_2 : 100% of RDN, N_3 : 125% of RDN) -

Nitrogen fertilizer was applied in three splits in *parpal* rice, 1/3 at transplanting, 1/3 three weeks after transplanting and the remaining 1/3 six weeks after transplanting, four Zn application treatments in sub plot (Zn₁: No Zn, Zn₂: soil Zn @ 50 kg ZnSO₄ ha⁻¹, Zn₃: foliar Zn @ 0.5% and Zn₄: soil Zn @ 50 kg ZnSO₄ ha⁻¹ + foliar Zn @ 0.5%) and two Fe treatments (Fe₁: No Fe and Fe₂: foliar Fe @ 0.5%). Foliar sprays of ZnSO₄.7H₂O and FeSO₄ @ 0.5% were applied at anthesis and milking stages of the crop. All the growth characters were recorded at harvest. Plant height was recorded from each plot at 10 different selected plants, it was taken from base of the plant to the last fully opened leaf. Periodic dry matter accumulation was recorded from each plot in which two spots were randomly selected and plant biomass taken from these spots to ensure the increase in biomass production overtime. The collected plant biomass was first sun dried and then oven dried at 60°C till constant weight, which was expressed as q ha⁻¹. Tillers were counted from one metre square area from each plot. Leaf area was recorded by using leaf area meter. Chlorophyll content was recorded by SPAD meter. Harvested crop produce from net plot was threshed manually and was recorded on whole plot basis. The observations on growth parameters and yield (pooled) were calculated by using CPCS software and the comparison were made at 5 per cent level of significance.

RESULTS AND DISCUSSION

Plant height: The plant height was significantly affected by different nitrogen levels. Application of 125% of RDN i.e N₃ was significantly higher than the level of 75% of RDN but was statistically at par with 100% of RDN i.e. N₂ (Table 1). Similar results were observed by Zadeh and Hashemi (2014) who reported that plant increased with increased level of nitrogen. Increase in plant height might be due to nitrogen application, which resulted in beneficial effect on cell division and cell elongation of the plant which might be attributed to the increase in plant height with increase in nitrogen level. Among zinc application treatments, the effect of zinc on plant height was non-significant. Similarly, foliar application of Fe had no significant effect on plant height.

Dry matter accumulation: Among different nitrogen levels, significantly higher dry matter accumulation was recorded with the application of 125% of RDN as compared to level of 75% of RDN. The treatment of 100% of RDN and 125% of RDN were statistically at par with each other during the study. Application of nitrogen impacted on photosynthesis exchange and plant growth and higher levels of nitrogen stimulated the vegetative growth, reduced storage carbohydrate and increased allocation of dry matter to the

plant. Among different Zn application treatments, significantly higher dry matter accumulation was in the treatment where soil Zn @ 50kg ZnSO₄ ha⁻¹ followed by foliar Zn @ 0.5% was applied (Table 1) as compared to foliar Zn @ 0.5% alone and no Zn application but was statistically at par with soil Zn @ 50kg ZnSO₄ ha⁻¹ alone. Zinc has a crucial role to play in various physiological aspects of plant such as carbohydrates and photosynthetic metabolisms, phytohormone activity and the proper functioning of a number of enzymes, which help in more dry matter accumulation (Cakmak 2000). The effect of foliar Fe application was non-significant.

No. of tillers m⁻²: The effect of nitrogen on the number of tillers was significant. The significantly higher number of tillers m⁻² was at 125% of RDN as compared to 75% of RDN. Tiller counts were statistically at par when 100% of RDN and 125% of RDN were applied during the study. This might be attributed that nitrogen promoted sprouting of lateral buds in plants because of more cell division and more production of auxin which promoted meristematic growth and cause vigorous vegetative growth, which might resulted in the increase in number of tiller m⁻² with increase in nitrogen levels. With respect to Zn application treatments, number of tillers were significantly affected by zinc application. Treatment of soil Zn @ 50kg ZnSO₄ ha⁻¹ followed by foliar Zn @ 0.5% was significantly higher than foliar Zn @ 0.5% alone and No Zn during the study. Treatments soil Zn @ 50kg ZnSO₄ ha⁻¹ followed by foliar Zn @ 0.5% and soil Zn @ 50kg ZnSO₄ ha⁻¹ were statistically at par with each other (Table 1). This might be attributed that adequate Zn supply affected the metabolic and enzymatic activity (Hafeez *et al.* 2010). Foliar Fe treatment on number of tillers was non-significant during the study. The enhancement in tiller number with the increase in micronutrient and nitrogen use availability was attributed to the rapid conversion of carbohydrates into proteins and consequently increased the number and size of growing cells which ultimately resulted in increase in number of tillers (Muhammad *et al.*, 2012). Interaction effects between nitrogen levels, zinc and iron application methods in respect to tillers m⁻² found to be non-significant.

Leaf area (cm²): The nitrogen had significant effect on leaf area, significantly higher leaf area was recorded with the application of 125% of RDN as compared to nitrogen level of 75% of RDN. The treatment of 100% of RDN and 125% of RDN were statistically at par with each other. Increase in leaf area may be attributed to the positive effect of nitrogen on leaf development. Among different treatments of Zn application, the application of soil Zn @ 50 kg ZnSO₄ ha⁻¹ followed by foliar Zn @ 0.5% produced significantly higher leaf area as

Table 1. Effect of N, Zn and Fe fertilizers on growth parameters, grain and straw yield of *pamal* rice

Treatments	Plant height (cm)	Dry matter accumulation (q ha ⁻¹)	Leaf area (cm ²)	No. of tillers m ⁻²	SPAD value	Grain yield (q ha ⁻¹)	Straw yield (q ha ⁻¹)
Nitrogen levels*							
N ₁ -75% of RDN	101.0	178.5	625.8	384.6	36.9	66.13	98.57
N ₂ -100% of RDN	103.7	185.8	644.7	400.0	39.0	70.46	105.46
N ₃ -125% of RDN	106.5	189.6	657.8	402.4	39.3	73.01	108.48
CD (p=0.05)	3.84	6.86	22.38	12.63	1.66	2.75	3.24
Zinc method of application and level							
Zn ₁ - No Zn	102.8	178.6	621.3	391.7	37.8	66.04	100.35
Zn ₂ - Soil Zn @50kg ZnSO ₄ ha ⁻¹	104.1	189.3	663.9	398.6	38.8	71.82	106.73
Zn ₃ - Foliar Zn @ 0.5%	102.9	178.9	621.5	391.9	38.0	67.51	101.21
Zn ₄ - Soil Zn @50kg ZnSO ₄ ha ⁻¹ + foliar Zn @ 0.5%	105.4	191.8	664.5	400.4	39.0	74.10	108.40
CD (p=0.05)	NS	10.14	36.78	NS	0.86	4.12	4.90
Iron							
Fe ₁ - No Fe	103.7	184.5	642.7	395.2	37.9	69.29	103.59
Fe ₂ - Foliar Fe @ 0.5%	103.8	184.7	642.9	396.1	38.9	70.45	104.75
CD (p=0.05)	NS	NS	NS	NS	0.41	NS	NS
Interaction CD (p=0.05)							
N × Zn	NS	NS	NS	NS	NS	7.12	8.49
N × Fe	NS	NS	NS	NS	NS	NS	NS
Zn × Fe	NS	NS	NS	NS	NS	NS	NS
N × Zn × Fe	NS	NS	NS	NS	NS	NS	NS

*RDN = Recommended dose

compared to foliar Zn @ 0.5% and no Zn. Application of soil Zn @ 50 kg ZnSO₄ followed by foliar Zn @ 0.5% and soil Zn @ 50 kg ZnSO₄ ha⁻¹ alone were statistically at par with each other and foliar Zn @ 0.5% and no Zn were statistically at par with each other. Significant increase in leaf area might be due to more availability of zinc by foliar sprays, which is actively involved in chlorophyll synthesis, cell division, meristematic activity of the tissue and expansion of cells.

SPAD value: Among the different nitrogen levels, nitrogen had significant effect on SPAD value, the application of 125% of RDN recorded significantly higher SPAD value as compared to 75% of RDN which was statistically at par with the application of 100% of RDN. This might be due to increased level of nitrogen, which increased the availability of nitrogen concentration in leaves which help in increasing the chlorophyll concentration in leaves. Among Zn application treatments, zinc had significant effect on SPAD value, soil Zn @ 50 kg ZnSO₄ ha⁻¹ followed by foliar Zn @ 0.5% application recorded significantly higher SPAD index value as compared to application of foliar Zn @ 0.5% and no Zn, which was statistically at par with soil Zn @ 50 kg ZnSO₄ ha⁻¹. The application of foliar Fe @ 0.5% recorded significantly higher SPAD value as compared to no Fe application. Iron play crucial role in plant metabolism particularly in chlorophyll synthesis, which is vital for plant photosynthesis and necessary for the biosynthesis of chlorophyll and cytochrome

as being it is a structural component of porphyrin molecules like cytochrome, hemes, ferrichrome (involved in photosynthesis).

Grain and straw yield: Different levels of nitrogen showed significant effect on grain and straw yield of *pamal* rice (Table 1). Grain yield was increased with increased level of nitrogen upto 125% of RDN which recorded significantly higher grain yield which was 10.40 per cent higher than 75% of RDN. Similarly, 100% of RDN recorded 6.55 per cent higher grain yield over 75% of RDN during the study. These were in line with the findings of Mahajan *et al.* (2011) and Singh *et al.* (2007). Higher grain and straw yield with higher nitrogen level might be due to more availability of nitrogen which increased cell division, cell elongation, promote auxin synthesis which helped in more growth of rice plant in terms of leaf area and dry matter accumulation etc., which might have captured more of solar energy and higher level of N also helped in more chlorophyll content in leaves, which helped in more photosynthesis due to more photosynthates synthesized and translocated from source to the sink (Singh *et al.*, 2007). Among Zn application treatments, soil application followed by foliar Zn recorded 12.20 per cent higher grain and straw yield over no Zn application but was statistically at par with soil Zn application.

Treatment Zn₄ and Zn₂ were statistically at par with each other and treatments Zn₃ and Zn₁ were statistically at

par with each other. These results are in accordance with the findings of Shivay *et al.* (2015) and Dhaliwal *et al.* (2011) who reported significant increase in grain yield over control due to soil plus foliar application (no Zn). Zn increased membrane integrity, synthesis of carbohydrates, cytochrome and nucleotide synthesis, auxin synthesis, chlorophyll synthesis and metabolism of N (Marschner *et al.*, 1996), thereby enhancing photosynthesis, photosynthates and their translocation to grain and increased the grain yield. The application of Fe through foliar spray had no significant effect on grain and straw yield. The interaction effect between N and Zn was significant during the study. From the results conclude that all the growth parameters and yield were significantly increased with increased level of nitrogen. Soil followed by foliar application of Zn significantly improved dry matter accumulation, leaf area, SPAD value, grain and straw yield.

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Received 21 November, 2015; Accepted 11 April, 2016



Molecular Diversity of *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV) Isolates using AFLP Markers

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Abstract: Amplified fragment length polymorphism (AFLP) was used to characterize five silkworm (*Bombyx mori* L. Nuclear Polyhedrosis Virus) BmNPV isolates. A total of 235 bands were scored for four AFLP primer combinations, of which 202 (86%) were polymorphic with an average of 50 polymorphic bands per primer combination. Polymorphism information content (PIC) ranged from 0.29 (for E-CAT/M-GTG & E-CAG/M-GTG) to 0.31 (E-CAG/M-GCC). Marker index (MI) ranged from 12.90 (E-CAA/M-GCC) to 17.98 (E-CAT/M-GTG). Primer E-CAG/M-GCC revealed higher values for both PIC (0.31) and MI (14.83). Cluster analysis based on AFLP markers exhibited concordant relationships among the isolates in relation to geographical origin with high bootstrap values. Survey of unique AFLP bands across five BmNPV isolates revealed correlation between number of unique bands noticed for each isolate and their existing genetic relationships. Mandya isolate was most distinct among five isolates and perhaps other four isolates have evolved from this due to higher sequence variation reflected from the number of unique bands.

Key Words: AFLP, *Bombyx mori* nuclear polyhedrosis virus, Genetic diversity

Sericulture is an important agro based cottage industry in Karnataka. However, cocoon production is prone to 30-40 per cent crop loss due to various diseases such as grasserie caused by *Bombyx mori* nuclear polyhedrosis virus (BmNPV) and flacherie by densovirus (BMNV) and infectious flacherie virus (IFV). BmNPV belongs to a diverse group of large dsDNA viruses with genome size ranging 88-520 kbp (Blissard and Rohrmann, 1990). Taxonomically there are three subgroups of baculoviruses A, B and C. BmNPV generally occurs as single nucleocapsid virus (SNPV) or as multiple nucleocapsid virus (MNPV) (Hayakawa *et al.*, 2000). The virions of Bangalore isolate (BmNPV-BGL) measures 300±5 nm in length and 35±3 nm in diameter (Palhan and Gopinathan, 1996).

In a detailed review on Baculovirus diversity, Blissard and Rhormann (1990) have discussed the highly variable nature of baculovirus isolates from different geographical regions, single agricultural fields and between different polyhedra in a single host. In addition, variation has also been noticed in pathogenicity and time of kill of these isolates. In order to understand molecular diversity prevailing among various insect NPVs, initially restriction fragment length polymorphism (RFLP) was used. Palhan and Gopinathan (1996) constructed a restriction map using *EcoRI* and *HindIII* enzymes for BmNPV-BGL isolate, which was similar to BmNPV T₃. Similarly, four genetically distinct isolates of BmNPV viz., BmNPV- K1, K2, K3 and K4 from sericultural farms in Korea were characterized by RFLP. The

EcoRI and *Sall* fragments showed similar overall patterns with minor differences for each isolate (Hong *et al.*, 2000). cDNA-AFLP analysis has been used to establish the identity of new viruses like a 4th human corona virus causing a respiratory disease (Van der hoek *et al.*, 2004) and new human pathogenic RNA/DNA viruses (Pyrce *et al.*, 2008). Similarly, cDNA-RAPD has been used for identification of Getah virus (Hu *et al.*, 2012).

The use of AFLP analysis in molecular characterization of BmNPV has not been reported so far. Hence, the present study was undertaken to demonstrate the application of AFLP technique in understanding the genetic relationships among five isolates of BmNPV collected from different sericulturally active geographical locations.

MATERIAL AND METHODS

The five different isolates of BmNPV were collected from Kolar, Melur, Hebbal, Devanahalli and Mandya districts of Karnataka. The diseased worms exhibiting typical symptoms of inter segmental swelling, white ooze and crawling on the periphery of rearing trays were selected. Isolates were given the same name as their place of collection. Viral DNA extraction and dialysis was done as per a standard protocol (Chandramohan *et al.*, 1999).

Estimation of the molecular weight of viral DNA using gel electrophoresis: The DNA was electrophoresed after restriction enzyme digestion (with catalogue # MBE3S, supplied by GeNei, Bangalore) using standard marker (with

catalogue # MBD1L, Lot # 12122, supplied by GeNei, Bangalore), to confirm the viral DNA by determining its molecular weight. Agarose gel of 0.7 % was prepared in 1X TAE buffer and mounted on a horizontal electrophoresis unit; 5µg of DNA was loaded into the well and electrophoresed at 25 V in a tank containing 1X TAE buffer till the dye front migrated nearly 3/4th of the gel. The gel was then observed after staining with ethidium bromide under transilluminator and the image was captured and documented.

A standard AFLP amplification protocol (Vos *et al.*, 1995) was used. The DNA fingerprint pattern was scored for the presence (1) or absence (0) of bands to form binary matrix. Only reproducible and polymorphic bands were considered for the analysis. Data sets were analyzed using SIMQUAL option to generate pair-wise Jaccard similarity coefficients (1908) using NTSYS-pc version 2.1 (Rohlf, 2000). The similarity matrices thus generated was utilized for construct of dendrogram based on combined data of AFLP marker system using UPGMA (Unweighted Pair Group Method with Arithmetic Average) algorithm and SAHN clustering. The robustness of dendrogram was evaluated by bootstrap analysis using the computer program WINBOOT (Yap and Nelson, 1996). Dendrogram was reconstructed 1000 times by repeated sampling with replacement. The informativeness of given primer combinations *i.e.* polymorphism information content (PIC) and marker index (MI) were calculated. PIC were calculated for all the primer combinations (Botstein *et al.*, 1980) using POWER MARKER program version, 3.0 (Liu and Muse, 2005).

RESULTS AND DISCUSSION

PCR amplification of genomic DNA of five isolates produced clear and reproducible banding patterns for AFLP analysis (Fig. 1). AFLP markers allowed clear and precise haracterization of BmNPV isolates. A total of 4 primer combinations E-CAT/M-GTG, E-CAG/M-GTG, E-CAG/M-GCC and E-CAA/M-GCC were screened that yielded 235 bands of which 202 were polymorphic. The per cent polymorphism varied from 79 (E-CAT/M-GTG) to 95 per cent (E-CAA/M-GCC) with an average of 87 per cent. Polymorphism information content (PIC) and marker index (MI) are the two best parameters to be considered while selecting best AFLP primers for viral characterization. In the present study, these parameters were used to test the infromativeness of different AFLP primer combinations used. The PIC values ranged from 0.29 (for E-CAT/M-GTG and E-CAG/M-GTG) to 0.31 (E-CAG/M-GCC) with an average of 0.29 per primer combination. Higher PIC values (0.31 and 0.30) were observed for the primer combinations E-CAG/M-GCC and E-CAA/M-GCC. Marker index values ranged



a. E-CAT/M-GTG; b. E-CAG/M-GCC; c. E-CAA/M-GCC and d. E-CAG/M-GTG (Lane 1-5 represents BmNPV isolates Kolar, Melur, Mandya, Hebbal & Devanahalli respectively)

Fig. 1. Amplification profiles of five BmNPV isolates using AFLP primer combinations

from 12.90 (E-CAA/M-GCC) to 17.98 (E-CAT/M-GTG) with an average of 15.06 per primer combination (Table 2). The primer combination E-CAG/M-GCC showed higher values of both PIC (0.31) and MI (14.83) and hence this primer is considered to be the most informative in revealing diversity in these isolates.

The results from AFLP amplification indicated that the NPV isolates examined in the present study were genetically different. The variations in banding patterns have possibly arisen due to single nucleotide changes, insertions, deletions and/or chromosomal rearrangements occurring in the viral genome. Goldberg *et al.* (2003) reported similar observations in viruses belonging to the family Iridoviridae. Unique AFLP bands were surveyed across five isolates of BmNPV. Of the 202 polymorphic bands, 104 bands were found to be unique sites in the viral genomes. E-CAT/M-GTG primer combination has detected maximum number of

Table 1. Adapter and primer sequences used for AFLP analysis

	Primer/Adapter name	Sequence
Adapters	Double stranded <i>Eco</i> RI adapter	5'-CTCGTAGACTGCGTAC-3' 5'-AATTGGTACGCAGTC-3'
	Double stranded <i>Mse</i> I adapter	5'-GACGATGAGTCCTGAG-3' 5'-TACTCAGGACTCAT-3'
Primers	<i>Eco</i> RI +C	5'-GACTGCGTACAATTCC-3'
	<i>Mse</i> I +G	5'-GATGAGTCCTGAGTAAG-3'
	<i>Eco</i> RI +CAT	5'-GACTGCGTACAATTCCAT-3'
	<i>Eco</i> RI +CAG	5'-GACTGCGTACAATTCCAG-3'
	<i>Eco</i> RI +CAA	5'-GACTGCGTACAATTCCAA-3'
	<i>Mse</i> I +GTG	5'-GATGAGTCCTGAGTAAGTG-3'
	<i>Mse</i> I +GCC	5'-GATGAGTCCTGAGTAAGCC-3'

unique bands (34), followed by E-CAG/M-GTG (28), E-CAA/M-GCC (27) and E-CAG/M-GCC (15). The maximum number of unique bands were specific to Kolar isolate (27), followed by Hebbal (25), Melur and Devanahalli (24) and the least number of bands were observed for Mandya isolate (4) (Table 3). Palhan and Gopinathan (1996) have also found distinct differences for restriction enzyme *Hind*III in Bangalore (BLG) and Dharwad (DHR) isolates of BmNPV in Karnataka, India. Four genetically distinct isolates of BmNPV using RFLP has been reported in Korea (Hong *et al.*, 2000).

A restriction endonuclease analysis (REN) was successfully used to characterize ten isolates of *Heliothis* NPV isolated from different geographical regions of the world with the enzymes *Bam*HI, *Eco*RI, and *Hind*III (Getting and McCarthy, 1982), *Spodoptera litura* (SiMNPV), *Spodoptera*

frugiperda (SfMNPV), *Epiphyas postvittana* (EpMNPV), HaMNPV, *Chrysodensis eriosoma* (CeMNPV), *Phthorimea operculella* GV (PoGV) and *Laspeyresia pomonella* (LpGV) with *Eco*RI (Burgess, 1983). Bilimoria (1983) studied the REN analysis of five isolates of SNPVs viz., the Guatemala isolate of *Pseudoplusia includes* (PiSNPV) (PGT), the Arkansas isolate of *Trichoplusia. Ni* (TnSNPV) (TAR), Alabama isolate of *T. ni* (TnSNPV) (TAL), the Michigan isolate of *Syngrapha selecta* (SMI) and the New York isolate of *T. ni* SNPV (TnSNPV) (TNY).

The polymorphic marker data of AFLP in the present study were used for UPGMA based cluster analysis. Dendrogram on the basis of pooled data produced a well separated group with higher bootstrap values. Similarity matrix values ranged from 0.28 between Mandya and Hebbal isolates to 0.47 between Hebbal and *Devanahalli* isolates. At 30 % genetic similarity, all the five isolates were clearly separated into two clusters (Table 4, Fig. 2). Cluster I comprised four BmNPV isolates from Kolar, Melur, Hebbal and *Devanahalli*, while Cluster II comprised only one isolate from Mandya. Cluster I was further divided into two sub-clusters at 40 % genetic similarity with 98 % bootstrap support value as evident from the dendrogram. Sub-cluster I comprised Kolar and Melur isolates with genetic similarity of 0.46 and bootstrap value of 83 %. Similarly, sub-cluster II comprised Hebbal and *Devanahalli* isolates having genetic similarity of 0.47 with 86 % bootstrap value. Mandya isolate remained as a separate node. It was clearly evident from

Table 2. Number of unique bands produced by different AFLP primer combinations in five BmNPV isolates

	Kolar	Melur	Mandya	Hebbal	Devanahalli	Unique bands/primer
E-CAT/M-GTG	12	12	1	7	2	34
E-CAG/M-GTG	9	1	0	9	9	28
E-CAG/M-GCC	2	3	0	9	1	15
E-CAA/M-GCC	4	8	3	0	12	27
Unique bands / isolate	27	24	4	25	24	104

Table 3. Degree of polymorphism and informativeness as revealed by AFLP primer combinations

AFLP primer combinations	TN	TP	NUB	% P	PIC	MI
E-CAT/M-GTG	78	62	34	79	0.29	17.98
E-CAG/M-GTG	57	49	28	88	0.29	14.50
E-CAG/M-GCC	55	48	15	87	0.31	14.83
E-CAA/M-GCC	45	43	27	95	0.30	12.90
Total	235	202	104	349	1.19	60.26
Average	59	50	26	87	0.29	15.06

Note* TN, Total number of bands; TP, Total number of polymorphic bands; NUB, Number of unique bands; %P, Percentage of polymorphism; PIC, Polymorphism information content; MI, Marker index; Resolving power

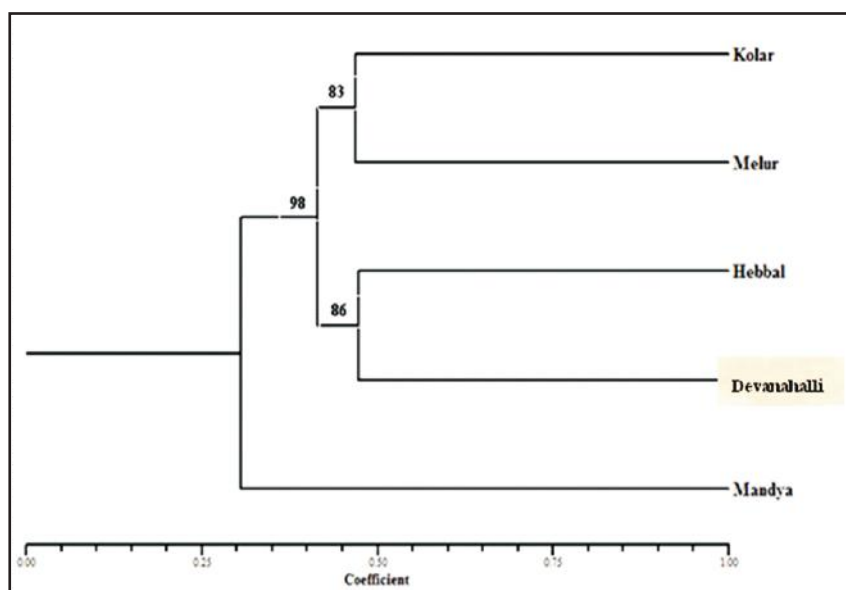


Fig. 2. Dendrogram representing relationships among five BmNPV isolates based on AFLP data

Table 4. Jaccard's similarity matrix as obtained based on AFLP data

	Kolar	Melur	Mandya	Hebbal	Devanahalli
Kolar	1.00				
Melur	0.46	1.00			
Mandya	0.29	0.34	1.00		
Hebbal	0.42	0.39	0.28	1.00	
Devanahalli	0.43	0.40	0.29	0.47	1.00

both similarity matrix and dendrogram that the Mandya isolate is more distinct and least similar to other isolates from Hebbal and Devanahalli (0.28 and 0.29, respectively), followed by those from Kolar and Melur (0.29 and 0.34, respectively). These results suggested that the viral isolates other than Mandya may have evolved as a variation of this isolate as reflected from the four unique bands detected in this isolate. It was interesting to note that number of unique bands could be correlated to the genetic distance and clustering. The Mandya isolate is least similar to Hebbal (similarity 0.28 and 25 unique bands) and Kolar isolates (similarity 0.29 and 27 unique bands), followed by Devanahalli (0.29 and 24 unique bands) and Melur (similarity 0.34 and 24 unique bands). These results showed the reliability of grouping of isolates based on AFLP data.

Comparing the grouping of isolates with their place of collection, there was a clear indication that the basis of grouping was mainly geographical. Isolates from Hebbal within Bangalore and Devanahalli, 45 Km away in north Bangalore grouped together. Similarly, isolates from Melur belonging to Chickballapur district and Kolar north of

Bangalore which are neighbouring districts grouped together in the other sub cluster and these locations are approximately 60 Km away from each other. Isolate from Mandya 200 Km west of Bangalore towards Mysore was placed separately.

Genotypic variation in baculovirus populations in *Heliothis armigera* MNPV, *H. armigera* SNPV, *H. zea* SNPV, *Spodoptera litura* and *S. frugipeda* has been detected at several ecological scales, from between geographical regions (Shapiro *et al.*, 1991; Vickers *et al.*, 1991) to within virus isolates (Smith and Crook, 1988; Maeda and Majima, 1990; Munoz *et al.*, 1999). Differences in pathogenicity have also been demonstrated between geographical isolates from within single agricultural fields (Hodgson *et al.*, 2001; Cooper *et al.*, 2003; Ebling and Kaupp, 1995) and between genotypes derived from the same isolate (Lynn *et al.*, 1993; Stiles and Himmerich, 1998). The present investigation is unique where AFLP was used for genetic diversity studies of a virus infecting silkworm *B. mori*, demonstrating a greater utility for this marker. Since viruses evolve more rapidly due to higher rate of mutations, this technique provides an excellent molecular tool for a quick scan and characterization of viral genomes for detection of newly evolved strains. Diversity studies using whole virus genome and viral genes like those encoding for the viral capsid have to be understood for a large number of isolates and such information has to be related to bioassay studies on the virulence/ avirulence of the virus.

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Influence of Biofertilizers on Growth, Yield, Quality and Nutrient Uptake in Onion (*Allium cepa* L.)

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Abstract: The *Azotobacter* was beneficial for improving plant height, leaf area and bulb diameter when applied along with recommended dose of fertilizers (RDF). Likewise *Azospirillum* along with RDF the (234.4 q ha⁻¹) which was 11.2 % higher bulb yield than the RDF. The application of *Azospirillum* along with 75 % recommended dose of nitrogen or *Azotobacter* along with VAM and 75 % N and 75 % P of the recommended dose of nitrogen and phosphorus recorded yield which was statistically at par with RDF. The *Azospirillum* also improved TSS and ascorbic acid. The uptake of nitrogen and potassium also improved with the application of *Azospirillum* along with RDF. *Azotobacter* along with VAM and 75 % dose of N and P saves the nitrogenous and phosphorus fertilizers without any significant reduction in yield. *Azospirillum* either along with RDF or with 75 % dose of N suggested for sustainable production of onion.

Key Words: *Azospirillum*, *Azotobacter*, Biofertilizers, Nutrient uptake, PSB, VAM

Onion (*Allium cepa* L.), a member of family Alliaceae is cultivated on an area of 1203 million hectare in the country (Anonymous, 2014). Being a shallow rooted crop, it is quite responsive to heavy dose of inorganic fertilizers, but higher doses of these fertilizers deteriorates the soil health. Beside this, increasing cost of inorganic fertilizers is making crop production an uneconomical particularly for small and marginal farmers. Hence renewable and low cost sources of plant nutrients are required for supplementing and complementing chemical fertilizers to make farming economically viable. Integrated nutrient management involving suitable combination of inorganic fertilizers, organic manures and biofertilizers can help in curtailing over dependence on inorganic fertilizers only.

Biofertilizers are cost effective and are considered important for sustainable production of crops. Free-living nitrogen-fixing bacteria like *Azotobacter* and *Azospirillum* have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis and also help to combat diseases. In the present investigation, the effect of biofertilizers (*Azotobacter*, *Azospirillum*, *Phosphate Solubilizing Bacteria* and *Vesicular-Arbuscular Mycorrhizae*), organic manures (farm yard manure and poultry manure) and synthetic fertilizers have been studied in different combinations to establish the morphological, biochemical and yield effects on onion.

MATERIAL AND METHODS

The present investigation was carried out at Punjab

Agricultural University, Ludhiana during the *rabi* season 2013-14. The onion cultivar Punjab Naroya comprised plant material. The soil was loamy sand in texture with pH 7.6. Soil was low in nitrogen, high in phosphorus and medium in potassium. The crop was sown in nursery beds during November and transplanted in January at a space of 15 X 7.5 cm between rows and plants, respectively. The dose of chemical fertilizers was manipulated at various levels in combination with different biofertilizers and organic manures as per the treatment schedule. The experiment was carried out in a randomized block design with fifteen treatments replicated thrice (Table 1).

The cultures of *Azotobacter*, *Azospirillum* and PSB @ 500g ha⁻¹ each were dissolved in 10 % jiggery and the onion seedlings were dipped for one hour. The culture of VAM @500 g ha⁻¹ was directly applied in soil before planting. Half dose of nitrogen in each plot and full dose of phosphorus and potash was applied at the time of transplanting. The remaining dose of N was applied after one month of transplanting. Observations were recorded on plants height at 30, 60 and 90 days after transplanting, number of leaves per plant, leaf area, neck thickness, bulb diameter, bulb weight and various quality parameters i.e. TSS, ascorbic acid, dry matter, bulb dry mass, plant dry mass and total mineral. Nutrient uptake was also recorded per hectare.

RESULTS AND DISCUSSION

The maximum plant height at 30 days after transplanting (DAT) were observed in the plot treated with *Azotobacter* along with recommended dose of N, P and K (100 Kg N ha⁻¹, 50 Kg ha⁻¹ P and 50 Kg ha⁻¹ K), followed by

Azospirillum along with recommended dose of fertilizers (RDF) (T_3). Both treatments resulted in significant increases in plant height as compared to the control plant at 30 DAT. *Azotobacter* along with RDF treated plants had more growth in terms of plant height as compared to plants treated with *Azospirillum* along with RDF. The plants treated with *Azotobacter* along with RDF were 41.9% higher than that of the plots where no fertilizer was applied.

Maximum leaf area (35.3 cm^2) attained with the T_1 which was significantly higher from all treatments except T_3 and T_7 . Among organic manure, maximum leaf area was recorded with poultry manure (29.1 cm^2) which was significantly higher from T_{11} and T_{12} and significantly lower from T_1 where maximum leaf area was recorded (Table 1). Application of T_3 resulted in significantly higher bulb weight of onion (94.3 g) than with T_{15} , T_6 , T_8 , T_{11} , T_{12} , T_{13} and T_{14} and was statistically at par with rest of treatments (Table 1).

The maximum bulb yield (234.4 q ha^{-1}) was attained with T_3 which was significantly higher than that attained with all other treatments except T_1 and T_9 . Among organic manures, maximum bulb yield (189.3 q ha^{-1}) was in T_{11} but the difference among other organic manure treatments was non-significant and it was significantly higher from control (T_{15}). The application of *Azospirillum* along with 75 %

recommended dose of nitrogen and *Azotobacter* along with VAM and 75 % N and 75 % P of the recommended dose of nitrogen and phosphorus recorded yield which was statistically at par with RDF (Table 1). There was saving of 25 % nitrogenous fertilizers.

Combined application of biofertilizers and fertilizers improved the growth of onion because these will increase the absorption of nutrients especially nitrogen to enhanced the cell division and cell elongation. *Azotobacter* and *Azospirillum* by fixing N improves the growth attributes (Parmar *et al.*, 2011). Similar results were reported in onion and cauliflower where the inoculation with *Azotobacter* along with synthetic fertilizers mostly increased the growth (Jayatilake *et al.*, 2002 and Singh and Singh, 2005). Brar *et al.* (2015) also recorded higher yield and better quality in onion with organic nutrition than inorganic sources.

Quality attributes: Quality parameters also showed a good response to the bacterial inoculants. Treatments of *Azospirillum* along with RDF resulted in significant increases in Total soluble solids (13.1° brix) and ascorbic acid ($15.6 \text{ mg/ fresh weight of bulb}$) as compared to the control (Table 2). With application of FYM along with VAM and *Azotobacter* maximum TSS (11.3° brix) was observed in T_{11} that was significantly higher than control but at par with rest of the treatments. Ascorbic acid attained with organic manure alone

Table 1. Effect of different treatments on yield contributing parameter and yield of onion

Treatment	Treatment detail	Plant height (cm)			Number of leaves per plant	Leaf area (cm^2)	Neck thickness (cm)	Bulb diameter (cm)	Bulb weight (g)	Total yield (q ha^{-1})
		30 DAT	60 DAT	90 DAT						
T_1	<i>Azotobacter</i> + RD NPK	36.2	57.1	79.2	10.5	35.3	1.4	5.8	87.0	218.9
T_2	<i>Azotobacter</i> + 75% N +RD PK	32.7	50.5	69.5	9.7	28.4	1.5	5.4	81.0	190.0
T_3	<i>Azospirillum</i> + RD NPK	35.6	55.7	79.8	10.5	34.5	1.3	5.9	94.3	234.4
T_4	<i>Azospirillum</i> + 75% N +RD PK	33.4	52.4	73.2	9.9	27.9	1.5	5.7	86.0	204.8
T_5	PSB+ RD NPK	34.5	54.9	78.5	10.0	27.0	1.6	5.7	84.3	201.5
T_6	PSB + 75% P +RD NK	33.0	50.3	67.3	9.9	29.9	1.5	5.4	74.3	187.8
T_7	VAM + RD NPK	34.3	54.2	76.3	9.9	34.5	1.5	5.7	86.7	209.3
T_8	VAM + 75% P +RD NK	32.3	51.4	71.3	9.8	30.7	1.6	5.1	75.3	182.4
T_9	<i>Azotobacter</i> +VAM + RD NPK	34.7	54.9	78.6	9.9	26.2	1.3	5.9	93.3	224.8
T_{10}	<i>Azotobacter</i> + VAM + 75% N + 75% P + RD K	31.0	49.6	69.3	9.7	33.7	1.5	5.4	82.7	197.4
T_{11}	FYM @ 20 t/ha + <i>Azotobacter</i> + VAM	33.7	52.9	74.5	10.0	27.8	1.5	5.2	78.3	189.3
T_{12}	FYM @ 20 t/ha	30.9	50.9	71.8	9.7	27.0	1.8	5.1	73.0	172.9
T_{13}	Poultry Manure @ 5t/ha	33.8	54.1	77.4	9.8	29.1	1.6	5.1	68.3	176.9
T_{14}	RD NPK	32.5	52.3	72.2	9.7	27.9	1.6	5.4	79.7	210.8
T_{15}	Control	25.5	44.4	67.1	8.7	25.2	1.7	4.9	65.7	151.1
CD ($p=0.05$)		4.7	6.0	8.6	0.8	0.8	NS	0.5	13.6	17.5

*RD: Recommended dose of fertilizers (100 Kg N ha^{-1} , 50 Kg P ha^{-1} , 50 Kg K ha^{-1}), DAT: Days After Transplanting

Table 2. Effect of different treatments on quality attributes of onion

Treatment	TSS (^o Brix)	Ascorbic Acid (mg 100 g ⁻¹ fresh wt)	Dry matter (%)	Bulb Dry mass (q ha ⁻¹)	Plant dry mass	Total minerals (mg100 g ⁻¹ dry weight)
T ₁	11.7	15.2	9.9	21.7	19.1	5.0
T ₂	11.5	13.8	9.5	18.1	19.2	5.5
T ₃	13.1	15.6	10.2	23.8	20.4	4.5
T ₄	10.3	12.4	9.5	19.4	18.2	4.0
T ₅	12.6	14.8	9.5	19.2	17.5	6.0
T ₆	9.7	11.4	9.4	17.7	15.2	5.5
T ₇	12.1	15.1	9.6	20.1	17.8	3.5
T ₈	9.9	13.8	9.3	16.9	14.4	4.0
T ₉	11.8	12.4	10.1	22.7	20.2	5.0
T ₁₀	11.9	13.8	9.5	18.9	16.5	3.5
T ₁₁	11.3	12.4	9.4	17.8	15.5	6.0
T ₁₂	9.8	15.7	8.5	14.7	16.5	5.5
T ₁₃	10.6	12.4	9.3	16.4	15.5	6.0
T ₁₄	9.0	12.9	9.1	19.1	15.3	6.0
T ₁₅	8.4	10.7	8.4	12.8	12.2	3.4
CD (p=0.05)	2.6	3.3	NS	2.9	3.3	NS

*RD: Recommended dose of fertilizers (100 Kg N ha⁻¹, 50 Kg P ha⁻¹, 50 Kg K ha⁻¹). See Table 1 for treatment details

or along with biofertilizers treatments was at par with each other. Increase in quality due to application of biofertilizers and chemical fertilizers could be attributed to the metabolic activities, which synthesis of higher amount of acids and contributed to synthesis of TSS %, acidity and ascorbic acid in vegetables (Yogita and Ram, 2012).

Nutrient content in onion bulb and leaves: The nitrogen content in the onion bulb improved with application of N, P and K in combination with biofertilizers (Table 3). Application of *Azotobacter* along with RDF (T₁) resulted in significantly higher nitrogen content (3.98 per cent) in bulb than that attained with T₁₅ (2.10 per cent). The maximum phosphorus content (0.44 per cent) was recorded with T₅ and it was significantly higher than with T₁₅ (0.13 per cent). The increased phosphorus content in the onion bulb may be due to the higher availability of phosphorus in the Phosphorus solubilising bacteria (PSB) when it is applied at higher concentration. PSB and VAM improve the P content in the onion bulb due to solubilization of P with PSB and VAM. Maximum P content was observed in treatments where PSB and VAM were applied. The maximum K content in onion bulb (4.69 per cent) attained with T₂, which was significantly higher than that recorded with T₁₅. *Azotobacter* improves the potassium content in the soil it may be due to the ability of bacterium to fix the nutrients in the soil. Potassium availability enhanced the availability of P and K in the soil and plant.

Application of T₁ resulted in significantly higher nitrogen content (3.57 per cent) in leaves than that in T₁₅ (2.15

per cent). Kavvadias *et al.* (2012) reported increased concentration of nitrogen with increasing nitrogen application. *Azospirillum* and *Azotobacter* increase the N availability to the plants. The P and K content of the plant was not significantly influenced by different treatments.

Nutrient uptake studies: Application of *Azospirillum* along with RDF (T₃) resulted in maximum total nitrogen uptake (162.6 Kg ha⁻¹), which was statistically at par with T₁ and T₉ but significantly higher than rest of the treatments (Table 3). Total N uptake attained with T₁₄ (105.7 kg ha⁻¹) was significantly higher than T₁₅. The application of full dose of nitrogen along *Azospirillum* and *Azotobacter* resulted in significantly higher total nitrogen uptake as compared to reduced nitrogen application. This could be due to more availability of nitrogen to plants because of N fixation by biofertilizers which is helpful for their vegetative and reproductive cycle. The maximum total phosphorus uptake of 13.6 kg ha⁻¹ was attained with T₉ followed by T₆, which was significantly higher than rest of treatments except T₁, T₃, T₅ and T₇. Application of *Azospirillum* along with RDF resulted in maximum total potassium uptake of 126.9 Kg ha⁻¹ (T₃), which was significantly higher than rest of treatments except T₁ and T₉ (Table 3).

CONCLUSION

Onion should be inoculated with *Azotobacter* in combination with recommended dose of fertilizers or with 75% dose of N fertilizers was best for vegetative growth.

Table 3. Effect of different treatments on nutrient uptake by onion and nutrient status of soil

Treatment	Nutrient content (%) in bulb			Nutrient content (%) in leaves			Nutrient uptake (Kg ha ⁻¹)		
	N	P	K	N	P	K	N	P	K
T ₁	3.98	0.35	4.25	3.19	0.19	1.45	154.0	11.5	119.9
T ₂	3.22	0.31	4.69	3.54	0.20	1.42	119.5	9.4	108.4
T ₃	3.84	0.35	4.13	3.40	0.20	1.40	162.6	12.4	126.9
T ₄	3.70	0.34	4.13	2.49	0.24	1.39	133.8	10.1	105.8
T ₅	2.31	0.44	4.06	2.35	0.16	1.47	87.7	12.6	102.1
T ₆	2.41	0.35	4.38	3.10	0.23	1.37	78.7	8.6	95.9
T ₇	2.68	0.40	4.00	2.45	0.19	1.38	109.2	12.0	104.9
T ₈	2.48	0.37	4.06	3.40	0.23	1.44	76.5	8.8	84.8
T ₉	3.26	0.39	4.25	2.40	0.13	1.36	142.1	13.6	125.4
T ₁₀	3.40	0.20	3.88	3.08	0.12	1.34	103.2	5.9	91.9
T ₁₁	3.12	0.18	3.69	3.52	0.15	1.44	103.1	5.1	82.8
T ₁₂	3.82	0.22	4.32	3.10	0.16	1.41	115.2	5.6	86.1
T ₁₃	3.02	0.25	4.13	2.99	0.13	1.37	98.8	6.5	84.0
T ₁₄	3.16	0.16	4.06	2.15	0.11	1.30	105.7	4.9	86.3
T ₁₅	2.10	0.13	3.44	3.57	0.21	1.47	53.3	2.9	59.8
CD (p=0.05)	0.83	0.15	0.58	0.68	NS	NS	24.5	3.3	13.4

*RD: Recommended dose of fertilizers (100 Kg N ha⁻¹, 50 Kg P ha⁻¹, 50 Kg K ha⁻¹). See Table 1 for treatment details

Azospirillum along with recommended dose of fertilizers or with 75% dose of N fertilizers was best for yield and quality. *Azospirillum* also improved the TSS, ascorbic acid and uptake of N, P fertilizers. *Azotobacter* along with VAM and 75% N, P fertilizers save 25% nitrogenous and phosphorus fertilizers. Hence, Application of *Azotobacter* or *Azospirillum* either along with RDF or with 75% dose of N may be recommended for cultivation of onion.

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Received 6 September, 2015; Accepted 4 April, 2016



Bioutilization of Agro-Industrial Waste to Produce Biopigment using *Monascus Purpureus* MTCC 369 by Solid State Fermentation

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Abstract: The main objective of the study was to optimize media and process parameters for biopigments extraction using *Monascus purpureus* MTCC 369 by solid state fermentation. Among different carbon substrates (wheat bran, rice bran, sweet potato peel powder, potato peel powder, kinnow peel powder), sweet potato peel powder (8%) was best source and among nitrogen sources (pea pod, taro leaves, soy okara, corn steep liquor faba beans hull and peptone), pea pod (4%) was best substrate with 55% moisture content. Biopigment production was carried out using solid state fermentation. To the selected substrates of 0.25 mm particle size, optimized salt solution of 3% (pH 5.5) was supplemented and it was fermented for 11 days at 30°C resulted in significant accumulation of pigment when extracted with ethanol (90%). After optimization of all media components (carbon and nitrogen substrates) and process parameters (particle size, moisture content, salt concentration, pH, temperature and incubation time) maximum yield obtained was 17.90 CVU/g using one-factor-at-a-time-technique.

Key Words: Biopigment, Agro-industrial waste, *Monascus purpureus*, Solid state fermentation

Color from synthetic origin is criticized because of their negative health effect (Carvalho *et al.*, 2006). Nature is filled with colors (Sehrawat *et al.*, 2016). Biopigments are natural source of color from micro-organism, plants or animals. Color from micro-organism also overcomes limitation of plant and animal sources like seasonal variations, non-availability throughout the year, price and ease of control over process parameters (Gulani *et al.*, 2012).

Monascus species is well known for the production of biopigments, which are being utilized for coloring food (Chinese cheese, fish paste, surimi, red wine sausage, ham, and tomato ketchup) (Dikshit and Tallapragada, 2011). *Monascus* species (*M. purpureus*, *M. pilosus* and *M. ruber*) produce edible pigments of red, yellow and orange color. Antimutagenic, antimicrobial and antiobesity are some of the biological activities exhibited by the pigments produced by the *Monascus* species (Wu *et al.*, 2011; Kamalam *et al.*, 2012). *Monascus* can grow over different substrates because it is most likely considered as a xerophilic fungus. Also utilization of agro-industrial wastes sorts out the problems of waste disposal and it also do not pollute the environment (Babitha *et al.*, 2006; Chavan *et al.*, 2015). Large volume of waste or residual material is being generated by industries and agriculture sector which pose a serious problem of disposal. Due to organic nature of these wastes contains important constituent like sugar, starch, proteins etc which could be utilized as substrate for growth of microflora. Solid state fermentation (SSF) has benefits of higher yield over submerged fermentation (SmF) also liquid waste generated in SmF need to be disposed off (Ali and Zulkali, 2011).

For this study *Monascus purpureus* MTCC 369 was utilized for biopigment production on different agro-industrial wastes by SSF and further optimization and purification was carried out.

MATERIAL AND METHODS

Procurement of culture, chemicals and raw materials: *M. purpureus* MTCC 369 was procured from Microbial Type Culture Centre (MTCC), Institute of Microbial Technology, Chandigarh. All reagents and chemicals used for experimental investigation were of analytical grade (Hi-media chemicals) and procured from Central Drug House (CDH) chemicals. Different natural carbon substrates wheat bran (local market Sangrur, India), rice bran (A.P Solvex Limited Dhuri, Sangrur, Punjab, India) sweet potato peel (Fruit and Vegetable Lab, Santlongowal institute of engineering and technology, Longowal, India), potato peel (Pepsico India Hoarding, Frito Lay division, Bhawanigarh, Sangrur, Punjab, India) and kinnow peel (local market, Sant longowal institute of engineering and technology, Longowal, Punjab) were procured. Different natural nitrogen substrates soy okara (Dairy Technology Lab, Sant Longowal Institute of Engineering and Technology, Longowal, India), faba beans hulls (Fruit and Vegetable Lab, Sant Longowal Institute of Engineering and Technology, Longowal, India), corn steep liquor (Sukhjit starch and chemicals Limited Phagwara, Punjab, India), taro leaves and pea pod (local market Sangrur, India) were procured.

Maintenance of culture and substrate preparation: *M. purpureus* MTCC 369 culture was maintained on slants of

Potato Dextrose Agar (PDA) medium, incubated at 30°C for 7 days and preserved at 4-5°C. It was conserved by sub culturing aseptically. The peels were washed vigorously with hot tap water (50°C), to reduce extraneous matter attached to peel. Small pieces of peels were made using knife, then dried in oven in 60°C for 48 h to reduce the moisture up to 5-6%. After drying the dried carbon sources were passed through a mixer and sieved using appropriate mesh size to get fine powder.

Optimization of media for biopigment production:

Screening of carbon source from agricultural or industrial waste and concentration. Different carbon sources such as wheat bran, rice bran, sweet potato peel powder, potato peel powder, kinnow peel powder and fructose (control) were supplemented individually (5g) in 2 ml salt solution containing potassium di-hydrogen phosphate (0.2g/100ml), ammonia nitrate (0.5g/100ml), sodium chloride (0.1g/100ml), Magnesium Sulphate (0.1g/100ml). The pH of medium was adjusted 6.5 by adjusting the pH of salt solution. Requisite amount of water was added to adjust the initial moisture content to 55%. Then media was autoclaved at 15 psi for 20 minute at 121°C. After cooling of the media it was inoculated with *Monascus purpureus* MTCC 369 spores (1.5×10^5 per mL) and incubated at 30°C for 11 days. Effect of selected carbon substrate at different concentration (5-10% w/w) was also analyzed.

Screening of nitrogen source from agricultural or industrial waste and concentration: To study effect of various nitrogen sources (Pea pod, taro leaves, soy okara, corn steep liquor faba beans hull and peptone) on mycelial growth, they were supplemented individually in basal media with selected carbon source and optimized concentration. Effect of different concentration (1-6% w/w) of the above screened best nitrogen source for the pigment production was also carried out.

Optimization of process parameters (particle size analysis, salt solution concentration, pH, temperature and incubation time) by considering one factor at a time:

Selected carbon source and nitrogen source with their optimised concentration were added in basal media. The particle size measurement was done by passing the dry substrate through series of sieves of different mesh size (12, 14, 30, 60, 72, 85 and 120 BSS) in the rotary shaker to obtain particle of (1.40, 1.20, 0.5, 0.25, 0.21, 0.17 and 0.12 mm, respectively) different sizes. Selected carbon source and nitrogen source after optimising there concentrations were added in basal media and moisture contents were varied from 50-70%.

To know the effect of hydrogen ion concentration on fermentation medium the pH of sal solution was varied 4.5-

8.5 using either 0.1N HCl or 0.1N NaOH. (Dikshit and Tallapragada, 2011). Different concentrations (1-5 ml/100ml) of salt solution were prepared and supplemented in the fermentation medium. The media was prepared by adding optimized carbon source and nitrogen source into the salt solution of different concentration with optimized pH and incubated at 30°C for 11 days. Further to optimize temperature effect of different temperature (25-40°C) on mycelia growth was monitored. Finally optimising all these process parameters by considering one factor at a time incubation time for the production of biopigment, was varied from 3 days to 13 days. Samples were drawn first on 3 day subsequently followed by 2 days intervals and analyzed by spectrophotometer.

Extraction of biopigments using different solvents and its estimation:

Pigment has been extracted from fermented mass by following the method of (Babitha *et al.*, 2006). The known amount of fermented mass was taken and dried in an oven at 60°C overnight. Then 1g fermented dry matter was mixed with 5 mL of 90% ethanol and kept on a shaker rotating at 100 rpm for 1 hour. Further the mixture has been allowed to stand for 15 minute. After filtration through Whatman No. 1 filter paper filtrate was analysed for spectrophotometric analysis. The pigment concentration was obtained by taking the absorbance at wavelength of 500, 400 and 460 nm for red, yellow and orange pigment respectively using UV-VIS spectrophotometer (DR 5000, HACH, Germany). The absorbance values were calculated into color value unit (Dikshit and Tallapragada, 2011).

RESULTS AND DISCUSSION

Screening of carbon source from agricultural or industrial waste and concentration: Sweet potato peel powder show maximum pigment production (4.5 CVU/g) followed by rice bran (4.1 CVU/g) and kinnow peel powder (4.0 CVU/g). Cheap agro-industrial wastes such as rice bran, potato peel, wheat bran, etc. were utilized to increase the yield by Babitha *et al.* (2006) but no work has been carried out on sweet potato peel to the best of knowledge. It has been reported by Kamalam *et al.* (2012) that the substrate rice bran (3.2 optical density) gave higher production than wheat bran (2.7 O.D) which are also in agreement with the results (Fig.1).

The sweet potato peel powder (8%) gave the maximum specific absorbance (6.2 CVU/g) on 11th day of incubation (Fig.2). So, 8% concentration was considered optimum for growth of pigment by *M. purpureus* MTCC 369 and was used in further experimentation. Nimnoi and Lumyong (2011) observed that corn meal (8%) with glucose enhanced the pigment production.

Screening of nitrogen source from agricultural or

industrial waste and concentration: Among cheap substrate, maximum pigment production was obtained with pea pod (8.7 CVU/g). The supplementation of pea pod was comparable with the peptone (9.0 CVU/g). Pea pod was selected as source of nitrogen because it gave highest pigment yield (Fig.3). The mycelia growth, vegetative growth and reproduction of fungus depends upon the ability to assimilate organic sources of nitrogen, nitrates etc (Ajdari *et al.*, 2011; Kaur *et al.*, 2013; Singh *et al.*, 2015). The effect of pea pod concentration (1-5%) was tested using sweet potato peel powder (8%) and salt solution (2 ml) at pH 6.5. The results in Fig.4 indicated that the concentration of pea pod (4%) showed maximum specific absorbance (9.5 CVU/g) as compared to other concentrations. So the pea pod concentration (4%) was used in further experimentation. According to Silveira *et al.* (2008), concentration of nitrogen substrate upto 3.5% exhibited a positive influence on biopigment production with grape as substrate.

Optimization of Process Parameters

Effect of particle size analysis: Fermentation media with particle size (0.25 mm, mesh size 60 μ m) was optimal for pigment production with the maximum specific absorbance (10.9 CVU/g) (Fig.5). This might be due to the presence of carbohydrates in higher amount compared to other substrate as sweet potato peel contain 65-70% potato peel 58.7%

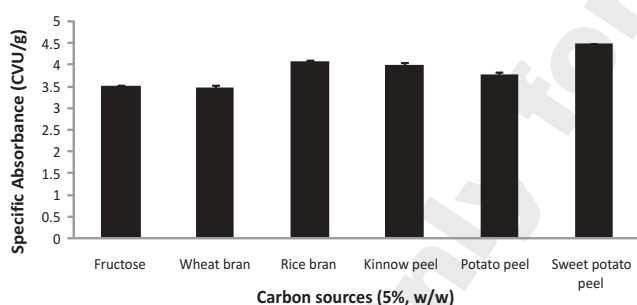


Fig. 1. Effect of different carbon sources on bio-pigment production

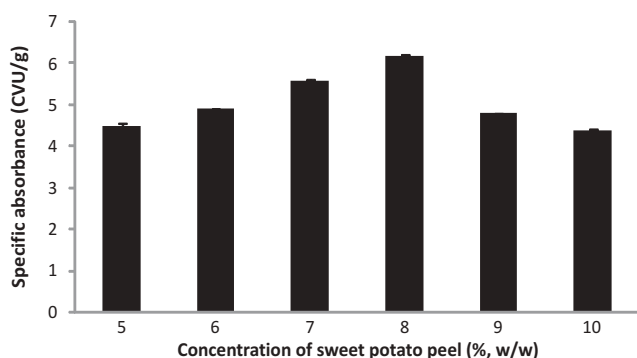


Fig. 2. Effect of sweet potato peel powder concentration on bio-pigment production

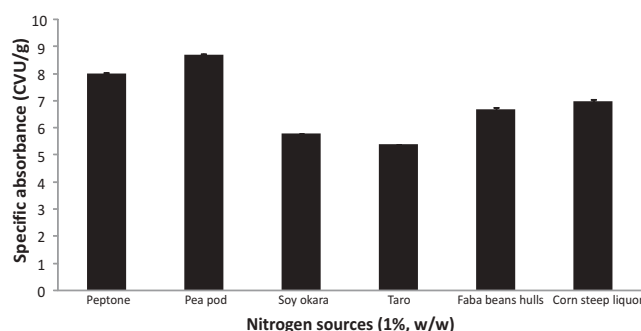


Fig. 3. Effect of different nitrogen sources on bio-pigment production

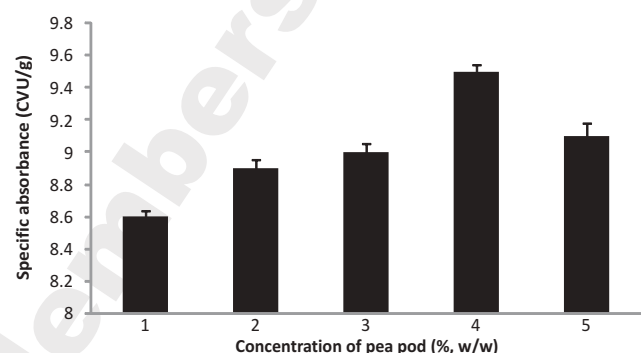


Fig. 4. Effect of pea Pod concentration on bio-pigment production

(Arapoglou *et al.*, 2009) and kinnow peel has (50-60%) because sugar and starch generally provide the energy for growth and secondary metabolism. In further experiments, this particle size of sweet potato peel powder was used for production of pigment. Generally, smaller particle size of substrate will enhance the surface area for growth of microbes. however, lump formation of substrate occur for too small particles size which might interfere with air circulation process and result in poor growth. Large particles provide aeration efficiency but may limit the surface area for the microbial growth. So, compromised particle size is necessary for microbial growth (Pandey *et al.*, 2000). Previous studies reported that 0.4-0.6 mm (jackfruit seed powder) particle size was optimal for pigment production under solid state fermentation (Babitha *et al.*, 2006). The particle size 0.8-2.0 mm for cassava bagasse gave pigment production (15.7AU/g) using solid state fermentation (Carvalho *et al.*, 2007).

Effect of moisture content: In solid state culture, moisture content of the source has a substantial influence on growth and metabolite production (Pandey, 1991). For the experiment distilled water was added (5-30 ml depending upon the desired moisture content) in the media. The moisture content was varied from 50-70%. It was observed that maximum pigment production was obtained at 55%

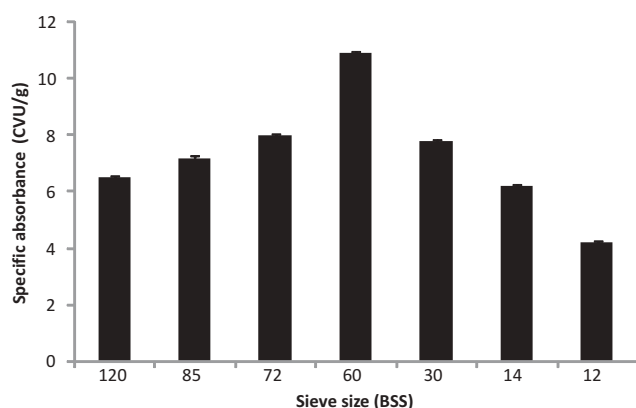


Fig. 5. Effect of particle size on bio-pigment production

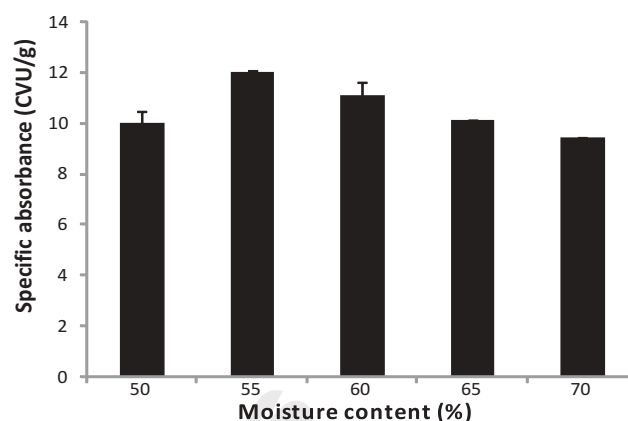


Fig. 6. Effect of moisture content on bio-pigment production

initial moisture content (Fig.6). Too high a moisture content may reduce substrate porosity and mass transfer of oxygen. While too low moisture content resulted in reduced accessibility of nutrients to the fungus, it may be happened due to the reduction in dissolution of nutrients into the media and less heat exchange and oxygen-transfer rate (Babitha *et al.*, 2007; Pandey, 2003). In most cases, fungal growth requires moisture in the range of 30-75%. The moisture content in the range of 50-60% has been reported to be good for pigment production with *Monascus* strain (Babitha *et al.*, 2007). 56% moisture content and pH 6 gave optimum pigment production by *Monascus purpureus* FRR using rice as substrate (Johns and Stuart, 1991).

Effect of salt solution concentration: Salt solution containing potassium dihydrogen phosphate (0.2g/100ml), ammonia nitrate (0.5g/100ml), sodium chloride (0.1g/100ml), Magnesium Sulphate (0.1g/100ml) was prepared. The media was prepared by adding optimized sweet potato peel (8%) and pea pod (4%), different concentrations (1-5 ml) of salt solution were taken & supplemented in the fermentation medium to find its effect on the pigment production and incubated at 30°C. Maximum pigment formation (14.0 CVU/g) was obtained with 3 ml salt concentration as shown in (Fig.7). It has been reported that the media containing corn meal supplemented with peanut meal gave the pigment production (19.4U/g) when added into 2 ml salt solution and incubated at 30°C for 14 days (Nimnoi and Lumyong, 2011). 10 g of different substrate like jackfruit seed powder, grape seed powder, soybean powder, wheat bran, rice bran, rice, and a salt solution (4 ml) were inoculated with the spore suspension containing 1×10^6 spores/ml of *Monascus ruber* and incubated at 30°C for 14 days (Kamalam *et al.*, 2012).

Effect of pH : Increase in pigment formation was observed in the range of 4.5-5.5 pH (Fig.8) and maximum production (15.7 CVU/g) at pH 5.5. Any change in media alkalinity or acidity hinders the growth of microorganism, thereby affects biopigment production. Growth has been reported over a

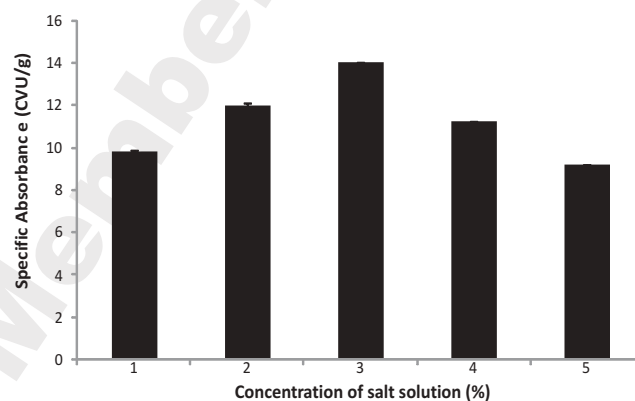


Fig. 7. Effect of salt solution on bio-pigment production

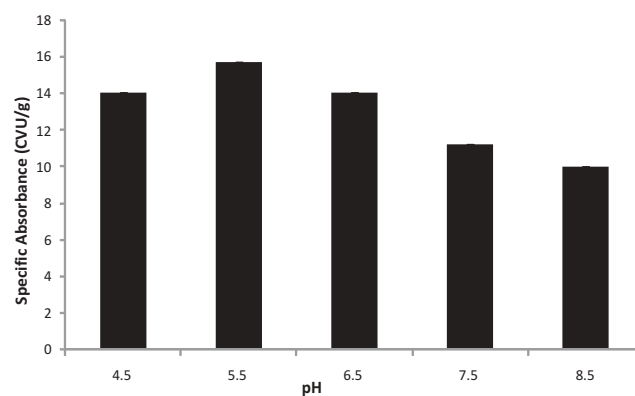


Fig. 8. Effect of pH on bio-pigment production

broad range of pH from 2.5 to 8.0 and optimal in between 4.0-7.0. Unless pH is controlled, the pH profile during cultivation depends significantly on the nitrogen source and to a lesser extent on carbon source used (Yongsmith *et al.*, 1993). At lower pH yellow pigments formation predominate whereas at higher pH red pigment was generated. Production of yellow pigments has been found to be increase with increasing pH until pH 5.5 (Mak *et al.*, 1990).

Effect of temperature: The effect of temperature on

biopigment formation was studied with sweet potato peel (8%) and pea pod (4%) at temperature range of 25-40°C for 11 days (Fig.9). The results indicated that optimum pigment growth was observed at 30°C temperature with specific absorbance (15.7 CVU/g). As temperature affects the activity of one or more enzymes involved in biopigment synthesis and it might be optimum at range of 28-32°C (Dikshit and Tallapragada *et al.*, 2011). Temperature affects the rate of biochemical reactions therefore affects the rate of biomass growth and product formation (Pirt, 1975). Optimal conditions for *Monascus* sp. was observed at 32°C at pH 6.0 after incubation of 8 days (Carvalho *et al.*, 2005) in a media containing rice & cassava bagasse. Maximum production of pigment at 30°C has been observed (Babitha *et al.*, 2007). Incubation at 30°C promotes the maximum pigment production with significant differences from other temperature (Nimnoi and Lumyong, 2009).

Effect of incubation time: The effect of incubation period on pigment production using *M. purpureus* MTCC 369 was investigated for 13 days. The samples were taken at regular intervals (3, 5, 7, 9, 11, 13 days) to estimate the optimum incubation period for pigment production. Mycelia growth

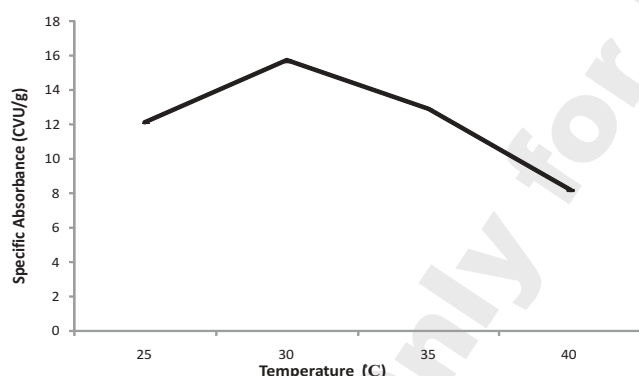


Fig. 9. Effect of temperature on bio-pigment production

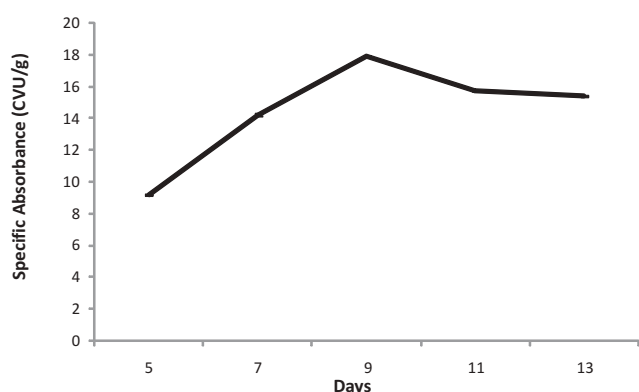


Fig. 10. Effect of incubation time on bio-pigment production

was observed on 3rd day and it continues to accumulate and maximum pigment production was observed on 9th day (17.9 CVU/g) and further pigment production declined (Fig.10). Non-availability of nutrients might have ceased the growth of fungus. Previous studies reported that pigment production by *Monascus* sp. is growth associated (Han and Mudgett, 1992). Optimum fermentation time for solid state fermentation in rice was 7 days (Carvalho *et al.*, 2007). The maximum pigment production was observed at 10th day after incubation using *M. purpureus* MTCC 1090 and *M. purpureus* NFCCI 1756 (Chatterjee *et al.*, 2009; Mukherjee and Singh, 2011). It could be attributed to strain specificity for different incubation time.

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Received 26 August, 2015; Accepted 15 March, 2016



Extent of Natural Parasitisation of Cocoons of *Cotesia marginiventris* (CRESSON), A Larval Parasitoid on *Helicoverpa armigera* (Hübner)

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Abstract: Pod borer, *Helicoverpa armigera* is a major pest on pigeonpea in Telangana State and *Cotesia marginiventris* (Cresson), a braconid is its key natural enemy. However, the efficacy of *C. marginiventris* as a natural enemy can be disrupted by hyperparasitism. Weekly observations on population of the pest and the parasitoid were recorded from their first appearance in the second fortnight October to early December. Cocoons of *C. marginiventris* were collected in late October each year coinciding with peak population of the pest (*H. armigera*) and maximum parasitisation by the braconid wasp. Results revealed that *C. marginiventris*, a dominant primary parasitoid on *H. armigera* caused 30.33% mortality of the pest. But its cocoons were hyperparasitised by three Hymenopterans viz., *Eurytoma braconidis*, *Mesochorus* sp. and *Tetrastichus cotesiae* to an extent 12.07%. Among these, the most dominating genus, *Mesochorus* caused 33.33-100% parasitisation followed by *T. cotesiae* (50.00 %) and *E. braconidis* (0-33.33 %) of the cocoons. This is a first report of the hyperparasitoids of *C. marginiventris* on *H. armigera* in pigeonpea ecosystem in Rajendranagar, Hyderabad.

Key Words: *Cotesia*, Hyperparasitoids, Pigeonpea

Helicoverpa armigera is a major pest on pigeonpea crop in Telangana State and *Cotesia marginiventris* (Cresson), a braconid is its key parasitoid. The braconidae is the second most important family of Hymenopteran parasitoid wasps used in biological control for larval stages of Lepidoptera, Coleoptera and Diptera, and even Hemimetabolous insects (Marchiori and Penteado-Dias, 2002). However, the effectiveness of *C. marginiventris* as a natural enemy can be disrupted by hyperparasitism (Nofemela, 2013). Hyperparasitism is a highly evolved fourth trophic level relationship that exists between entomophagous insects referring to the development of a secondary insect parasitoid or hyperparasitoid at the expense of a primary insect parasitoid (Sullivan and Völkl, 1999). But life-time fecundity and intrinsic rate of increase of hyperparasitoids are generally lower than those of their primary hosts (Sullivan and Völkl, 1999), which offers some hope for natural control of pests. Hyperparasitism can disrupt natural biological control of pests and hence assumes an important role in integrated pest management. Information on hyperparasitoids of *C. marginiventris* in this area is scant. Therefore, the present study was undertaken to understand the degree of hyperparasitism and the composition of the hyperparasitoids of this solitary endoparasitoid wasp.

MATERIAL AND METHODS

The study was conducted for two years from 2010-11 and 2011-12 in five unsprayed pigeonpea fields of seed production plots of the College Farm, Rajendranagar,

Hyderabad. Weekly observations on population of the pest and the parasitoid were recorded from their first appearance in October to early December. Cocoons of *C. marginiventris* were collected in late October each year coinciding with peak population of the pest (*H. armigera*) and maximum parasitisation by the braconid wasp. They were brought to the laboratory and placed in glass vials of length 5cm and diameter 2.5cm to observe for the emergence of *C. marginiventris* or its hyperparasitoids. Adult stages were sent for identification to The National Coordinator, Network Project on Insect Biosystematics, Indian Agricultural Research Institute, New Delhi. Even empty cocoons were collected and observed for parasitisation based on the nature and shape of the exit hole on the cocoon. The shape of the exit hole of primary parasitoids (Fig. 1) was different from that made by hyperparasitoids (Fig. 2). Hofsvang *et al.* (2014) observed that primary parasitoids (Braconidae: Hymenoptera) always made a circular exit hole with smooth edges and in most cases, the lid was still present, while hyperparasitoids made a saw-edged exit hole with the lid missing. Per cent parasitization of cocoons was worked out (Number of cocoons parasitized by a single genus/ Total number of cocoons parasitized) X 100. The extent of hyperparasitism was worked out for the all genera together and also each genus individually.

RESULTS AND DISCUSSION

Results of the present study revealed that *C. marginiventris*, a dominant primary parasitoid on *H.*



Fig. 1. Parasitized cocoons of *C. marginiventris* with irregular emergence holes and without the lids



Fig. 2. Adult of *Eurytoma braconidis*



Fig. 4. Adult of *Tetrastichus cotesiae* sp.



Fig. 3. Adult of *Mesochorus* sp.

armigera caused around 30.33% mortality of the pest. Other parasitoids recorded on the pest were Tachinids, *Goniophthalmus halli* (Mesnil) and *Senometopia* (Eucarcelia) *illota* (Curran) and an Ichneumonid, *Campoletis chloridae*, which parasitized 48.9, 3.6 and 17.3% of *H.armigera* larvae respectively. However, cocoons of *C.marginiventris* were hyperparasitised by three Hymenopterans to an extent 12.07%. The three secondary parasitoids (Fig. 3-5) were identified as *Eurytoma braconidis* (Hymenoptera: Eurytomidae), *Mesochorus* sp. (Hymenoptera: Ichneumonidae) and *Tetrastichus cotesiae* (Hymenoptera: Eulophidae). Among these, *Mesochorus* caused 33.33-100% parasitisation, *T. cotesiae* upto 50.00%, while *E. braconidis* parasitized 0-33.33% of the cocoons (Fig.6.). There could be heavy competition between these three hyperparasitoids for the host insect resources since all of them co-existed in the field throughout the observation

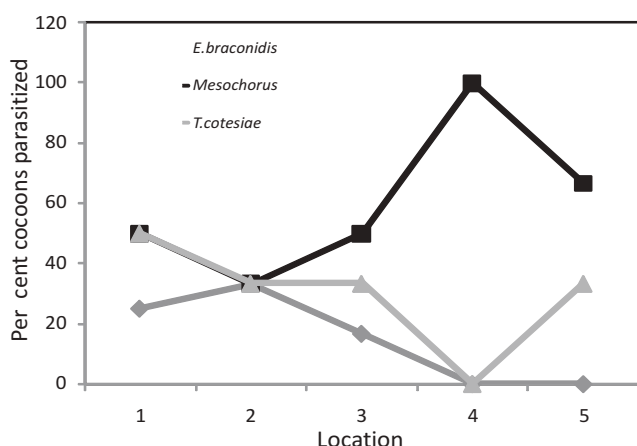


Fig. 5. Extent of parasitisation on *C. marginiventris* in different experimental fields

period. It was observed that from each mass of silk cocoons of *C. marginiventris*, only one genus of hyperparasitoids emerged i.e., parasitisation of cocoons was in an 'all or none' manner and no sharing of the cocoon masses between the different genera was observed. This is a first report of the hyperparasitoids of *C. marginiventris* on *H. armigera* in pigeonpea ecosystem in Rajendranagar, Hyderabad. Bouchier and Nealis (1992) recorded 50-90% mortality of *Cotesia melanoscela* by 16 hyperparasitoids interfering with the biological control of the pest, *Lymantria dispar* on trees in North America. Nofemela (2013) recorded *Mesochorus* sp. (Ichneumonidae) and *Eurytoma* sp. (Eurytomidae) as Hyperparasitoids on *Cotesia vestalis*, a primary parasitoid on diamond back moth (*Plutella xylostella*) on cabbage in South Africa.

The present study was aimed at understanding the

extent of parasitisation of *C. marginiventris* in the pigeonpea ecosystem. This information is required to know the level of natural control offered by *C. marginiventris*. Hyperparasitoids reduce host numbers and in this case delay natural biological control of the pest. They could be the main hurdles for successful establishment of the bioagent in new areas where it is introduced. Hence, indiscriminate introduction of any parasitoid into an area should be avoided because generalist hyperparasitoids could render the primary bioagent ineffective. In depth studies on the quantitative impact of the hyperparasitoids on parasitisation of *H. armigera* by *C. marginiventris* are underway and they could throw some more light on the effectiveness of *C. marginiventris* as a bioagent.

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Monitoring of Alate Mustard Aphid, *Lipaphis erysimi* Kalt on Yellow Sticky Traps

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Abstract: The alate mustard aphid (*Lipaphis erysimi* Kalt.) population was monitored for three years using sticky traps. The first appearance of alate aphid was recorded as early as in the 46th standard week during 2009-10, the 49th standard week during 2010-11 and latest in the 47th standard week during 2011-12. The initial average population ranged from 0.2 to 0.6 aphids per trap and reached to peak variably depending upon the weather parameters from 7th to 10th standard week in different years of study.

Key Words: Alate mustard aphid, yellow sticky trap, weather parameters

Rapeseed and mustard crops are highly vulnerable to the mustard aphid (*Lipaphis erysimi* Kalt.) that is the key pest. Alate forms determine the initiation of infestation and intensity of attack on the *Brassica* crops. The aphid multiplies gradually and reaches to a peak during December to March in different agro-ecological regions of the country. Kulta *et al.* (1997) found that in Nagpur, a combination of ambient maximum temperature (26.4 to 29°C), minimum temperature (8.4 to 12.6°C) and high relative humidity ranging from 75 to 85% in the month of January favoured aphid multiplication, whereas the activity of the aphid ceased at relative humidity of 65% and below.

Singh *et al.* (2012) reported average temperature 23.3 to 34.3°C (maximum) and 7.3 to 15.7°C (minimum) coupled with 64 to 74% relative humidity was found effectively for aphid moment. For effective and economical management of this serious pest in eastern Rajasthan, the alate population was observed and correlated to climate factors prevailing during the study period.

MATERIAL AND METHODS

The experiment was conducted at Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur (Rajasthan) for three consecutive years i.e. from 2009-10 to 2011-12. Traps were made from empty tin boxes of 1kg capacity and painted with yellow colour paint to attract the alate aphids. These traps were placed with the help of iron pole at the height of 2.0 meters above the ground level. The traps were fixed on the bunds/boundaries at the four side's viz. East, west, north and south directions of the field. A thin layer of transparent grease was traps were observed daily at 10AM for the count of alate aphids, which adhered on these traps. Daily weather parameters were also recorded from the

Directorate of Rapeseed-Mustard Research observatory situated near the sticky traps installed and weekly means were worked out. The observation was recorded from October to August regularly on yellow sticky traps.

Population data of different insect pests thus obtained were subjected to statistical analysis to find out the coefficient of correlation and multiple regression line with average temperature (X_1), relative humidity (X_2) and rainfall (X_3) which were obtained from the Meteorological Observatory, Directorate of Rapeseed-Mustard Research, Sewar. Following formula was used for calculating correlation coefficient:

$$r_{xy} = \frac{\sum XY - \frac{\sum X \sum Y}{n}}{\sqrt{\left[\sum X^2 - \frac{(\sum X)^2}{n} \right] \left[\sum Y^2 - \frac{(\sum Y)^2}{n} \right]}}$$

Where,

r_{xy} = Simple correlation coefficient

X = Variable i.e. abiotic component.
(Average temperature, relative humidity and rainfall, etc.)

Y = Variable i.e. mean number of insect pests

n = Number of paired observations

The correlation coefficient (r) values were subjected to the test of significance using t-test:

$$t = \frac{r}{\sqrt{1-r^2}} \times \sqrt{n-2}$$

The calculated t-value obtained was compared with tabulated t-value at 5% level of significance.

RESULTS AND DISCUSSION

The alate mustard aphid differed in the timing of

their appearance during the 3years study. The alate aphid population initiated in the 46th SW (2008-09), 46th SW (2010-11) and 47th (2011-12). The corresponding initial populations were 0.0.2, 0.4, and 0.6 aphid traps. Similarly, the peak alate aphid population was recorded during the 7th SW (2009-10) with 530.2 aphid/trap , 8th SW (2010-11) with 36.3 aphid/trap and 10th SW (2011-12) with 550.5 aphid/trap.

The population record clearly indicated an increase every alternate year, but second year decrease population abiotic factor not favour in aphid population. It was evident that abiotic factors had a key role in regulating the aphid population reported the role of temperature and relative humidity on the incidence of mustard aphid (Chakravarty and Gautam, 2002; Gami *et al.*, 2002; Prasad, 2003; Singh and Sharma, 2009; Singh *et al.*, 2012). Average temperature 18 to 26°C (maximum) and 5.3 – 10°C (minimum) coupled 65 to

70% relative humidity was found conducive for rapid multiplication of mustard aphid (Singh *et al.*, 2012). The present study conforms to the earlier work that weather parameters affect appearance and the intensity of mustard aphid population in eastern Rajasthan.

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Table 1. Monitoring of alate mustard aphid on yellow sticky traps 2011-12

Std. Week	Meteorological observation							Mean alate aphid
	Temperature (°C)		Mean RH (%)	Rain fall (mm)	Sun shine (hrs)	Evapo. (mm day ⁻¹)	Wind speed (km hr ⁻¹)	
	Max.	Mini.						
47	27.2	13.8	75.4	0.0	4.9	13.3	1.2	0.6
48	26.3	10.7	68.5	0.0	6.4	10.9	1.3	1.9
49	28.0	12.5	71.4	0.0	6.1	13.4	1.4	4.9
50	23.5	5.4	66.5	0.0	6.8	6.1	1.4	2.2
51	20.5	4.5	73.6	0.0	5.5	4.6	0.6	2.0
52	20.3	2.8	73.8	0.0	5.5	3.3	0.7	1.9
1	18.5	10.2	88.5	24.3	2.8	0.7	1.6	1.2
2	17.1	3.7	73.9	0.0	5.1	2.6	1.5	1.6
3	19.5	5.7	78.1	0.0	5.3	1.0	1.5	3.9
4	20.2	3.6	66.8	0.0	8.4	1.9	2.0	3.6
5	21.0	4.6	63.6	0.0	7.7	2.1	1.4	14.7
6	19.4	5.1	68.7	0.0	7.3	2.5	3.3	28.1
7	22.4	5.7	62.9	0.0	6.3	2.5	2.7	51.8
8	27.8	9.1	63.8	0.0	8.6	3.4	2.8	77.4
9	28.2	8.1	51.9	0.0	8.9	4.4	3.2	197.8
10	29.7	10.2	59.9	0.0	7.2	4.2	3.3	550.5
11	30.5	10.9	52.4	0.0	7.7	5.4	3.8	201.7
12	34.2	12.8	40.1	0.0	7.2	5.9	4.9	32.2
13	36.9	16.5	44.3	0.0	6.6	7.5	4.7	17.6
14	39.3	21.1	39.9	0.0	6.6	7.0	4.0	2.2
Simple correlations (r)	0.256	0.0789	-0.255	-0.106	0.348	-0.998	0.3495	-

Table 2. Monitoring of alate mustard aphid on yellow sticky traps 2010-11

Std. Week	Weather parameter							Mean alate aphid
	Temperature (°C)		RH (%)	Rainfall (mm)	Sunshine (hrs)	Evapo. (mm day ⁻¹)	Wind Speed (km hr ⁻¹)	
	Max.	Mini.						
45	30.2	14.8	62.2	0.0	5.3	2.8	1.9	0.0
46	27.7	18.9	77.7	19.7	0.7	1.9	2.5	0.4
47	23.9	14.4	77.1	13	5.1	1.3	2.5	1.1
48	23.4	10.7	69.8	0.0	7.7	1.6	1.3	1.7
49	22.4	7.4	72.0	0.0	6.7	1.1	0.9	2.6
50	22.2	5.7	65.1	0.0	7.6	1.6	2.1	4.4
51	23.3	4.4	69.2	0.0	8.1	1.4	1.4	6.1
52	20.5	7.5	80.1	1.8	4.0	0.9	1.7	7.3
1	14.8	2.2	89.9	0.0	3.5	0.7	2.4	1.5
2	17.6	3.2	76.3	0.0	5.2	1.0	1.7	2.1
3	21.1	3.3	67.4	0.0	8.5	2.0	3.0	6.2
4	22.1	4.5	70.0	0.0	7.4	1.7	2.5	7.0
5	22.5	4.9	71.5	0.0	7.5	1.5	3.1	8.1
6	26.6	7.6	67.1	0.0	8.8	2.6	1.9	9.5
7	24.4	10.2	73.7	2.0	7.2	1.9	6.1	22.9
8	23.3	9.1	73.5	1.0	8.2	1.9	5.5	36.3
9	24.9	10.9	71.2	1.0	6.7	2.5	2.9	22.6
10	28.2	10.6	71.0	0.0	8.4	4.3	3.3	18.4
11	32.0	12.1	73.7	0.0	8.7	4.4	3.5	16.6
12	34.7	14.6	65.9	0.0	7.4	5.3	2.8	9.7
13	35.9	16.6	45.0	0.0	8.4	6.6	4.3	3.8
Simple correlations (r)	0.120	0.018	0.054	-0.219	0.425	0.173	0.726	-

Table 3. Monitoring of alate mustard aphid on yellow sticky traps 2009-10

Std. Week	Weather parameter							Mean alate aphid
	Temperature °C		RH (%)	Rainfall (mm)	Sun Shine (hrs)	Evapo. (mm day ⁻¹)	Wind Speed (km hr ⁻¹)	
	Max.	Mini.						
46	24.3	17.3	82.6	4.0	1.1	1.4	3.0	0.2
47	25.3	8.8	61.8	0.0	8.3	1.4	1.5	1.0
48	26.2	8.6	64.8	0.0	6.9	1.2	0.5	4.4
49	25.8	9.4	68.2	0.0	6.8	1.0	0.4	9.4
50	22.8	11.2	74.3	1.0	2.1	1.0	0.6	4.0
51	21.6	6.6	69.9	0.0	6.3	0.9	0.6	9.4
52	22.6	5.1	72.2	0.0	6.2	1.0	0.2	6.4
1	17.9	5.8	84.5	0.0	4.9	1.0	0.1	5.6
2	14.3	6.0	91.0	10.0	1.1	0.8	0.3	4.2
3	14.9	5.4	91.0	0.0	3.3	0.7	0.5	11.8
4	21.8	7.8	81.5	0.0	5.0	0.6	0.3	134.4
5	24.5	6.7	71.0	0.0	7.9	1.2	1.0	310.6
6	25.7	10.2	73.0	2.0	6.2	1.5	2.3	492.8
7	24	10.0	74.0	0.0	5.5	1.3	1.5	530.2
8	28.1	9.4	64.0	1.5	9.0	1.8	2.2	111.4
9	33.2	13.3	57.5	0.0	8.7	2.6	2.1	63.6
10	30.4	12.0	60.5	0.0	7.2	2.8	2.9	52.8
11	34.6	13.8	57.5	0.0	8.2	3.4	2.2	8.8
12	39.7	16.2	41.5	0.0	8.3	4.7	3.6	1.0
13	40.1	19.8	33.5	0.0	7.9	5.0	3.8	0.8
Simple correlations (r)	0.320	0.035	-0.185	-0.041	0.387	-0.063	0.271	-

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Received 16 October, 2015; Accepted 25 February, 2016



Bio-Efficacy of Newer Insecticides and Biopesticides against Thrips, *Thrips tabaci* in Garlic

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Abstract: The present study was conducted to find out the effective and alternate management of thrips in garlic crop during rabi, 2011-12. All the treatments were significantly effective against garlic thrips, *Thrips tabaci* as compared to control. On the mean basis, the maximum reduction in thrips population was recorded in imidacloprid 17.8 SL followed by acetamiprid 20 SP and fipronil 5 SP and these were statistically at par with each other in their efficacy. The treatments of malathion and spinosad were found moderately effective with 70.58 and 67.03% reduction, respectively in thrips population. The treatments of azadirachtin 0.03 EC, *B. bassiana* (1 kg ha⁻¹) and neem seed kernel extract (NSKE) (5 ml lit.⁻¹) were found least effective, which exhibited 55.86, 51.69 and 41.00% reduction, respectively in thrips population, however, NSKE was significantly superior over azadirachtin. The maximum clove yield of 260 q ha⁻¹ was obtained in the plots treated with imidacloprid followed by acetamiprid (257 q ha⁻¹) and fipronil (254 q ha⁻¹).

Key Words: Garlic, *Thrips tabaci*, IPM, Neonicotinoids, Azadirachtin

India ranks second after China in area (230.5 lakh ha) and third in production (1251.88 thousand metric tonnes) of garlic with an average productivity of 5.43 t ha⁻¹ (www.nhb.gov.in). The major garlic producing states of the country are Madhya Pradesh, Orissa, Rajasthan, Karnataka and Gujarat. In Rajasthan, garlic is extensively grown in 45.02 lakh ha with an annual production of 218.42 lakh tonnes (www.nhb.gov.in). The insect pests viz., thrips, *Thrips tabaci* Lindeman; maggot fly, *Delia antiqua* (Meigen); tobacco caterpillar, *Spodoptera litura* (Fabricius); caliothrips, *Caliothrips indicus* (Bangall) are responsible for low productivity of garlic (Rajavel and Suresh, 2007). Among them thrips (*T. tabaci*) is the most important pest causing 15.30 to 46.82% yield loss (Mishra *et al.*, 2014). Chemical insecticides are only the alternative that works as frontline defense sources to combat the problem of any insect pests in any crop. The insecticidal recommendations have been made by various workers from time to time against thrips on garlic crop (Bhargava and Bhatnagar, 2005; Kumar and Ameta, 2005) but, their wide scale application creates problems like development of resistance and resurgence of pest, besides causing environmental pollution. Keeping these points in view and production of quality crop, the investigation was undertaken to test the bio-efficacy of newer safe insecticide molecules and bio-pesticides against thrips on garlic crop.

MATERIAL AND METHODS

The present investigations were conducted at S.K.N. College of Agriculture, Jobner (Rajasthan) on garlic crop during Rabi, 2011-12. Jobner is located at longitude of

78°28' East latitude, 26°26' North and at an elevation of 427 meter from mean sea level (MSL) in Jaipur district of Rajasthan. The experiment was laid out in a simple randomized block design with nine treatments including untreated control (Table 1.) and each replicated thrice. The local variety of garlic was sown on 29th October, 2011 in plot size of 1.5 x 1.5 m², keeping row to row and plant to plant distance of 15 and 10 cm, respectively. The recommended package of practices was followed to raise the crop.

All the insecticides/biopesticides were applied as a foliar spray. Sandovit at one ml per litre of spray solution was also used as surfactant. The spraying was done using a pre-calibrated knapsack sprayer. The first insecticidal spray was given on 22nd February 2012 and the second after 20 days of first spray. The quantity of spray solution used was 600 liters per hectare in each spray application. The observations on thrips population were recorded at one day before and 1, 3, 7 and 10 days after application of insecticides in both the sprays. The clove yield of garlic was recorded on whole plot basis. The data thus obtained were corrected for mortality in untreated plots by calculating the per cent reduction over untreated plots.

RESULTS AND DISCUSSION

One day after treatment (DAT), all the insecticides reduced the thrips population significantly over untreated control, however, there existed a considerable difference among them. The maximum reduction of 85.45% in thrips population was in imidacloprid (0.005%) followed by acetamiprid (0.005%) and fipronil (0.001%) and are statistically at par with each other. The treatment of malathion

Table 1. Details of treatments used

Common Name	Trade name	Formulation	Concentration (%) or dose
Imidacloprid	Confidor	17.8 SL	0.005
Acetamiprid	Pride	20 SP	0.005
Fipronil	Regent	5 SC	0.001
Spinosad	Success	2.5 SL	0.009
Beauveria bassiana	Racer-BB	1.15 WP	2 g l ⁻¹
Azadirachtin	Nimbecidine	0.03 EC	5 ml l ⁻¹
NSKE	-	-	5 ml l ⁻¹
Malathion	Cythion	50 EC	0.05
Control	-	-	-

NSKE: neem seed kernel extract

and spinosad were moderately effective against thrips. The azadirachtin, *B. bassiana* and NSKE were found least effective. At 3 DAT, all the treatments reduced thrips population significantly over untreated control. The maximum reduction of 92.25% in thrips population was recorded in imidacloprid followed by acetamiprid and fipronil and these were statistically at par with each other and significantly superior over rest of the treatments. Azadirachtin, *B. bassiana* and NSKE were found least effective, which exhibited 50-65.16% reduction, respectively in thrips population; however, NSKE differed significantly with both the treatments. Similar trend of per cent reduction in thrips population was observed after seven days of insecticidal application.

At 10 DAT of insecticidal application, the maximum reduction in thrips population was imidacloprid (73.41%) followed by acetamiprid and fipronil, which resulted in 71.22 and 69.44 per cent reduction, respectively and these were found statistically at par with each other in their efficacy. The treatments of malathion and spinosad gave 60.50 and 56.94% reduction in thrips population, respectively and were found at par with each other. Azadirachtin, *B. bassiana* and NSKE were least effective.

The order of effectiveness of insecticides on the basis of mean per cent reduction of thrips population was imidacloprid (0.005%) > acetamiprid (0.005%) > fipronil (0.001%) > malathion (0.05%) > spinosad (0.009%) > azadirachtin (5.0 ml l⁻¹) > *B. bassiana* (2.0 g l⁻¹) > NSKE (5.0 ml l⁻¹). The results are in agreement with Verma *et al.* (2012); Naruka *et al.* (2010)

After second spray, the data revealed (Table 2) that all the insecticidal treatments reduced thrips population significantly over untreated control 1 DAT. The maximum reduction of 88.15% in thrips population was in imidacloprid followed by acetamiprid and fipronil and these were found statistically at par with each other in their efficacy. Azadirachtin, *B. bassiana* and NSKE were found least

effective. The non-significant difference was between azadirachtin and *B. bassiana* and both were significantly better than NSKE. The per cent reduction in thrips population 3 and 7 DAT followed the same trend as in first spray. AT 10 DAT, imidacloprid gave maximum reduction of 78.69% in thrips population followed by acetamiprid and fipronil. Azadirachtin, *B. bassiana* and NSKE were least effective with non-significant differences. On the mean basis, the maximum reduction of 88.05% was observed in imidacloprid followed by acetamiprid and fipronil with 82.26-85.06% reduction. The present findings corroborate with that of Gajera *et al.* (2010) and Joshi *et al.* (2010) who found spinosad given most effective against thrips in garlic and they also reported that use of *B. bassiana* was shown least effective.

All the treatments showed significant increase in yield over untreated control. Highest mean clove yield of garlic was recorded in imidacloprid (260 q ha⁻¹) followed by acetamiprid (257 q ha⁻¹) and fipronil (254 q ha⁻¹) and these were found statistically at par with each other. The mean clove yield of 247 and 244 q ha⁻¹ recorded in malathion and spinosad treated plots, respectively, whereas, the lowest yield was observed in untreated control (223 q ha⁻¹). Similarly, Kumawat (2009) and Verma *et al.* (2012) reported the higher clove yield of garlic due to imidacloprid and fipronil treatments whereas, Joshi *et al.* (2010) and Gajera *et al.* (2010) recorded highest clove yield in spinosad and *B. bassiana* treated plots. Rajavel and Suresh (2007) recorded highest clove yield of garlic in the NSKE treated plots and these results contrary to the present findings in garlic. The highest benefit cost ratio was noticed in fipronil and acetamiprid treatments followed by imidacloprid and malathion treatments though spinosad recorded higher seed yield compared to other treatments however, this treatment combination has given low benefit cost than others due to high cost of the insecticide.

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Table 2. Bioefficacy of newer insecticides and biopesticides against thrip (*T. tabaci*) on garlic crop during Rabi, 2011-12

Treatments	Conc. (%) / dosage	Per cent reduction in thrips population days after spray										Clove yield (q ha ⁻¹)
		I st Spray					II nd Spray					
		1	3	7	10	Mean	1	3	7	10	Mean	
Imidacloprid 17.8 SL	0.005	85.45 (67.58)	92.25 (73.84)	80.21 (63.59)	73.41 (58.96)	82.33 (65.52)	88.15 (69.86)	94.65 (76.00)	90.71 (72.25)	78.69 (62.51)	88.05 (69.78)	260
Acetamiprid 20 SP	0.005	83.22 (65.82)	90.35 (71.90)	78.28 (62.22)	71.22 (57.56)	80.77 (63.99)	85.25 (67.41)	92.39 (73.99)	87.18 (69.02)	75.41 (60.27)	85.06 (67.26)	257
Fipronil 5 SP	0.001	80.16 (63.55)	88.47 (70.15)	76.41 (60.94)	69.44 (56.44)	78.62 (62.46)	82.10 (64.97)	90.18 (71.74)	84.15 (66.54)	72.61 (58.44)	82.26 (65.09)	254
Spinosad 2.5 SL	0.009	70.22 (56.93)	77.40 (61.61)	63.54 (52.86)	56.94 (50.14)	67.03 (54.96)	76.45 (60.97)	83.80 (66.27)	76.16 (57.54)	62.20 (51.27)	74.65 (59.77)	244
Beauveria bassiana 1.15 WP	2 g l ⁻¹	56.31 (48.63)	61.64 (51.73)	54.67 (47.45)	34.15 (35.76)	51.69 (45.97)	60.53 (51.08)	67.15 (55.03)	63.41 (52.21)	42.10 (40.45)	58.30 (49.78)	232
Azadirachtin 0.03 EC	5 ml l ⁻¹	60.74 (51.20)	65.16 (53.82)	58.69 (50.00)	38.91 (38.59)	55.86 (48.37)	63.22 (52.67)	70.19 (56.91)	67.92 (55.80)	45.10 (42.19)	61.61 (51.71)	236
NSKE	5 ml l ⁻¹	41.16 (39.91)	50.05 (45.03)	42.57 (40.73)	30.21 (33.34)	41.00 (39.82)	46.31 (42.83)	59.10 (50.24)	54.31 (47.47)	35.66 (36.67)	48.85 (44.34)	227
Malathion 50 EC	0.05	73.15 (58.79)	80.15 (63.54)	68.51 (55.86)	60.50 (51.06)	70.58 (57.15)	79.90 (63.36)	86.23 (68.22)	78.65 (61.16)	67.50 (55.24)	78.07 (62.08)	247
S.Em ±	-	2.03	2.24	2.12	1.84	1.47	2.28	2.67	1.93	2.09	1.53	2.16
CD (p=0.05)	-	5.08	5.70	5.34	4.50	4.40	5.83	5.97	5.41	4.80	4.60	6.49

Figures in the parentheses are angular transformation values

Table 3. Assessment of losses caused by thrip (*T. tabaci*) on garlic crops and increase in yield over untreated control during Rabi 2011-12

Treatments	Concentration (%) /dosage	Mean yield (q ha ⁻¹)	Total avoidable losses (q ha ⁻¹)	% avoidable losses	Total increase in yield over control (q ha ⁻¹)	% increase yield in over control	Cost of increased yield (RS)	Total cost of management (Rs)	Net profit Rs ha ⁻¹	Benefit cost ratio
Imidacloprid 17.8 SL	0.005	260*	0.00	0.00	37	16.59	55500	1878	53622	29.55
Acetamiprid 20 SP	0.005	257	3.00	1.15	34	15.25	51000	1680	49320	30.36
Fipronil 5 SC	0.001	254	6.00	2.31	31	13.90	46500	1512	44988	30.75
Spinosad 2.5 SL	0.009	244	16.00	6.15	21	9.42	31500	14784	16716	2.13
Beauveria bassiana 1.15 WP	2 g l ⁻¹	232	28.00	10.77	9	4.04	13500	2004	11496	6.77
Azadirachtin 0.03 EC	5 ml l ⁻¹	236	24.00	9.23	13	5.83	19500	2760	16740	7.07
NSKE	5 ml l ⁻¹	227	33.00	12.69	4	1.79	6000	2160	3840	2.78
Malathion 50 EC	0.05	247	13.00	5.00	24	10.76	36000	1320	34680	27.27
Control	-	223	37.00	14.23	0.0	0.00	0.00	0.00	0.00	0.00

*Cost of garlic cloves= Rs.1500 q⁻¹; Labour charges @ 120 day⁻¹ labour⁻¹Cost of insecticides per two applications ha⁻¹=Imidacloprid (Rs.918); Acetamiprid (Rs.720); Fipronil (Rs. 552); Spinosad (Rs.13824); Beauveria bassiana (Rs.1044); Azadirachtin (Rs.1800); NSKE (Rs.1200); Malathion (Rs.360)

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Received 22 January, 2016; Accepted 10 May, 2016



Role of Abiotic Factors in Population Fluctuation of Mango Hopper (*Amritodus atkinsoni* Leth.)

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Abstract: The population of mango hopper, *Amritodus atkinsoni* was higher during flower initiation or panicle emergence to the stone-sized fruits stage. Highest population of hopper (8.46 hoppers panicle⁻¹) was observed on 14th standard week (SW) coinciding with stone sized fruit stage. Elaborate role of ELT and weather parameter was positively ($r = 0.594$) influenced by maximum temperature, sunshine and evaporation and negatively ($r = -0.594$) by evening relative humidity, wind velocity and rainfall. Higher population of hopper was observed on 14th SW coinciding with stone sized fruit stage of the crop.

Key Word: Hopper, *Amritodus atkinsoni* Leth. Abiotic factor, Population dynamics

Mango is known as king of fruits not only in the Indian sub-continent but also the world over. According to the Indian mythology, it is considered to be a transformation of God's own creation. It has also been described as "Kalpa-Vriksha" i.e. wish granting tree in the Vedic literature. Its leaves, flowers and wood are indispensable to religious ceremonies and often used as offerings to the various deities. The Mango, *Mangifera indica* (Linn) is the most important member of family *Anacardiaceae*. It is regarded as the delicious fruits and is one of the important fruit crops in tropical and subtropical regions of the world. Mango is cultivated in about 87 countries. India has third position in mango production in the world, next to Brazil and USA. It grows equally well under tropical and subtropical climates. It is utilized at all stages of its development i.e. from immature to the mature stage. The unripe fruit are used for making pickles and chutney. Mango juice is served as a soft drink. Ripe fruits are used in preparing squash, jam, custard powder toffees etc. The seeds of mango are used for medicinal purpose. The wood is used in many ways like timber and furniture. Mango is grown in almost all stages of India and comprises about 42 percentage area under fruit. Several insect- pests cause a considerable damage to mango crop every year. Among the mango hopper, *Amritodus atkinsoni*, *Idioscopus clypealis* and *I. niveosparsus* are one of the most limiting factors in mango cultivation. Damage is caused by nymph and adult by sucking sap from inflorescences as a consequence of which inflorescence and fruit if set, fall prematurely. Excessive cell sap sucking from buds and splitting of leaf midrib leads to shrivelling and dryness of the bud and leaves get curling, respectively.

MATERIAL AND METHODS

A field experiment was conducted at Regional Horticultural Research Station, Navsari Agricultural University, Navsari, Gujarat during 2009-11 on twelve randomly selected 15 year old mango (cv. Kesar) trees in 1 ha. Orchard, which was kept free from insecticide application. Ten terminal twigs and panicles were selected randomly from lower canopy of each tree during vegetative (April-December) and flowering stages (January-March) at weekly interval for counting mango hopper population (nymphs and adults or both), respectively. Important meteorological data viz., temperature (maximum and minimum), relative humidity (morning and evening), rainfall, rainfall days, sun shine and wind velocity were recorded at weekly interval during October 2009- June 2011.

RESULT AND DISCUSSION

The result indicated significant difference in the incidence of hopper at different periods of mango tree stages. The hopper population was maximum in 14th (2-8 April) standard weeks (SW) while its higher activity ($> \text{ETL} = 5$ hoppers twig⁻¹ or panicle) was observed during 9-17 SW (26 February – 29 April) (5.20-7.30 per panicle) in 2009-10, 11-16 SW (12 March – 22 April) (5.02 – 6.42) in 2010-11 and 10-17 SW (5.20 – 6.14) in pooled results. However, lower activity (< 1 hopper per twig or panicle) of the pest was noticed during 23-29 SW (4 June – 22 July) in 2009-10 and 2010-11, while in pooled results, 23-28 SW (4 June – 15 July) had the lowest population to the tune of 0.18, 0.16 and 0.21 hoppers twig⁻¹ during 26 (25 June – 1 July), 27 (2-8 July) and 26 SW in respective years and pooled results, respectively (Table 1 and Fig. 1).

In the entire period of study, the higher population of

Table 1. Seasonal abundance of mango hopper

Std. week	Std period	Crop stage	Hoppers/twigs or panicle		
			2009-10	2010-11	Pooled
48	26 Nov- 2 Dec 2009	Bud/bud burst	2.30	2.12	2.21
49	3-9 Dec	Bud/bud burst	2.18	2.04	2.11
50	10-16 Dec	Bud/bud burst	1.85	1.54	1.70
51	17-23 Dec	Bud/bud burst	1.52	1.32	1.42
52	24-31 Dec	Bud/bud burst	1.30	1.20	1.25
1	1-7 Jan 2010	Flowering	2.00	1.85	1.93
2	8-14 Jan	Flowering	2.30	2.02	2.16
3	15-21 Jan	Flowering	2.52	2.12	2.32
4	22-28 Jan	Flowering	2.67	2.28	2.48
5	29 Jan- 4 Feb	Peak flowering	2.73	2.35	2.54
6	5-11 Feb	Peak flowering	2.92	2.67	2.80
7	12-18 Feb	Pea/Marble	3.33	3.12	3.23
8	19-25 Feb	Pea/Marble	4.62	3.83	4.23
9	26 Feb-4 March	Pea/Marble	5.20	4.12	4.66
10	5-11 March	Pea/Marble	5.58	4.82	5.20
11	12-18 March	Stone size	5.82	5.02	5.42
12	19-25 March	Stone size	6.12	6.96	6.54
13	26 March-1 Apr	Stone size	6.56	9.72	8.14
14	2-8 Apr	Stone size	6.78	10.13	8.46
15	9-15 Apr	Stone size	8.82	7.52	8.17
16	16-22 Apr	Stone size	8.21	6.42	7.32
17	23-29 Apr	Stone size	7.30	4.97	6.14
18	30 Apr-6 May	Stone size	4.23	3.15	3.69
19	7-13 May	Fruiting	3.33	2.18	2.76
20	14-20 May	Fruiting	2.12	2.10	2.11
21	21-27 May	Ripening	1.82	1.84	1.83
22	28 May-3 June	Rip/Harvest	1.44	1.12	1.28
23	4-10 June	Harvest	0.98	0.95	0.97
24	11-17 June	Harvest	0.45	0.66	0.56
25	18-24 June	Vegetative	0.32	0.42	0.37
26	25 June-1 July	Vegetative	0.18	0.24	0.21
27	2-8 July	Vegetative	0.35	0.16	0.26
28	9-15 July	Vegetative	0.74	0.47	0.61
29	16-22 July	Vegetative	0.92	0.86	1.12
30	23-29 July	Vegetative	1.24	1.31	1.48
31	30 July-5 Aug	Vegetative	1.82	1.72	2.06
32	6-12 Aug	Vegetative	2.46	2.30	2.47
33	13-19 Aug	Vegetative	2.54	2.48	2.64
34	20-26 Aug	Vegetative	2.69	2.74	2.94
35	27 Aug-2 Sep	Emerge new flush	3.16	3.18	3.20
36	3-9 Sep	Emerge new flush	3.28	3.24	3.33
37	10-16 Sep	Emerge new flush	3.48	3.38	3.47
38	17-23 Sep	Emerge new flush	3.72	3.46	3.62
39	24-30 Sep	Emerge new flush	3.80	3.52	3.74
40	1-7 Oct	Emerge new flush	3.62	3.68	3.58
41	8-14 Oct	Emerge new flush	3.84	3.54	3.67
41	15-21 Oct	Emerge new flush	3.92	3.50	3.18
43	22-28 Oct	Emerge new flush	3.78	2.44	3.04
44	29 Oct- 4 Nov	New twigs	3.28	2.30	2.77
45	5-11 Nov	New twigs	2.82	2.26	2.54
46	12-18 Nov	New twigs	2.54	2.20	2.35
47	19-25 Nov	New twigs	2.32	2.16	2.24

hoppers (> ETL=5 hoppers/twig or panicle) was observed between 9-17 SW (26 March-29 April), which had its peak (8.46 hoppers per panicle in pooled observation) on 14th standard week (2-8 April) coinciding with stone sized fruit stage of the crop. Rehman and Singh (2004) reported lowest hopper population during first week of February; however, population gradually increased from March and reached its peak during April, later on decreased. Vijaya *et al.* (2010) reported peak incidence of mango hopper during 8th and 9th standard weeks. Kumar (2015) recorded the population of hoppers was maximum in the first week of April which continuously to decreased and reached to minimum in the last week of May.

Effect of abiotic factors on population build-up of mango hopper: In pooled results (2009-11), the population showed significant and positive correlation with maximum temperature ('r' = 0.4731), sunshine ('r' = 0.4322) and evaporation ('r' = 0.3723), but it was significant and negative with evening relative humidity ('r' = -0.2628), average relative humidity ('r' = -0.2163) and wind velocity ('r' = -0.2262) (Table 2 and Fig. 2).

The multiple correlation coefficients (R) were significant in 2009-10 (R = 0.6230), 2010-11 (R = 0.6598) as well as in pooled results (R = 0.5938). The regression equations developed (check Table 2 for parameter details) are:

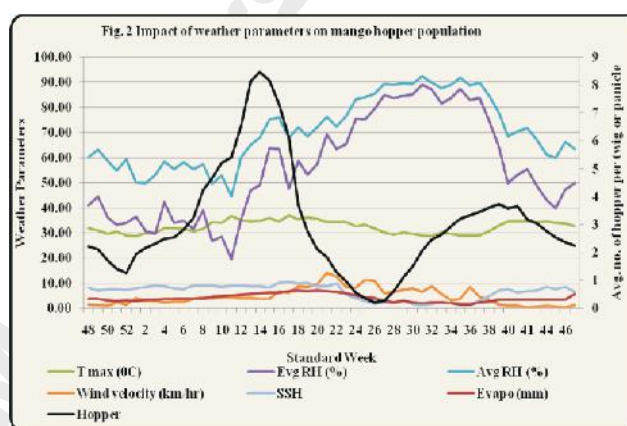
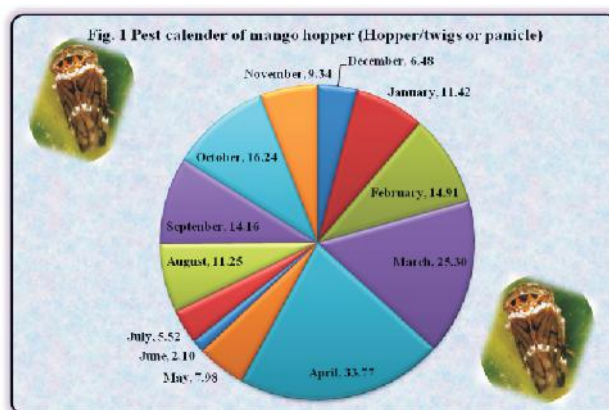
$$\text{2009-10 : } Y = -12.2407 + 0.4075 (X_1) + 0.0092 (X_5) + 0.4471 (X_8) + 0.0059 (X_9) - 0.3682 (X_{10})$$

$$\text{2010-11 : } Y = -2.2229 + 0.1067 (X_1) - 0.3898 (X_7) - 0.1612 (X_8) + 1.0412 (X_{10})$$

$$\text{Pooled : } Y = -10.4438 + 0.2100 (X_1) - 0.0469 (X_5) + 0.1111 (X_6) - 0.1643 (X_7) + 0.2478 (X_8) + 0.1386 (X_{10})$$

Total contributions of all the abiotic factors on population buildup of mango hopper was to the tune of 32.16, 38.73 and 31.26 % in 2009-10, 2010-11 and pooled observations, respectively.

So, looking to the relationship of weather factors on overall abundance of mango hopper complex, it may be concluded that abundance of hoppers was directly influenced by maximum temperature, sunshine hours and evaporation whereas, minimum temperature, evening relative humidity, wind velocity and rainfall had a negative bearing. This implies that as maximum temperature, sunshine hours and evaporation increased, hopper population increased further and vice-versa, whereas, hopper population decreased as relative humidity, wind velocity and rainfall increased or vice-versa. This interpretation is substantiated by the fact that hopper



population remained higher i.e. 2.00-8.82, 2.02-10.13 and 2.16-8.46 twig⁻¹ or panicle during 1-20 (1 Jan. - 20 May), 2-20 (8 Jan. - 20 May) and 2-20 SW in 2009-10, 2010-11 and pooled observations at pea sized fruit stage of the crop growth when maximum temperature and sunshine hours were in the range of 29.83- 39.03°C and 6.20-10.86 hours, respectively, and there were no rains. The total hopper population increased from 2.00 to 8.82, 2.02 to 10.13 and 2.11 to 8.46 twig⁻¹ or panicle during 1-20 (2009-10), 2-20 (2010-11) and 2-20 (pooled results) SW; whereas, relative humidity (evening and average), wind velocity and rainfall had a negative impact indicating higher population of mango hopper when their values were lower.

Xie and Xie (2000) observed temperature range of 18-28°C and more than 95 % RH favourable for the multiplication of mango leaf hopper. Pandey *et al.* (2003) on the other hand, recorded highest hopper population when temperature was more than 28°C. They further stated that fortnightly rainfall of more than 100 mm. had washing effect on hopper population although the temperature ranges were optimum with total contribution of abiotic factors ranged between 36 and 61 %. Rahman and Singh (2004) reported negative relationship with rainfall and wind velocity. Maximum temperature and sunshine hours caused buildup of the pest population, while RH, rainfall and rainy days had adverse

Table 2. Correlation and regression coefficients of mango hopper population with meteorological factors

Weather parameters	Correlation coefficient ('r')			Regression coefficient		
	2009-10	2010-11	Pooled	2009-10	2010-11	Pooled
Maximum temperature (X_1)	0.5324**	0.4255**	0.4731**	0.4075	0.1067	0.2100
Minimum temperature (X_2)	-0.1140	-0.1147	-0.1057	--	--	--
Average temperature (X_3)	0.1643	0.0866	0.1250	--	--	--
Morning relative humidity (X_4)	-0.0154	-0.1522	-0.0778	--	--	--
Evening relative humidity (X_5)	-0.3036*	-0.2401	-0.2628*	0.0092	--	-0.0469
Average relative humidity (X_6)	-0.2287	-0.2233	-0.2163*	--	--	0.1111
Wind velocity (X_7)	-0.2004	-0.2832*	-0.2262*	--	-0.3898	-0.1643
Sunshine hours (X_8)	0.5209**	0.3714**	0.4322**	0.4471	-0.1612	0.2478
Rainfall (X_9)	-0.2806*	-0.1858	-0.1781	0.0059	--	--
Evaporation (X_{10})	0.3785**	0.3756**	0.3723**	-0.3682	1.0412	0.1386
R^2	--	--	--	0.3216	0.3873	0.3126
Variation explained (%)	--	--	--	32.16	38.73	31.26
R	--	--	--	0.6230	0.6598	0.5938
Constant (A value)	--	--	--	-12.2407	-2.2229	-10.4438

* Significant at 5 % level ** Significant at 1 % level

effect on the pest population (Sushil Kumar *et al.*, 2005). Kannan and Rao (2006) reported negative relationship between minimum temperature, evening RH and rainfall. Varshneya and Rana (2008) reported maximum temperature was positively affected whereas RH and rainfall had negative effect on mango hopper. Kumara *et al.* (2009) reported negative correlation of mango hoppers with RH (morning and evening) and rainfall. In the present investigation, the population increased when temperature was increasing and RH was decreasing. Thus, the present investigation is in close agreement with the earlier reports.

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Effect of Water-Logging on Morphological Parameters and Yield in Maize Hybrids

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Abstract: Water-logging for six days decreased the plant height and number of leaves by 15.92 and 15.07%, respectively over control on 46th days of sowing (DAS) and 16.88 and 18.50% on 90th DAS (*i.e.* 10 days after relieving water-logging stress). Anthesis Silking Interval (ASI) and nodes bearing adventitious roots (NBAR) were increased by 21.37 and 50.62% on 46th DAS, respectively over control. Water logging decreased the kernel yield by 48.26% over control. Lakshmi-2277 recorded higher plant height number of leaves and nodes bearing adventitious roots and lower ASI apart from higher yield followed by Bharati-99, whereas, CN-117 recorded lower values of all the above parameters.

Key Words: ASI, NBAR, Maize hybrids, Number of leaves, Plant height, Water-logging

Maize (*Zea mays* L.) is the third most important cereal crop after rice and wheat and is known as "queen of cereals" because it has the highest genetic yield potential among the cereals. It is grown in diverse agro climatic conditions ranging from sub-tropical to cooler temperate regions. It is a staple food of major portion of world population and is consumed in the form of pop, sweet, waxy and baby corn. Maize ranks first in world production (868 Mt from 168 M ha) followed by wheat (691 Mt) and rice (461 Mt). This represents 38% of the total grain production as compared to 30 % for wheat and 20% for rice (Farmers Portal 2015). Andhra Pradesh ranks 5th in area (0.79 M ha) and has recorded the highest production (4.14 M t) and productivity (5.26 t ha⁻¹) in the country. Andhra Pradesh contributes 20.9% of the total maize production in the country (Anonymous 2014-15). Among the various abiotic stresses, water-logging, caused by contingent flooding, continuous rainfall and inadequate drainage or a high water table, is one of the most important constraints for maize production in Asia and many other parts of the world. In South and Southeast Asia alone, over 18% of the total maize growing areas are frequently affected by floods and water-logging problems. In South and Southeast Asia alone over 18% of the total maize growing areas are frequently affected by floods and water-logging problems (Zaidi *et al.*, 2009). Out of the total 6.6 M ha area of maize in India about 2.5 M ha is affected by excess soil moisture (ESM) problem that causes on an average 25-30% loss of national maize production almost every year (Rathore *et al.*, 1996). Water-logging decreased plant height number of leaves delayed ASI and increased NBAR in maize genotypes (Ajaz and Warsi, 2009). Savita and Shekhar (2008) reported that water-logging in maize significantly

reduced the maize yields as well as its nutrient compositions. Water-logging also reduced the uptake of N, P, K, Fe, Mn and Zn by the crop. Keeping these aspects in view, the present study was taken up to study the effect of water logging on morphological parameters and kernel yield in maize hybrids.

MATERIAL AND METHODS

Field experiment was conducted at Agricultural College Farm Bapatla located on the eastern side of the peninsular India at 80°3' East longitude, 15°54' North latitude, 5.49 m above mean sea level and 7 km away from Bay of Bengal in Krishna - Godavari Agro-climatic zone of Andhra Pradesh, in sandy clay loam soil in a split plot design with two main treatments M₁ control; no water-logging and M₂ ; Water logging was imposed at 30 DAS for 6 days and eight maize hybrids as sub plot treatments with three replications during *Kharif* 2014-15 (Table 1). The plot size was 4m x 3m with spacing of 60cm x 20cm. Water-logging stress was imposed at knee height stage *i.e.* at 30 DAS for six days (from 30 to 35 DAS) and control plants were maintained under normal irrigation. Water-logging was administered by applying heavy irrigation to the plots and the soil was kept saturated with water above field capacity by continuous flooding usually every day twice to create an oxygen deficient environment. The crop was grown following the recommended package of practices and timely plant protection measures (Vyavasaya Panchangam, 2014). The data on plant height number of leaves nodes bearing adventitious roots and anthesis silking interval were recorded from five representative tagged plants from each plot at 30 DAS (*i.e.* before imposition of water logging) and at

Treatment Abbreviations	Treatment details
Main Plot Treatments	
M ₁	No water logging
M ₂	Water logging for 6 days
Sub plot treatments	
S ₁	33A96
S ₂	King-3063
S ₃	Bharati-99
S ₄	Sandya-666
S ₅	Lakshmi -2277
S ₆	CN-117
S ₇	JKMH-4545
S ₈	SY-280

RESULTS AND DISCUSSION

Table 2. Effect of waterlogging on number of leaves per plant and kernel yield (Kg ha^{-1}) in maize

Name of the Hybrid	No. of leaves per plant											
	30 DAS				46 DAS				90 DAS			
	Control	Waterlogging	Mean	Control	Waterlogging	Mean	Control	Waterlogging	Mean	Control	Waterlogging	Mean
33A96	880	10 07	9 43	12 87	11 33	12 10	11 67	9 80	10 73	4640 28	2011 94	3326 11
King-3063	933	9 33	9 33	13 53	10 93	12 23	11 93	9 53	10 73	4873 61	1841 11	3357 36
Bharati-99	927	9 47	9 37	12 87	11 33	12 10	11 40	9 80	10 60	4848 61	2875 56	3862 08
Sandya-666	927	9 40	9 33	12 80	11 20	12 00	11 47	9 47	10 47	4192 78	1727 78	2960 28
Lakshmi-2277	967	9 00	9 33	13 33	11 20	12 27	12 07	9 60	10 83	4741 39	3018 89	3880 14
CN-117	827	7 87	8 07	12 00	9 27	10 63	10 93	7 80	9 37	4076 11	1795 83	2935 97
JKMH-4545	887	9 67	9 27	13 20	11 20	12 20	11 60	9 93	10 77	4566 67	2548 33	3557 50
SY-280	867	9 80	9 23	12 93	11 47	12 20	11 47	9 47	10 47	4560 83	3065 00	3812 92
Mean	902	9 33		12 94	10 99		11 57	9 43		4562 53	2360 56	
CD (p=0.05)	Main	Hybrids	Interaction	Main	Hybrids	Interaction	Main	Hybrids	Interaction	Main	Hybrids	Interaction
	NS	NS	NS	0 92	0 90	NS	0 63	0 77	NS	237 61	483 09	NS
CV	11 96	9 90		10 86	6 32		8 47	6 15		9 72	11 71	

Table 3. Effect of waterlogging on plant height (cm) in maize.

Name of the Hybrid	Plant height (cm)					
	30 DAS		46DAS		90DAS	
	Control	Waterlogging	Mean	Control	Waterlogging	Mean
33A96	165 80	182 07	173 93	237 36	215 87	226 61
King-3063	180 60	172 13	176 37	245 33	206 60	225 97
Bharati-99	170 73	152 27	161 50	249 60	211 13	230 37
Sandya-666	162 40	158 53	160 47	229 33	192 47	210 90
Lakshmi-2277	175 20	167 67	171 43	253 27	208 20	230 70
CN-117	159 53	143 40	151 47	225 07	175 73	200 40
JKMH-4545	167 27	174 73	171 00	246 80	202 13	224 47
SY-280	165 53	171 67	168 60	244 47	211 60	228 03
Mean	168 38	165 31		241 40	202 97	
	Main	Hybrids	Interaction	Main	Hybrids	Interaction
	NS	NS	NS	9 82	15 99	NS
CD (p=0.05)				6 24	6 04	
CV	10 19	8 01				
				15 74	15 47	NS
				9 89	5 76	
				Main	Hybrids	Interaction

Water-logging treatment reduced the number of leaves at 46 and at 90 DAS by 15.07 and 18.50 per cent, respectively over control plants. Number of leaves was affected by increase in the water level because maximum water level inhibits the growth of plant which ultimately reduced the number of leaves and leaf area. Lakshmi-2277 recorded higher number of leaves and it was on par with all other hybrids except CN-117. The lower number of leaves was recorded in CN-117. The Anthesis Silking Interval increased after water-logging treatment. The maximum increase in ASI was found in waterlogged plants. Significant differences were observed between the main treatments and maize hybrids with respect to ASI and interaction was also significant (Table 4).

Water-logging treatment showed significant increase in ASI (4.26 days) over control plants. Increase in Anthesis Silking Interval in water-logging treatment was due to delayed silking and reduced photosynthesis in susceptible hybrids under excessive moisture stress which apparently reduced the assimilates for silk growth and cob development. Similar results were also reported in maize (Ajaz and Warsi, 2009 and Zaidi *et al.*, 2003). Lakshmi-2277 recorded lower increase in ASI and it was on par with Bharati-99 JKM-4545 and SY-280. The higher increase in ASI was recorded in CN-117 (4.27 days). Under water-logging, Lakshmi-2277 and SY-280 showed lower ASI where as CN-117 higher ASI. Under non waterlogged condition lower ASI in King-3063 and Bharati-99 and higher in Sandya-666.

Water-logging treatment significantly increased the number of nodes bearing adventitious roots at 46 DAS over control plants by 50.62 per cent due to water-logging stress (Table 3). Plants growing under water-logging condition develop adventitious roots and aerenchyma formation. The aerenchyma promotes gas exchange between shoots and roots that supports plant to survive under waterlogged condition. Ethylene stimulation would be involved in the physiological mechanisms for aerenchyma formation in adventitious roots under hypoxia (Haque *et al.*, 2010). Lakshmi-2277 recorded more number of nodes bearing adventitious roots and was on a par with Bharati-99, King-3063 and SY-280 at 46 DAS. Among the interactions under water-logging condition Lakshmi-2277 and Bharati-99 maintained more number of nodes bearing adventitious roots where CN-117. Under non-water-logging condition Lakshmi-2277 and SY-280 maintained more number of nodes bearing adventitious roots where as lower in Bharati-99. Significant differences were observed between water-logging treatments and maize hybrids with regards to kernel yield but the interaction was non-significant (Table 3). The kernel yield was reduced by 48.3 per cent due to water-logging over control. Water-logging decreased the kernel yield by 48.26

Table 4. Effect of waterlogging on anthesis silking interval (days) and nodes bearing adventitious roots (NBAR) in maize.

Name of the Hybrid	Anthesis silking interval				Nodes bearing adventitious roots			
	46 DAS		30 DAS		46 DAS			
	Control	Waterlogging	Mean	Control	Waterlogging	Mean	Control	Waterlogging
33A96	3 53	4 53	4 03	1 27	1 47	1 37	2 47	3 40
King-3063	3 40	4 67	4 03	1 33	1 33	1 33	2 47	3 67
Bharati-99	3 40	3 87	3 63	1 27	1 40	1 33	2 20	4 13
Sandya-666	3 73	4 47	4 10	1 40	1 27	1 33	2 27	3 60
Lakshmi-2277	3 53	3 67	3 60	1 33	1 47	1 40	2 67	3 93
CN-117	3 53	5 00	4 27	1 47	1 27	1 37	2 27	3 33
JKMH-4545	3 47	4 07	3 77	1 47	1 33	1 40	2 53	3 40
SY-280	3 47	3 80	3 63	1 40	1 27	1 33	2 53	3 80
Mean	3 51	4 26		1 37	1 35		2 43	3 66
				Main	Hybrids	Interaction	Main	Hybrids
CD (p=0.05)				0 20	0 25	0 12	0 13	0 25
CV				7 20	5 38	11 25	6 22	6 89
						NS	Interaction	
						NS	0 09	

per cent over control. Water-logging stress induced low oxygen concentration in soil (hypoxia) or complete absence of oxygen (anoxia) which affected the nutrient uptake, synthesis and translocation of growth regulators, photosynthesis, respiration and carbohydrate partitioning and decreased the yield of crops (Ferreira *et al.*, 2008). The maize hybrid Lakshmi-2277 recorded higher kernel yield (3880.1 Kg ha⁻¹) followed by Bharati-99 and SY-280. Higher kernel yield in Lakshmi-2277 might be due to higher leaf number and leaf area, more plant height, quick recovery of photosynthesis after water-logging and more translocation of assimilates to reproductive parts. It also showed lesser increase in ASI (3.60 days).

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Received 27 February, 2016; Accepted 1 April, 2016

Oh y for Members



Influence of Site-Specific Nutrient Management on Productivity, Nutrient Use Efficiency and Economics in Rice-Rice Cropping System

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Abstract: The maximum *kharif* rice yield ($5498.40 \text{ kg ha}^{-1}$) was obtained with the application of $\text{N}_{150}\text{P}_{60}\text{K}_{80}\text{S}_{20}\text{Zn}_8$, which was 44% and 46% higher as compared to state recommendation and farmers' practice, respectively. Similarly maximum yield of *boro* rice was found with the application of $\text{N}_{150}\text{P}_{60}\text{K}_{80}$ nutrients ($6355.56 \text{ kg ha}^{-1}$), which was at par with $\text{N}_{150}\text{P}_{60}\text{K}_{80}$. Highest system net return of Rs. 43,645 ha^{-1} was recorded in $\text{N}_{150}\text{P}_{90}\text{K}_{80}\text{S}_{20}\text{Zn}_8$ followed by $\text{N}_{150}\text{P}_{60}\text{K}_{80}\text{S}_{20}\text{Zn}_8$. Balanced nutrient application resulted higher partial factor productivity as well as agronomic efficiency of applied N, P and K.

Key Words: Agronomic efficiency, Partial factor productivity, Rice-rice cropping system, Site specific nutrient management

Global demand for food is rising because of population growth, increasing affluence and changing dietary habits. The UN/FAO forecasts that global food production will need to be increased over 40% by 2030 and 70% by 2050. Rice is the most important food crop of the developing world and the staple food for more than 60% of the Indian populace. It is estimated that the demand for rice will be 121.2 million tonnes by the year 2030, 129.6 million tonnes by the year 2040 and 137.3 million tonnes by the year 2050 for internal consumption (Anonymous 2013). In order to achieve the target, the productivity of rice has to be brought to the level of 3.3 tonnes per ha (2.4 tonnes at present). Rice-rice cropping system is one of the most important food production systems of India and covers about 6 million ha (Singh *et al.*, 2009). Rice crop removes about 15 kg N, 4 kg P_2O_5 and 24 kg K_2O to produce 1 ton of grain with an equal amount straw from the soil (Hegde, 1996).

The common tendency for farmers is to practice N-driven fertilizer management neglecting emerging deficiencies of other nutrients. Such unbalanced fertilizer use not only aggravates the extent of soil fertility depletion, but is also harmful in terms of low nutrient use efficiency, poor quality of produce, and enhanced susceptibility of crops to biotic and abiotic stresses (Singh *et al.*, 2009). Incidence and expansion of multi-nutrient deficiencies in the soils under intensive cropping in general, and in rice-based cropping systems in particular, can be linked to inadequate and unbalanced nutrient input. This is also considered as major reason for observed decline in productivity. Blanket fertilizer recommendations are often prevailing over large areas

without taking into account the wide variability and site- and season-specific crop nutrient requirements within each recommendation domain. This inadvertently promotes unbalanced fertilization as they fail to account for crop yield goals or emerging multi-nutrient deficiencies. Hence, development of a site-specific nutrient supply system seems to be the only way to increase nutrient use efficiency and arrest the ever-increasing occurrences of soil nutrient deficiencies (Dobermann *et al.*, 2004).

Therefore, in the present study an attempt was made to find out site specific nutrient management including macro and micronutrients on yield and economics of rice-rice cropping system on an Inceptisol located in the hot humid subtropical region of India under lower Gangetic Plains of West Bengal, India.

MATERIAL AND METHODS

The field experiment was carried out in *kharif* (wet) and *boro* (summer) seasons of three consecutive years from 2008-11 with rice-rice cropping system located at Research Farm, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India ($23^{\circ}08' \text{N}$ latitude, 88°E longitude and 15 m above mean sea level) along the new alluvial zone of the state. The climate of the area is hot, humid subtropics. The experimental site received an average rainfall of approximately 1480 mm and experienced mean annual minimum and maximum temperatures of 12° and 36.2°C , respectively. The soil is sandy clay loam (Inceptisol) with 56% sand, 24% silt and 20% clay, having neutral in reaction (pH 7.4), low in available N (168.4 kg ha^{-1}), medium in available P

(23.8 kg ha⁻¹), medium in available K (176.72 kg ha⁻¹), available S 37.12 kg ha⁻¹ and available Zn 1.50 kg ha⁻¹ at the beginning of experiment in 0-20 cm soil layer.

Treatments and crop management: The two rice crops were grown annually with necessary plowing to a depth of 15 to 20 cm using a power tiller. The rice variety IET-4094 was cultivated in rainy season and high yielding variety IET-4786 in summer season during the experimental periods. The experiment was laid out in a randomized block design with twelve treatments, ten on the basis of soil test values (SSNM) along with two other treatments, state agricultural departments' recommended (SR)-N₈₀P₄₀K₄₀Zn₂ in *kharif* followed by N₁₂₀P₆₀K₆₀ in *boro*, and farmers' practice (FP)-N₁₀₀P₃₀K₃₀ in *kharif* followed by N₁₂₀P₄₀K₄₀ in *boro*. On the basis of soil test values S and Zn were applied in rice hybrid (PHB-71) only, whereas N, P and K were applied during both seasons' rice crops in SSNM treatments. The SSNM treatments are N₀P₆₀K₈₀S₂₀Zn₈, N₁₅₀P₆₀K₈₀S₂₀Zn₈, N₁₅₀P₀K₈₀S₂₀Zn₈, N₁₅₀P₃₀K₈₀S₂₀Zn₈, N₁₅₀P₉₀K₈₀S₂₀Zn₈, N₁₅₀P₆₀K₀S₂₀Zn₈, N₁₅₀P₆₀K₄₀S₂₀Zn₈, N₁₅₀P₆₀K₁₂₀S₂₀Zn₈, N₁₅₀P₆₀K₈₀S₀Zn₈ and N₁₅₀P₆₀K₈₀S₂₀Zn₀. Each treatment was replicated four times with 10 m X 8 m plots. Crops were raised under optimum management conditions. Apart from differences in nutrient application rates, all other management practices were the same for all plots.

Yield and profitability: Yield of grain and straw of each crop under various treatments were measured by hand-harvest of 20 m² area. Farm gate price at the harvesting time was used for calculation gross return. Net return or profit was calculated by subtracting production cost from the gross value of the produce (*kharif* + *boro*), including straw value or gross return. The benefit: cost ratio (BCR) was calculated by dividing gross return by the production cost.

Nutrient use efficiency (NUE): Nutrient use efficiencies of applied nutrient was measured in terms of Partial Factor Productivity (PFP) and Agronomic Efficiencies (AE) of fertilizer nutrient, a ratio of the grain yield to applied nutrient is a useful measure of nutrient use efficiency as it provides integrative index that quantifies total economic output relative to the utilization of resources in the system including soil nutrients and nutrients from applied fertilizers. It is possible to increase the PFP by increasing the amount, uptake and utilization of indigenous nutrients, and by increasing the efficiency with which applied nutrients are taken up by the crop and utilized to produce grain (Cassman *et al.*, 1996).

PFP and AE of N (PFP_n/AE_n: kg grain kg⁻¹ of native+applied fertilizer) of SSNM treatments were calculated as:

$$PFP_n \text{ (Kg grain Kg}^{-1} \text{ N)} = \frac{YTi}{150}$$

$$AE_n \text{ (Kg grain Kg}^{-1} \text{ N)} = \frac{YTi - YN_0}{150}$$

Where, YTi is the yield of *kharif*/*boro* rice under different treatments of SSNM, YN₀ is the yield of N omission plot and 150 is the amount of applied N in *kharif*/*boro* season. Similar process was followed for PFP and AE of P and K.

Data obtained over the years from same experimental site followed the homogeneity test, pooling was done over the seasons and mean data are given.

RESULTS AND DISCUSSIONS

Yield: The yield of both *kharif* and *boro* rice increased with higher doses of NPK along with use of S and Zn as compared to farmers' practice and state recommended dose. In case of *kharif* rice the highest yield was obtained in N₁₅₀P₆₀K₈₀S₂₀Zn₈ (5498.40 kg ha⁻¹) followed by N₁₅₀P₉₀K₈₀S₂₀Zn₈ (5496.23 kg ha⁻¹), N₁₅₀P₆₀K₁₂₀S₂₀Zn₈ (5100.27 kg ha⁻¹), N₁₅₀P₃₀K₈₀S₂₀Zn₈ (5009.64 kg ha⁻¹) and N₁₅₀P₆₀K₄₀S₂₀Zn₈ (4981.35 kg ha⁻¹) and these were observed at par. Application of N₁₅₀P₆₀K₈₀S₂₀Zn₈ recorded 44% and 46% higher *kharif* rice yield as compared to State recommended and Farmers' Practice respectively. In *boro* rice N₁₅₀P₉₀K₈₀S₂₀Zn₈ (6355.56 kg ha⁻¹) recorded the highest yield and was at par with N₁₅₀P₆₀K₈₀S₂₀Zn₈ (5979.49 kg ha⁻¹), N₁₅₀P₆₀K₄₀S₂₀Zn₈ (5956.67 kg ha⁻¹), N₁₅₀P₆₀K₈₀S₀Zn₈ (5942.36 kg ha⁻¹), N₁₅₀P₆₀K₁₂₀S₂₀Zn₈ (5940.69 kg ha⁻¹) and N₁₅₀P₃₀K₈₀S₂₀Zn₈ (5882.50 kg ha⁻¹). N₁₅₀P₆₀K₈₀S₂₀Zn₈ treatment in *boro* rice recorded 12% higher yield as compared to State recommended as well as Farmers' practice (Table 1). Mauriya *et al.* (2013) observed higher yield of rice with the application of higher dose of NPK along with S, B, Mn and Zn. Four years experiment revealed that combined application of macro- and micronutrients (N₁₅₀ P₆₀ K₁₂₀ S₄₀ B₅ Mn₂₀ Zn₂₅) significantly increased the rice yield showing, 4.1 and 2.1 t ha⁻¹ increase over the farmers' practice (N₉₀P₃₀) and state recommendation (N₁₂₀ P₆₀K₆₀ Zn₂₅) respectively (Yadav and Kumar, 2009). Sing *et al.* (2009) reported that SSNM out yielded farmers' practice by 1.03 (+20%) and 1.57 t ha⁻¹ (+31%) during *kharif* and *rabi* season respectively, whereas state recommended out yielded by 0.45 t ha⁻¹ (+9%) during *kharif*.

Omission of N from the SSNM, where same levels of PKSZn were applied, reduced the rice yield by 125% and 139% in *kharif* and *boro* season, respectively. Reduction or omission of phosphorus dose from SSNM treatment resulted in reduction of rice yield by 10-30% and 2-9% in *kharif* and *boro* season, respectively. Similarly, when potassium was reduced or omitted from SSNM reduced the rice yield by 10-24% and 4-7% in *kharif* and *boro* season, respectively.

Omission of S and Zn from SSNM also reduced the *kharif* rice yield by 22% and 20% respectively, whereas this reduction of residual effect of S and Zn in *boro* rice was 6.2% and 5.9% respectively (Table 1). Mauriya *et al.* (2013) observed phosphorus omission or reduction decreased the rice yield by 9-21.3%, whereas, omission of potassium reduced the yield by 16% and omission of zinc and sulphur resulted in a decrease of yield by 9.4 and 10%, respectively.

Probably a positive effect was exerted on the development of source and sink strength of the plants when levels of nutrients were increased, which ultimately improves the yield attributing traits. A combined application of macro- and micronutrients might have influenced different metabolic process in positive direction. Nutrient particularly nitrogen is a very important constituent of cell and plays a major role in the production of protein. Higher dose of nitrogen might lead to more shoot growth and chlorophyll synthesis, ultimately increasing the yield (Shangguan *et al.*, 2000). P is responsible for better root development and subsequent absorption of N, whereas K is involved in N metabolism in cereals as well as imparts resistance to pest and disease and helps to escape the plants from other abiotic stresses. Singh and Singh (2014), Ghosh *et al.* (2004) and Jat *et al.* (2011) also reported that balanced NPK application is essential to achieve the target yield. Sulphur improves the growth of plant. Zinc is known to influence the translocation of metabolites and improving sink strength in plant. It is a constituent of several enzymes, which are responsible for better photosynthesis and auxin metabolism directly affecting source strength leading to better tillering, growth, yield attributing characters like panicle number per unit area,

panicle length, number of grains per panicle and grain weight, which ultimately increased crop yield.

Economics: The variations in the cost of cultivation under different treatments were resulted due to application of different amount of macro- and micronutrients. Grain and straw yield of rice were the important factors for causing difference in gross and net returns. The highest cost of cultivation (Rs. 69528 ha⁻¹), net return (Rs. 43645 ha⁻¹) as well as B:C ratio (1.63) were recorded in N₁₅₀P₉₀K₈₀S₂₀Zn₈. However, no significant difference was observed among N₁₅₀P₉₀K₈₀S₂₀Zn₈, N₁₅₀P₆₀K₈₀S₂₀Zn₈, N₁₅₀P₃₀K₈₀S₂₀Zn₈, N₁₅₀P₆₀K₄₀S₂₀Zn₈, N₁₅₀P₆₀K₁₂₀S₂₀Zn₈ and N₁₅₀P₆₀K₈₀S₀Zn₈ regarding net return and B:C ratio. SSNM treatment N₁₅₀P₉₀K₈₀S₂₀Zn₈ involved an additional expenditure of Rs. 8014 ha⁻¹ and Rs. 7271 ha⁻¹ over farmers' practice and state recommendation respectively due to additional fertilizer application and resulted Rs. 17532 ha⁻¹ and Rs.17801 ha⁻¹ extra net return. Similarly an additional expenditure of Rs. 6514 ha⁻¹ and Rs. 5771 ha⁻¹ was incurred for SSNM treatment N₁₅₀P₆₀K₈₀S₂₀Zn₈ over farmers' practice and state recommendation, respectively and earned additional net return of Rs.15234 ha⁻¹ and Rs. 15503 ha⁻¹. Omitting of S and Zn reduced the net return by Rs. 7918 ha⁻¹ and Rs. 9425 ha⁻¹, respectively. P and K omission also caused a reduction of net return by Rs. 13161 and Rs. 11182 ha⁻¹, respectively. Yadav and Kumar (2009) also reported similar type findings while working in rice-wheat cropping system using site-specific nutrient management. Singh *et al.* (2009) reported much more net return with SSNM as compared to farmers' practice and state recommendation.

Table 1. Yield and economics of *kharif* and *boro* rice (pooled over 3 years)

Treatment	Treatment details	Kharif rice yield (kg ha ⁻¹)	Boro rice yield (kg ha ⁻¹)	System cost of cultivation (Rs. ha ⁻¹)	System net return (Rs. ha ⁻¹)	System B:C ratio
T ₁	N ₀ P ₆₀ K ₈₀ S ₂₀ Zn ₈	2443.17	2501.39	64773	-17192	0.73
T ₂	N ₁₅₀ P ₆₀ K ₈₀ S ₂₀ Zn ₈	5498.40	5979.49	68028	41347	1.61
T ₃	N ₁₅₀ P ₀ K ₈₀ S ₂₀ Zn ₈	4198.83	5494.58	65028	28185	1.43
T ₄	N ₁₅₀ P ₃₀ K ₈₀ S ₂₀ Zn ₈	5009.64	5882.50	66528	37415	1.56
T ₅	N ₁₅₀ P ₉₀ K ₈₀ S ₂₀ Zn ₈	5496.23	6355.56	69528	43645	1.63
T ₆	N ₁₅₀ P ₆₀ K ₀ S ₂₀ Zn ₈	4453.46	5590.56	66424	30165	1.45
T ₇	N ₁₅₀ P ₆₀ K ₄₀ S ₂₀ Zn ₈	4981.35	5956.67	67226	37317	1.56
T ₈	N ₁₅₀ P ₆₀ K ₁₂₀ S ₂₀ Zn ₈	5100.27	5940.69	68830	36481	1.53
T ₉	N ₁₅₀ P ₆₀ K ₈₀ S ₀ Zn ₈	4497.15	5942.36	67128	33429	1.50
T ₁₀	N ₁₅₀ P ₆₀ K ₈₀ S ₂₀ Zn ₀	4571.93	5646.94	66028	31922	1.48
T ₁₁	SR	3808.75	5335.97	62257	25844	1.42
T ₁₂	FFP	3760.32	5355.74	61514	26113	1.42
	CD (p=0.05)	737.45	621.11	-	10881	0.16

N.B. Price of different fertilizers: Urea- Rs.5.00 kg⁻¹, single super phosphate- Rs.5.00 kg⁻¹, muriate of potash- Rs.6.00 kg⁻¹, elemental sulphur- 45.00 kg⁻¹, zinc sulphate- 50.00 kg⁻¹; Farm gate price of raw rice: *kharif* rice- Rs.8.50 kg⁻¹, *boro* rice- Rs.9.20 kg⁻¹ and rice straw- Rs.0.50 kg⁻¹

Partial factor productivity: Partial factor productivity (PFP) of N was significantly influenced by the level of nutrients and proved to be higher under balanced nutrient application with NPKSZn as compared to removal of any of these nutrients. The highest PFP_N (36.66 kg grain kg^{-1} N) was in $N_{150}P_{60}K_{80}S_{20}Zn_8$ followed by $N_{150}P_{90}K_{80}S_{20}Zn_8$ (36.66 kg grain kg^{-1} N) for *kharif* rice, whereas in *boro* rice the highest PFP_N was recorded in $N_{150}P_{90}K_{80}S_{20}Zn_8$ (42.37 kg grain kg^{-1} N) followed by $N_{150}P_{60}K_{80}S_{20}Zn_8$ (39.86 kg grain kg^{-1} N). The lowest values of PFP_N 27.99/36.63 kg grain kg^{-1} N respectively for *kharif* and *boro* rice were in $N_{150}P_0K_{80}S_{20}Zn_8$, where P was not applied (Table 2). Ravisankar *et al.* (2014) reported PFP_N can be increased to 54.6% with balanced application of NPK over N application alone. Partial factor productivity of P and K declined with the increase in its level of application in both crop. The highest PFP_Ps 160.69 kg ha^{-1} and 192.43 kg ha^{-1} during *kharif* and *boro* season, respectively were recorded with the application of 30 kg ha^{-1} P (Fig. 1 and Fig. 2). Similarly, the highest PFP_Ks, 120.02 kg ha^{-1} and 146.62 kg ha^{-1} during *kharif* and *boro* season, respectively were observed under 40 kg ha^{-1} K. In both the seasons, a gradual decrease in PFP_P and PFP_K values were

noticed with the increase of their levels (Fig. 3 and Fig. 4).

Agronomic efficiency: In general marginal farmers are more interested in applying N fertilizer rather balanced dose with PK and other deficient secondary and micronutrients. Agronomic efficiency of N (AE_N) was significantly influenced by different levels of nutrients. In *kharif* rice significantly higher AE_N (20.37 kg grain kg^{-1} of N) was with $N_{150}P_{60}K_{80}S_{20}Zn_8$ followed by $N_{150}P_{90}K_{80}S_{20}Zn_8$ (20.35 kg grain kg^{-1} of N) and were at par; whereas lower values of AE_N viz. 11.70, 13.40, 13.69 and 14.19 kg grain kg^{-1} of N were recorded with the omission of P, K, S and Zn, respectively. Similarly in *boro* rice the highest AE_N (25.69 kg grain kg^{-1} of N) was observed in $N_{150}P_{90}K_{80}S_{20}Zn_8$ and the lowest value was recorded in $N_{150}P_0K_{80}S_{20}Zn_8$ (19.95 kg grain kg^{-1} of N) (Table 2). Ravisankar *et al.* (2014) also reported that AE_N can be increased to 238.9% in rice-rice system by applying the recommended quantity of N with recommended quantity of P and K instead of N alone. Agronomic efficiency of phosphorus (AE_P) and potassium (AE_K) were influenced significantly by their different levels. Maximum AE_P 26.39 and 11.02 kg grain kg^{-1} of P for *kharif* and *boro* rice respectively were recorded with 30 kg P_2O_5 . Similarly maximum AE_K 13.99 and 8.97 kg grain kg^{-1}

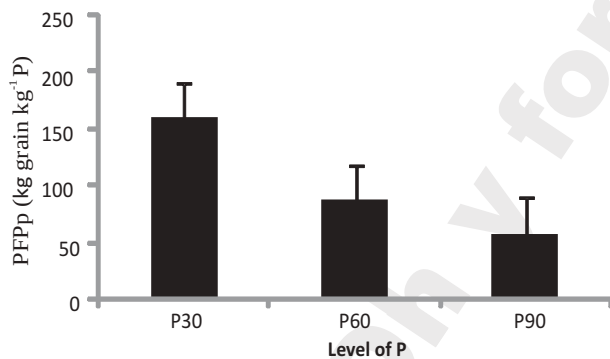


Fig. 1. Partial factor productivity of P in *kharif*

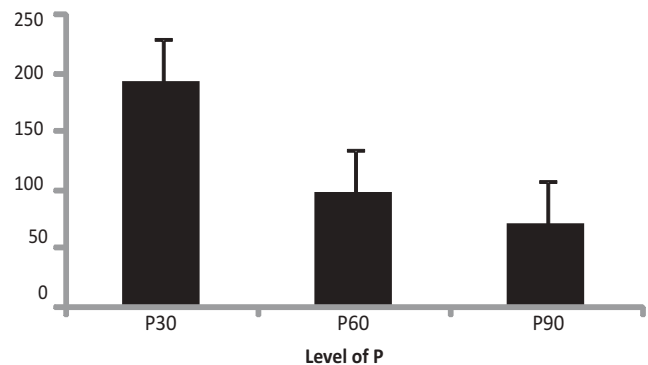


Fig. 2. Partial factor productivity of P in *boro*

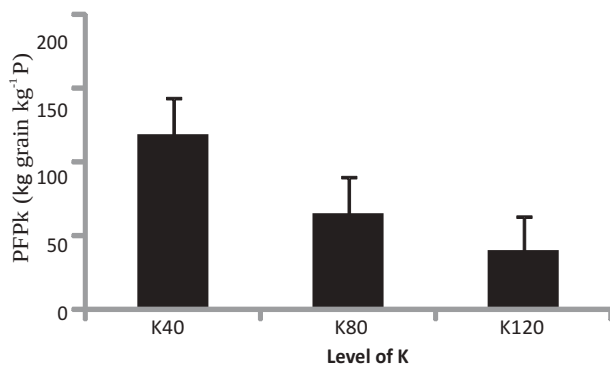


Fig. 3. Partial factor productivity of K in *kharif*

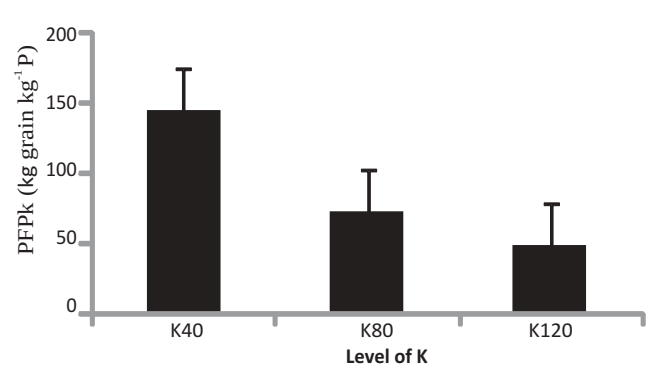
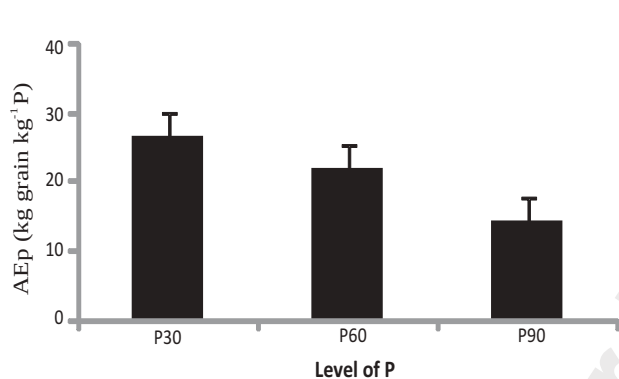
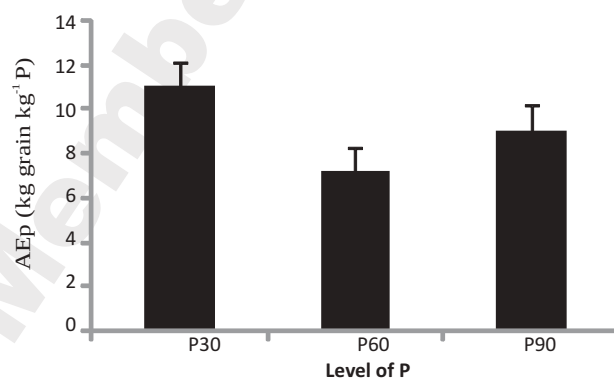
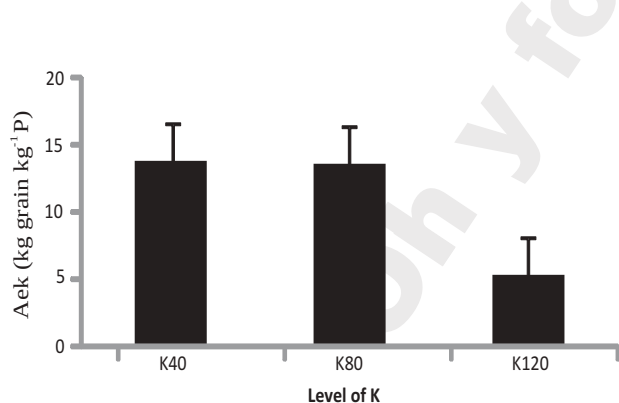
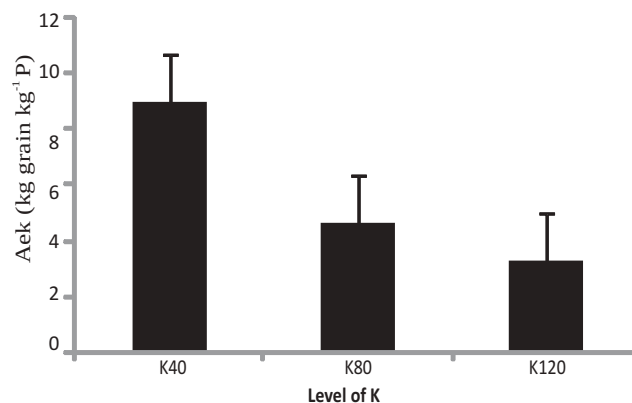


Fig. 4. Partial factor productivity of K in *boro*

Table 2. Partial factor productivity and agronomic efficiency of N (pooled over 3 years) of SSNM treatments

Treatment	Treatment	PFP _n (Kg grain Kg ⁻¹ N)		AE _n (Kg grain Kg ⁻¹ N)	
		<i>Kharif</i>	<i>Boro</i>	<i>Kharif</i>	<i>Boro</i>
T ₁	N ₀ P ₆₀ K ₈₀ S ₂₀ Zn ₈	0	0	0	0
T ₂	N ₁₅₀ P ₆₀ K ₈₀ S ₂₀ Zn ₈	36.66	38.72	20.37	23.19
T ₃	N ₁₅₀ P ₀ K ₈₀ S ₂₀ Zn ₈	27.99	36.15	11.70	19.95
T ₄	N ₁₅₀ P ₃₀ K ₈₀ S ₂₀ Zn ₈	33.40	37.33	17.11	22.54
T ₅	N ₁₅₀ P ₉₀ K ₈₀ S ₂₀ Zn ₈	36.64	40.52	20.35	25.69
T ₆	N ₁₅₀ P ₆₀ K ₀ S ₂₀ Zn ₈	29.69	36.59	13.40	22.30
T ₇	N ₁₅₀ P ₆₀ K ₄₀ S ₂₀ Zn ₈	33.21	37.44	16.92	23.04
T ₈	N ₁₅₀ P ₆₀ K ₁₂₀ S ₂₀ Zn ₈	34.00	38.42	17.71	22.93
T ₉	N ₁₅₀ P ₆₀ K ₈₀ S ₀ Zn ₈	29.98	38.56	13.69	22.94
T ₁₀	N ₁₅₀ P ₆₀ K ₈₀ S ₂₀ Zn ₀	30.48	36.95	14.19	20.97
	LSD (P=0.05)	2.65	4.89	2.71	2.99

**Fig. 5.** Agronomic efficiency of P in *kharif***Fig. 6.** Agronomic efficiency of P in *boro***Fig. 7.** Agronomic efficiency of K in *kharif***Fig. 8.** Agronomic efficiency of K in *boro*

of K for *kharif* and *boro* rice, respectively were obtained with 40 kg K₂O. AE_p and AE_k showed declining trend with the increase in their levels and lowest value of AE_p (14.35 and 9.06 kg grain kg⁻¹ of P for *kharif* and *boro* rice, respectively) and AE_k (5.47 and 3.23 kg grain kg⁻¹ of K for *kharif* and *boro* rice, respectively) were given by 90 kg P₂O₅ and 120 kg K₂O, respectively (Fig. 5 to 8). It might be due to decline in incremental grain yield at each successive levels of P and K.

According to Barthwal *et al.* (2013), AE_p and AE_k values were found to be declined with the increase in P and K levels.

Thus, it may be concluded that site-specific nutrient management approach may increase the yield of rice-rice system significantly through judicious exploitation of available nutrients by plants. Nutrients applied to the first rice crop have definite carry over beneficial effects on enhancing yield of succeeding rice. This indicates for application of S

and Zn in *kharif* rice only for system productivity and profitability.

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Received 1 February, 2016; Accepted 15 May, 2016



PGPR Mediated Induction of Systemic Resistance in Chickpea Against *Fusarium Oxysporum* and Growth Promotion under Field Condition

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Abstract: Seed bacterization with rhizobacterial isolates Ps14c and B20d induced significant protection against the pathogen, *Fusarium oxysporum*, in chickpea and reduced the wilt incidence to 85.7 and 73.3%, respectively, as compared to 92% in uninoculated control. Bacterial inoculation also caused systemic protection against wilt besides increasing the plant growth under field condition. Time course analysis for the induction of defense related enzymes and PR proteins showed higher accumulation of phenols, Peroxidase, Poly Phenol oxidase, phenyl ammonia lyase and total proteins in chickpea roots as compared to uninoculated control. Higher accumulation of phenols, total protein and peroxidase activity was observed on 45th day which declined on 60th day after germination, while polyphenol oxidase and phenyl ammonia lyase activity was higher on 60th day as compared to 45th day in inoculated plants.

Key Words: Biochemicals, Chick pea, Enzymes, Fusarium, Resistance

Plant growth-promoting rhizobacteria (PGPR)—mediated induced systemic resistance (ISR) has received considerable attention since biological approaches to manage pests and diseases are more ecologically sustainable than the use of synthetic chemicals. Several mechanisms involved in the induction of systemic resistance include production of siderophores, salicylic acid, antibiotics and induction of pathogenesis related proteins (PR's). The production of enzymes related to pathogenesis (PR-proteins) such as chitinases and glucanases by strains of rhizobacteria can lyse the fungal cell wall and considered as an important trait for antagonism (Saikia *et al.*, 2004). Apart from PR-proteins, the plants also produce other defense enzymes, including peroxidases, phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO). Metabolic changes involved in the defense mechanism of plants are correlated with changes in activity of key enzymes in primary and secondary metabolism. Peroxidase and PPO catalyse the formation of lignin while PAL is involved in the formation of phytoalexins and phenolics (Figueiredo *et al.*, 2010). Activation of defense offering sleeping genes in plants on prior inoculation with rhizobacteria offer an attractive alternative, providing a natural, safe, effective, persistent, and durable type of protection. However, in most of the cases, ISR has been studied mainly in the laboratory and greenhouse. In the present study, an attempt was made to study the effect of seed bacterization on induction of defense response against *Fusarium oxysporum* and enhancement of growth in chickpea under field condition.

MATERIAL AND METHODS

Bacterial strains: Two rhizobacterial strains isolated from chickpea rhizosphere, B20d and Ps14c, previously characterized as antagonists of *Fusarium oxysporum* f.sp. *ciceris* (Kumari, 2015) under controlled conditions and maintained at Bacteriology Laboratory of the Microbiology Department, Punjab Agricultural University, Ludhiana, India were used in the present study.

Field experiment: Field experiment was conducted in wilt sick plot of row length 4 m and spacing 40 cm at Punjab Agricultural University, Ludhiana. Experiment was in Randomized Block design with three replications on wilt sensitive chickpea variety JG-62. The seeds were thoroughly soaked in the bacterial cultures, Ps14c and B20d (10^8 cells ml^{-1}) separately to ensure uniform coating of the surface. Seeds were sown on 15th November 2013 @ 45g plot^{-1} . Observations on wilt incidence, induction of defense response and plant growth promotion were recorded at 15 days interval starting from 45 days after sowing.

Sample collection and analysis of defense-related proteins: Plants were carefully uprooted at 45 and 60 days after sowing without causing any damage to root tissues for estimation of defense related proteins. Fresh roots were washed in running tap water and used for biochemical analysis.

Estimation of phenol: Phenol content in roots was assayed by the method given by Chen *et al.* (2000) using catechol as standard.

Assay of peroxidase: Peroxidase activity was assayed as

per the method described by Ramanathan *et al.* (2000). The peroxidase activity was expressed as change in absorbance at 420nm min⁻¹g⁻¹ fresh weight.

Assay of phenol peroxidase (PPO): PPO activity was determined as per the procedure given by (Mayer *et al.*, 1965). The activity was expressed as changes in absorbance at 495 nm min⁻¹g⁻¹ fresh weight.

Estimation of phenyl ammonia lyase (PAL) activity: PAL activity was measured by the method described by Sujatha and Ammani 2011. The enzyme activity expressed as n moles of cinnamic acid min⁻¹g⁻¹ fresh weight.

Total protein: Total protein content in roots of chickpea plant was determined by the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria mediated induction of systemic resistance in plants to fight against the pathogens has recently been introduced as a substitute to synthetic pesticides. However, the successful application of ISR has been studied mainly in the laboratory and greenhouse. The present study was carried out to study the effect of seed bacterization in managing wilt of chickpea through induction of defense response and enhancing growth under field condition.

Effect of seed bacterization on wilt incidence in chickpea under field condition: Seed bacterization with two rhizobacterial antagonists, Ps14c and B20d offered protection against the pathogen, *Fusarium oxysporum*, under field condition. Although the protection exhibited by rhizobacterial antagonists was not highly remarkable, isolates reduced the wilt incidence to 85.7 and 73.3% respectively as compared to 92% in uninoculated control (Table 1). The inconsistency of rhizobacterial isolates under glasshouse and field condition observed has been attributed to a range of factors including weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil that may affect their growth and exert their effect on the plant (Ahmad *et al.*, 2008). However, there are reports of successful application of bioantagonists in suppressing disease incidence under field condition. Kaur *et al.* (2007) reported that seed treatment with 2 isolates of *Pseudomonas fluorescens* H-Pf5 and C7R12 showed 3.4 and 8.7% wilt incidence in chickpea.

Table 1. Effect of seed bacterization on wilt incidence in chickpea under field condition

Treatments	Wilt incidence (%)		
	45 th day	75 th day	90 th day
Ps 14c	15.0	77.20	85.7
B20d	27.8	71.48	73.3
Control	37.9	86.6	92.0

Induction of rhizobacterial induced defense response in chickpea under field condition:

There are very less reports on induction of systemic resistance under field condition. The present study puts an insight into this phenomenon. Time course analysis on induction of defense response in chickpea roots against the wilt pathogen revealed accumulation of phenolics in plants bacterized with rhizobacterial isolates Ps14c (362.2 µg g⁻¹) and B20d (565.0 µg g⁻¹) on 45th day after germination which was significantly higher when compared to uninoculated control (249.5 µg g⁻¹). Although phenol content in chickpea roots exhibited drastic reduction on 60th day suggesting the successful infection by pathogen, it remained higher in bacterized plants as compared to control (Table 2). Singh *et al.* (2003) in their study also recorded maximum amount of total phenolics in all parts of 4-week-old plants of chickpea bacterized with two *Pseudomonas* strains Pf4 and Pag where their combined inoculation provided significant higher protection against *Sclerotium rolfsii* as compared to single inoculation. Phenols may be the natural constituents of plants where constitutive phenols function as preformed inhibitors associated with non-host resistance. However, others are induced in response to ingress of pathogen or other microorganisms where their emergence is considered a part of defense mechanism. They may directly function as phytoalexins which inhibit the growth of plant pathogenic fungi or be incorporated into the structural barriers such as phenol conjugated, lignified or suberized cell wall appositions (Groenewald, 2006).

Similarly, significantly higher accumulation of proteins and peroxidase activity was observed on 45th day which subsequently declined by 60th day. B20d recorded maximum accumulation of proteins and peroxidase activity in roots (20.8 mg g⁻¹ and 38.1? abs/gfw/min, respectively) which was significantly higher than in control (10.8 mg g⁻¹ and 14.12 ? abs/gfw/min, respectively). In contrast to this, higher activity of polyphenol oxidase and phenyl ammonia lyase was observed on 60th day as compared to 45th day. Although PAL recorded higher activity on 60th day, it was not significant when compared to uninoculated control. Contrary to this, PAL activity on 45th day was significantly higher in bacterized plants compared to that in control. Maximum PAL activity was recorded with B-20d (22.5 µm α-ketobutyrate/gfw/h) 60 days after sowing (Table 2). PAL is thought to be involved in the formation of phytoalexins and phenolics (Figueiredo *et al.*, 2010). In similar manner, PPO activity reached its peak on 60th day in all the treatments. However, this increase in PPO activity was significantly higher in bacterized plants in comparison to control (0.117 abs/gfw/min). Isolate Ps14c recorded highest PPO activity (0.367abs/gfw/min) followed

Table 2. Induction of defense response in chickpea under field condition (days after sowing)

Treatments	Phenolics ($\mu\text{g/gfw}$)		Peroxidase activity (?abs/gfw/min)		Polyphenol oxidase activity (? abs/gfw/min)		Protein (mg/gfw)		PAL ($\mu\text{m } \alpha\text{-ketobutyrate/gfw/h}$)	
	45	60	45	60	45	60	45	60	45	60
Ps14c	362.2	254.9	26.0	8.2	0.10	0.367	19.1	6.02	16.2	31.5
B20d	565.0	246.2	38.15	11.4	0.26	0.360	20.8	16.1	22.5	44.5
Control	249.5	165.0	14.12	6.6	0.07	0.117	10.8	1.83	13.1	37.1
CD (p=0.05)	31.4	16.8	5.0	0.35	0.04	0.066	0.79	1.5	1.07	9.4

by B20d (0.360 abs/gfw/min). However, on 45th day, only isolate B20d exhibited significant increase in PPO activity (Table 2). The increased expression of the PO and PPO enzymes could be correlated with resistance in plants as these enzymes are involved in the biochemical reaction necessary for lignification, which protect the plants from the fungal pathogen. Induction of defense-related enzymes and proteins in plants on prior inoculation with rhizobacterial isolates could offer protection against the pathogen, however, the durability of resistance by PGPR differs from crop to crop and also due to different bacterial strains. Nayar (1996) indicated that induction of defense mechanisms using *P. yuorescens* persisted up to 60 days by seed-treatment, 30 days by root-dipping and 15 days by foliar spray. In cucumber, PGPR-mediated ISR resulted in protection of cucumber from anthracnose disease for five weeks from the time of sowing. But the consistency of ISR over time varies with PGPR strains. In sugarcane, induction of resistance by PGPR persists for 90 days of crop growth. Application of PGPR as sett-treatment induced systemic resistance against *C. falcatum* in addition to enhanced sett germination, tillering and growth of the cane both under controlled conditions as well as field conditions (Viswanathan, 1999). These studies clearly show that PGPR-mediated ISR and plant growth promotion can operate under field conditions.

Plant growth promotion under field condition: Effect of seed bacterization on plant growth was recorded in terms of plant height and plant weight. Rhizobacterial isolates significantly enhanced the plant height as compared to uninoculated control. Isolate Ps-14c recorded maximum plant height (34 cm) followed by B20d (29 cm). Significant increase in plant weight was also recorded in bacterized plants, maximum being with isolate Ps14c (12.1g) compared to 11.8g with B20d and 7.1g with uninoculated control (Table 3). The significant increase in growth parameters on seed bacterization may be associated with systemic protection of plants against the pathogen. Kloepper *et al.* (1993) reported that PGPR strains, viz., *P. putida* strain 89B-27, *S. marcescens* strain 90-166 and *Flavomonas oryzae* strain INR-5 caused systemic protection against angular leaf spot and bacterial wilt besides increasing the plant growth

under field condition. Barriuso *et al.* (2008) revealed that bacterization of chickpea seeds with a siderophore-producing fluorescent *Pseudomonas* strain RBT13 and an antibiotic-producing *B. subtilis* strain AF1, isolated from tomato rhizoplane and pigeon pea rhizosphere, respectively increased the shoot height, root length, fresh weight, dry weight and grain yield in soils infested with *Fusarium oxysporum* f. sp. *ciceris*. Seed bacterization also resulted in a significant reduction in chickpea wilt caused by the same pathogen.

Table 3. Effect of seed bacterization on growth parameters of chickpea under field condition

Treatments	Plant height (cm)	Plant weight (g)
Ps14c	34.0	12.1
B20d	29.0	11.8
Control	23.0	7.1
CD (p=0.05)	2.3	1.6

Inoculation with rhizobacterial isolates B20d and Ps14c offered systemic protection to chickpea plants against wilt which was also associated with plant growth promotion under field condition. However, more rigorous field studies are required to exploit these isolates as potential candidate of bio-inoculating agents.

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Received 23 November, 2015; Accepted 29 March, 2016



Dynamic Population of *Macaca fascicularis* due to Construction of Dam from 2010-2015

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Abstract: Jatibarang Dam construction started in 2010 to prevent flooding in central Java, Indonesia. The associated land clearing had significantly affected the ecosystem and degraded the habitat of 248 *Macaca fascicularis* (Mf). Number of individual and the change of troops data were obtained through seven until ten days-long follows/year and then analyzed using google earth software. The location of sleeping and food areas were recorded by GPS and mapped. *Macaca fascicularis* have lost more than 80% of their habitat including feeding area. Number of individual in 2010 were 201 in 2 troops. In 2015, the troops increased to four. Daily range of groups varied and density of *Macaca fascicularis* was 41.69 individuals ha⁻¹. Conservation activities are listed for consideration in the paper as well.

Key Words: Dam, Habitat loss, *Macaca fascicularis*, Population

Economic growth and a rapidly expanding human population have resulted in increasingly widespread encroachment on forest habitat, resulting in burgeoning human/non-human primate conflict due to crop-raiding by the macaques and, more recently, pest behavior in urban environments as they exploit garbage and other human food sources (Twigg, 2008). Kinnaird and O'Brien (1996) reported that ecotourism had deleterious influences and behaviour. Similar case was recorded in the construction of Jatibarang dam in Semarang, Central Java, Indonesia. Although the construction is very important to prevent flooding, which occurred every year, as tourist project, it would be predicted to get a complex problem since there was a troop of *Macaca fascicularis* (Mf) in that location. More than 80% habitat of *M. fascicularis* will be inundated and there are many problems such as survival on the same location since they were an icon for tourist project, human conflict and zoonotic diseases such as *Giardia duodenalis* in gorillas (Graczyk *et al.*, 2002a; Johnston *et al.*, 2010; Salzer *et al.*, 2007) and *E. coli* in chimpanzees (Goldberg *et al.*, 2007) and gorillas (Rwego *et al.*, 2008). In this activity, monitoring of *M. fascicularis* should be done for about 5 years, starting from 2010 when Jatibarang Dam as ecotourism site was constructed until finished in the middle of 2015. Hopefully, *M. fascicularis* will habituate to prolong construction of dam as similar as *Macaca* when they prolonged exposure to vibrations (Becnoche, 2014), dynamites exploded (Novak 2014). Alternatively, as the researchers suggest, making a stimulus predictable may decrease the stress response of an animal (Westlund *et al.*, 2012).

Although many aspects for conservancy such as daily activities, behaviour, analysis preference has been done, this publication just focused on profile number of individual and population. The research objective is to conduct monitoring number of *M. fascicularis*, the change number of troops from 2010-2015 and give recommendation for future.

MATERIAL AND METHODS

The study site is located in the Semarang region, central of Java, Indonesia (Fig.1). The study site covers an area of 189 ha, including the territories and landholdings of agricultural communities (Talunkacang district).

Situation of Jatibarang between before and after construction of dam has shown in Fig. 2. Generally, longtailed macaque in Jatibarang dam tourism project distributed across several habitat type, such as riverbank, ravine, natural forest, and also human made habitat such as, parking area, road/stairs to the Kreo Cave, ricefield and settlement.

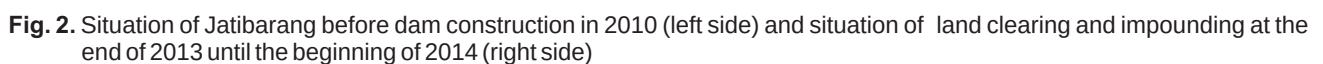
Macaca fascicularis lived in area of Jatibarang dam were used in this research. To ensure human safety, the tourist object were closed for visitor start from 2011 until the last of December 2013. During this time, the animals fed came from Tourism agency and Project Developer everyday such as: 10 kg of casava, 5 kg of corn, and 5 kg of sweet potato. Beside giving food, they look for food from visitor and people who visit the tourist project (unless the tourist project was closed for visitors). Feeding for *M. fascicularis* should be done to prevent them to expand the local community and



General activity: The First step, information of group size, estimation of number population, sleeping tree (sleeping area) and food sites (food area) on *M. fascicularis* will be obtained by interviewing with local communities. The position of sleeping and food area will be recorded by Global Positioning System (GPS).

daily activities and some other ecological aspects are basic information when we consider the measures for long-tail macaque conservation.

Counting number of individu and troops: According to condition of area of the Dam, such as small and no flat with certain area for moving and doing activity, track and count methods (Steiner, 1999; NRC, 1984) was used in this methods. In the first step, researcher will follow the troops and counting individual in their feeding and sleeping sites. It was lucky, when the tourist projects would be stopped for visitors, feeding were obtained by developer and tourism agency, so that census was much easier to conduct while they ate in certain area. Estimation of population (Structure of population) will be done during the seven different days. The



highest data of population number will be estimated as density and excessive population of long-tailed macaque.

Infant are marked by smaller body frame, black, and breast-fed by its parent. While the juvenile are gray, actively running but still at the same area with its parent. Adult are gray color. Adult male are marked with testes, moustache, have feed pocket, while the female adult have mamary gland. As a special mark, in one group a unique mark were needed especially for the alpha male like big body frame, scar mark on legs, etc. This was conducted to reduce or annihilate miscounting.

RESULTS AND DISCUSSION

During 2010, before construction of dam, it mainly two groups of longtailed macaque in Kreo Cave Tourism (Jatibarang dam) namely “Kreo” and “Parkiran” were identified. Before following the groups and counting individual, estimation of population on *Macaca fascicularis* were obtained by interviewing with local communities then it compared with scientific counting as demonstrated in Table 1.

Using track and count methods, total 201 individual of the *Macaca fascicularis* (Mf) were estimated, which consist of 143 individual for Kreo and 83 individual of Parkiran group. Furthermore, structure population for Kreo groups was consist of 15% infant, juvenile 54%, adult male 10% and adult female 21% (Fig. 3). For Parkiran group, number of individual was less than Kreo group, the highest percentage of individual was also juvenile (42%) then adult female (25%), adult male (18%) and infant (15%) (Fig. 4). Comparison of percentage of individual between Kreo and Parkiran are showed in Fig. 5.

Futhermore, counting number of individual had been done every year from 2011 till 2015 where the project finished. It would be noticed that number of *M. fascicularis* from year to year varied considerably i.e., 234 in 2011; 219 in 2012; 222 in 2013; 248 in 2014 and 2015 (Table 2 and Fig. 6).

Surprisingly, there was a new small troops in 2013, which derived from Kreo group, so that the name is Kreo-1 (Table 2; Fig. 7). In the middle of 2015, again, there was an another new troop, which derived from Parkiran group so total number of group from two in 2010 become four in 2015.

Composition of each group from 2010 until 2015 were shown in Table 3. It could be noticed that number of infant were getting decreased from time to time.

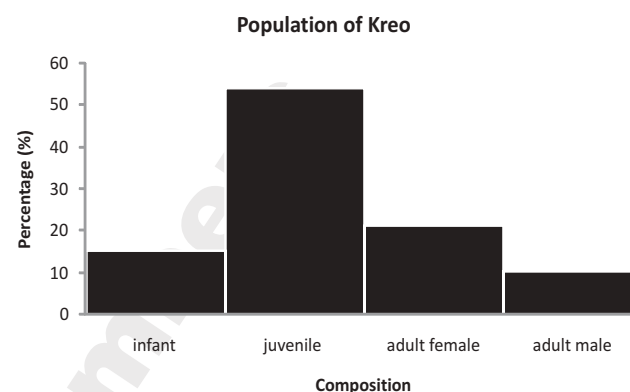


Fig. 3. Population composition of Kreo, which contains 15% infant, 54% juvenile, 10% adult male and 21% adult female

In 2010, it was observed that the composition of population was dominated by juvenile, it meant that rate of reproduction was growing well. Based on external condition, population were healthy as indicator of wounds, scars and bot fly lesion. Westin (2007) evaluated the health of subjects through observations of external indicators of poor health such as wounds, scars, and bot fly lesions, and through the analysis of non-invasively. Even though, the activity of dam construction could affect the activity or the rate of reproduction because of the occurrence of stress in some individual. Brook and Marshall (2000) reported that stress will

Table 1. Comparison counting between local communities and track and count method in area before construction of Jatibarang Dam

Group ID	Local count (all)	all	Adult female	Adult male	Juvenile	Infant
Parkiran_obs1	500	13+	4	3	4	3
Kreo_obs1		83+	40	37	33	9
Parkiran_obs2	300	51	12	11	19	9
Kreo_obs2		100	29	14	44	18
Parkiran_obs3	400	65	27	8	21	9
Kreo_obs3		120	27	27	46	20
Parkiran_obs4	400	63	20	11	23	9
Kreo_obs4		133	27	14	72	20
Parkiran_obs5	300	67*	17	12	28	10
Kreo_obs5		134*	28	14	72	20

*maximum number, + minimum number

suppress ovulation so that the growth of reproduction become zero. Previous researcher also reported that unpredictable construction activity may initially cause some changes in monkey behavior some of which seems to be related to decreased activity. However, because many of these changes are reversed over time, they appear to habituate to prolonged exposure to vibrations (Becnoche,

Table 2. Number of Individual in each group from 2010 - 2015

Number Individual from 2010 - 2015						
Group	2010	2011	2012	2013	2014	2015
Kreo	134	144	144	114	110	112
Parkiran	67	90	75	98	94	78
Kreo-I	-	-	-	20	34	37
Parkiran-I	-	-	-	-	-	21
Total	201	234	219	222	248	248

Table 3. Composition of individual

	2010	2011	2012	2013	2014	2015
Kreo						
Infant	20	7	6	3	3	4
Juvenile	72	24	30	18	14	13
Adult	42	113	108	93	93	95
Parkiran						
Infant	10	4	9	6	4	2
Juvenile	28	26	26	44	45	35
Adult	29	60	40	48	45	41
Kreo-1						
Infant				2	4	3
Juvenile				8	10	13
Adult				10	20	21
Parkiran-1						
Infant						1
Juvenile						10
Adult						11

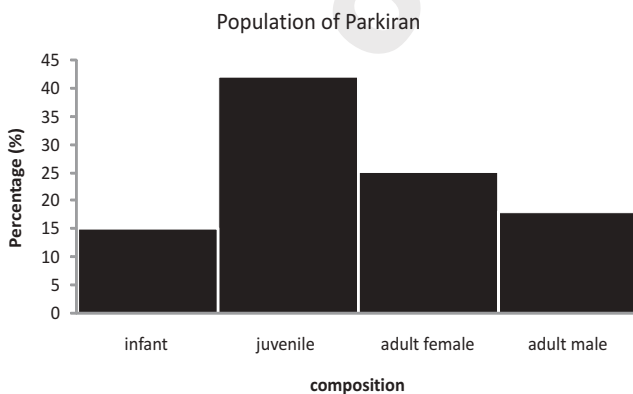


Fig. 4. Population composition of Parkiran in 2010, which contains of 15% infant, 42 % juvenile, 25% adult male and 18% adult female

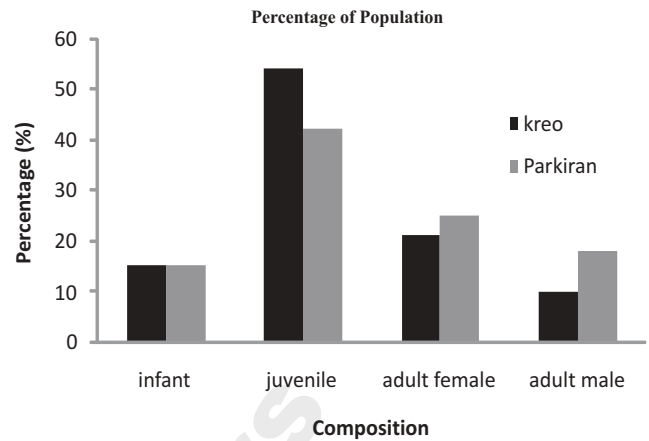


Fig. 5. Percentage of population between Kreo and Parkiran (%) in 2010

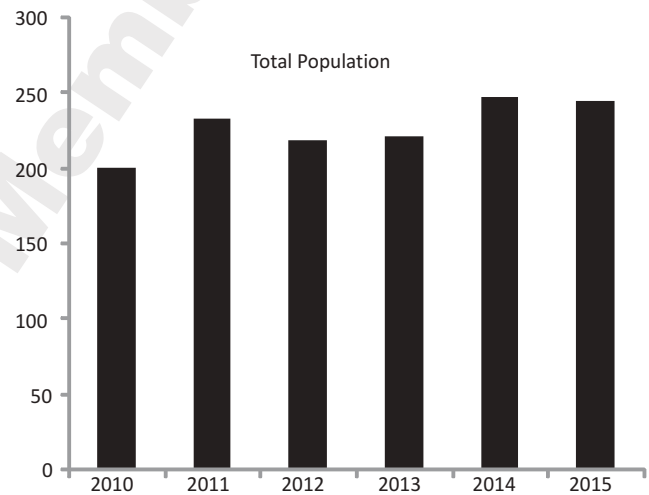


Fig. 6. Number of individual from 2010-2015

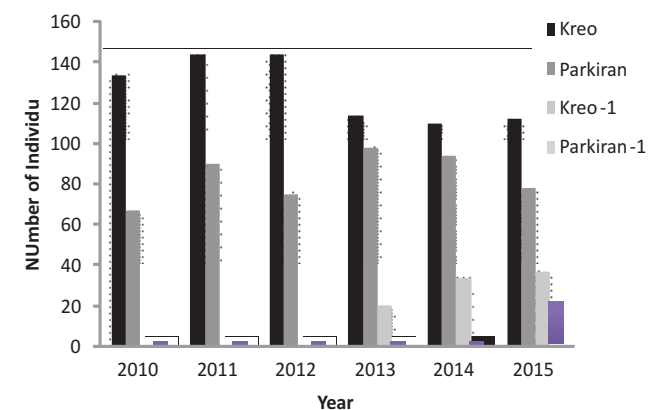


Fig. 7. The change in number of troops from two in 2010 to four in 2015

2014). In the early 2011 until the end of 2015, feeding were conducted by developer and agency tourism to prevent expansion of animal to the urban area and disturbing the local community even though it was against the idea of conservation. It would be stopped gradually and will be returned to the natural food after the project was finished. As information, existing of *M. fascicularis* in monkey had been allocated in the same area to prevent human conflict, to conserve eco tourism and spread of zoonotic.

An important information was recorded in 2013, when landclearing and inundated was conducted in 2014, the monkeys had already lost about 80% of their habitats, so that daily range of groups varied and, density of *M. fascicularis* from 11,46 individuals ha^{-1} in 2010 become 41.69 individuals ha^{-1} . In that period, number of group both in Parkiran and Kreo has changed from 2 to 6 lower group such as Parkiran (PG), Kreo (KG), Kreo 1 (KG-1), (PG1), Dam Group (DG), and smaller group from KG1. The three of last group were still not clearly separated, sometimes they still foraging together then separated when sleeping. It was only Kreo-I that was permanently separated. Lehman (2006) reported, groups of monkeys have a clear social system and has a strong bond between the group members, but if the group size is too large it will result in a lack of the role of individuals in providing inter-individual contribution in the social system, so that cohesion among depressed individuals in the group be decreased as well. Unharmonic event had been reported by Karimullah and Shahrul (2011) who stated that *Macaca* prefer to exist in a large group for the purpose of safety and food ability. Subsequently they have learned to associate with food and human.

Although number of individual in each group have been increasing from time to time, but it must be noted that number of infant was decreasing (Table 2 and 3). Chapman *et al.* (2000); Paulus (2007) reported habitat destruction resulted in losses in animals. It had effects on primate population decades later. Populations of blue monkeys, *Cercopithecus mitis*, and red-tailed monkeys, *C. ascanius*, continued to decline in response to heavy logging. This condition is similar as most wild populations of primates, which are relatively small and their reproductive cycles are protracted (with low reproductive rates relative to most mammals of similar size), so they are particularly vulnerable to population decrease, including those that result from human activities (Elizabeth *et al.*, 2010). Based on experience, managing the population of *M. fascicularis* should be done to help the population to survive. Therefore, during the development of Jatibarang dam, social and ecology of this species have to be regularly monitored to assess the viability of Long-tailed macaque.

At the end of 2010 until last of 2013, it was forbidden to visit area of dam then were reopened for public in 2014. According to increasing of people to visit the dam, interaction between monkey and people also more frequent, dissemination conservation of *Macaca*, zoonotic diseases for society and other agency should be done especially for the dam's merchandise who hits monkeys. How important for human being, the environment and animal together in harmony should be disseminated. For visitors, many notice board should be put on to inhibit conflict between visitor and monkey and to prevent zoonotic diseases.

CONCLUSION

Macaca fascicularis in area of Jatibarang dam are very adaptable primates. Even the loss of huge habitat, they still survived, but it must be noted that number of infant was decreasing. Conservation action should be discussed clearly among expert and implemented so that *M. fascicularis* population, human and environment can be harmonized. Responsibility of *M. fascicularis* feeding by parties involved must be evaluated and stopped gradually after the tourist project has finished and would be changed by natural feeding.

ACKNOWLEDGMENT

This study was supported by funds received from CTI Foundation, We are grateful to student (Nining), who help working in the field and the guidance in conducting this study. As always, were indebted to our family members for their cheer, goodwill and unflagging enthusiasm for all.

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Quaternary Quinolinium Salts as a New Antifungal Agent for Protection of Rice Crop

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Abstract: Quinoline was firstly prepared by Skraup's reaction and then series of quinoline and its derivatives based quaternary ammonium salts have been synthesized by condensing quinoline and quinaldine separately in the presence of ethanol using different alkyl halides having varying carbon chain length in the ratio of (1:14) equivalents. The structure of the final compounds were confirmed on the basis of salt analysis, conductivity measurements and their spectral studies. The synthesized quaternary quinolinium salts were examined for antifungal activity against two fungal strains of rice i.e. *Drechslera oryzae* and *Fusarium verticilloides*. The results of bioassay showed that some of the synthesized quaternary quinolinium salts emerged with excellent per cent spore inhibition at higher concentration against above mentioned organisms.

Key Words: Quaternary quinolinium salts, Rice, *D. oryza*, *Fusarium verticilloides*

Rice is the agricultural commodity with the third highest worldwide production after maize and sugarcane and it is consumed by almost half of the world's population. From last two decades it is being widely exploited by several phytopathogens, which effects its production and economy in diverse ways such as reduction in quality and quantity of crop. A wide range of control options such as green manures, soil manures, soil amendments, crop rotation, fungicidal applications and biological control agents were already in use, but they are mainly relied on host resistant varieties, which is more environment friendly and practical way to control the diseases. As there is huge challenge to use resistant varieties due to their cost and availability. So, the development of fungicides against disease is thus main focus for plant breeders and researchers.

Quinoline is a heterocyclic scaffold of paramount importance to human race. Several quinoline derivatives isolated from natural resources or prepared synthetically are significant with respect to medicinal chemistry and biomedical use. Indeed quinoline derivatives are some of the oldest compounds, which have been utilized for the treatment of a variety of diseases. The bark of cinchona plant containing quinine was utilized to treat palpitations, fevers and tertians since more than 200 years ago. Compounds containing quinoline moiety are most widely used as anti-malaria (Lamontagne *et al.*, 1982), antibacterials (Fang *et al.*, 2000), antifungals (Kumar and Bawa, 2009) and anticancer agents (Gatto *et al.*, 1999). They are also used as polymers, catalysts, corrosion inhibitors, preservatives, and as solvent for resins and terpenes. Furthermore, these compounds find applications in chemistry of transition metal catalyst for uniform polymerization and luminescence

chemistry (Smirnov *et al.*, 1973; Diwedi *et al.*, 2014). Quinoline derivatives also act as antifoaming agent in refineries (Calus *et al.*, 2007).

Several pesticides containing quinoline moiety have been designed for the development of new fungicides with different chemical base because of its broad range of bio-activities to broaden the spectrum and to retard the resistant development (Schafer *et al.*, 2008; Rogers and Cooke, 2011; Long *et al.*, 2012). But due to the mutations in the pathogens to overcome the host defence. There is an urgent need for new antifungal remedies with novel mode of action and minimal non-ecological hazards for better yield of staple food and healthier environment. As quaternary ammonium salts are the emerging new bio-potential molecules and they have proven their efficacy in the fields of pharmaceuticals, as anticancer, antiviral, anti-parasitic, antifungal and antibacterial agents, as agro-chemicals and as potential wood preservatives (Clarke and Davis, 1941). Present work was aimed to incorporate the different alkyl halides in quinoline moiety to obtain their quaternary salts to evaluate their effectiveness as fungicides against pathogens of rice.

MATERIAL AND METHODS

Various analytical techniques were used to characterize the synthesised analogues such as thin layer chromatography and conductivity measurements. Salt analysis were also done for the confirmation of synthesized salts. All these experiments were performed in organic chemistry laboratory at Punjab Agricultural University, Ludhiana. Structure elucidation was done by ¹H NMR and ¹³C NMR from Central Instrumentation Lab, Punjab University Chandigarh.

Synthesis of quinoline and their quaternary salts:

Quinoline was synthesized by heating and stirring the mixture of 10g of ferrous sulphate, glycerol (2.35 moles, 108g), aniline (0.6 moles, 27g), nitrobenzene (0.4 moles, 17g) and sulphuric acid on hot plate for 1 hour. Distillation was done in the presence of 40 % solution of sodium hydroxide and distillate was collected. The purity was checked by TLC (Badwai *et al.*, 1983).

After the successful synthesis of quinoline, their quaternary salts were prepared by condensing quinoline and 2-methyl quinoline separately in the presence of ethanol with methyl iodide, propyl bromide, isopropyl bromide, butyl bromide, 2-bromo butane, pentyl bromide, hexyl bromide, heptyl bromide, octyl bromide, decyl bromide, allyl bromide, hexadecyl bromide in the ratio of (1:14 equivalents) for 48 hours on sand bath. The excess of ethanol was distilled off. The residue obtained were washed 4-5 times with dry ether (Clarke and Davis, 1941). to get N-methyl quinolinium iodide, N-propylquinolinium bromide, N-butylquinolinium bromide, N-pentylquinolinium bromide, N-isopropylquinolinium bromide, N-secbutylquinolinium bromide, N-hexylquinolinium bromide, N-heptylquinolinium bromide, N-octylquinolinium bromide, N-decylquinolinium bromide, N-allylquinolinium bromide, N-hexadecylquinolinium bromide, N-methylquinaldinium iodide, N-decylquinaldinium bromide and N-isopropylquinaldinium bromide.

Source isolation and maintenance of cultures: Diseased samples of *brown spot* and *foot rot of rice* were collected from experimental areas of Punjab Agricultural University, Ludhiana and washed in running tap water for 5-10 min. The infected parts were cut into small pieces with the help of sterilized blade and were sterilized with mercuric chloride solution (0.1%) for one minute followed by three washings with sterilized distilled water. The sterilized infected pieces were transferred to test tubes containing potato dextrose agar (PDA) medium (200 g peeled potato, 20 g dextrose and 20 g agar in 1 litre water) under aseptic conditions. These test tubes were incubated at $25\pm 1^\circ\text{C}$ in order to get good growth of fungus. The cultures so obtained were purified and maintained by further sub culturing on PDA slants and stored in refrigerator till further use.

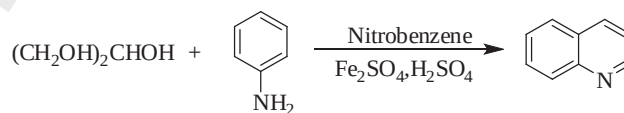
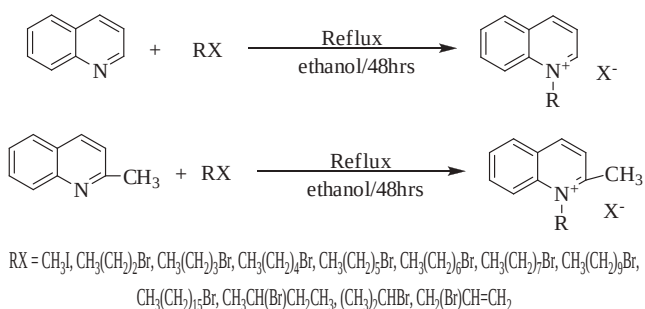
Preparation of stock solution: The stock solution (2000 $\mu\text{g/ml}$) of synthesized compounds and standard fungicides i.e. Tilt 25 EC (Propiconazole) were prepared by dissolving the chemical (20 mg) in 1 ml of Tween 20 (Polyoxyethylene sorbitan) and volume was made 10 ml with sterilized distilled water separately. The required dilutions of 1000, 500, 250, 100, 50 and 25 $\mu\text{g/ml}$ were subsequently made from the stock solution. All the test compounds were screened against two phytopathogenic fungiviz. *D. Oryzae* and *F.*

verticilloides by using spore germination method (Marek *et al.*, 2012).

Spore germination method: Spore suspension was made by adding sterilized distilled water to the fresh culture of *D. Oryzae* and *F. verticilloides*. Suspension was filtered through three layers of sterilized cheese cloth in order to remove mycelia under aseptic conditions. Small droplets (0.02 ml) of test solution and spore suspension in equal amount were seeded in the cavity of the cavity slides. These slides were placed in Petri plates lined with moist filter paper and were incubated for 72 hrs at $25\pm 1^\circ\text{C}$ for *D. oryzae* and 24 hrs at $20\pm 1^\circ\text{C}$ for *F. verticilloides*. Observations on spore germination were taken at 2 hours interval and per cent spore germination inhibition was calculated using following formula devised by Vincent (Nene and Thapliyal, 1993).

RESULTS AND DISCUSSION

Fifteen quaternary quinolinium salts (1-15) were synthesized by reacting different alkyl halides chains with tertiary amines viz. quinoline and quinaldine under the experiment conditions. General schemes of preparation of tertiary amine and quaternary quinolinium salts are given in Scheme-1 and Scheme-2. The physical characteristics of quinoline and their salts are given in Table 1 and Table 2.

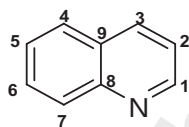
**Scheme 1****Scheme 2****Table 1.** Physical characterization of quinoline

Physical properties	Values
Molar mass	129.16g/mol
Appearance	Yellowish oil liquid
Density	1.093g ml ⁻¹
Boiling point	237 °C
Solubility	Less soluble in the water but in more soluble in alcohol and ether
Yield	70%

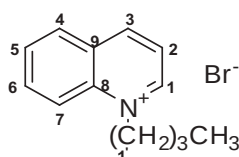
Table 2. Physical characterization of quaternary ammonium salts of quinoline and its derivatives (1-15)

Quinolinium salts				
Salts	Mol. wt.	Yield	Appearance	M P (°C)
C-1quinoliniumiodide	144.19	70	Viscous liquid	-
C-3quinoliniumbromide	172.25	68	Viscous liquid	-
C-4quinoliniumbromide	186.13	68	Viscous liquid	-
isoC-3quinoliniumbromide	200.30	62	Viscous liquid	-
C-5quinoliniumbromide	172.25	60	Viscous liquid	-
secC-4quinoliniumbromide	186.27	65	Viscous liquid	-
C-6quinoliniumbromide	214.13	60	Viscous liquid	-
C-7quinoliniumbromide	228.35	58	Viscous liquid	-
C-8quinoliniumbromide	242.38	52	Solid	66-68
C-10quinoliniumbromide	270.43	48	Solid	48-50
C-16quinoliniumbromide	354.59	30	Solid	90-92
Allylquinoliniumbromide	170.23	50	Viscous liquid	-
Quinaldinium salt				
C-1quinaldiniumiodide	158.22	68	Solid	65-70
C-10quinaldiniumbromide	284.46	60	Viscous liquid	-
isoC-3quinaldiniumbromide	186.13	50	Solid	50-55

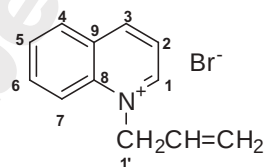
Characterization of quinoline: The ^1H NMR (400MHz, $\text{CDCl}_3/\text{DMSO}$, 25°C) spectrum of quinoline contained seven protons multiplet ($\text{C}_1\text{-C}_7$) between 8.8-7.26 ppm due to aromatic protons. There are three protons at C_7 , C_{11} , C_3 having chemical shifts at 8.05, 8.1 and 8.8 ppm that represent bicyclic nature of the compound as well as the presence of electronegative atom attached with C_1 of the ring system. The mass spectrum of the product contained molecular ion peak at 130.1 which confirmed its formation.



Characterization of quaternary quinolinium salts: Compound (3) showed signal in its ^1H NMR (400MHz, $\text{CDCl}_3/\text{DMSO}$, 25°C) spectrum at 9.5-8.0 due to aromatic protons. Another signal at 1.3 due to the methyl group attached to the nitrogen of quinoline. The ^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{DMSO}$, 25°C) spectrum signals appeared in the range of 1450-120 ppm due to nine aromatic carbons. Another signal was appeared in the range 50-20 ppm due to four aliphatic carbons. The most justified signal appeared at 53.4 ppm due to attachment of methylene group to the nitrogen of the quinoline. On the basis of data obtained this confirmed the compound (3) N-butylquinolinium bromide.



N-allylquinolinium bromide: The ^1H NMR (400MHz, $\text{CDCl}_3/\text{DMSO}$, 25°C) spectrum of compound (12) contained seven protons multiplet ($\text{C}_1\text{-C}_7$) between 9.5-8.0 due to aromatic protons. The chemical shift for two aliphatic protons attached to the nitrogen of quinoline appeared at 2.0. The ^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{DMSO}$, 25°C) spectrum of compound (12) contained nine carbons ($\text{C}_1\text{-C}_9$) having δ -values in the range of 145-120ppm that confirmed the presence of aromatic carbon. There are three other carbons present that have δ -values in the range of 132-55 ppm that confirmed the presence of unsaturated aliphatic carbons. The chemical shift for carbon attached to the nitrogen of quinoline appeared at 59 ppm. All other compounds structural determination were done similarly.



Antifungal activity against *fusarium verticilloides* and *Drechslera oryzae*: Quinoline and their quaternary salts were evaluated *in vitro* for their antifungal potential against *F. verticilloides* and *D. oryzae* by spore germination method at different concentrations i.e. 1000, 500, 250, 100 and 50 $\mu\text{g ml}^{-1}$ and per cent spore inhibition was calculated for the synthesized compounds. Then evaluation of fungi-toxicity of all compounds was done by calculating the ED_{50} and ED_{90} values (effective dose at which 50 and 90 per cent inhibition taken place). Analysis of data was done statistically using (2-way ANOVA) C-pcs software.

The information of the statistical analysis and per cent spore inhibition is given in Table 5 and Table 6. The correlation between all concentrations and synthesized quinolinium salts and the obtained values of per cent spore inhibition were significant at different concentrations. But few of the compounds at higher concentration were also found statistically at par. At 1000 $\mu\text{g/ml}$ concentration, decreasing order for the per cent spore inhibition comes out as C-16quinolinium bromide > C-8quinolinium bromide > C-7quinolinium bromide > C-10quinolinium bromide for *F. verticilloides* and for *D. oryzae* the decreasing order varies as C-8quinolinium bromide > C-6quinolinium bromide > C-7quinolinium bromide > C-10quinolinium bromide. The quinolinium salt that contain unsaturated hydrocarbon chain i.e. compound (12) was found more effective against *D. oryzae* but not in the case of *F. verticilloides*. In case of *D. oryzae*, quinolinium salts those contains very small hydrocarbon chain from C-1 to C-5 showed zero inhibition at 50 $\mu\text{g ml}^{-1}$ but they are effective against *F. verticilloides* at 50 $\mu\text{g ml}^{-1}$.

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Received 21 September, 2015; Accepted 16 November, 2015



Determining Genetic Architecture of Upland Rice (*Oryza Sativa* L.) Genotypes under Suboptimal Rainfed Ecosystem

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Abstract: Analysis of variance revealed significant difference among genotypes for all parameters under study, except for days to flowering and crop duration, indicating physiological threshold of the traits in the prevailing environments. Heritability estimation revealed that one tenth of variability, as measured by σ^2 , was due to environment and nine tenth was genetic in origin for yield factors. The present study reports high heritability and low genetic advance for economic yield *viz.*, economic yield (94% and 0.77, respectively), biological yield (97% and 1.99) and harvest index (86% and 23.31); which specifies that the character is predominantly governed by non-additive gene action (dominance and epistasis). Among variability coefficients, phenotypic and genotypic proportion was maximum for economic yield (51.57 and 50.26) followed by biological yield (41.88 and 41.36), denoting probability of genetic manipulations among test populations. Grain yield was found to coinherit with plant height (0.438**), panicle length (0.695**), and biological yield (0.908**) and harvest index (0.416**).

Key Words: Assimilates partition, Heritability, Genetic advance, Rainfed ecology, Water stress

Nearly, hundred million people depend on the upland rice as their daily staple food globally (Aditya and Bhartiya, 2013), of which two third are Asian. In India, the total area under upland rice is 6 mha (13% of total rice). Uniqueness of upland rice is entirely dependency upon the monsoon and production under rainfed conditions. Variable levels of water deficit in upland rice cultivation cause negative alteration in crop architecture resulting lowered down of root and shoot biomass and eventually grain yield (Lafitte *et al.*, 2006; Wang *et al.*, 2009). Moreover, poor productivity of existing cultivars, making rice production in upland regions risky and unattractive due to low yield of 1200 kg ha⁻¹ (Sarma and Pattanayak, 2009; Toshimenla and Changkija, 2013) as compare to 3600 kg ha⁻¹ for wet season lowland rice. Consequently, the quest for further understanding of water deficit tolerance for increased productivity has continued to attract intensive research in upland paddy, which still remain scientifically untouched (Adewusi and Nassir, 2011).

Traditional upland cultivars has lesser phenotypic liking and poor yielder but are highly adapted to rainfed ecology however, they tend to lodge under unfavorable conditions and hence not suitable for higher input management. Since no variety is available for typical upland ecosystem, the need for improving productivity by exploiting existing variability has been long felt. Further, looking for difficulty to adopt lowland genotype upland region, improvement in yield can be achieved by selection among early upland genotypes only. Therefore, the work was undertaken with 33 local and early genotypes and 4 national released varieties to understand responsive genetic makeup to upland ecology and set research protocols for this emerging rainfed rice research.

MATERIAL AND METHODS

Thirty three broadly adapted traditional, four improved and elite lines varieties were used as parent material. Data from field experiments and weather data was recorded from S. G. College of Agriculture and Research Station, Jagdalpur, Chhattisgarh, India (19.05°N, 81.57°E, 549 MSL). The soil type of station was sandy loam with pH of 6.7. Average annual rainfall is 1100 mm. Experiment was conducted with Complete Randomized Block Design with three replications of 15 sqm plot size. Crop was grown in upland research block where no water accumulated for longer time span and trial was maintained completely rainfed i.e. no irrigation was given in entire life cycle, even no starter dose. Observation was recorded for nine quantitative characters *viz.* early vigour, plant height, panicle length, flag leaf area, panicle length, days to flowering, economic yield, biological yield and harvest index. Phenotypic, Genotypic and environmental variances were done using the formula of Uguru (2005). Heritability estimates were done according to Burton and Devane (1953). Phenotypic and genotypic correlation coefficients were worked out by the formulae suggested by Al Jibouri *et al.* (1958). The significance of phenotypic correlation coefficients were tested against *r* values from the *r* table of Fisher and Yates (1963) at *P* = 0.05 and *P* = 0.01.

RESULTS AND DISCUSSION

Analysis of variance revealed significant difference among genotypes for all the traits, except days to flowering and crop duration, indicating ample scope for the selection of different quantitative traits for rice improvement. Onset of South West monsoon in first fortnight of June and termination

in mid September are the probable factors behind this durational adaption. The studies on genotypic and phenotypic coefficients of variation evidenced high amount of variation and environmental role on trait expression. There was narrow margin between two coefficients for flowering period, crop duration and yield complex (Fig. 1), showing lesser environmental play. However, for characters like effective tillers per plant and panicle length, magnitude of phenotypic coefficients of variation was significantly higher than genotypic coefficients, may be due to higher degree of interaction of genotypes with the prevailing environment (Zahid *et al.*, 2006 and Pandey *et al.*, 2010). The maximum genotypic coefficient of variation was recorded for economic yield followed by biological yield (50.26 and 41.27, respectively). Traits like harvest index (HI), panicle length, tillers per plant and plant height also exhibited comparatively higher value for genotypic coefficient of variation. The higher values clearly indicated a high degree of variability in these quantitative characters and suggested the possibility of yield improvement through selection of these traits (Rangare *et al.*, 2012). Heritability measures relationship between parent and progeny and has been widely used to assess the degree to which a character may be transmitted from parent to progeny. It also indicates the relative importance of heritability and environment in the expression of character (Srivastava *et al.*, 2014). In the present study, economic traits like biological yield, economic yield, days to flowering and crop duration showed higher amount of heritability (BS) (Fig. 2). This nature of inheritance reveals that selection can procedure can be simplified by going through yield directly. Higher values of heritability and minimal range of PCV and GCV difference could mean that characters are being regulated by genetic factors and selection will be rewarding. However, question arise that if yield is highly heritable that where exactly water stress operates? This needs intense research and further discussion. High estimates of heritability coupled with moderate or high value of genetic advance was

observed for harvest index and flowering period. This suggested the preponderance of additive gene action for the expression of these characters, which could be improved through direct selection methods (Pfukrei *et al.*, 2011; Koli *et al.*, 2013). Traits like economic and biological yield exhibited high heritability but lowered genetic advance which implies for predominance of non-additive factors.

Identifying yield architecture: Significant positive correlation among majority of the morphological characters evaluated was observed except for early vigour. Grain yield was found to be positively associated with plant height, panicle length, biological yield and harvest index. Optimum plant stature is essential for sufficient dry matter accumulation and proper translocation of assimilates which eventually enhances number of spikelet per panicle and ultimately panicle length. In present investigation, 1:2 ratio of economic yield to biological yield was observed i.e. 50 per cent of harvest index, which stands for better utilization of food reserve. Flowering phenomenon was positively and significantly correlated with crop duration and total biomass (Table 1 and 2), which means delay of reproductive phase promotes better vegetative growth and in turn higher

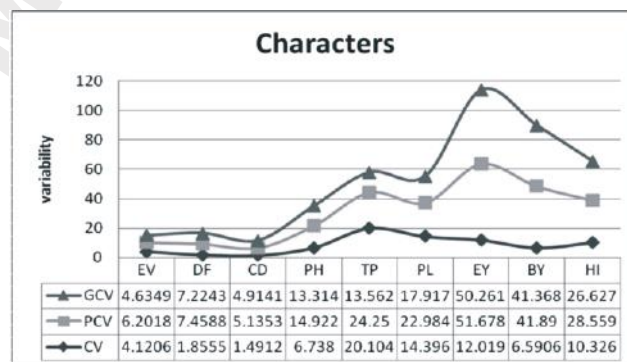


Fig. 1. Variability pattern among traits

(Where: EV-Early vigour, DF – Days to flowering, CD – Crop duration, PH – Plant height, EY – Economic Yield; BY – Biological yield; HI – Harvest Index)

Table 1. Genotypic correlation coefficients among traits

	EV	DF	CD	PH	TP	PL	EY	BY	HI
EV	1.0000	-0.1163	-0.0302	-0.0373	-0.1706	-0.0570	-0.0328	-0.0445	-0.0771
DF	-	1.0000	0.8168**	0.1641	0.0011	0.0914	0.2265*	0.2296**	0.0746
CD	-	-	1.0000	0.0595	-0.0043	-0.0212	0.2470**	0.3509**	-0.1232
PH	-	-	-	1.0000	-0.5445	0.6950**	0.4380**	0.4680**	0.1709
TP	-	-	-	-	1.0000	0.3195**	0.0819	0.0491	0.1254
PL	-	-	-	-	-	1.0000	0.5838**	0.5873**	0.2809**
EY	-	-	-	-	-	-	1.0000	0.9085**	0.4166**
BY	-	-	-	-	-	-	-	1.0000	0.0397
HI	-	-	-	-	-	-	-	-	1.0000

Critical value of 'r' at 5%= .1761 and that at 1%= .2289; *, ** - significant at 5 % and 1 % level respectively

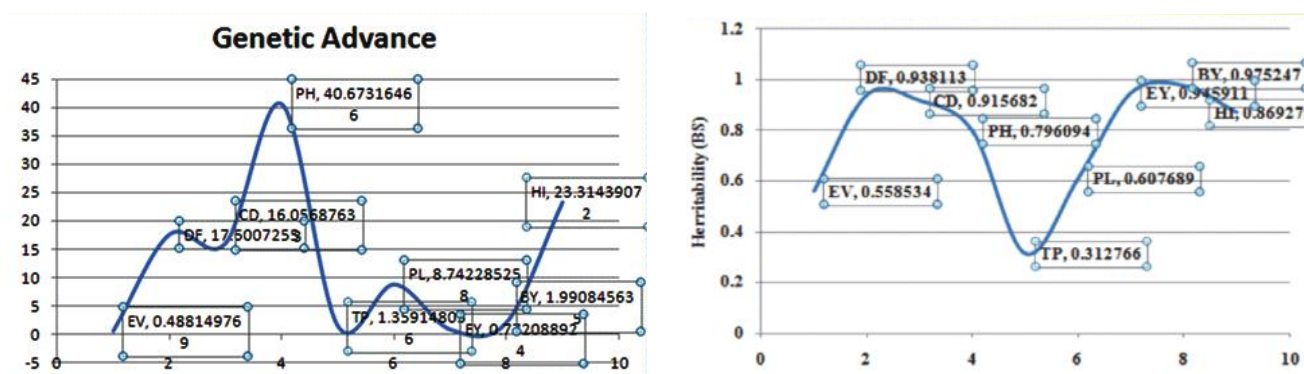


Fig. 2. Genetic advance and heritability pattern of test population

Table 2. Phenotypic correlation matrix

	EV	DF	CD	PH	TP	PL	EY	BY	HI
EV	1.0000	-0.0379	-0.0872	-0.0278	-0.0465	-0.0254	-0.0439	-0.0351	-0.0931
DF	-	1.0000	0.7622**	0.1940*	0.0379	0.0384	0.2231*	0.2152*	0.0908
CD	-	-	1.0000	0.0470	0.0437	-0.0187	0.2257*	0.3272**	-0.1106
PH	-	-	-	1.0000	-0.2560*	0.4682**	0.3998**	0.4022**	0.1819*
TP	-	-	-	-	1.0000	0.0332	0.0514	0.0151	0.0782
PL	-	-	-	-	-	1.0000	0.4205**	0.4582**	0.1814*
EY	-	-	-	-	-	-	1.0000	0.8956**	0.4427**
BY	-	-	-	-	-	-	-	1.0000	0.0447
HI	-	-	-	-	-	-	-	-	1.0000

photosynthesis, greater panicle length, spikelet L x B ratio and grain yield finally. This could be probable reason for poor productivity of early varieties in comparison to late durational as well. Similar explanations implies when relationship of crop duration and biological yield is discussed i.e. greater the crop duration – higher the biomass. In continuation with previous discussion by Lestari *et al.* (2011), plant height was positively correlated with panicle length, grain yield and biological yield but showed negatively relationship with tillers/plant. However, contrasting results for negative association of plant height and crop duration to paddy yield have also been reported (Lanceras *et al.*, 2004 and Abarshahr *et al.*, 2011). Hence clear evidence that, better plant height with sufficient number of productive leaves and good sized panicle, enhances the genotypic productivity while excessive length reduces the emergence of tillers and cause lodging in upland cultivars. Therefore, economic yield and crop biomass can be selected directly while considering early flowering and optimum statured plant type. Inheritance behavior of major traits displayed positive sign for future research agenda. Present study concludes that crop stature, reproductive transition and harvest index are critical to work in rainfed ecology.

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Received 30 October, 2015; Accepted 10 February, 2016



Character Association Studies in Ridge Gourd (*Luffa Acutangula* Roxb. L.) With Reference to Yield Attributes

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Abstract: The nature and magnitude of genetic diversity were estimated in eighteen ridge gourd genotypes on twenty quantitative traits to identify promising traits on which selection can be based. The high phenotypic and genotypic coefficients of variation were found for fresh fruit yield per vine followed by fruit length, fruit diameter and number of fruits per vine, indicating high genetic variability in these traits. High heritability coupled with high genetic advance was observed for chlorophyll content, proline content of leaf, fruit length, fruit yield per vine, fruit diameter and fruit weight indicating the possible role of additive gene action. The traits like fruit weight, fruit diameter, chlorophyll content of leaf and proline content of leaf as it has positive correlation with fruit yield per vine and the days to first female flowering and sex ratio has the negative correlation with the fruit yield per vine.

Key Words: Ridge gourd, Heritability, Genotypic and phenotypic coefficient of variation, Genetic advance

The genus *Luffa* (family Cucurbitaceae) includes about sixty species that are native to Indo-Myanmar and India. Among all the species, *Luffa acutangula* L. (ridge gourd) and *Luffa cylindrica* L. (sponge gourd) are cultivated largely due to its nutritional, availability of genetic variability (Reddy *et al.*, 2013) and medicinal properties (Satkar *et al.*, 2013). There is a large diversity in ridge gourd for fruit shape (spindle shaped, elliptical, oblong, long cylindrical, top shaped, and globular), color (dark green, light green) and fruit surface (ridged and non-ridge). Genotypic and phenotypic coefficients of variation are useful in detecting the amount of variability present in the available genotypes. The main purpose of estimating heritability and the genetic parameters that compose the heritability and the genetic parameters that compose the heritability estimate is to compare the expected gains from selection based on alternative selection strategies (Holland *et al.*, 2003). Correlation analysis is a biometrical technique to find out the nature and degree of associations among various traits. Therefore, information on variability and heritability of plant characters and association among yield and quality characters are of vital importance in plant breeding programme. The present study was under taken to ascertain the magnitude and extend of genetic variability, heritability, genetic advance and the association of twenty characters in ridge gourd genotypes.

MATERIAL AND METHODS

The experiment was conducted at Kittur Rani Channamma College of Horticulture (Karnataka) India during the summer season of 2012-14. The experimental material comprised of 18 diverse genotypes collected from different parts of India. The experiment was laid down in a randomized

block design (RBD) with three replications. The crop was managed as per recommended package of practices to raise the crop during summer season. In each replication, seeds of each genotype were sown in 9m long rows at 90cm distance between lines and 60 cm between plants. Five plants of each genotype were randomly selected from each replication for recording horticultural traits at 45 and 90 DAS (Table 1). Genotypic and phenotypic coefficients of variation were estimated using the procedure suggested by Burton and De Vane (1953) and heritability in broad sense and genetic advance expressed in percent of mean were calculated (Burton, 1952). The correlations were worked out as per methods suggested by Al-Jibouri *et al.* (1958) and path analysis was calculated according to Dewey and Lu (1959).

RESULTS AND DISCUSSION

The analysis of variance for twenty quantitative characters revealed that mean squares were highly significant for all the characters indicating enough variability in genotypes. However, the absolute variability in different characters does not permit identification of characters showing the higher degree of variability. The higher degree of variation was observed in phenotypic and genotypic variance among the characters studied (Table 1). A close proximity in the phenotypic and genotypic coefficients of variability was observed indicating a little influence of environment in the expression of various horticultural traits studied. Maximum variation was shown by total chlorophyll content in leaf at 45 and 90 days and proline content in leaf at 45 and 90 DAS followed by total yield per vine. Low variance was observed for tendril length, days to first female flowering, days to 50 per cent flowering, days to first harvest and days to last harvest.

Table 1. Phenotypic and genetic variability for various parameters among the different ridge gourd genotypes

Particulars	Vine length (cm)		Number of branches		Number of leaves		Tendrill length (cm)	
	Days after sowing							
	45	90	45	90	45	90	45	90
Deepthi	93.83	265.50	2.50	4.50	30.50	57.50	22.33	26.67
Pusa Nasadar	93.73	254.33	<u>1.83</u>	5.00	23.33	61.00	20.17	28.83
Mudigere local	139.33	280.17	3.33	5.67	36.67	69.17	25.17	29.67
Jaipur long	125.19	245.00	2.33	4.83	30.50	66.00	21.33	28.50
Khanapur local	96.73	232.33	2.00	5.00	22.33	63.00	22.50	27.33
Mandya local	84.80	206.33	<u>1.83</u>	2.33	27.00	55.83	22.00	25.50
Gadag local	126.07	210.83	2.67	3.17	30.17	<u>50.83</u>	23.50	28.17
Chintamani local	74.83	224.67	2.50	5.17	22.67	59.17	23.50	25.83
Srinivasapur local	120.13	251.50	2.00	4.83	20.33	61.33	22.33	26.50
Ghataprabha local	120.77	199.50	2.67	3.00	29.50	48.67	22.00	26.50
Green long	119.67	277.33	2.83	3.83	<u>32.33</u>	67.17	23.83	26.83
Arka Sujata	104.53	206.50	2.83	5.17	<u>28.33</u>	61.17	21.83	24.83
Dalasanur local	130.13	272.67	2.50	5.17	25.67	63.83	23.17	24.17
Selection 4-12	111.00	235.17	2.67	5.17	25.50	62.17	23.17	29.00
AHRG-1	130.03	217.00	2.67	5.00	28.33	67.50	18.67	27.50
Arabhavi local	147.97	290.50	3.83	6.17	40.33	73.67	26.00	30.83
Kolar local	110.53	264.17	2.83	5.33	33.83	64.50	21.67	28.17
Arka Sumeet	110.08	255.00	2.67	5.00	31.00	66.17	22.33	27.00
CD (p=0.05)	12.49	0.43	0.54	0.43	4.59	5.17	2.49	1.62
Mean	113.29	243.80	2.58	4.68	28.79	62.15	22.52	27.32
Range	74.83- 147.97	199.5 - 290.5	<u>1.83</u> - 3.83	2.33- 6.17	20.33- 40.33	48.6 - 73.67	18.6 - 26.00	24.17 - 30.83
GV	352.80	775.59	0.21	0.88	23.98	35.52	2.03	2.60
PV	409.44	880.35	0.32	0.98	31.65	45.24	4.28	3.55
PCV (per cent)	17.86	12.17	21.92	21.42	19.54	10.82	9.18	6.90
GCV (per cent)	16.58	11.42	17.89	20.34	17.00	9.59	6.32	5.90
h² (per cent)	86.00	88.00	7.00	90.00	76.00	79.00	47.00	73.00
GA	35.92	53.85	78.00	18.40	88.07	10.88	2.02	2.84
GAM	31.70	22.09	30.07	39.80	30.49	17.51	8.97	10.40

Table 1. Continued....

Particulars	Days to first female flowering	Days to 50per cent flowering	Days to first harvest	Days to last harvest	Sex ratio (M:F)	Total Chlorophyll (mg.g ⁻¹ fresh wt)		Proline (µg.g ⁻¹ fresh wt)	
						45 DAS	90 DAS	45 DAS	90 DAS
Deepthi	46.83	57.00	63.50	89.67	28.33	2.36	2.30	2.32	13.14
Pusa Nasadar	50.83	55.50	59.83	88.83	22.67	1.21	1.88	13.82	22.56
Mudigere local	47.00	58.33	69.83	92.50	19.17	2.38	2.23	22.37	51.01

Jaipur long	54.00	57.00	70.67	94.83	22.50	0.42	1.23	4.64	26.13
Khanapur local	49.83	59.33	66.50	92.17	28.83	1.62	2.17	6.23	17.63
Mandya local	47.83	57.00	58.83	88.33	25.00	1.03	1.15	10.10	39.13
Selection 4-12	53.33	55.50	60.17	89.17	25.50	1.61	0.94	7.34	12.08
Gadag local	49.33	57.00	65.83	90.83	24.00	0.52	0.64	9.95	37.78
Chintamani local	46.67	55.83	68.67	90.50	25.17	0.30	0.63	10.29	37.00
Srinivasapur local	49.67	59.66	69.33	89.50	26.67	1.04	0.99	20.58	22.46
Ghataprabha local	51.33	59.00	71.33	92.50	27.50	1.61	2.09	20.68	17.49
Green long	50.50	54.83	60.00	94.17	28.83	2.12	1.40	7.34	29.52
Arka Sujata	49.17	55.16	59.00	88.17	22.83	1.27	0.46	9.71	32.75
Dalasanur local	53.33	58.50	60.17	87.50	25.67	0.84	0.67	11.79	22.95
AHRG-1	50.50	57.66	69.33	91.33	24.67	1.52	2.18	12.46	28.21
Arabhazi local	46.33	54.00	58.33	87.33	18.33	2.47	2.39	23.53	56.71
Kolar local	49.83	54.33	63.50	93.00	25.67	1.31	1.19	11.01	21.35
Arka Sumeet	47.67	54.33	58.83	92.00	28.50	1.29	1.41	21.64	55.12
CD (p=0.05)	4.25	3.08	2.94	2.95	2.89	0.08	0.18	1.41	4.80
Mean	49.78	56.17	64.07	90.69	24.99	1.38	1.44	9.42	30.16
Range	46.33-54.00	54.00 - 59.66	58.33 - 71.33	87.3-94.83	18.33-28.83	0.30-2.47	0.46-2.39	2.32-23.53	12.08-56.71
GV	4.81	2.22	21.28	4.07	8.33	0.43	0.44	42.22	182.86
PV	8.26	2.23	24.43	7.23	11.37	0.43	0.45	42.94	191.22
PCV (per cent)	5.77	2.66	7.71	2.97	13.49	47.38	46.42	52.24	45.84
GCV (per cent)	4.41	2.65	7.20	2.22	11.55	47.24	45.80	51.80	44.83
h ² (per cent)	58.00	100	87.00	56.00	73.00	99.00	97.00	98.00	96.00
GA	3.45	3.07	8.87	3.12	05.09	1.34	1.34	13.27	27.24
GAM	6.93	5.47	13.85	3.44	20.37	97.03	93.09	105.82	90.30

Table 1. Continued.....

Particulars	Number of fruits per vine	Average fruit weight (g)	Fruit length (cm)	Fruit diameter (mm)	Flesh thickness (cm)	Rind thickness (mm)	Fruit yield per vine (g)
Deepthi	5.50	126.54	19.70	26.55	2.12	1.86	696
Pusa Nasadar	5.50	132.35	21.40	25.92	2.19	1.82	728
Mudigere local	7.83	179.83	25.48	31.70	2.54	2.23	1408
Jaipur long	6.50	144.43	29.38	25.73	1.87	1.45	938
Khanapur local	6.16	130.28	21.68	25.65	2.18	1.58	802
Mandya local	6.50	139.79	22.27	27.73	2.30	1.59	909
Gadag local	6.16	144.87	19.92	27.80	2.11	1.59	892
Chintamani local	6.33	150.67	23.77	22.30	2.34	1.39	954
Srinivasapur local	7.00	158.08	23.40	24.76	2.11	1.38	1107
GPB local	6.50	131.08	22.62	29.03	2.31	1.60	853
Green long	6.50	125.03	27.27	24.68	2.13	1.75	813
Arka Sujata	7.50	163.80	32.27	31.92	2.39	1.93	1229
Dalasanur local	6.83	133.68	26.07	28.41	2.33	2.02	913

Selection 4-12	6.00	125.70	22.07	27.30	2.24	1.46	754
AHRG-1	6.16	156.48	26.35	24.92	2.25	1.47	963
Arabhavi local	9.66	182.25	33.03	30.50	2.69	2.29	1761
Kolar local	6.00	151.27	27.02	25.36	2.12	1.49	908
Arka Sumeet	8.16	175.54	32.53	31.05	2.53	2.29	1432
CD (p=0.05)	0.99	28.42	3.44	4.88	0.37	0.53	208.22
Mean	6.71	147.31	25.34	27.29	2.26	1.73	1004.48
Range	5.50-9.66	125.03-182.25	19.70-33.03	22.29-31.92	1.87-2.68	1.38-2.28	696-1761
GV	0.94	284.84	15.56	4.38	0.03	0.08	75630.00
PV	1.28	489.60	23.09	26.46	0.06	0.12	91377.28
PCV (per cent)	16.86	15.02	18.95	18.85	10.53	20.17	30.09
GCV (per cent)	14.49	11.45	15.56	7.66	7.31	16.47	27.37
h ² (per cent)	73.00	58.00	67.00	16.00	48.00	66.00	62.00
GA	1.72	0.26	6.67	1.75	0.23	0.48	515.39
GAM	25.66	18.00	26.33	6.42	10.46	27.72	51.30

GCV = Genotypic coefficient of variation

h² = Heritability (broad sense)

PV= Phenotypic variance

GAM = Genetic advance (per cent mean)

GA = Expected genetic advance

PCV = Phenotypic coefficient of variation

GV= Genotypic variance

DAS = Days after sowing

In the present investigation, all the characters except diameter of fruit showed narrow differences between the values of GCV and PCV (Table 1) indicating variability due to genetic constitution. This indicates better scope of selection through these traits for improvement in ridge gourd on the basis of phenotypic characters alone with equal probability of success. The heritability was very high for all the traits studied indicated less influence of environment in the expression of these traits. The higher estimates of heritability coupled with higher genetic advance only for vine length, total yield per vine and proline content of leaf whereas high heritability with moderate genetic advance only for number of leaves and number of branches indicated that heritability of these traits is mainly owing to additive effects and consequently a high genetic advance is expected from selection under such situations. High heritability accompanied by low genetic advance for days to first female flowering, tendril length, total chlorophyll content of leaf, days to first harvest, days to last harvest, fruit diameter, fruit length, fruits per vine, average fruit weight, flesh thickness and rind thickness is indicative of non-additive gene action; therefore selection in early generation for these traits may not be effective due to linkage (Islam *et al.*, 2009; Kundu *et al.*, 2012; Singh *et al.*, 2013; Reddy *et al.*, 2014 and Singh *et al.*, 2014). Variability studies provide information on the extent of improvement in different characters, but they do not throw light on the extent and nature of relationship existing between

various characters. Fruit yield per vine had positive and significant correlation (Table 2) with the vine length at 45 DAS (0.513), number of leaves at 45 DAS (0.670), number of leaves at 90 DAS (0.471), number of branches at 45 DAS (0.575), number of branches at 90 DAS (0.561), tendril length at 45 DAS (0.496), proline content in leaf at 45 DAS (0.578), proline content in leaf at 90 DAS (0.866), number of fruits per vine (0.966), average fruit weight (0.930), fruit length (0.742), fruit diameter (0.629), flesh thickness (0.766), rind thickness (0.670).

Negatively correlated with days to first female flowering (-0.512), days to 50 per cent flowering (-0.473) and sex ratio (-0.461). Hence, correlation study has greater significance and could be effectively utilized in formulating an effective selection scheme. Many of these yield contributing characters are interact in desirable and undesirable direction. Therefore, knowledge of association between the traits can greatly help in avoiding inversely related compensation effects during selection (Singh *et al.*, 2013; Singh *et al.*, 2014; Reddy *et al.*, 2014) for yield per vine.

The direct and indirect effects obtained at genotypic level were different from those at the phenotypic level (Table 3), which might be due to varying degree of influence of environment. This was supported by results of component variance analysis and correlation at the environmental level. Vine length had moderately positive direct effect on fruit yield per vine (0.041). In the same way, number of fruits (0.583),

Table 2. Correlation for earliness, biochemical and yield contributing traits in ridge gourd

	Fruit yield per vine (g)	Significance level
Fruit yield per vine (g)	1.0000	
Vine length at 45 DAS	0.5129*	*
Vine length at 90 DAS	0.3434	
Number of leaves at 45 DAS	0.6707	***
Number of leaves at 90 DAS	0.4706	*
Number of branches at 45 DAS	0.5745	**
Number of branches at 90 DAS	0.5605	**
Tendrill length at 45 DAS	0.4955	*
Tendrill length at 90 DAS	0.3259	
Days to first female flowering	-0.5120	*
Days to 50per cent flowering	-0.4733	*
Days to first harvest	-0.1873	
Days to last harvest	0.0707	
Sex ratio (M:F)	-0.4606	*
Total Chlorophyll (mg.g ⁻¹ fresh wt) at 45 DAS	0.3838	
Total Chlorophyll (mg.g ⁻¹ fresh wt) at 90 DAS	0.2372	
Proline (µg.g ⁻¹ fresh wt) at 45 DAS	0.5784	**
Proline (µg.g ⁻¹ fresh wt) at 90 DAS	0.8659	***
Number of fruits per vine	0.9658	***
Average fruit weight (g)	0.9298	***
Fruit length (cm)	0.7422	***
Fruit diameter (mm)	0.6288	**
Flesh thickness (cm)	0.7656	***
Rind thickness (mm)	0.6700	***

average fruit weight (0.411), fruit length (0.013), days to first female flowering (0.003), proline content (0.023) and days to first harvest (0.038) also showed the positive direct effects with the total fruit weight per vine. Path coefficient analyses did not account for all variation in fruit yield as indicated by the magnitude of residual effect indicating that there are other traits also that contributed to fruit yield. The low residual effect (0.0241) at genotypic level indicates that all the important characters correlated with fruit yield in ridge gourd (Dey *et al.*, 2006; Husna *et al.*, 2011; Yadav *et al.*, 2013 and Singh *et al.*, 2014).

There was adequate genetic variability within the genotypes evaluated for improvement of fruit yield and growth related traits. The genetic variation suggested that a positive response to direct selection is possible for all the

Table 3. Direct (diagonal) and indirect genotypic path effects of different characters towards total fruit yield per vine in ridge gourd

	Vine length (cm)	Number of leaves per vine	Number of fruits per vine	Average Fruit weight (g)	Fruit length (cm)	Fruit diameter (mm)	Days to first female flowering	Total chlorophyll (mg.g ⁻¹ fresh weight)	Proline (µg.g ⁻¹ fresh weight)	Days to first harvest	Days to last harvest	Yield
Vine length (cm)	0.041	0.028	0.016	0.018	0.014	0.022	0.008	0.013	0.009	0.019	0.018	0.448
Number of leaves	-0.024	-0.035	-0.018	-0.018	-0.021	-0.014	-0.019	-0.009	-0.014	-0.016	-0.011	0.548**
Number of fruits	0.222	0.306	0.583	0.471	0.439	0.384	0.268	0.127	0.401	0.428	0.346	0.965**
Average Fruit weight	0.181	0.209	0.333	0.411	0.274	0.212	0.131	0.138	0.247	0.254	0.234	0.932**
Fruit length (mm)	0.004	0.008	0.009	0.009	0.013	0.006	0.005	-0.001	0.006	0.007	0.005	0.741**
Fruit diameter (mm)	-0.004	-0.003	-0.005	-0.004	-0.003	-0.007	-0.001	-0.001	-0.004	-0.003	-0.002	0.633**
Days to first female flowering	-0.001	0.002	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.002	0.001	0.433
Total Chlorophyll (mg.g ⁻¹ fresh weight)	0.009	0.008	0.007	0.010	-0.002	0.001	0.009	0.030	0.002	0.009	0.008	0.308
Proline (µg.g ⁻¹ fresh weight)	0.005	0.010	0.018	0.016	0.011	0.015	0.009	0.001	0.023	0.017	0.011	0.693**
Days to first harvest	0.018	0.017	0.028	0.023	0.019	0.018	0.023	0.011	0.023	0.038	0.031	0.746**
Days to last harvest	-0.005	-0.004	-0.007	-0.007	-0.004	-0.004	-0.005	-0.003	-0.005	-0.009	-0.011	0.630**

traits studied. Correlation and path coefficient analyses indicated that selection for more fruits and average fruit weight could be criteria for simultaneously increasing fruit yield in ridge gourd. Therefore, due attention should be paid to improve these traits while selection of high yielding genotypes or for choosing desirable parents for heterosis breeding.

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Diallel analysis for Combining Ability in China aster (*Callistephus chinensis* [L.] Nees)

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Abstract: Field study with diallel analysis (including reciprocals) in China aster indicated that the mean squares due to GCA, SCA and RCA was significantly higher for all the characters. The high magnitude of GCA mean square than SCA and RCA mean square were found in almost all the characters except leaf area per plant and seed yield per plant. This indicated variation in GCA of the parents and SCA and RCA of the crosses are significant combinations of additive and non-additive gene effects in the expression of the characters. Estimates of general combining ability effects showed that parents Violet Cushion and AAC-1 were good general combiners for most of the growth, flowering, yield and quality traits. The specific combining ability effects for yield and its components showed that the best cross was AAC-1 x Poornima, while for reciprocal combining ability effects Violet Cushion x AAC-1 was found superior for yield and quality parameters.

Key Words: Diallel analysis, General combining ability, Reciprocal combining ability, Specific combining ability

China aster (*Callistephus chinensis* [L.] Nees.) is one of the most important cut as well as loose flower in India. It is grown for landscaping and occupies an even increasing demand in medicinal and industrial sector. However, information on breeding aspect is meager. For increasing the quality production, exploitation of hybrid vigour associated with combining ability analysis is a need. Now a day's combining ability is becoming more and more important as a tool of crop improvement among plant breeders. Diallel crossing techniques is one of the most common biometrical procedures to study the combining ability of the diverse parents for heterosis breeding. Therefore, the present investigation was undertaken to estimate the general, specific and reciprocal combining ability for various vegetative, yield and quality attributes.

MATERIAL AND METHODS

The present investigation was conducted at College of Horticulture, Arabhavi on China aster during 2014-15. The experimental material consisted of five parents and its twenty crosses of China aster, parents collected were maintained at Arabhavi. One and half month old, healthy, vigorous and uniform plants were selected for transplanting with an spacing of 30 x 30cm. Experiment was laid out in a randomized block design and was replicated twice. Out of twenty-five plants per row, five plants were selected for taking observation. Five prominent parents of China aster namely Kamini, Phule Ganesh Purple, Poornima and Violet Cushion and one accession AAC-1 were used for crossing in all possible combination including reciprocals. Data was recorded on fifteen characters

RESULTS AND DISCUSSION

The mean square for GCA, SCA and RCA were highly significant for all the characters indicating both additive and non-additive genetic variance played role in the expression of these characters. The mean square of GCA was of higher order than those for SCA and RCA except leaf area per plant and seed yield per plant. Griffing (model I) suggested that higher values of mean square of GCA includes, besides additive gene action, additive x additive epistasis also played role (Table 1). For recombination breeding approach, the information on combining ability of the parents offers useful clues regarding the choice of productive parents and hybrid for further use. Combining ability analysis is believed to be the quickest and most efficient method to evaluate parental lines for their usefulness in crosses (Singh and Misra, 2010).

Among the five parents, Violet Cushion was found to be the best combiner for number of leaves, number of branches, duration of flowering, number of flowers per plant, individual flower weight, flower yield per plant, flower yield per hectare, flower stalk length, vase life and seed yield per plant. AAC-1 and P.G. Purple attained highest values for plant spread and plant height, respectively. Estimating the flowering behavior, where early types are preferred, the parent Kamini was best combiner for earliness followed by P.G. Purple.

The hybrid AAC-1 x Poornima attained maximum SCA effects for number of leaves, leaf area per plant, duration of flowering, number of flowers per plant and flower yield per plant. The hybrid P.G. Purple x Poornima attained highest SCA effects for plant height, plant spread. Whereas,

Table 1. Variance due to general, specific and reciprocal combining ability and their ratio for fifteen characters in China aster

Source	Mean sum of square				Component of variance				
	GCA	SCA	RCA	Error	σ^2_g	σ^2_s	σ^2_t	gca:sca	
Degrees of freedom	4	10	10	24					
Plant height (cm)	160.28**	36.41**	20.94**	8.51	86.72	278.97	124.27		0.31
No. of leaves	54.59**	8.96**	26.48**	5.93	27.81	30.30	205.50		0.91
Plant spread (cm ²)	120573.68**	46326.43**	16266.72**	12244.71	61902.27	340817.20	40220.10		0.18
Leaf area per plant (cm ²)	863819.39**	700527.20**	1134680.88**	197134.19	380962.97	5033930.10	9375466.90		0.07
No. of branches	59.63**	4.39**	1.04	0.84	33.59	35.42	1.96		0.94
Days for 1 st flower bud initiation (DAT)	73.11**	17.99**	4.49**	1.96	40.66	160.30	25.30		0.25
Days for 1 st flower (DAT)	108.92**	22.41**	5.06**	2.21	60.98	202.00	28.50		0.30
Duration of flowering	15.80**	4.78**	3.28**	0.4992	8.74	42.81	27.81		0.20
No. of flower per plant	390.45**	59.07**	16.87**	1.26	222.39	578.10	156.10		0.38
Individual flower weight (g)	0.37	0.05	0.10	0.01	0.20	0.40	0.89		0.51
Flower yield per plant (g)	10197.34**	824.18**	484.35**	77.46	5782.79	7467.20	4068.90		0.77
Flower yield per hectare (t/ha)	12.31**	49.42**	12.16**	0.95	6.49	484.70	112.10		0.01
Flower stalk length (cm)	6.22**	2.39**	3.08**	1.24	2.85	11.50	18.40		0.24
Vase life (days)	6.01**	1.21	0.09	0.04	3.41	11.65	0.55		0.29
Seed yield per plant (g)	0.11	0.12	0.06	0.01	0.06	1.05	0.48		0.12

*Significant at 5 per cent level

**Significant at 1 per cent level

DAT- Days after transplanting

Table 2. Specific combining ability (sca) effects for different traits in China aster

Cross	Plant height (cm)	No. of leaves (no.)	Plant spread (cm ²)	Leaf area plant ⁻¹ (cm ²)	No. of branches (no.)	Days for flower bud initiation	Days for 1st flowering (DAT)	Duration of flowering	No. of flowers plant ⁻¹	Individual flower weight (g)	Flower yield plant ⁻¹ (g)	Flower yield ha ⁻¹ (t ha ⁻¹)	Flower stalk length (cm)	Vase life (days)	Seed yield plant ⁻¹ (g)
AAC-1 x Poornima	-0.09	3.09*	14.80	979.48**	0.25	1.47	1.22	1.71**	6.77**	0.07	26.55*	1.90**	0.18	-0.53**	0.15*
P.G. Purple x Poornima	7.68**	-0.14	308.58**	-228.88	1.83**	-4.23**	-4.89**	0.87*	4.03**	-0.11	8.03	0.84	-1.62*	-0.01	0.02
AAC-1 x P.G. Purple	-1.20	0.63	24.14	84.08	1.44*	-1.90*	-2.05*	-0.24	-4.44**	0.24**	-1.65	-0.36	-0.20	0.47**	-0.16*
Kamini x Violet Cushion	3.57*	1.33	53.49	323.59	1.95**	1.47	2.02*	1.16**	3.35**	0.14	17.86**	-1.15	1.31	0.30*	-0.24**
AAC-1 x Kamini	3.69*	1.20	67.05	711.48*	-0.12	2.54**	2.55**	-0.03	2.98**	0.02	9.16	1.99**	-0.36	0.25	0.21**
Kamini x P.G. Purple	2.66	1.27	4.62	-454.59	0.51	0.64	0.94	0.63	2.84**	0.11	14.76**	-1.85**	0.04	0.97**	-0.07

Table 3. Reciprocal combining ability (rca) effects for different traits in China aster

Cross	Plant height (cm)	No. of leaves (no.)	Plant spread (cm ²)	Leaf area plant ⁻¹ (cm ²)	No. of branches (no.)	Days for flower bud initiation	Days for 1st flowering (DAT)	Duration of flowering	No. of flowers plant ⁻¹	Individual flower weight (g)	Flower yield plant ⁻¹ (g)	Flower yield ha ⁻¹ (t/ha)	Flower stalk length (cm)	Vase life (days)	Seed yield plant ⁻¹ (g)
Violet Cushion x Kamini	0.15	-0.50	-41.44	728.98*	-0.10	2.35*	2.50*	-1.01	-0.20	-0.02	-1.87	5.88**	0.35	0.40*	0.38**
Violet Cushion x P.G. Purple	0.80	-0.20	-13.12	-394.77	-0.05	0.70	1.00	-0.20	-0.20	0.22*	13.60*	4.08**	-0.52	0.15	0.25**
Violet Cushion x Poornima	2.55	0.90	-87.52	-411.44	-0.10	0.20	0.60	1.98**	0.15	0.10	7.18	7.23**	-0.40	0.00	0.23**
P.G. Purple x Kamini	-2.05	0.40	-46.26	650.30*	1.70*	0.30	0.25	-1.06*	-0.90	-0.19	-13.83*	1.50*	-0.68	-0.10	0.00
Violet Cushion x AAC-1	-5.55*	-7.60**	37.02	277.99	0.25	0.45	2.00	-0.60	1.95*	-0.20*	-5.61	5.12**	3.13**	0.15	0.17*

it attained least SCA days for first flower bud initiation and \ days for first flowering. The cross AAC-1 x P.G. Purple had significant higher SCA effects for individual flower weight of branches and vase life. The cross between Kamini x Violet Cushion was good specific combiner for plant height, number of branches, duration of flowering, number of flowers plant⁻¹, flower yield plant⁻¹ and vase life. The hybrid AAC-1 x Kamini showed good SCA effects for plant height, leaf area per plant, number of flowers plant⁻¹, flower yield hectare⁻¹ and seed yield per plant (Table 2). The hybrid of Kamini x P.G. Purple has shown highest SCA effect for vase life, number of flowers per plant and flower yield per plant. Thus, the above results are in accordance with findings of Kumar *et al.* (2004).

The cross Violet Cushion x Kamini attained maximum RCA effects for leaf area per plant, vase life and seed yield per plant and significantly higher SCA effects for flower yield hectare⁻¹ (Table 3). The hybrid of Violet Cushion x P.G.Purple had significant higher SCA effects for individual flower weight, flower yield plant⁻¹ and flower yield hectare⁻¹. The cross Violet Cushion x Poornima was a good specific combiner for plant height, flower yield hectare⁻¹ and duration of flowering. The P.G. Purple x Kamini attained significantly

good SCA effects for number of branches, leaf area plant⁻¹ and flower yield hectare⁻¹. The hybrid of Violet Cushion x AAC-1 showed significantly high GCA effects for stalk length, number of flowers plant⁻¹ and flower yield hectare⁻¹.

It is apparent from the above studies that parents Violet Cushion and AAC-1 were good general combiners for growth and yield characters. Crosses P.G.Purple x Poornima, AAC-1 x Poornima and Kamini x P.G. Purple were the best cross combinations for growth, flowering, yield and quality traits, respectively. Majority of the crosses had High x Low type of general combining ability of parents, which is significant for exploitation of heterosis. Hence, for attempting successful hybridization economically, it is worthwhile to start with High x Low type of crosses for exploiting heterosis.

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Correlation and Path Coefficient Analysis of Yield Attributes in Okra (*Abelmoschus esculentus* (L.) Moench.)

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Abstract: At genotypic level, okra fruit yield per plant had direct and positive correlation with number of fruits, fruit length, fruit diameter and fruit weight in both F_2 and F_3 populations and plant height, number of branches in phenotypic level. In path coefficient analysis, fruit weight recorded highest direct effect (0.8852) followed by number of fruits per plant (0.3618), plant spread E-W (0.0837) and fruit length (0.0311) in F_2 population. In F_3 population, fruit weight recorded highest direct and positive effect (0.8732) followed by number of fruits per plant (0.3787) and plant height (0.0374).

Key Words: Biparental mating, Genotypic & phenotypic correlation, Okra, Path analysis

Selection based on multiple traits is always better than selection based on yield alone. Yield is quantitative character controlled by many genes, therefore an adequate knowledge about the magnitude and degree of association of yield with its attributing characters is of great significance to the breeders, through which they can clearly understand the strength of correlated traits. The success of any breeding programme depends upon selection of a proper plant. The efficiency of selection depends on the magnitude and nature of genetic variation in a specific population for effective breeding program. Yield is polygenic in nature and influenced by environmental factors, which complicate the selection process thus, the knowledge of correlation of the traits is necessary for effective selection process. Therefore, before aiming an improvement in yield, it is necessary to have the knowledge of correlation with yield. Correlation coefficient analysis measures the mutual relationship between two plant characters and determines component characters in which selection can be based for genetic improvement in yield. The association of these characters due to their direct effect on yield or is a consequence of their indirect effects via other component characters may be answered through path coefficient analysis. Such information reveals the possibility of simultaneous improvement of various attributes and also helps in increasing the efficiency of selection of complex inherited traits. Keeping this in view, the present investigation was aimed at correlation of various characters, and direct/indirect path coefficient of independent components on okra fruits yield in F_2 and F_3 populations.

MATERIAL AND METHODS

The experiment was conducted in *kharif* season of

2013 at K. R. C. College of Horticulture Arabhavi, Karnataka. The base experimental material comprised of commercial private hybrids as parents *Viz.*, C_1 (Bhendi 10 X Mahyco) and C_2 (Indus X Harshita). C_1 and C_2 crosses were advanced to F_2 and F_3 progenies. F_2 plants were derived by selfing of F_1 and F_3 -progenies were derived from selfing of selected F_2 plants of both the crosses. Selected populations of F_2 and F_3 were evaluated for yield and yield attributing traits studied on correlation and path analysis. The recommended cultural practices were followed. Observations were recorded on five randomly tagged plants of F_2 and F_3 populations. Yield and yield attributing characters were selected for the observation *viz.*, plant height (cm), number of branches per plant, fruit length (cm), fruit width (cm), number of fruits per plant, plant spread East-West (cm), plant spread North-South (cm), fruit weight (g) and fruit yield per plant ($g\ plant^{-1}$). Mean values were subjected to analysis of variance, genotypic and phenotypic correlation coefficient and path coefficient was computed by using the formula of Dewey and Lu (1959). The biometrical analysis of the data was carried out using SPAR and Indostat programmes. The test of significance for association between characters was done by comparing table 'r' values at $n-2$ error degrees of freedom for phenotypic and genotypic correlations with estimated values, respectively.

RESULTS AND DISCUSSION

The phenotypic and genotypic correlation coefficient were determined to obtain information on the relationship among 10 quantitative characters in both F_2 and F_3 populations of okra (Table 1). The magnitude of genotypic

correlation coefficients exhibited higher than the phenotypic ones for all the traits, which established inherent relationships among the characters in okra.

Correlation co-efficient analysis revealed that the genotypic correlations in general were higher in magnitude than their corresponding phenotypic correlations for all the

pairs of traits indicating that genotypes are superior but their expression are lessened under the influence of environment (Bello *et al.*, 2006). The correlation analysis has showed highly significant and positive correlation for fruit yield per plant in both the populations. In F_2 population, for phenotypic correlation, characters like fruit length (0.980), fruit diameter

Table 1. Phenotypic and genotypic*** correlation coefficients for growth and yield contributing traits in F_2 and F_3 populations of okra

Characters	Populations	Number	Fruit	Fruit	Fruit	Number	Plant	Plant	Fruit
Phenotypic									
Plant height	F_2	0.167	0.069	0.287	0.222	0.026	0.871**	0.857**	0.037
	F_3	0.452*	0.227	0.192	0.097	0.489*	0.867**	0.230	0.509*
Number of branches	F_2	1.000	0.439*	0.142	0.080	0.106	0.0838	0.0763	0.203
	F_3	1.000	0.050	0.240	0.504*	0.300	0.269	-0.020	0.479*
Fruit length	F_2		1.000	0.221	0.418*	0.529*	-0.736	-0.127	0.980**
	F_3		1.000	-0.043	0.010	0.122	-0.090	-0.078	0.598*
Fruit diameter	F_2			1.000	0.295	0.695**	0.128	0.117	0.907**
	F_3			1.000	0.960**	0.970**	-0.105	-0.071	0.984**
Fruit weight	F_2				1.000	0.900**	-0.078	-0.089	0.982**
	F_3				1.000	0.418*	-0.091	-0.030	0.832**
Number of fruits per	F_2					1.000	-0.062	-0.070	0.934**
	F_3					1.000	-0.051	-0.023	0.918**
Plant spread (N-S)	F_2						1.000	-0.039	-0.089
	F_3						1.000	-0.045	-0.087
Plant spread (E-W)	F_2							1.000	0.995**
	F_3							1.000	0.214
	F_3								1.000
Genotypic									
Plant height	F_2	0.1766	-0.106	0.2159	-0.0628	0.0257	0.8720**	0.8716	0.1140
	F_3	0.2662*	-0.101	-0.1637	-0.0682	-0.0885	0.8669**	0.2305 *	-0.1140
Number of branches	F_2	1.000	0.1623	-0.0565	0.0600	0.0348	0.0839	0.0764	0.0976
	F_3	1.000	-0.109	0.2907*	0.0544	0.2463*	0.2694*	-0.0203	0.1742
Fruit length	F_2		1.000	-0.1812	0.3961**	0.1116	-0.1736	-0.1671	0.4502*
	F_3		1.000	-0.0430	0.0970	0.1112	-0.0909	-0.0728	0.4763*
Fruit diameter	F_2			1.000	0.2886*	0.4064**	0.1280	0.1174	0.3242*
	F_3			1.000	0.4468**	0.3632**	-0.1054	-0.0719	0.3071*
Fruit weight	F_2				1.000	0.6471	-0.0779	-0.0893	0.7165*
	F_3				1.000	0.4186	-0.0914	-0.0309	0.4135*
Number of fruits per	F_2					1.000	-0.0623	-0.0699	0.9317*
	F_3					1.000	-0.0516	-0.0235	0.9189*
Plant spread (N-S)	F_2						1.000	0.9952**	-0.0899
	F_3						1.000	0.2146	-0.0879
Plant spread (E-W)	F_2							1.000	-0.0939
	F_3							1.000	-0.0500

F_2 : Second generation population of C_1 and C_2 ;

F_3 : Third generation population of C_1 and C_2

C_1 (Bhendi 10 X Mahyco) and C_2 (Indus X Harshita)

*and** indicates significant values at C.D. at 5 % and 1 % respectively.

***Genotypic correlation in parentheses

(0.907), fruit weight (0.982) and number of fruits per plant (0.934) were significant and positive correlation with fruit yield per plant. For phenotypic correlation in F_3 population, plant height (0.509) and number of branches (0.479) and fruit length (0.598) has shown positive and significant correlation with fruit yield per plant in okra. To increase fruit yield, selection might be directed towards plants having higher number of fruits with large fruit size (Hazra and Basu, 2000; Abhishek, 2014). Since, these association characters are in the desirable direction, selection for these traits may improve the yield per plant.

For genotypic correlation in both F_2 and F_3 populations, the characters number of fruits per plant (0.4502 and 0.4763), fruit length (0.3242 and 0.3071), fruit diameter (0.7165 and 0.4135) and fruit weight (0.9317 and 0.9189) has shown positive and significant correlation with fruit yield per plant.

Path analysis reveals the direct and indirect association, which is the most reliable method in addition to the correlation coefficient and genetic variation (Singh *et al.*, 2014). In the path coefficient analysis, there was correspondence between direct effect and phenotypic correlation for most of the component characters with fruit yield per plant in the F_2 and F_3 populations (Table 2). Further

in F_2 population it revealed that, fruit weight has recorded high direct effect (0.8852) followed by number of fruits per plant (0.3618), plant spread E-W (0.0837) and fruit length (0.0311). However, plant height exhibited highest negative direct effect with fruit yield per plant (-0.0071) followed by fruit diameter (-0.0064) and plant spread N-S (-0.0543). Looking to the indirect effects, high positive indirect effect was found in case of number of fruits per plant via number of branches per plant, plant height and fruit length. Therefore, traits like fruit weight and number of fruits per plant had high positive direct effect on fruit yield per plant. So, selection based on fruit weight and number of fruits per plant will be effective in advanced generations. In F_3 population, fruit weight showed more positive direct effect (0.8732) for fruit yield per plant followed by number of fruits per plant (0.3787), plant height (0.0374), fruit length (0.0048) and fruit diameter (0.0081). Hence, desirable improvement may be brought about by selecting genotypes with more number of fruits per plant, fruit diameter and fruit weight. All characters mentioned earlier, which contributed directly and positively to fruit yield per plant possesses significant correlations suggesting that the association between these traits is perfect and it was due to genetic factors only.

The information obtained from the present study

Table 2. Direct (diagonal) and indirect path effects of different characters of F_2 and F_3 population towards fruit yield per plant in okra

Parameters	Plant height	Number of branches	Number of fruits per plant	Fruit length	Fruit diameter	Fruit weight	Plant spread N-S	Plant spread E-W	Fruit yield per plant
Plant height	-0.0071	-0.0012	0.0008	-0.0015	0.0004	-0.0002	-0.0062	-0.0062	0.0114
Number of branches	0.0017	0.0096	0.0016	-0.0005	0.0006	0.0003	0.0008	0.0007	0.0976
Number of fruits per plant ¹	-0.0387	0.0587	0.3618	-0.0656	0.1433	0.0404	-0.0628	-0.0605	0.4502
Fruit length	0.0067	-0.0018	-0.0056	0.0311	0.009	0.0126	0.004	0.0036	0.3242
Fruit diameter	0.0004	-0.0004	-0.0025	-0.0018	-0.0064	-0.0041	0.0005	0.0006	0.7165
Fruit weight	0.0227	0.0308	0.0988	0.3598	0.5728	0.8852	-0.0552	-0.0619	0.9317
Plant spread N-S	-0.0474	-0.0046	0.0094	-0.007	0.0042	0.0034	-0.0543	-0.0541	-0.0899
Plant spread E-W	0.0730	0.0064	-0.014	0.0098	-0.0075	-0.0059	0.0833	0.0837	-0.0939
Plant height	0.0374	0.0100	-0.0038	-0.0061	-0.0026	-0.0033	0.0325	0.0086	-0.1140
Number of branches	-0.0002	-0.0008	0.0001	-0.0002	0.0000	-0.0002	-0.0002	0.000	0.1742
Number of fruits per plant ¹	-0.0384	-0.0413	0.3787	-0.0163	0.0367	0.0421	-0.0344	-0.0276	0.4763
Fruit length	-0.0008	0.0014	-0.0002	0.0048	0.0021	0.0017	-0.0005	-0.0003	0.3071
Fruit diameter	-0.0006	0.0004	0.0008	0.0036	0.0081	0.0034	-0.0007	-0.0002	0.4135
Fruit weight	-0.0773	0.2150	0.0971	0.3171	0.3655	0.8732	-0.0451	-0.0206	0.9189
Plant spread N-S	-0.0338	-0.0105	0.0035	0.0041	0.0036	0.0020	-0.0390	-0.0084	-0.0879
Plant spread E-W	-0.0003	0.000	0.0001	0.0001	0.000	0.000	-0.0003	-0.0015	-0.0500

F_3 population in parantheses

could be exploited in breeding programmes to develop horticultural superior genotypes under the scenario of climate change.

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Received 15 September, 2015; Accepted 23 February, 2016



Assessment of Genetic Diversity of China aster (*Callistephus chinensis* [L.] Nees) Genotypes using SRAP Markers

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Abstract: Sequence Related Amplified Polymorphism (SRAP) is a new molecular marker technology developed based on PCR. SRAP analysis was carried out using twenty five primer combinations out of which, eleven primer combinations were polymorphic and revealed a total of 98 scorable bands, among which 89 were polymorphic with an average of 7.27 polymorphic bands per pair of primers. The similarity matrix coefficient ranged from 6 to 64 per cent, suggesting a lower to very higher genetic variation within China aster genotypes. On comparing, the genetic diversity as revealed by the dendrogram and PCA, it was evident that all genotypes used in the experiment was quite distinct from each other.

Key Words: China aster, Genetic diversity, Genotypes, SRAP marker

China aster (*Callistephus chinensis* [L.] Nees) is an important ornamental plant prized for its beauty of flowers as well as longer vase-life and is one of the most attractive and popular flowering shrub, known for its majestic flower stalk possessing attractive, elegant and delicate flowers. As the number of China aster cultivars is continuously increasing, the newly constituted cultivars need to be more intensively monitored for novelty, distinctness, uniformity, stability and molecular markers useful for the protection of Plant Breeder's Rights. Moreover, proper characterization and assessment of genetic relationships among diverse genotypes allow breeders to select desirable varieties for further breeding programme. As one of the DNA-based markers, Sequence-Related Amplified Polymorphism (SRAP) is designed to amplify open reading frames (ORFs) based on specially designed primer pairs (Li and Quiros 2001). The forward primers preferentially amplify exonic regions and the reverse primers preferentially amplify intronic regions and regions with promoters. Compared with other marker systems, SRAP markers have been successfully used to study genetic diversity and relationships because of its simplicity, reproducibility and disclosure of multiple markers from a single two-primer reaction, when compared with other marker systems..

MATERIAL AND METHODS

The seventeen China aster genotypes materials were planted in randomized block design with three replications at Department of Floriculture and Landscape Architecture, K. R. C. College of Horticulture Arabhavi (UHS Bagalkot) Karnataka during 2013-14.

Genomic DNA was isolated from young leaves of

seventeen China aster genotypes. About two hundred milligrams of young leaf tissue were ground into a fine powder using 1 ml preheated extraction buffer (consisting of 100 mM Tris HCl of 8 pH, 2M NaCl, 25 mM EDTA, 2% CTAB, 2 % PVP and 0.2% β - Mercaptoethanol) in Pistle and Mortar. Then the homogenate was transferred 2 ml Eppendorf tubes and were incubated at 60°C for 1 hour, then the tubes were cooled to the room temperature, and extracted two times with equal volume of chloroform : Isoamyl alcohol (24:1) solution, and then centrifuged at 8000 rpm for 15 minutes. The supernatant was transferred to fresh 1.5 ml tubes then 300 μ l isopropanol (IPA) was added and centrifuged at 8000 rpm for 15 min at 10°C. The pellet formed after centrifugation was washed with 70% (v/v) ethanol for 30 minutes. Then alcohol was decanted and pellets were dried at least for 30 minutes till there was no alcohol smell. After drying the pellet was later dissolved in (150 μ l) T10E1 buffer Tris- EDTA (10-1mM). RNase treatment a required quantity (3 μ l) of RNase was mixed to the DNA sample and tubes were incubated on water bath at 37°C for one hour and 50°C for five minutes to remove the RNA present in the DNA. The re-suspended DNA was then diluted in sterile distilled water to 100 ng/ μ l concentration for use in amplification reactions. Again dilution was made up to 10 ng/ μ l of genomic DNA.

PCR Amplification of selected cultivars using SRAP (Sequence Related Amplified Polymorphism) marker:

DNA was essentially extracted for Polymerase chain reaction for SRAP profiling and carried out in 20 μ l reaction volume. A reaction mixture contained 10 ng/ μ l of genomic DNA, 3.0 unit of Taq DNA polymerase, 2mM of each dNTPs, 25 mM MgCl₂ and 3 μ M each of forward and reverse primers. The SRAP amplification programme (Mishra *et al.*, 2011) was 5 minutes

initial denaturation at 94°C; 5 cycles consisting of 1 minute denaturation at 94°C, 1.20 minutes primer annealing at 35°C and 2 minutes extension at 72°C followed by 30 cycles consisting of 1 minute denaturation at 94°C, 1.20 minutes primer annealing at 50°C and 2 minutes extension at 72°C and final extension of 10 minutes at 72°C. The amplification products were separated on 2% agarose gel, stained with 6 µg/100 ml ethidium bromide. DNA molecular weight marker, 1kb plus DNA ladder was loaded in each gel to estimate the sizes of bands and wells were loaded with 20 µl of reaction mixture mixed with 4 µl of loading buffer. The gels were photographed under UV light by using Alpha Digi Doc Gel Documentation System (Fig.1).

Data analysis: Each SRAP band was visually scored as '1' for presence, and '0' for absence in a lane, and the binary data were used for statistical analysis. The scored band data was subjected to statistical analysis using the computer programme NTSYS-PC Ver. 2.2 software (Rohlf, 2000). The

resultant similarity matrix was used to generate a tree by UPGMA (Unweighted Pair Group Method with Arithmetical averages). The primers combination used in the experiment are presented in the table 2.

RESULTS AND DISCUSSION

Genetic diversity of seventeen China aster genotypes using eleven primer combinations revealed a total of 98 scorable bands, among which 89 were polymorphic with an average of 8.90 polymorphic bands per pair of primers (Table 3). The number of bands scored for each primer combination varied from 2-17 with an average of 7.41 bands per primer. This was comparable with Cai *et al.* (2011) in *Dendrobium loddigesii* (231 bands from 17 primer combinations with an average of 13.5 bands per primer combinations), Xue *et al.* (2010) in *Dendrobium* spp. (98 bands from 10 primer combinations with an average of 9.8 markers per primer pair). Among eleven primer combinations

Table 1. Details of genotypes used in the experiment

Genotypes	Flower type	Flower color	Stem color
Kamini	Double	Bright pink	Slight pink
Poornima	Double	Creamy white	Slight green
Shashank	Double	Milky white	Dark green
Violet cushion	Double	Violet	Slight brown
PG- Pink	Double	Bright pink	Dark pink
PG- Purple	Double	Purple	Dark pink
PG- Violet	Double	Violet	Slight brown
PG- White	Double	Milky white	Dark green
AAC-1	Double	Bright dark red	Slight pink
AAC-2	Double	Dark pink	Slight pink
AAC-3	Semi double	Purple	Dark brown
AAC-4	Semi double	Bright pink	Slight pink
AAC-5	Single	Light pink	Slight pink
AAC-6	Semi double	Pink	Slight pink
AAC-7	Single	Milky white	Slight green
AAC-8	Double	Bright Pink	Slight pink
AAC-9	Semi double	Dark purple	Dark brown

Table 2. Sequences of SRAP forward, reverse primers and primer combinations used for fingerprinting and diversity analysis in China aster genotypes

Forward primer		Reverse primer		Primer combinations	
Name	Sequence	Name	Sequence	Forward	Reverse
ME2	TGAGTCCAAACCGGAGC	EM2	GACTGCGTACGAATTGTC	ME2	EM2/EM3/EM4/EM5/EM7
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTGAC	ME3	EM2/EM3/EM4/EM5/EM7
ME4	TGAGTCCAAACCGGACC	EM4	GACTGCGTACGAATTGTA	ME4	EM2/EM3/EM4/EM5/EM7
ME5	TGAGTCCAAACCGGAAG	EM5	GACTGCGTACGAATTAAC	ME5	EM2/EM3/EM4/EM5/EM7
ME7	TGAGTCCAAACCGGACG	EM7	GACTGCGTACGAATTCCA	ME7	EM2/EM3/EM4/EM5/EM7

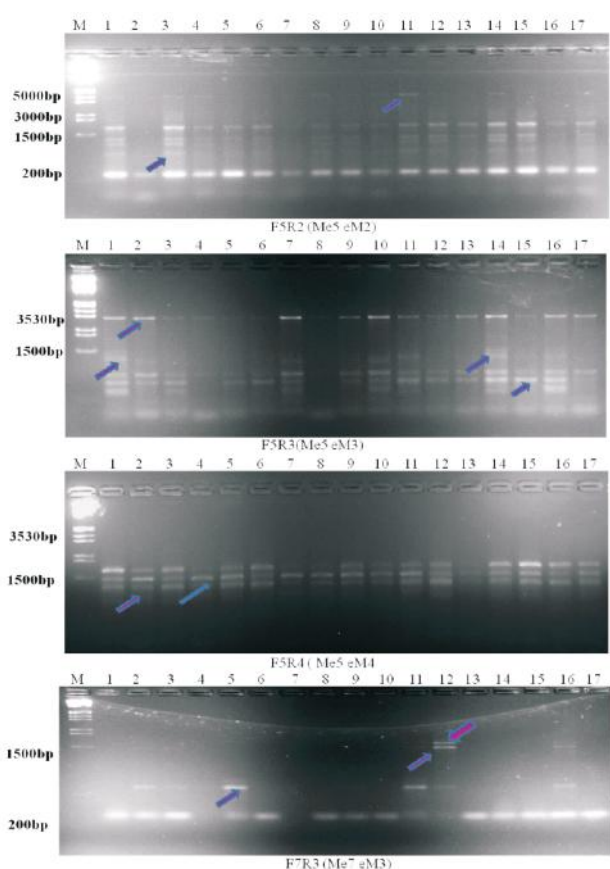


Fig. 1. Gel profile of 17 China aster genotypes using different SRAP primer combination (lane 1-17 represents as genotypes listed in table 1)

screened, Me5 + Em3 scored maximum number of polymorphic bands (16 bands) followed by Me7 + Em4 (15 bands) and Me1+Em3 (10 bands) while Me7 + Em2 and Me8 + Em3 produced minimum number of bands (02 each). The combinations of primer Me5 + Em3, Me5 + Em4, Me7 + Em2 and Me7 + Em5 produced highest polymorphism of 100 per cent each, followed by Me5 + Em2 (92.30%), where as primer combination Me7 + Em3 produced least polymorphism of 75 per cent. Similarly Xue *et al.* (2010) analyzed ten polymorphic primer combinations out of eleven primer combinations in *Dendrobium* spp.

The bands that were unique could be used as the special marker to distinguish the genotypes. For instance, PG-Pink and AAC-6 variety each had one special amplified unique bands generated by Me7/Em3. AAC-7 and AAC-4 each had one special amplified unique bands generated by Me5/Em7 where as PG-Purple and PG-White each had one special amplified unique bands generated by primer combination Me5/Em5 and Me7/Em7 primer combinations, respectively. The similarity matrix of coefficient ranged from 6 to 64 per cent, suggesting a lower to higher genetic variation within China aster genotypes (Table 4).

Table 3. Per cent polymorphism as revealed by SRAP primer combinations in China aster genotypes

Primer combination	No. of polymorphic bands	Unique bands	Percentage polymorphism
Me 5 + Em 2	12	1	92.30
Me 5 + Em 3	16	0	100.00
Me 5 + Em 4	8	0	100.00
Me 5 + Em 5	8	1	88.88
Me 5 + Em 7	7	2	77.77
Me 7 + Em 2	2	0	100.00
Me 7 + Em 3	6	2	75.00
Me 7 + Em 4	15	2	88.23
Me 7 + Em 5	9	0	100.00
Me 7 + Em 7	4	1	80.00
Me 8 + Em 3	2	0	100.00
Total	89	9	
Mean	7.27	-	91.10

The highest genetic similarity of 64 per cent was observed between the AAC-1 and AAC-2, followed by AAC-4 and AAC-5 (56%), while least (6 %) was between AAC-8 and Violet cushion followed by AAC-7 and Violet cushion, AAC-6 and Violet cushion (8 % each) this could have happened due to highly similar morphological nature of the crop. Similar results were reported by Pragya *et al.* (2010) in gladiolus revealed that in cluster analysis by RAPD marker, the cultivar 'Pusa Lohit' branched out from the dendrograms, confirming that it is quite different from all other genotypes. The dendrogram based on UPGAM clustering resulted in the identification of two major clusters (Fig 2). Majority of the genotypes in major cluster I were collected from many parts of the country like local collections; AAC-1, AAC-2 almost originated in Belgaum area hence those were grouped together and AAC-4, AAC-5, AAC-7, AAC-8 and AAC-9 were collected from Namdhari seeds private company and were grouped together in same cluster. PG-White and PG-Violet were collected from MPKV Rahuri and hence grouped in same cluster. Major cluster II consists of Violet cushion, Poornima, Shashank and Kamini were collected from IIHR, Bangalore and apart from that genotypes, Violet cushion and Shashank were having same parents and hence grouped together in same cluster. PG-Purple and PG- Pink had collected from MPKV, Rahuri because of their morphological characters like presence of hairs on leaf and on laterals, leaf shape, stem color and their spreading habit and natural mutation they were grouped together in major cluster II. Majority of sub-groups were grouped according to origin and some of the genotypes grouped based on their parents involved in hybridization (Shashank and Violet cushion) and

Table 4. Similarity co-efficient matrix of China aster genotypes based on SRAP marker

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1.00																
2	0.49	1.00															
3	0.40	0.33	1.00														
4	0.44	0.54	0.45	1.00													
5	0.34	0.31	0.36	0.45	1.00												
6	0.27	0.24	0.37	0.31	0.41	1.00											
7	0.21	0.18	0.19	0.18	0.28	0.40	1.00										
8	0.23	0.22	0.28	0.27	0.32	0.43	0.37	1.00									
9	0.21	0.22	0.22	0.23	0.23	0.37	0.42	0.53	1.00								
10	0.19	0.23	0.22	0.23	0.21	0.33	0.33	0.36	0.64	1.00							
11	0.16	0.19	0.12	0.18	0.13	0.21	0.28	0.26	0.38	0.46	1.00						
12	0.18	0.23	0.12	0.20	0.16	0.21	0.20	0.23	0.33	0.46	0.52	1.00					
13	0.22	0.14	0.14	0.16	0.18	0.21	0.20	0.19	0.24	0.38	0.47	0.56	1.00				
14	0.19	0.14	0.08	0.17	0.12	0.15	0.21	0.20	0.32	0.43	0.40	0.38	0.30	1.00			
15	0.17	0.12	0.08	0.19	0.15	0.14	0.14	0.22	0.20	0.33	0.41	0.44	0.42	0.42	1.00		
16	0.20	0.19	0.06	0.15	0.17	0.13	0.25	0.18	0.24	0.35	0.41	0.34	0.33	0.42	0.40	1.00	
17	0.20	0.16	0.12	0.18	0.18	0.17	0.13	0.27	0.30	0.29	0.38	0.36	0.33	0.41	0.53	0.42	1.00

Genotypes: 1. Kamini, 2. Poornima, 3. Shashank, 4. Violet cushion, 5. PG-Pink, 6. PG-Purple, 7. PG-Violet, 8. PG-White, 9. AAC-1, 10. AAC-2, 11. AAC-3, 12. AAC-4, 13. AAC-5, 14. AAC-6, 15. AAC-7, 16. AAC-8, 17. AAC-9

the rest of them grouped incongruently. The similar results were found by Sehrawat *et al.* (2003) in chrysanthemum. Although major emphasis of the present investigation was to characterize the genotypes using SRAP marker, the data also revealed the genetic relationship among the genotypes. Molecular markers have been proved to be a fundamental and reliable tool for fingerprinting of genotypes.

PCA of 17 genotypes based on SRAP markers:

China aster genotypes were scattered on the PC plot, which is a reflection of moderate genetic base. The results of PCA showed a clear-cut separation (Fig. 3). However, some of the genotypes appear to be overlapping with each other depicting redundancy in these genotypes. Genotypes Violet cushion, PG-Purple, PG-White, PG-Violet, AAC-1 and AAC-2 were found at the periphery of the PCA and were quite distinct and forming separate entities. In general, the results obtained from the PCA are in agreement with the

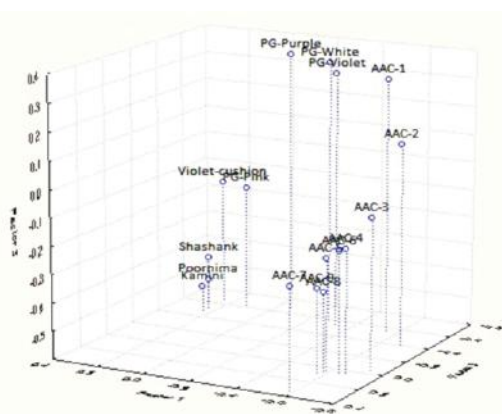


Fig. 3. Principal Component Analysis (PCA) of 17 China aster genotypes based on SRAP markers

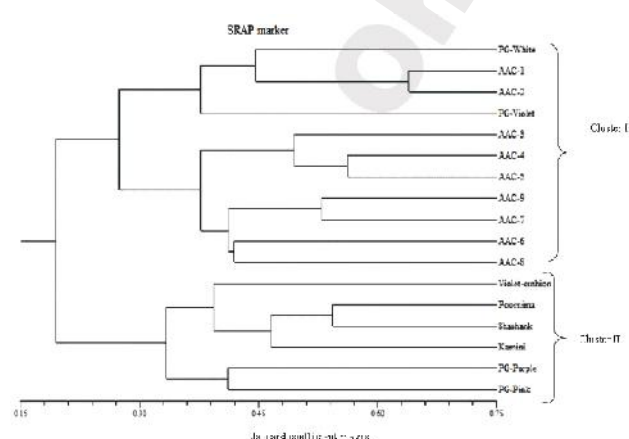


Fig. 2. Dendrogram showing genetic relationship among 17 China aster genotypes based on SRAP molecular data using unweighted pair group method with arithmetic average (UPGMA) analysis

dendrogram generated by Jaccard's method, is a further confirmation of the genetic relationships delineated by cluster analysis. On comparing the genetic diversity as revealed by the dendrogram, it was evident that all genotypes used in the experiment was identified quite distinct each other.

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Received 10 October, 2015; Accepted 26 January, 2016



Correlation and Path Analysis of Morpho-physiological Traits with Yield of Soybean (*Glycine max* L. Merrill) Genotypes under Variable Photoperiods

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Abstract: An experiment was conducted with fifteen soybean genotypes grown under longer (early June) and shorter (early July) photoperiods. The correlation analysis showed significant positive relationships between most of the traits with seed yield. Path coefficient analysis revealed that under longer photoperiod the maximum direct effect was observed in crop growth rate at 45-60 days after sowing with seed yield ($r=1.649$). Similarly, maximum value of direct relationship was observed between transpiration rate at flowering and seed yield ($r=1.884$) under shorter photoperiod. The present study explored the importance of morpho-physiological traits viz., leaf area index at vegetative, leaf area index at flowering, specific leaf weight at vegetative, crop growth rate at 30-45 DAS, relative growth rate at 30-45 DAS and 45-60 DAS, net photosynthetic rate at vegetative and flowering stage, for the selection of superior genotypes in soybean.

Key Words: Soybean, Correlation coefficient, Path coefficient analysis, Photoperiod, Seed yield

Soybean (*Glycine max*. L. Merrill) is one of the most important leguminous crops in the world. India ranks fifth in the world after USA, Brazil, Argentina and China in soybean production and accounts for 11.5 million metric tons (3%) of total world production in the year 2012-13 (Soy Stats, 2013). However, average yield of soybean in India is less than that of world average which needs to be improved through genetic manipulations. Grain yield of soybean is a complex character which is dependent on a number of components. In breeding programme to improve yield of crops, breeder has the choice to select yield directly or indirectly through yield related traits. Morpho-physiological traits, if correlated with grain yield in soybean, can be used as indirect selection for yield. The path coefficient analysis helps to partition correlation coefficient in to direct and indirect effects (Saleem *et al.*, 1999) and can be used to supplement information on correlation coefficients. The correlations of yield with yield components and morphological traits has been studied extensively and used as a tool to improve seed yield of soybean (Arshad *et al.*, 2006; Malik *et al.*, 2006). But correlations among different character can vary under different environments. Keeping these points in view, the present study has been planned, to establish the correlation between yield and morpho-physiological traits under variable photoperiods and to determine the direct and indirect effect of morphological traits on grain yield in soybean genotypes grown under variable photoperiods in order to find out a suitable trait that could be used for the yield improvement.

MATERIAL AND METHODS

The field experiment was conducted at the research area of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, during *kharif* season 2012. Ludhiana is situated in the sub-tropical zone of Punjab at about 247m above sea level on latitude 30° 54'N and longitude 75° 48'E. The soil at the experimental site is sandy loam and experimental material consisting of 15 soybean genotypes were sown in randomized block design with three replications. Each block consisted of 15 plots, each of which measured 5m x 4m, giving a total of 45 plots, and the spacing between plants and rows were 5cm and 45cm respectively, with one meter alleys between the blocks. Data were recorded on 5 plants of each plot for morpho-physiological traits viz., Leaf area index, specific leaf weight, crop growth rate, relative growth rate, photosynthetic rate, transpiration rate and seed yield (g plant^{-1}) and average was calculated.

RESULTS AND DISCUSSION

Correlation coefficient analysis: Top diagonal matrix elucidates all parameters were positively and significantly correlated with seed yield under longer photoperiodic conditions (Table 2). Leaf area index at vegetative stage was highly correlated with seed yield per plot ($r = 0.89$) followed by leaf area index at flowering, net photosynthetic rate (and transpiration rate at vegetative growth stage). Likewise,

Table 1. Correlation matrix of yield and Morpho-physiological traits of soybean genotypes under longer photoperiod (top of diagonal) and shorter photoperiod (bottom of diagonal)

	LAIV	LAIF	LAIP	SLWV	SLWF	SLWP	CGR1	CGR2	RGR1	RGR2	NPRV	NPRF	NPRP	TRV	TRF	TRP	SY
LAIV	1	0.787**	0.757**	0.735**	0.716**	0.657**	0.755**	0.760**	0.759**	0.764**	0.784**	0.767**	0.791**	0.724**	0.753**	0.620*	0.890**
LAIF	0.684**	1	0.740**	0.557*	0.784**	0.524*	0.596*	0.523*	0.637*	0.639*	0.611*	0.697**	0.661**	0.669**	0.590*	0.377	0.825**
LAIP	0.611*	0.528*	1	0.543*	0.721**	0.682**	0.688**	0.643**	0.858**	0.857**	0.658**	0.534*	0.671**	0.596*	0.557*	0.580*	0.713**
SLWV	0.707**	0.751*	0.613*	1	0.664**	0.645**	0.293	0.294	0.472	0.495	0.732**	0.688**	0.708**	0.523*	0.788**	0.525*	0.741**
SLWF	0.531*	0.398	0.203	0.391	1	0.677**	0.518*	0.377	0.580*	0.530*	0.524*	0.641*	0.673**	0.564*	0.635*	0.518*	0.701**
SLWP	0.407	0.247	0.142	0.27	0.182	1	0.462	0.461	0.610*	0.630*	0.584*	0.710**	0.540*	0.295	0.608*	0.675**	0.553*
CGR1	0.603*	0.298	0.144	0.486	0.384	0.326	1	0.961**	0.833**	0.821**	0.606*	0.475	0.649*	0.672**	0.51	0.630*	0.668**
CGR2	0.644**	0.503	0.229	0.552*	0.588*	0.401	0.879**	1	0.803**	0.822**	0.605*	0.515*	0.601*	0.603*	0.503	0.572*	0.607*
RGR1	0.773**	0.759**	0.505	0.852**	0.402	0.373	0.641*	0.732**	1	0.963**	0.772**	0.487	0.651**	0.723**	0.48	0.729**	0.737**
RGR2	0.718*	0.589*	0.412	0.772**	0.585*	0.353	0.776**	0.882**	0.799**	1	0.767**	0.528*	0.623*	0.619*	0.473	0.712**	0.752**
NPRV	0.604*	0.821**	0.549*	0.792**	0.156	0.204	0.504	0.601*	0.868**	0.652**	1	0.638*	0.744**	0.835**	0.693**	0.526*	0.785**
NPRF	0.665**	0.846**	0.548*	0.779**	0.452	0.524*	0.268	0.501	0.766**	0.694**	0.719**	1	0.747**	0.45	0.830**	0.436	0.636*
NPRP	0.419	0.636*	0.141	0.530*	0.051	0.473	0.271	0.336	0.574*	0.482	0.48	0.666**	1	0.738**	0.942**	0.505	0.768**
TRV	0.646**	0.615*	0.579*	0.703**	0.262	0.048	0.409	0.51	0.821**	0.524*	0.825**	0.505	0.213	1	0.611*	0.371	0.717**
TRF	0.642**	0.703**	0.455	0.649**	0.351	0.524*	0.661**	0.868**	0.775**	0.789**	0.783**	0.683**	0.49	0.602*	1	0.434	0.672**
TRP	0.611*	0.747**	0.214	0.697**	0.351	0.252	0.673**	0.691**	0.723**	0.667**	0.815**	0.599*	0.478	0.613*	0.706**	1	0.660**
SY	0.909**	0.772**	0.576*	0.805**	0.386	0.202	0.544*	0.568*	0.775	0.673**	0.705**	0.662*	0.516*	0.725**	0.629*	0.731**	1

Note: *, ** indicates the correlation significant at the 0.05 and 0.01 probability level.

LAIV=Leaf Area Index at Vegetative, LAIF=Leaf Area Index at Flowering, LAIP=Leaf Area Index at Pod, SLWV= Specific Leaf Weight at Vegetative, SLWF=Specific Leaf Weight at Flowering, SLWP = Specific Leaf Weight at Pod, CGR1= Crop Growth Rate at 30-45 days after sowing, CGR2= Crop Growth Rate at 45-60 days after sowing, RGR1= Relative Growth Rate at 30-45 days after sowing, RGR2= Relative Growth Rate at 45-60 days after sowing, NPRV= Net Photosynthetic Rate at vegetative, NPRF= Net Photosynthetic Rate at Flowering, NPRP= Net Photosynthetic Rate at Pod, TRV= Transpiration Rate at vegetative, TRF= Transpiration Rate at Flowering, TRP= Transpiration Rate at Pod, SY= Seed Yield.

Table 2. Direct (diagonal) and indirect (off diagonal) effects of 16 characters on seed yield of soybean genotypes under longer photoperiod

	LA1V	LAIF	LAIP	SLWV	SLWF	SLWP	CGR1	CGR2	RGR1	RGR2	NPRV	NPRF	NPRP	TRV	TRF	TRP
LA1V	1.082	0.570	-0.412	0.043	-0.331	-0.151	1.244	-1.514	0.639	0.097	0.294	-0.373	-0.318	-0.603	0.658	-0.037
LAIF	0.852	0.725	-0.403	-0.033	-0.362	-0.120	0.983	-1.060	0.536	0.081	0.229	-0.338	-0.266	-0.557	0.516	-0.022
LAIP	0.819	0.537	-0.544	0.032	-0.333	-0.157	1.135	-1.280	0.722	0.109	0.247	-0.260	-0.270	-0.496	0.486	-0.035
SLWV	0.795	0.404	-0.296	0.059	-0.307	-0.148	0.483	-0.586	0.397	0.063	0.275	-0.334	-0.285	-0.436	0.688	-0.031
SLWF	0.775	0.568	-0.392	0.039	-0.462	-0.155	0.855	-0.751	0.489	0.067	0.196	-0.311	-0.271	-0.470	0.554	-0.031
SLWP	0.711	0.379	-0.371	0.038	-0.313	-0.230	0.761	-0.919	0.514	0.080	0.219	-0.345	-0.217	-0.246	0.531	-0.040
CGR1	0.817	0.432	-0.375	0.017	-0.240	-0.106	1.649	-1.916	0.701	0.104	0.227	-0.231	-0.261	-0.559	0.445	-0.037
CGR2	0.822	0.386	-0.350	0.017	-0.174	-0.106	1.586	-1.992	0.676	0.104	0.227	-0.250	-0.242	-0.502	0.439	-0.034
RGR1	0.821	0.462	-0.467	0.028	-0.268	-0.140	1.373	-1.600	0.842	0.122	0.289	-0.237	-0.262	-0.602	0.419	-0.043
RGR2	0.827	0.463	-0.466	0.029	-0.245	-0.145	1.353	-1.637	0.810	0.127	0.288	-0.256	-0.251	-0.515	0.413	-0.042
NPRV	0.849	0.443	-0.358	0.043	-0.242	-0.134	0.999	-1.206	0.649	0.098	0.375	-0.310	-0.299	-0.695	0.605	-0.031
NPRF	0.830	0.505	-0.291	0.040	-0.296	-0.163	0.783	-1.027	0.410	0.067	0.239	-0.486	-0.301	-0.375	0.725	-0.026
NPRP	0.856	0.479	-0.365	0.041	-0.311	-0.124	1.070	-1.197	0.548	0.079	0.279	-0.363	-0.402	-0.615	0.823	-0.030
TRV	0.784	0.485	-0.324	0.031	-0.261	-0.068	1.108	-1.201	0.609	0.079	0.313	-0.219	-0.297	-0.833	0.533	-0.022
TRF	0.815	0.428	-0.303	0.046	-0.293	-0.140	0.841	-1.002	0.404	0.060	0.260	-0.403	-0.379	-0.508	0.873	-0.026
TRP	0.671	0.274	-0.316	0.031	-0.239	-0.155	1.038	-1.141	0.613	0.091	0.197	-0.212	-0.155	1.038	0.379	-0.060

See Table 1 for details

specific leaf weight at flowering was positively and significantly linked with crop growth rate at 30-45 DAS ($r=0.518$). Specific leaf weight at flowering was positively and significantly correlated with net photosynthetic rate at flowering and pod stage. Further, net photosynthetic rate at vegetative stage was positively and significantly associated with transpiration rate at vegetative, flowering and pod stage. Similarly, leaf area index at vegetative, flowering and pod stage were positively and significantly linked with net photosynthetic rate at vegetative, flowering and pod stage. The highest correlation was obtained between relative growth rate at 30-45 DAS and relative growth rate at 45-60 and was found significant. The correlation results showed significant positive relationships between most of the traits with seed yield. Since the leaves are better exposed to solar radiation owing to the broader leaf area, photosynthesis processes thus increase on the leaf surface with the resultant increase in productivity. The leaf area index at harvest has positive significant correlation with seed yield during both the photoperiodic condition. This suggests that the maintenance of high leaf area index, particularly at the time of flowering stage is necessary for higher productivity in soybean. The results of correlation studies indicated a significant positive association of crop growth rate (30–45 DAS and 45-60 DAS) with seed yield during both the photoperiods. Likewise, bottom diagonal of matrix showed that all parameters were positively correlated with seed yield under shorter photoperiodic condition (Table 1). Leaf area index at vegetative stage showed the highest correlation with seed yield ($r=0.909$) than other parameters. Specific leaf weight at vegetative stage associated positively and significantly with net photosynthetic rate at vegetative, pod and flowering stages. Specific leaf weight at vegetative stage had correlation coefficient of 0.805 with seed yield which was significant. Similarly, leaf area index at vegetative stage was positively and significantly related with net photosynthetic rate at vegetative and flowering stage. Likewise, leaf area index at flowering stage linked with net photosynthetic rate at vegetative, flowering and pod stage and net photosynthetic rate at vegetative, flowering and pod stage along with transpiration rate at vegetative, flowering and pod stages were positively and significantly correlated with seed yield under shorter photoperiodic condition. Besides, net photosynthetic rate was also positively and significantly correlated with transpiration rate at vegetative, flowering and pod stage. Photosynthetic rate have significant positive correlation with seed yield, thereby clearly indicating the importance of this parameter in improving the yield potential of genotypes. It is obvious that higher photosynthetic rate and transpiration rate leads to higher production of dry matter and

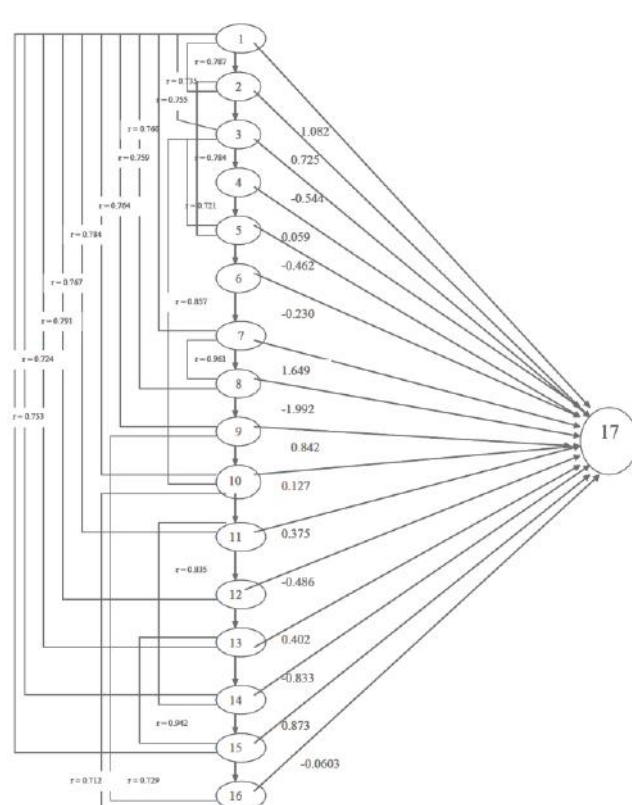


Fig. 1. Path diagram showing the path coefficients and correlation coefficients of seed yield and morpho-physiological traits in soybean genotypes under longer photoperiod

yield and this has also been proved from the results of the present investigation.

Path coefficient analysis: The leaf area index at pod initiation stage, specific leaf weight at flowering, specific leaf weight at pod, crop growth rate at 45-60 DAS, net photosynthetic rate at flowering and pod, transpiration rate at vegetative and pod stage had negative direct effect on seed yield. This suggested that selection on the basis of these traits might lead to loss in term of soybean yield. However, leaf area index at vegetative, leaf area index at flowering, specific leaf weight at vegetative, crop growth rate at 30-45 DAS, relative growth rate at 30-45 DAS and 45-60 DAS, net photosynthetic rate at vegetative and flowering showed the positive direct effect with seed yield. Maximum direct effect was observed in crop growth rate at 45-60 days after sowing

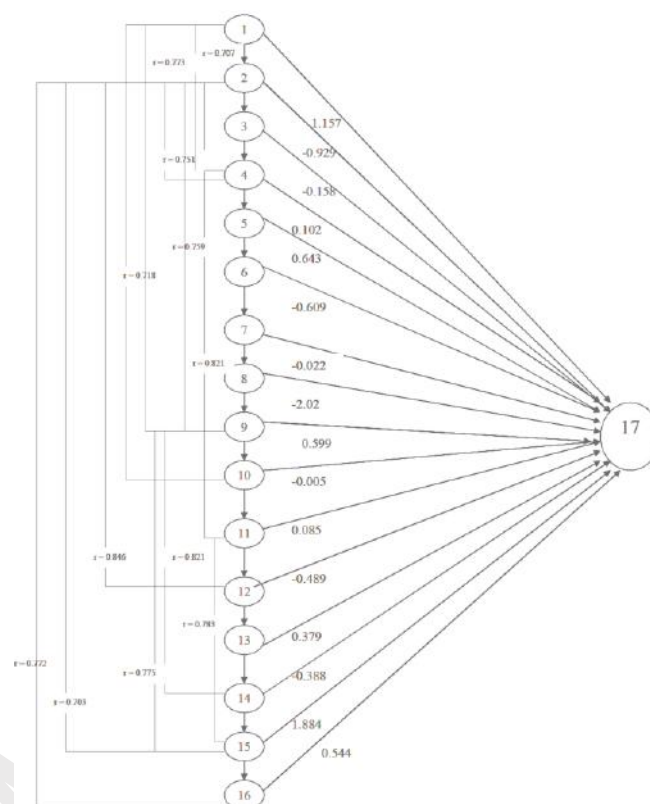


Fig. 2. Path diagram showing the path coefficients and correlation coefficients of seed yield and morpho-physiological traits in soybean genotypes under shorter photoperiod. (See Figure 1)

on grain yield. In the same way, under shorter photoperiod leaf area index at flowering and pod, specific leaf weight at pod, crop growth rate at 30-45 DAS and 45-60 DAS, net photosynthetic rate at flowering and transpiration rate at vegetative exerted negative direct effect on seed yield (Table 3). In contrary, leaf area index at vegetative, specific leaf weight at vegetative and flowering, relative growth rate at 30-45 DAS, net photosynthetic rate at vegetative and pod stage, transpiration rate at flowering and pod stage demonstrated positive direct effect on seed yield. Maximum value of direct relationship was observed between transpiration rate at flowering and seed yield (1.884). The results of this experiment are also supported by other researchers (Ball *et al.*, 2001; Arshad *et al.*, 2006; Tyagi *et al.*, 2013).

From present study, it suggest that the leaf area index at vegetative and flowering stage, crop growth rate at 30 - 45 DAS, net photosynthetic rate at vegetative and transpiration rate at vegetative and flowering stage should be considered while selecting for high yielding soybean genotypes under variable photoperiods. Additionally, path coefficient results explicitly indicate that the leaf area index at vegetative, leaf area index at flowering, specific leaf weight at vegetative, crop growth rate at 30-45 DAS, relative growth

Table 3. Direct (diagonal) and indirect (off diagonal) effects of 16 characters on seed yield of soybean genotypes under shorter photoperiod

	LA1V	LA1F	LA1P	SLWV	SLWF	SLWP	CGR1	CGR2	RGR1	RGR2	NPRV	NPRF	NPRP	TRV	TRF	TRP
LA1V	1.157	-0.636	-0.096	-0.073	0.342	-0.247	-0.013	-1.296	0.463	-0.004	0.051	-0.325	0.016	-0.251	1.201	0.333
LA1F	0.791	-0.929	-0.084	0.077	0.256	-0.15	-0.007	-1.016	0.455	-0.004	0.07	-0.414	0.241	-0.238	1.317	0.406
LA1P	0.706	-0.49	-0.158	0.063	0.131	-0.086	-0.003	-0.46	0.302	-0.002	0.047	-0.268	0.053	-0.224	0.852	0.116
SLWV	0.818	-0.697	-0.097	0.102	0.251	-0.164	-0.011	-1.12	0.51	-0.005	0.067	-0.381	0.210	-0.272	1.213	0.379
SLWF	0.614	-0.369	-0.032	0.04	0.643	-0.110	-0.008	-1.193	0.24	-0.004	0.013	-0.221	0.019	-0.101	0.657	0.191
SLWP	0.47	-0.229	-0.023	0.028	0.116	-0.609	-0.007	-0.807	0.224	-0.002	0.017	-0.257	0.179	-0.019	0.982	0.137
CGR1	0.697	-0.277	-0.023	0.05	0.247	-0.198	-0.022	-1.781	0.384	-0.005	0.043	-0.131	0.103	-0.159	1.249	0.367
CGR2	0.745	-0.468	-0.037	0.057	0.379	-0.244	-0.02	-2.020	0.439	-0.005	0.051	-0.245	0.127	-0.197	1.636	0.376
RGR1	0.894	-0.706	-0.08	0.088	0.259	-0.228	-0.014	-1.470	0.599	-0.005	0.074	-0.376	0.217	-0.319	1.467	0.394
RGR2	0.831	-0.547	-0.065	0.079	0.376	-0.214	-0.017	-1.783	0.479	-0.005	0.056	-0.34	0.187	-0.204	1.651	0.362
NPRV	0.699	-0.764	-0.087	0.081	0.101	-0.125	-0.012	-1.221	0.521	-0.004	0.085	-0.352	0.182	-0.321	1.477	0.443
NPRF	0.769	-0.787	-0.087	0.08	0.291	-0.319	-0.006	-1.009	0.459	-0.004	0.061	-0.489	0.252	-0.197	1.289	0.327
NPRP	0.485	-0.592	-0.022	0.055	0.032	-0.288	-0.006	-0.677	0.345	-0.003	0.041	-0.327	0.379	-0.083	0.918	0.259
TRV	0.748	-0.572	-0.092	0.072	0.169	-0.293	-0.009	-1.037	0.493	-0.003	0.07	-0.248	0.081	-0.388	1.128	0.334
TRF	0.743	-0.654	-0.072	0.067	0.226	-0.318	-0.013	-1.748	0.467	-0.005	0.067	-0.335	0.186	-0.233	1.884	0.385
TRP	0.708	-0.694	-0.034	0.072	0.227	-0.154	-0.015	-1.393	0.434	-0.004	0.069	-0.294	0.181	-0.238	1.133	0.544

See Table 1

rate at 30-45 DAS and 45-60 DAS, net photosynthetic rate at vegetative and flowering which directly contributed substantially towards seed yield, should be indices for selection in soybean.

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Received 6 February, 2016; Accepted 12 May, 2016

Tribal Fruits and Vegetable of Udaipur District of Rajasthan

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Abstract: Survey was conducted in five tribal *tehsils* viz Jhadol, Kotada, Kherwada, Sarada and Salumber of Udaipur to identify the fruits and vegetables grown wild and consumed in tribal region Udaipur District, Rajasthan, India.. The people residing in the tribal region of Udaipur consume a wide variety of fruits, green leafy vegetables, other vegetables and roots and tubers available in their locality. About 49- fruits, 35- green leafy vegetables, 34- other vegetables and 29- roots and tubers were identified in the tribal region of Udaipur and these were consumed during season and availability. Among them, 17 fruits and vegetables were available round the year. Total 16 fruits, 11 green leafy vegetables, 07 other vegetables and 09 roots and tubers were utilized for the treatment of various health problems, such as sexual disability, kidney stones, digestive problems etc.

Key Words: Food Security, Fruits and vegetables, Nutrition, Tribal, Wild

The Aravalli ranges possess an abundant population of tribals. The main tribes of the study area are Bheel, Meena, Garasia and Kathodi, which forms more than 16 per cent of total tribal population of state and approximately 50 per cent of the district's population (Census, 2011). Aravalli, spreading through Rajasthan have thick vegetation cover rich "green bank" of many medicinal plants. The variability in climate, edaphic, and topographic conditions causes diversity of vegetation in the Aravalli ranges. These plants have been collected from wild for food and therapeutic use in various diseases by tribal populations inhabiting the Aravallis (Pandey and Singh, 1998). Forests play an important role in many food systems, either through direct and indirect provisioning for human nutrition, particularly in developing countries (Vinceti *et al.*, 2008; Sunderland, 2011, Chauhan *et al.*, 2010). Forests and trees-outside forests contribute to the

livelihoods of more than 1.6 billion people (FAO, 2010).

Increased agricultural and forest biological diversity leading to a more varied diet, which in turn improves specific health outcomes (Lutaladio, 2010). Forest ecosystems seem to provide more diversified wild foods compared to other land uses within the same region. Many of the plant species are believed to possess edible value and not documented yet. So there is an urgent need to explore, and document the wild foods consumed by the tribal region of Udaipur of Rajasthan.

MATERIAL AND METHODS

The field survey was conducted in the five tribal *tehsils* i.e. Jhdol, Kotra, Kherwada, Sarada and Salumber of Udaipur district of Rajasthan (Fig. 1) during October 2011 to February 2012.

The main tribes of the study area were Bheel,



Fig. 1. Study Area

Meena, Garasia and Kathodi. In order to collect information, Participatory Rural Appraisal (PRA) technique with group/individual discussion were used. For collecting information open end perform was used. For effective communication, the help of Forest Guards and Van Mitra were also taken. A group of five to ten people and a total 50 groups (i.e. ten groups from each tehsil) were studied. Total of 450 tribal people were contacted to gather required information.

RESULTS AND DISCUSSION

The result of the present study shows that, people residing in the tribal region of Udaipur District of Rajasthan consume a wide variety of fruits, vegetables, and roots and tubers found around the forest area. Present study revealed that tribal utilizes these wild fruits and vegetables for the treatment of various diseases (Table 1 to 4). It was observed

that 49- fruits, 35- green leafy vegetables, 34- other vegetables and 29- roots and tubers were consumed by the tribal of the studied area. Total 149 species were consumed by tribals of the studied area and among them 17 fruits and vegetables were available round the year. The people of these tribal localities were utilizing these forest produce not only just consuming but also selling in the local market to generate income. Sasi *et al.* (2011) documented indigenous knowledge on wild edible plant resources from the tribal area at Nilgiri Hills. It was observed that tribals were partially or fully dependent on the wild resources for their nutritional requirements. The tribal communities fulfill their food deficiency by supplementing wild food plants in their daily diet. Mahapatra and Panda (2012) studied the collection, consumption, sale and income from edible forest fruits in 49 tribal villages spread over five districts of Orissa State in

Table 1. Fruits grown wild and consumed in the tribal region of Udaipur

Botanical name	Local name	Season of availability	Remark
<i>Aegle marmelos</i>	Bel	January - July	Used in the treatment of sexual debility, abdominal pain and chronic dysentery, habitual constipation and dyspepsia.
<i>Alangium salvifolium</i>	Ankol	Feb- June	Roots extract are used as abortifacient antidote.
<i>Annona reticulate</i>	Ramphal	April – August	-
<i>Annona squamosa</i>	Sitaphal	April – August	-
<i>Citrus medica</i>	Khata limba/ Bijaura	Round the year	Sour, refrigerant, digestive, haemostatic and toxic and are used after sun drying in the form of powder as <i>churan</i> .
<i>Cochlospermum religiosum</i>	Girnar	January-April	-
<i>Cordia gharaf</i>	Choti gundi	April – December	Helpful in constipation
<i>Cordia dichotoma</i>	Gundi	March-May	Mature fruits are eaten raw. Immature fruits are use in pickle formation.
<i>Diospyros melanoxylon</i>	Timru	Summer and rainy season	Used in treatment of urinary and heart diseases, anti-dysenteric.
<i>Diospyros montana</i>	Timru	Summer and rainy season	Dried fruits are used in off season
<i>Ensete superbum</i>	Jangli kala	August - October	Helps in raise uterus.
<i>Feronia limonia</i>	Kotabdi	March – October	Used as liver and cardio-tonic and as astringent in diarrhea, dysentery, sore throat and gum diseases.
<i>Ficus benghalensis</i>	Bad	Round the year	Used in treatment in regain sexual potency.
<i>Ficus carica L.</i>	Anjir	Round the year	Used in boils, eruption and other skin diseases.
<i>Ficus hispida</i>	Gullar/ Umbar / Umbio	January-December	Refrigerant, astringent, anti-dysenteric, anti-inflammatory and haemostatic.
<i>Ficus palmate</i>	Umbiya	February-July	Mature receptacles are eaten like <i>Ficus carica</i> (Anjir)
<i>Ficus racemosa</i>	Gullar/ Umbar / Kasumati	Round the year	-

<i>Ficus religiosa</i>	Pipal / Peepali	Round the year	-	
<i>Flacourtia indica</i> (Burm. F.) Merrill	Kankair	January-May	-	
<i>Gerwia orientalis</i>	Gengachi	September - October	-	
<i>Gerwia sclerophylla</i>	Dhaman	April to July	-	
<i>Gerwia tenax</i>	Kankera	January - December	-	Root powder is used in diarrhea and bone fracture.
<i>Gmelina arborea</i> Roxb.	Sivan	March-August	-	
<i>Grewia damine</i> Gaertn.	Gangaran	January-October	-	
<i>Grewia flavescens</i> A. Juss.	Gangas / Gangeshri	August - November	-	
<i>Grewia subinaequalis</i> DC	Phalsa	November - May	-	
<i>Grewia tiliifolia</i> Vaha	Jhujhana/ Dhamni	May-August	-	
<i>Holoptelea integrifolia</i>	Chural / Bandar batti / Kanaj / papan	December to May	-	Root is used in syphilis. Bark and leaf paste of the plant are applied externally on the white patches or leucoderma. The tender flowers are made into paste and applied locally to cure skin diseases and also for expulsion of guineaworm.
<i>Ipomoea pes-tigridis</i>	Panchpatri	August-December	-	Only ovary portion of the fruits are eaten raw
<i>Manilkara hexandra</i>	Rayana	April to June	-	Used as refrigerant
<i>Mimusops elengi</i> L.	Maulsari/ Khirni	April to June	-	
<i>Nelumbo nucifera</i> Gaertn.	Kamal kokari	August - February	-	
<i>Opuntia elatior</i> Mill.	Kantella thour	January - June	-	
<i>Phoenix sylvestris</i>	Jangli khajuor	February - August	-	
<i>Phyllanthus emblica</i>	Awla	March - November	-	Cure body ache, cough and dyspepsia.
<i>Pithecellobium dulce</i>	Jangal Jalabi/Kikar	March-August	-	
<i>Rhus mysurensis</i> G. Don.	Dansaria	June-October	-	Eaten especially by the children and ladies while collecting firewood from the forest. Dried and preserved for off season.
<i>Salvadora oleioides</i> Decne.	Pilu	February - June	-	Preserved for off season after drying
<i>Salvadora presica</i> L.	Mithi-Pilu	December - June	-	
<i>Spondias mangifera</i> Willd	Parangi	April- July	-	
<i>Sterculia urens</i>	Kadaya	December - May	-	Resin used by tribal to cure leucoderma and peptic ulcer
<i>Syzygium heyneanum</i>	Jamun	July-October	-	
<i>Tamarindus indica</i>	Imli	November - April	-	Mature fruits are soaked in water and little sugar and chilly are added to make a soft drink which is taken orally by the tribal community as blood purifier, to cure enlarged liver, jaundice, indigestion, constipation and sunstroke. Tribal ladies chew the fruits as such or eat as vegetable, to regularize menstrual disorders. Seed paste is applied locally as an antidote in scorpion sting and snakebite and also taken orally to cure piles. Semi-roasted seeds are eaten to cure diarrhea.

<i>Tribulus terrestris</i>	Gokhru / Kanti	August-December	The seeds are powdered and mixed with the flour of grains to make <i>chappati</i> . Immature fruits are sweet in taste and are eaten raw. Powder is used for urinary disorders. The infusion of whole plant is taken orally by the tribal men to cure gonorrhea and increase sexual power.
<i>Ziziphus glabrata</i> Heyne ex Roth.	Bor	December - January	Fruits are eaten raw and sold in the market
<i>Ziziphus mauritiana</i> Lam.	Bor/ Bara bor/ Pemli bor	September - February	Fruits are eaten raw and sold in the market
<i>Ziziphus nummularia</i> (Burm. F.) Wt. & Arn.	Jhari ber	July-January	Fruits are eaten raw and sold in the market
<i>Ziziphus trinervia</i> Roxb	Jungli ber	August - January	-
<i>Ziziphus xylopyrus</i> (Retz.) Willd.	Gat ber	February - April	The inner part of the seed is eaten especially by the children of the tribal locality.

Table 2. Green leafy vegetables grown wild and consumed in the tribal region of Udaipur

Botanical name	Local name	Season of availability	Remark
<i>Amaranthus caudatus</i>	Dandi Chandloi	Throughout year	Tender leaves and shoots are cooked as vegetable. Use to cure constipation and loss of appetite.
<i>Amaranthus spinosus</i>	Kantili Cholai	Throughout year	-
<i>Amaranthus viridis</i>	Jangali Chouli	March-October	-
<i>Asphodelus tenuifolius</i>	Piyagi / Wanzooa	December - March	Decoction of leaves is taken orally by the tribal women to cure toxemia and to remove kidney stone.
<i>Boerhavia diffusa</i>	Kanejada	Throughout year	Decoction of leaves is taken orally by the tribal women to cure toxemia and to remove kidney stone.
<i>Brassica campestris</i>	Sarson ki bhagi	November-December	-
<i>Cassia tora</i>	Puwariya	August-November	Leafy vegetable is given to the patients of anemia for one month.
<i>Cassia obtusifolia</i>	Marbga	August - November	-
<i>Celosia argentea</i>	Garkha	August - November	-
<i>Centella asiatica</i>	Brahami buti / Mandukparni	Throughout year	Leaves and tender shoots are cooked as vegetable. Leaves are also given in mental disorder.
<i>Chenopodium album</i>	Cheel / Bathua	October-April	Use as laxative.
<i>Chenopodium murale</i>	Chamar Bathua	November-April	Considered very nutritive and laxative. It is stored for future use after sun-drying
<i>Cicer arietinum</i>	Liliya / Lilwa / Chick pea	December - March	Cooked as vegetable and also in the form of <i>chutany</i>
<i>Colocasia esculenta</i>	Tera ki bhaji/ Jangali arvi	September - December	-
<i>Commelina benghalensis</i>	Pani vala / Jal nala/ Kallni/ Mokta / Bogana	July-October	Leaves and tender shoots are cooked as vegetable. Whole plant is purgative. The <i>pakor</i> made by mixing leaves and tender shoots with <i>besan</i> (gram flour) are given to the patients of leprosy.
<i>Cordia dichotoma</i>	Gunda ka more	March- May	The tender leaves of this tree are cooked as vegetable. In local tribal language it is often referred to as "Gundi ka more" which translate as top or crown of the branch. Tender leaves and flower are cooked as vegetable. This vegetable is prepared with Kadhi.
<i>Digera muricata</i> (L.) Mart.	Lehasua	Throughout year	Leaves are cooked as vegetable.
<i>Emilia sonchifolia</i> (L.) DC.	Meethi dodi	Throughout year	Leaves are as salad.

<i>Euphorbia chamaesyce</i> L.	Dudhi / Fhag	Throughout year	Leaves are cooked as vegetable.
<i>Euphorbia royleana</i>	Thor	January - April	If not cooked properly then it may cause stomach problem. The usual practice of tribal people was wash the vegetable, boil it in water then the water is discarded and the boiled leaves are once again washed and cooked as like the other green leafy vegetables.
<i>Leucas nutans</i> (Roth) Spreng.	Chatia	October-January	Cooked as vegetable.
<i>Marsilis oegyptiaca</i>	Jhalod ri Bhaji	Throughout year	Leaves are cooked as vegetable by the tribal community especially when there is no other vegetable is available.
<i>Marsilis minta</i>	Jhalod ri bhaji	Throughout year	Leaves are cooked as vegetable by the tribal community especially when there is no other vegetable is available.
<i>Medicago sativa</i>	Rajka ki bhaji	Throughout year	For fodder purpose it is cultivated. Mostly consumed by the <i>bheel</i> tribe. Given to the patient suffering from eye problem.
<i>Melilotus alba</i> Medik. Ex descr.	Mokhoundo	Almost throughout year	Leaves are cooked as vegetable.
<i>Melilotus Indica</i>	Moda ki bhaji / Pilisangi	January - April	Leaves are rubbed on the skin by the tribal to cure skin problems. The decoction of whole plant is taken orally to cure body ache. Tribal also believe that if the leaves are cooked as vegetable and taken once or in the whole year then they may not suffer from fever.
<i>Oxalis corniculata</i>	Khata limba ki bhaji	Throughout year	The plant sap is used to cure scurvy and other skin diseases. The leaves are chewed by the tribal as a refrigerant in stomach disorders, fever and acute headache.
<i>Pedaliun murex</i> L.	Bada gokhru	Rainy Season	-
<i>Polygonum galbrum</i> Willd.	Ghar ki sabji	September - May	-
<i>Portulaca oleracea</i>	Lunakiya	August - December	Leaves are beneficial in renal stone
<i>Portulaca pilosa</i>	Lunka	August – January	-
<i>Portulaca quadrifida</i>	Lunakiya	September - December	-
<i>Trianthema portulacastrum</i> L.	Sathi	August - December	-
<i>Trichodesma indicum</i> L.	Chhota kulpha	July - November	-
<i>Tribulus terrestris</i>	Gokhru / Kanti	August-December	-

Table 3. Other vegetables grown wild and consumed in the tribal region of Udaipur

Botanical name	Local name	Season of availability	Part used	Remark
<i>Acacia nilotica</i>	Babul fali	September- March	Pods (Fruits)	Gum is used in various preparations. Powder of unripe pods use to cure impotency among men.
<i>Acacia Senegal</i>	Kumat	July – February	Seeds and Pods	Seeds are cooked as vegetable along with the fruits of <i>Capparis decidua</i> (Ker) and pods of <i>Prosopis cineraris</i> (Sangari). Some times with pieces of unripe fruits of <i>Mangifera indicum</i> (Kari) and fruits of <i>Cordia dichotoma</i> (Gunda) these mix are also called as <i>Pachkutta</i> .
<i>Aloe barbadensis</i>	Aloe vera flowers / Sinduri	September-November	Flower buds	Leaves and immature flowers are cooked as vegetable.
<i>Averrhoa carambola</i>	Kamrakh	August - October	Fruits	Fruits are cooked as vegetable and sometimes in the form of chutney.

<i>Bambusa arundinacea</i>	Bamboo shoots	August - October	Shoots	-
<i>Bauhinia racemosa</i>	Jinja / Hitari	April- July	Pods	Fruit pulp is given after every motion in dysentery.
<i>Bauhinia variegata</i>	Kanchnar	May - November	Pods	Power of pods are mixed with wheat flower and make <i>chappati</i> of it.
<i>Bombax ceiba</i>	Samble / Hemlu dodhi	February - April	Flower buds	Flower is given to cure sexual diseases like leucorrhoea, gonorrhoea and to cure irregular menstrual cycle. The people believe that extract of flowers or powder of dried flowers with honey when taken orally is very effective in curing haematuria and leucorrhoea.
<i>Capparis deciduas</i>	Ker	March - June	Unripe fruits	Cooked as vegetable and also pickled after sun-drying for use in off season
<i>Carissa congesta</i>	Jangli karonda	April- June	Fruits	Ripe fruits are eaten raw and sold in the market. Unripe ones are pickled and also used as vegetable
<i>Cissus quadrangula</i>	Hadjood	Almost throughout year	Shoots	Tender shoots are cooked as vegetable. Cooking is done in lots of ghee. If not cooked properly the cause irritation in throughout. Especially given in fractures
<i>Cissus repanda</i>	Bhansatat	May- August	Shoots	-
<i>Coccinia grandia</i>	Tinda / Tindori	Almost throughout year	Unripe fruits	Cooked as vegetable and are sold in the market by the tribal community.
<i>Cosmostigma racemosa</i>	Sani dodi/ rai dodi	June- August	Buds	Vegetable cooked from the green pods is given to diabetic patient to maintain the blood sugar level.
<i>Crotalaria juncea</i>	Han / Hun/ san	September- February	Flower and tender shoots	Dried and stored for future use as vegetable.
<i>Cucumis callosus</i>	Kachri / Kachrio	August- November	Ripped fruits	The fruits are sweet, acrid, cooling, appetizer, laxative, galactagogue, diuretic, aphrodisiac tonic and are eaten raw and cooked as vegetable.
<i>Dendrocalamus strictus</i>	Bans / Karel	Rainy season	Tender shoots	-
<i>Holarrena antidysenterica</i> Wall.	Koorwa	May - September	Unripe fruits and flowers buds	Unripe fruits and flowers are used as vegetable. Latex is used as instant curdling of milk.
<i>Lagenaria siceraria</i> (Molina) Standl.	Tumbo/ Dhodha	August - February	Fruits	-
<i>Lagerstroemia parviflora</i> Roxb.	Kakarwa	May - December	Fruits	Young fruits are cooked as vegetable after boiling in water.
<i>Lannea coromandelica</i> (Hout.) Merrill	Godal/ Gojala	January - July	Unripe fruits	Unripe fruits are eaten raw during scarcity of foods.
<i>Leptadina reticulata</i>	Shani dodhi	Almost Throughout year	Unripe Fruits	Unripe fruits are eaten raw with salt and cooked as vegetable
<i>Luffa acutangula</i> (L.) Roem.	Kali torai / Karvi torai	July - October	Unripe fruits	Unripe fruits are cooked as vegetable. Dry fibrous mesocarp of mature fruits is chiefly used as a brushed for bathing and cleaning the utensils in the tribal area.
<i>Luffa cylindrical</i> (L.) M. Roem.	Ghia torai	July - October	Unripe fruits	The fruits are cooked as vegetable by removing the rind and frying the pulp in oil without adding any water.
<i>Madhuca indica</i>	Mahua	March - June	Flowers and fruits	Powder of dried flowers and fruits is mixed in ladoos and eaten to cure tuberculosis. Flowers are given to cure jaundice. Flower extract is also given in cough, cold and pneumonia. Flowers are also given to the insomnia patients.
<i>Momordica balsamina</i>	Jangali karela	July- October	Unripe fruits	The consumption in higher quantity may cause vomiting, stomach ache etc.
<i>Momordica dioico</i>	Kinkoda	August- October	Unripe fruits	Unripe fruits are cooked as vegetable and sold in the market by the tribal.

<i>Moringa concanensis</i>	Jangli sargua/ Sainjana	Almost throughout year	Unripe fruits and flowers buds	Unripe pods and flower buds and are cooked as vegetable.
<i>Moringa oleifera</i> Lam.	Sainjana / Sargua	Almost throughout year	Unripe fruits flowers buds	Flowers and immature fruits are cooked as vegetable. The pods are also sold in the market.
<i>Mucuna pruriens</i> (L.)	Kaunch	August - April	Unripe fruits	Unripe fruits are eaten during famine.
<i>Prosopis cineraria</i>	Khejra/ Sangeri	March - June	Unripe fruits	Unripe fruit are cooked as vegetable. They are dried and preserved for future use as vegetable. Gum is used in various sweet preparations.
<i>Solanum nigrum</i> L.	Kali papotan/ Makao/ Mukko/ Chirpoti	September - December	Fruits	Mature fruits are eaten raw by children. The tribal cook tender shoots and leaves as a vegetable.
<i>Typha angustata</i> Bory & <i>Chaub.</i>	Ara	August - June	Tender stems	The rhizome and tender stem are cooked as vegetable by bhils during scarcity of food.
<i>Vigna trilobata</i> (L.) Verd.	Mungi	August - October	Unripe fruits	Unripe fruit are cooked as vegetable.

Table 4. Roots and tubers grown wild and consumed in the tribal region of Udaipur

Botanical name	Local name	Season of availability	Part used	Remark
<i>Amorphophallus bulbifer</i>	Jangali Suran	July- September	Tubers	-
<i>Arisaema tortuosum</i>	Suran	July -August	Tubers	-
<i>Asparagus adscendens</i>	Dholi musli	July - February	Roots	Eaten raw and are cooked as vegetable. Infusion of roots and leaves is taken as a tonic for vitality.
<i>Asparagus racemosus</i>	Satavar	August - March	Tubers	-
<i>Butea monosperma</i>	Khakra	March - June	Root	Eaten raw. Dried flower powder is taken orally in diabetes.
<i>Ceropegia attenuata</i>	Khadula	July- October	Tubers	Eaten raw to quench their thirst, to protect themselves from hot winds and to promote fertility and vitality. Half cup of tuber extract is taken orally early in the morning for three days in urinary complaint as well as in kidney stone problem.
<i>Ceropegia bulbosa</i>	Khadula	July -November	Tubers	Eaten raw for vitality and considered as refrigerant where as leaves are cooked as vegetable
<i>Ceropegia hirsute</i>	Khilara	July- October	Tubers	As above
<i>Ceropegia tuberosa</i>	Vagryo	July -November	Tubers	Leaves and tubers are edible.
<i>Ceropegia vincaefolia</i>	Khilara	July- October	Tubers	Leaves and tubers are edible. Tubers are eaten raw for vitality and considered as refrigerant where as leaves are cooked as vegetable
<i>Chlorophytum borivillianum</i>	Safad musali / Dholi musli	June- September	Tubers	Cooked as vegetable. One tea spoon powder of tubers is taken orally along with milk in anemia, rheumatism and also to promote fertility and vitality.
<i>Chlorophytum laxum</i>	Safad musali ka patta/ Dholi musli	June- September	Tubers	Helps in regaining the strength.
<i>Chlorophytum tuberosum</i>	Safed musli	June- October	Tubers	Tubers are eaten raw for vitality and are also used in various food preparations. Tubers are cooked as vegetable. Dry tubers are also sold in market. A good source of income for poor tribals.

<i>Costus speciosus</i>	Maha lakdi	October - December	Rhizomes	-
<i>Curculigo orchioides</i> Gaertn.	Kali-musali	August - November	Roots	<i>Chappati</i> is prepared from the flour of dried roots after mixing with maize flour. Tender leaves are cooked as vegetable and are used as flouring agent also.
<i>Curcuma amada</i> Robx	Amba haldi	August - October	Rhizomes	Used for pickle and vegetable whereas dried powdered rhizomes are used as a spice. Leaves are simmered with paste of gram flour and then cooked in steam and then roasted.
<i>Curcuma angustifolia</i> Robx.	Haldi	June - August	Rhizomes	As above
<i>Curcuma aromatic</i> Salisb.	Jungali haldi	July - November	Rhizomes	As above
<i>Dioscorea</i> Sp.	Aamliya kand	July -August	Tubers	Roots and bulbils are cooked as vegetable.
<i>Dioscorea</i> Sp.	Alitha kand	July -August	Tubers	-
<i>Dioscorea alata</i>	Vakarkand	July -August	Tubers	-
<i>Dioscorea pentaphylla</i>	Suwari	July -August	Tubers	Tubers and bulbils are edible. Powdered tubers are given with milk and sugar in dysentery.
<i>Dioscorea hispida</i> Dennt	Kandu	July - October	Tubers	The roots are good for health and it gives strength. Generally tribal consume it in rainy season only as it gives good taste in that period only.
<i>Dioscorea esculanta</i>	Aamchai	July -August	Tubers	Boiled tubers are also used as salad.
<i>Dioscorea tomentosa</i>	Jangli kanda	July - October	Tubers	Tubers are sliced and steeped in stream overnight and cooked next day as vegetable.
<i>Eulophia ochreata</i> Lindl.	Salma mishri	May - September	Tubers	Tubers are used for the treatment of impotence.
<i>Pueraria tuberosa</i>	Modi	February - April	Tubers	Tubers are eaten by the tribals especially by children who like the sweet and cooling flavour tremendously. They also allay thirst when eaten raw.
<i>Urginea indica</i> (Roxb.) Kunth.	Jungli kanda	February - May	Root	Detoxified pieces of bulbs (after washing in water repeatedly) are used as a vegetable. In urgent needs, slices are detoxified by boiling in water for some time.
<i>Zingiber roseum</i> Rosc. Jangli Adrak	Jungli adrak	July - October	Rhizomes	Rhizomes are cooked as vegetable. Dried rhizomes are used as spices.

eastern India. Indigenous fruits formed part of the family diet with average annual consumption of 73 kg per household. Sale of wild fruits contributed 15 per cent of income for tribal households. These indigenous fruits and vegetables plays important role in meeting the nutritional requirement of tribal population in remote parts of the country (Sundriyal and Sundriyal, 2004). Studies on Ethno-food and ethno-medicinal plants have been carried out all over the world in tribal areas. Various researches have emphasized on the diversity and value of traditional vegetables. Most of traditional fruits and vegetables have a potential for income generation but fail to compete with exotic vegetables at present due to lack of awareness (Dhyani *et al.*, 2007; Kala, 2007; Jansenvan *et al.*, 2004). Consumption of traditional diets known to these societies are said to have many beneficial effects such as prevention of some age related

degenerative diseases – arteriosclerosis, stroke, etc. (Lindeberg *et al.*, 2003). Despite these advantages, most traditional plant foods are generally uncultivated and underutilized (Kunwar *et al.*, 2006).

The people from tribal area instantly collect vegetables from the surrounding at the time of weeding at agriculture farm or grazing their cattle in forest and cook it in the form of snack "*Paaniya*". In this recipe maize flour is the basic ingredient. Tribal collect green leafy vegetable and add in maize flour to make this traditional and famous snack item. Tribal make *Paaniya* especially in winter and rainy season. The tribal's had their own traditional processing methods to consume the vegetables. Tribal of the studied area consumed certain roots after processing especially the roots of *Dioscorea* family. The practices were adopted to make the food palatable or to make it free from natural toxicants. The

tribal consume *Urginea indica*, a root which is toxic if consumed as such. They wash the slices of *Urginea indica* (Jahari Kanda) over night in running water like river or water stream and then cook them as vegetable. In the case of urgent need, they boiled the slices of *Urginea indica* for detoxification and then cooked for consumption.

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Comparative Analysis of Eco-zone of Great Himalayan National Park, India

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Abstract: There has been an increase of about 9 km² area under habitation/agriculture/orchard category with a corresponding decline of about 4 km² area under cultivation. The change had been correlated with the human population dynamics and fuel/fodder consumption patterns. It is observed that the fuel wood/fodder consumption has increased nearly 80% in the period between 1961 and 1991. Total population of 11715 in EZ along with 13 revenue villages and 4281 households is needed full attention by forest and wildlife managers for better conservation planning and implementation.

Key Words: Conservation, Change detection, Eco-Zone

The UNESCO in June, 2014 has declared Great Himalayan National Park as world heritage site, which depicts its faunal and floral diversity (UNESCO, 2014). Great Himalayan National Park Conservation Area (GHNPCA) is one of the important protected Areas (PAs) for the conservation of the biological diversity in Western Himalayan region but scanty scientific data are available (Gaston and Garson, 1992). According to the Bio-geographic Classification, GHNP falls under north-western Himalayan biotic province i.e. 2A (Rodgers *et al.*, 2000). The species and plant communities of western Himalayas have also been studied (Khan *et al.*, 2012; Kumar and Sharma, 2016). Vegetation in park area is mainly temperate, sub-alpine and alpine. Vegetation is the single most important parameter for evaluation and conservation of biodiversity. Therefore, qualitative and quantitative status of the vegetation is basic requirements for strategy formulation and future monitoring.

Watershed i.e. Eco-Zone (EZ) is one of the important sub unit of Great Himalayan National Park Conservation Area. This is the main habitation zone within the conservation area. The area bears maximum biotic pressure and puts pressure in GHNPCA too in the form of migratory sheep and goat grazing, extraction of medicinal plant, collection of mushroom, fuel wood etc. For management point of view it is essential to monitor the area regularly. Zubair (2006) stated that Land use is land management strategy placed on the land cover by land managers to exploit the land cover and reflects human activities such as industrial zones, residential zones, agricultural fields, grazing, logging and mining, etc. Land cover is defined by the attributes of the earth's land surface captured in the distribution of vegetation, water, desert and ice and the immediate subsurface, including biota, soil,

topography, surface and groundwater and it also includes those structures created solely by human activities such as mine exposures and settlement (Khan *et al.*, 2012; Baulies *et al.*, 1997).

Landuse/cover map having importance due to its application as habitat map or ecosystem characterization and are the source for further description of unique habitats in terms of their biological richness and disturbance. These maps have various applications at different levels in man-made and natural ecosystems and very important aspects in conservation strategies for long term monitoring. Remote sensing offers multi-temporal data which is most crucial to evaluate the success of the management measures. For EZ, the landuse/landcover mapping has been done to assist Park Management to turn up with critical conservation strategies, specially seen in the context of biotic pressure from adjacent settlements for MFP collection, cattle grazing, illegal felling, poaching, encroachment etc. The socioeconomic data has also been studied to monitor change in population from 1961 to 1991.

MATERIAL AND METHODS

The Great Himalayan National Park Conservation Area (GHNPCA) in Himachal Pradesh encompass nearly 1171 km² area from 1344 to 6248 m altitude. Being one of the important entity of park in EZ broadly, three season can be recognised viz. summer, Rainy and winter. The temperature of the area varies from minus 10C to 35C. According to Champion and Seth's classification (1962) the 11 vegetation types have been recognised in the study area; form Sub-tropical Pine Forest (9/C1b) to Alpine Pastures in successive manner. The fauna of the park comprises 31 species of mammals (Gaston and Garson 1992), floral diversity studied

by Singh and Rawat (1999), 183 species of birds (Prakasam, 2010) and more than 125 species of invertebrates (Singh and Rawat, 2000). The methodology used in detecting changes in landuse/landcover is shown in Fig. 1.

Land use and people of the area: There are 13 revenue villages with 4281 households with total population of 11715 in EZ of GHNP (2011 census) is shown in Table 1 and the watershed map along with villages depicted in Fig 2.

The main occupation of these people is agriculture along with horticulture. Villages mostly grow Maize (*disambiguation*) and Ogal (*Fagopyrum esculentum*) in rainy season, wheat and barley grows on lower areas whereas in winters only maize. Rearing sheep and goat fetches a good income. The social Structure of the EZ is depicted in Table 2. The medicinal plants and mushrooms extractions are means of income and in some cases even contribute about 70% of the total income. Horticulture is becoming more popular raising orchards of apple, plum, walnut and cherry etc. The main pressures on the area are due to the collection of herbs, edible mushrooms, grazing of sheep and goat in summers (Lambin *et al.*, 2003). The Mushroom (*Morchella esculanta*) is mainly collected from February to May in the lower altitude because of their high price and value (Saxena, 1998). The fuel wood, fodder and grasses collection is being done by

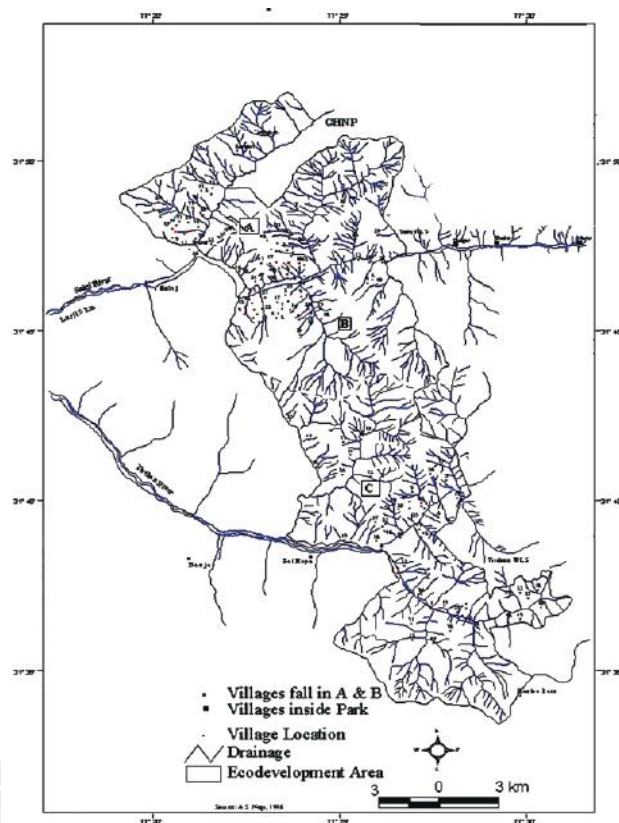


Fig. 2. Great Himalayan National Park Conservation Area (GHNPCA) eco-zone watershed: human settlement

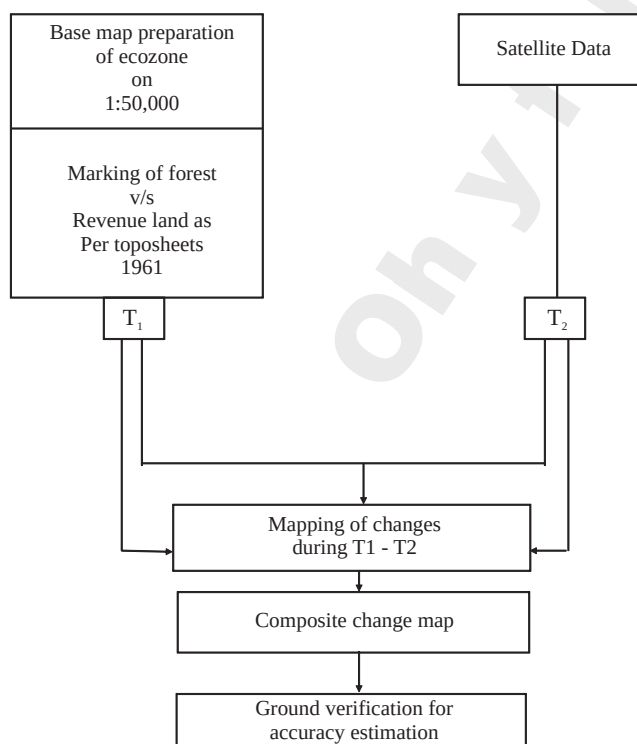


Fig.1. Flow chart of change detection in landuse/cover mapping

those villages which are close to PA boundary.

Materials and data used: False Color Composites (FCC) of IRS -1B LISS II Geo-coded data for the period of September/October of 1993 on 1:50,000 scales. The complete landuse/cover mapping was done using visual interpretation techniques with the help of interpretation key and classification scheme. For the Comparative Analysis the following data sources were used: SOI Topo-sheet 1961,

Table 1. Revenue villages, households and total population of eco-zone (2011 census)

Tehsils/Waziri	Kothi	Phati	House-holds (2011)	Total Population (2011)
Banjar/Inner Seraj	Tung	Chipni	405	2145
"	Nohanda	Pekhri	275	1477
"	Plach	Srikot	113	648
"	Sarchi	Shili	245	1243
Sainj/Inner Seraj	Banogi	Suchen	401	1994
"	Shangarh	Shangarh	200	1064
Sainj/Rupi	Sainshar	Sainshar	566	2681
Kulu/Rupi	Balhan	Railla	980	4704
Total	8	13	4281	21487

Source: Census of India 2011

Table 2. Social structure of eco-zone

Name of Village	total population	males	females	sex ratio	total sc population	sc males	sc females	% of scs to total population	total population	total literates	male literates	female literates	% of literate to total population
Chipni	1537	793	744	938	536	247	262	35	0	490	352	138	32
Mashyar	1280	647	33	978	376	200	176	29	4	230	171	59	18
Pekhri	1098	577	521	903	165	92	73	15	6	396	293	103	36
Tinder	577	364	313	860	96	50	48	14	1	219	169	50	32
Srikot	417	230	187	813	84	49	35	20	0	174	135	39	42
Kulwari	1132	537	595	1108	412	199	213	36	0	518	322	196	46
Shill	812	432	380	879	175	97	78	21	0	223	177	45	27
Suchen	1212	636	576	905	392	199	193	32	0	396	279	117	33
Shangarh	618	321	297	925	224	109	115	36	0	223	170	53	36
Lapah	222	108	114	1055	26	15	11	12	0	63	54	9	28
Sainshar	1606	829	777	937	887	458	429	55	0	468	345	123	29
Garaparli	592	312	280	897	175	93	82	29	0	27	27	0	4
Raila	2822	1462	1360	930	644	321	323	23	11	959	689	270	34
Total	13925	7248	6777	933	4192	2129	2038	23	22	4386	3183	1202	

Source: Computed from Census Data 1991 Block Development Office, Banjar, Kullu District

Satellite Imageries 1993 and Census Data of 1961, 1991 and 2011. The revenue land has been considered as landuse/cover for 1961 (T1 time period). Year 1993 (T2 period), satellite data of IRS LISS II was used to delineate landuse/cover. With the help of Geographical Information System (GIS), overlay analysis has been performed to observe the temporal changes. The overlay analysis is basically the draping of two temporal spatial data and extraction of spatial extent of the resultant polygon. Using this approach the composite changed map was produced. In order to understand the nature and quantum of these changes a comparative analysis was carried out as part of this study.

The population growth statistics of 13 villages have also been calculated using the compound base growth rate calculation method for 20 years base period. The linier population progression was discouraged owing to the fact that population growth is never linier and base year keeps changing for calculation purpose.

The following formula has been used for the population growth rate;

$$\text{Pop future} = \text{Pop present} \times (1 + r/100)^n$$

Where;

Pop future = Projected population

Pop present = Present population

r = Compound growth rate

n = Number of years

RESULTS AND DISCUSSION

Significant changes in land use patterns and population dynamics have been occurred in the EZ in the last three decades. In landuse/cover mapping, eleven forest and seven non-forest types have been delineated. The landuse/landcover map of EZ is given in Fig. 3.

The area estimation under different categories in EZ has been provided in Table 3. The habitation/agriculture/orchard areas in EZ in 1961 and 1993 are shown in Fig. 4 and Fig. 5, respectively.

The. Of which 26.07 km² area from the total area of EZ (265.6 km²) is under man made feature and remaining is under forest and non-forest classes. The mixed coniferous forest comprises of 73.49 km², whereas, the total grasslands are 34.70 km². The maps related to comparative analysis has been given in Fig.5. Increase in area of manmade features (Habitation /Agriculture/Orchard) was estimated 8.48 km², while 4.31 km² was found not cultivated as was practiced in 1961. The overall change in area was about 12.79 km². There has been an increase of about 9 km² in the areas under habitation/agriculture/orchard and a decline of about 4 km² of forest area during the period 1961 to 1993. It was also

Table 3. Areal estimation under different landuse/landcover categories

Type	Area in sq.km
Conifer (pinus roxburghii)	2.08
Mixed conifer	73.49
Conifer and broad leaved mixed	13.48
Broad leaved	33.03
Broad leaved and conifer mixed	48.29
Riparian	0.13
Slope grasses	26.09
Grasslands/ Blanks (Temp. sub Alpine & Alpine)	8.61
Secondary scrub	16.50
Alpine scrub	6.72
Plantation	0.16
Habitation/Agriculture/Orchards	26.07
Exposed rocks with slope grasses	8.35
Alpine exposed rocks with slope grasses	0.07
River	1.03
Escarpsments	1.34
Landslide	0.07
Total	265.6

observed that deterioration of vegetal cover is unidirectional i.e. towards forest. Change detection in land use and land cover can be performed on a temporal scale such as a decade to assess landscape change caused due to anthropogenic activities on the land (Gibson and Power 2000). The area has been correlated with population dynamics, fuel and fodder consumption from 1961 – 1991 as shown in Table 4.

The number of households in EZ has increased from 1364 (year 1961) to 2465 (Year 1991) and the fuel wood consumption has also registered 78% increase during this period. Similarly, the fodder consumption has also increased during this period.

The above table describes the biomass consumption in the EZ of Great Himalayan National Park Conservation Area. Although it is assumed that consumption of biomass (Fuel wood/Fodder) per household has not changed substantially in the area, however due to high increase in the total no. of households the total quantities of biomass required by the households has changed substantially.

The table reveals that the fuel wood consumption in the area experienced a significant increase of near about

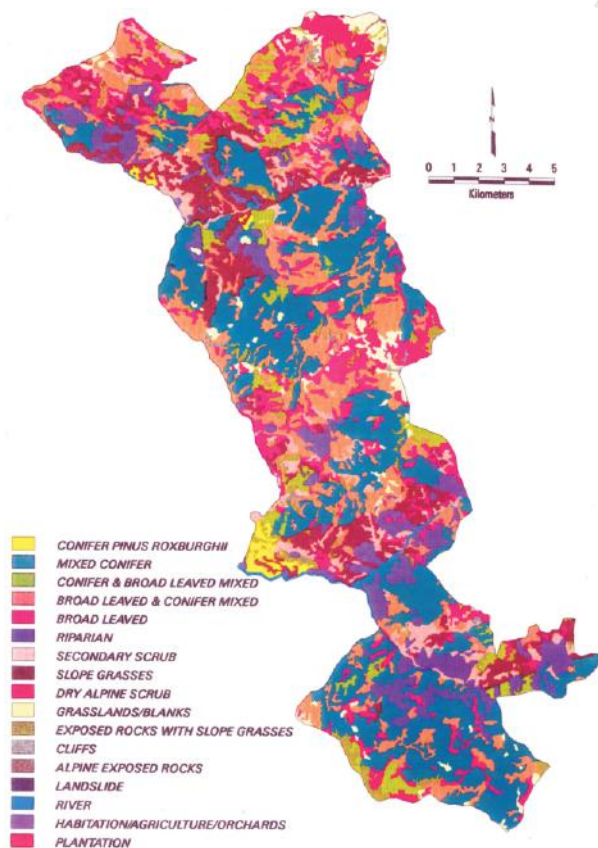
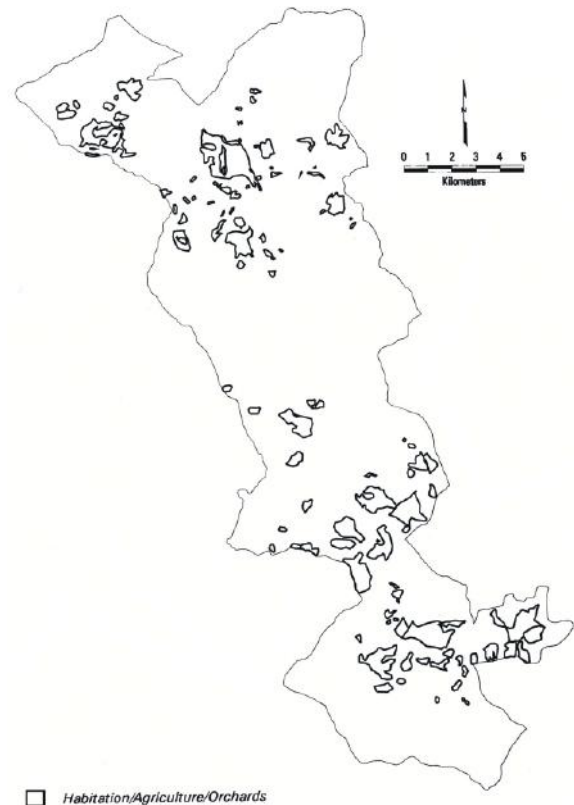
**Fig. 3.** Land use/cover map, eco-zone watershed as per IRS 1B LISS II, 1993**Fig. 4.** Habitation map, eco-zone watershed, 1961 survey

Table 4. Population dynamics, fuel and fodder consumption in all watersheds

Watershed Sub Water Shed	No. of Household, 1961	No of Household, 1991	Fuel Wood consumption, 1961(00 Kg)	Fuel Wood consumption 1991(00 Kg)	%change in Fuel wood consumption Between 1961-1991	Fodder Consumption 1961 (00kg)	Fodder Consumption 1991 (00kg)	% change in fodder consumption between 1961-1991
Jiwa sws	512	930	74393	135725	82.44	58522	106299	81.63
Sainj sws	213	350	30948.9	50855	64.33	24346	40005	64.31
Tirthan sws	639	1185	92846.7	172180.5	84.44	73038	135446	85.44
Total	1364	2465	198188.4	358760.5		82941	281750	

Source: Calculated from census data 1961 & 1991 per house hold, fodder and fuel wood consumption by Saxena (1998)

80%. In other watersheds, the Tirthan experienced the highest increase of near about 85% in fodder consumption while Sainj showed the minimum increase of 64% over this period. The table also reveals that fuel wood consumption has experienced almost similar trend in increase over this period.

The population growth statistics for the period 1991-2011 and projections for the year 2021 are given in Table 5. The population growth statistics was calculated as depicted in table 4. However there were important observations in reference villages Mashyar and Lapah. In Mashyar the growth rate is high as compared to the other villages, may be because of site suitability (slope and aspect with sun illumination side), availability of sufficient agriculture fields for production and infrastructure development in the village. Where as in the Lapah, the negative growth rate was observed may be because of factors just apposite as mentioned in the case of Mashyar along with heavy migration, gender disparity and other social and demographic constraints.

Table 5. Population growth statistics of eco-zone

Revenue Villages	Total population (1991)	Total population (2011)	Growth rate/year (%)	Total projected population 2021
Chipni	1537	2145	1.68	2534
Mashyar	1280	1716	9.49	4249
Pekhri	1098	1477	1.49	1712
Tinder	677	1048	2.21	1304
Srikot	417	648	2.23	808
Kalwari	1132	1539	1.55	1795
Shili	812	1243	2.15	1538
Suchen	1212	1994	2.52	2557
Shangurh	618	1064	2.75	1396
Lapah	222	203	-0.45	194
Sainshar	1606	2681	2.60	3466
Garaparli	592	1025	2.78	1348
Raila	2822	4704	2.59	6075
Total	13991	21487		

Source: Census of India 1991-2011

**Fig. 5.** Habitation map, eco-zone watershed as per IRS 1B LISS II, 1993

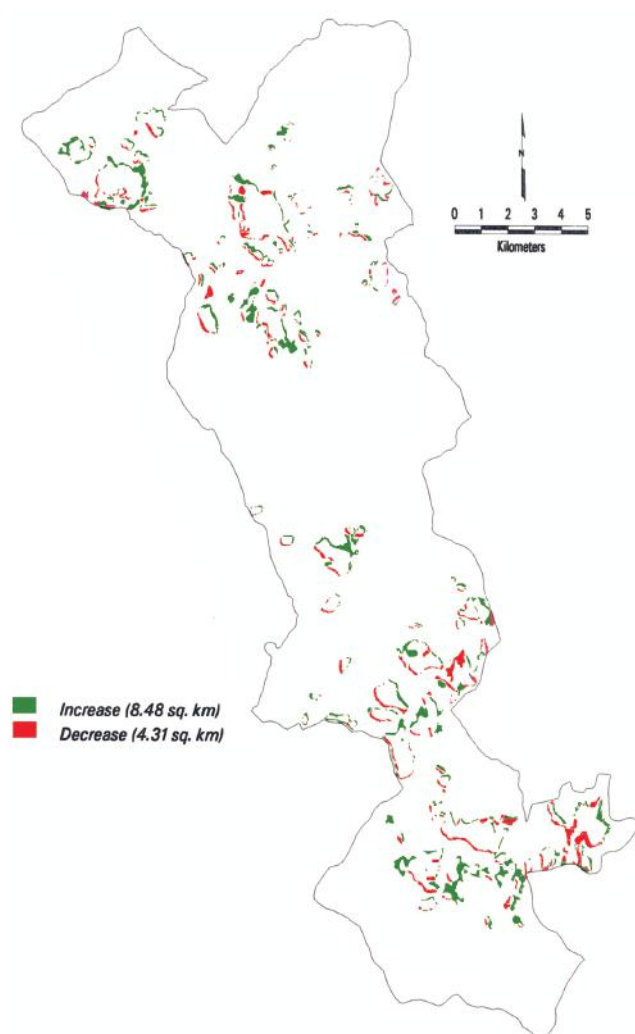
The table 5 shows that in the 30 years (1961-91), there has been nearly 9 km² increase in the area under the Habitation/Agriculture/Orchards in the EZ among the different watersheds. This change is maximum (5km) in Tirthan sub-watershed compare to only (1.16sqkm) in the Jiwanal sub-watershed, However, if we see in proportion Jiwa has experienced a maximum increase of 4.93% followed by Tirthan (3.99%) and Sainj (2.17%).

The table further shows that in this period there has been some area, which was in use in 1961 but was not found in use in 1991(1.6%). The table reveals that there has been

Table 6. Population change dynamics in ECO-ZONE

EZ Population 1961	EZ Population 1991	Name of the Watersheds	Total Area Sq.km	Area Changed between 1961-91 sq.km	% Increase (area)	Area not Currently Under cultivation As used in	% Decrease (area)	% Increase Population (1961-91)
3041	2710	Jiwa	23.5	1.16	4.93	0.34	1.44	-38.00%
1235	2052	Sainj	106.23	2.31	2.17	1.28	1.20	1.71%
4155	6953	Tirthan	125.27	5.01	3.99	2.69	2.14	1.73%
8431	11715	Total	255.00	8.48	3.7	4.31	1.59	1.10%

Source: Population change calculated by using census data of 1961 and 1991 of Kullu District. Change detection in area under Habitation/Agriculture/Orchards calculated by using ARC/INFO GIS domain from 1961 SOI sheets and 1993 FCC IRS IB LISS II Data. The study has been conducted before the award of new area.

**Fig. 6.** Change detection, eco-zone watershed

near about 1.10% increase in the total population of EZ from 1961 to 1991. Out of this, Tirthan watershed has experienced maximum (1.73%) closely followed by Sainj watershed (1.71%) Strangely enough the Jiwa watershed has

experienced a negative population (-38.0%); this seems to be due to many related constraints in the area.

CONCLUSION

Eco-zone has a very complex land use. IRS satellite data have been used successfully to map the landcover and landuse as per study objectives. The technology provides quick assessment of landcover and landuse for conservation planning, decision making and management and to know the current status of the vegetation. Habitation in EZ is associated with Agriculture and Orchards and relatively gentle slopes (fan-shaped fluvial deposits) and land with soil are under cultivation (unlike forested areas which have rock crop-outs, boulders and stones). About 2.18% of the area is under this land use.

The negative population was experienced in Jiwa watershed seemed to be because of heavy migration. The eleven forest and six non forest types in EZ, shows the rich diversity in the area needs more attention towards conservation strategies and monitoring of the area. The study is likely to give valuable and significant inputs into management efforts aimed at wildlife habitat management and its monitoring, to evaluate the landuse/land cover changing scenario, population dynamics, and biotic pressure in and around conservation area.

ACKNOWLEDGEMENT

We express our deep sense of gratitude to Director, Project Tiger, Government of India for sponsoring the WII-Forestry Research Education and Extension Programme (FREEP) Research Project under which this study was carried out, Director, Wildlife Institute of India (WII) for providing encouragement and logistic support. We also express our sincere gratitude to Dr. Sarnam Singh, Scientist, Indian Institute of Remote Sensing (IIRS) Dehradun, Forest Department, Himachal Pradesh and frontline staff for study support and assistance.

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Effect of Lopping on Stand Structure and Tree Species Composition of *Quercus glauca* Thunb. Forests of Himachal Pradesh, India

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Abstract: A study to quantitatively describe the effect of lopping on *Quercus glauca* growth and tree species composition of lower Himalaya revealed density decrease from low to high diameter classes. Lopping, pollarding and selective logging had a significant effect on stand structure and species composition of *Quercus glauca* forests. Basal area and volume showed much in-consistency along diameter classes. Due to lopping, high volume ($38.0\text{m}^3\text{ha}^{-1}$) of wood loss was recorded at Oachghat forest and the lowest volume ($1.8\text{m}^3\text{ha}^{-1}$) of wood loss was recorded at Shili forest.

Key Words: Diameter class, Lopping, *Quercus glauca*, Species composition, Stand structure

Covering an area of 55,673 sq km, Himachal Pradesh is found at the western foothills of Himalaya. Its climate varies from semi-arctic to semi-tropical making it suitable for the growth of diverse types of vegetation. Oak bearing forests are amongst the most conspicuous forest types in the middle and lower Himalayan region. *Quercus leucotrichophora*, *Quercus floribunda*, *Q. semecarpifolia* are among the dominant oak species of the region. *Quercus glauca*, on the other hand, is found in restricted areas usually in moist and shady areas of river basins and deep valleys (Ito *et al.* 2007). Because of their in-consistent occurrence and lower coverage; very low emphasis has been given to them for scientific studies. Oaks are fully exposed to human and animal pressures that make them more vulnerable to extinction.

Depletion of oak forest and lack of reproduction through natural regeneration was widely reported by different researchers from time to time (Chauhan and Verma, 1995). Singh and Rawat (2012) associated the phenomenon with Anthropogenic effects mainly of fuelwood collection, grazing, and fodder collection (lopping) and climate change are the main courses for their depletion (Shrestha, 2003; Maren and Vetaas, 2007; Rawat *et al.*, 2011).

There are reports that moderate disturbance being beneficial for the growth and reproduction of oaks (Thadani and Ashton, 1995). However, lopping, which removes majority of the crown, directly affects the growth and reproduction capacity of trees. Lopping will also reduce fertility of the soil by affecting nutrient turnover to the forest floor. Pokhriyal *et al.* (2012) reported the decline in tree density and basal area with increasing disturbance while Semawal *et al.* (2009) reported low nutrient contents in disturbed forest soils. However, lopping and pollarding, on

the other hand, create favourable condition for sun light to rich in to the forest ground. Lhotka (2013) emphasized the importance of available light (canopy opening) in determining stand structure and species composition of mixed broadleaf forest ecosystems. Lof *et al.* (1998) identified the interactive effect of light intensity and soil water content in influencing the growth of oak seedlings, while, Dech *et al.* (2008) reported that canopy cover and soil fertility determined the composition of under story plant communities. The purpose of this study was to assess stand structure, tree species composition and the effect of lopping on the growth of *Quercus glauca* forests of Himachal Pradesh.

MATERIAL AND METHODS

The present investigation was carried out at six *Quercus glauca* bearing forests lying in Sirmor and Solan districts of Himachal Pradesh, India. The basic categories of these forests sites are presented in table 1. From each site three plots of 20m x 20m were randomly taken for data collection of tree related traits. Diameter at breast height (d) was measured by tree caliper.

Crown width (cw) was measured on the ground directly below the overhead crown. Two measurements at right angle were made for diameter and crown width and the average was taken as tree diameter and tree crown width. Tree height (h), h_1 (height at which diameter becomes half of the diameter at breast height) and crown length (cl) was measured by multimeter. The data was used to calculate form factor (f), basal area (BA), and volume (V) of *Quercus glauca* trees.

Relative density, relative basal area and relative frequencies were calculated as the ratio of density, basal area and frequency of a species to the total density, basal

Table 1. Details of the study sites (Solan and Nahan Forest Divisions, HP)

Forest area	Forest Range	Latitude	Longitude	Altitude	Forest type	Access
Shili	Solan	30°54.563N	77°07.172E	1467	Protected	Restricted
Oachghat	Solan	30°52.055N	77°07.432E	1220	Private	Open (to owner)
Deothal	Nareg	30°49.767N	77°09.538E	1052	Communal	Open
Nareg	Nareg	30°49.385N	77°10.251E	1127	Protected	Restricted (loose)
Chakla	Sarahan	30°48.352N	77°10.581E	1165	Communal	Open
Tikri	Sarahan	30°48.024N	77°09.894E	1096	Communal	Open

area and frequency of all the species in a forest stand and expressed in percentile. Importance value index (IVI) was calculated as the average of the sum of relative density, relative basal area and relative frequency of a species.

RESULTS AND DISCUSSION

A total of 25 tree species that belonged to 24 genus and 19 families were recorded from the study sites (Table 2). The highest number of tree species (11) was recorded in Nareg and Tikri forests and the lowest number of tree species (5) was recorded at Deothal forest site. The lowest number of tree species in Deothal (5) and Chakla (7) was associated to anthropogenic disturbance, i.e. lopping, grazing and selective logging because of their management. Thapa and Chapman (2010) also reported low density, small basal area and low diversity and species richness in forests that were subjected to resource extraction.

The IVI values of *Quercus glauca* indicated that it was dominant tree species in all sites excepting Shili forest where it appeared as a codominant species following the dominant *Quercus leucotrichophora* species. From relative basal area distribution, it can be suggested that *Quercus glauca* was a dominant species in Shili and it lose its dominance overtime to *Quercus leucotrichophora*. Ito *et al.* (2007) reported the early-successional behavior of the species. The species was commonly associated to *Ailanthus excelsa*, *Ficus palmata*, *Machilus duthiei*, *Myrica esculenta*, *Pinus roxburghii*, *Punica granatum*, *Pyrus pashia*, and *Quercus leucotrichophora*. Troup (1921) also reported close association of *Quercus glauca* with *Quercus incana*, *pinus longifolia*, *Rhododendron arborium*, *Pieris ovalifolia*, *Pistacia integerrima*, *Pyrus pashia* and *Olea cuspidata*.

Tree growth parameters along diameter distribution of *Quercus glauca* trees: Analysis of growth characteristics along diameter classes indicates that tree density decreased from lower diameter classes to higher diameter classes in all sites except Deothal and Chakla (Table 3). The 30.0-39.9 diameter class in Deothal and the 10.0-19.9 and 60.0-69.9 diameter classes in Chakla had lower density than their successor, which might be due to selective logging.

The average diameter distribution of stands is a

direct reflection of diameter classes and it increased from lower to higher diameter classes forming inversed 'J' shape (Loewenstein *et al.*, 2000). The highest average diameter (75.3 cm) was recorded in Chakla, while the lowest average diameter (12.7 cm) was recorded in Deothal and Chakla forest sites. Diameter distribution has direct relation with age of the tree indicating stands with higher diameter trees (classes) to be an old stand (Loewenstein *et al.*, 2000).

The average height distribution along diameter classes show a general increase from the lower to the higher diameter class, excepting for Shili, Chakla and Tikri forests that showed slight variation from the trend, which may be due to high biotic pressure at these sites. The average crown diameter and average crown length also showed a general increasing trend with increasing diameter class. Minor deviations for crown diameter at Chakla and crown length at Shili were recorded. However, crown length was highly inconsistent in Tikri forest, which may be due to the effect of lopping and pollarding.

Both basal area and volume per hectare are results of a function of number of trees per hectare, diameter and height of each diameter class. However, these growth parameters have opposite trend along diameter classes. Thus, basal area and volume are expected to show some sort of inflation in the mid diameter classes and this characteristics is taken as a characteristics (normality) of managed uneven-aged forest (Loewenstein *et al.*, 2000; Babic *et al.*, 2010). However, most sites didn't show these characteristics, except Tikri forest. In contrast to higher human interference, Tikri forest showed some sort of normality of the forest, which may be due to even distribution of lopping and selective logging along diameter classes.

Effect of lopping on density, basal area and volume of *Quercus glauca* trees: All growth parameters were found significantly affected by forest sites that have different lopping intensities. However, difference for these growth parameters along diameter classes was an inherent characteristic of the forest. The higher standard deviations along variables were a direct reflection human interference. Lopping is a major factor that influences the growth of *Quercus glauca* trees. Maximum number of trees lopped

Table 2. Tree species composition and their importance value of the study sites

Site	Species	Family	Relative density	Relative basal area	Relative frequency	Importance value index
Shili	<i>Ailanthus excelsa</i>	Simaroubaceae	0.7	16.1	5.9	7.6
	<i>Albizia spp</i>	Fabaceae	0.7	18.4	5.9	8.3
	<i>Celtis australis</i>	Cannabaceae	2.1	6.4	5.9	4.8
	<i>Melia azedarach</i>	Meliaceae	1.4	3.3	11.8	5.5
	<i>Myrica esculenta</i>	Myricaceae	16.2	8.0	17.6	13.9
	<i>Pinus roxburghii</i>	Pinaceae	12.7	17.3	11.8	13.9
	<i>Quercus glauca</i>	Fagaceae	26.8	12.9	17.6	19.1
	<i>Quercus leucotrichophora</i>	Fagaceae	38.7	10.5	17.6	22.3
	<i>Rhododendron arboreum</i>	Ericaceae	0.7	7.1	5.9	4.6
Oachghat	<i>Albizia spp</i>	Fabaceae	0.9	2.4	7.7	3.6
	<i>Bauhinia variegata</i>	Fabaceae	1.7	9.1	7.7	6.2
	<i>Celtis australis</i>	Cannabaceae	0.9	14.0	7.7	7.5
	<i>Dalbergia sissoo</i>	Fabaceae	2.6	16.5	7.7	8.9
	<i>Ficus palmata</i>	Moraceae	1.7	2.4	7.7	3.9
	<i>Machilus duthiei</i>	Lauraceae	5.2	8.1	15.4	9.6
	<i>Myrica esculenta</i>	Myricaceae	0.9	19.6	7.7	9.4
	<i>Pinus roxburghii</i>	Pinaceae	0.9	11.2	7.7	6.6
	<i>Pistacia integerrima</i>	Anacardiaceae	1.7	7.1	7.7	5.5
	<i>Quercus glauca</i>	Fagaceae	83.6	9.7	23.1	38.8
Deothal	<i>Ehretia acuminata</i>	Boraginaceae	2.3	10.1	12.5	8.3
	<i>Punica granatum</i>	Lythraceae	6.8	12.6	25.0	14.8
	<i>Pyrus pashia</i>	Rosaceae	2.3	12.5	12.5	9.1
	<i>Quercus glauca</i>	Fagaceae	86.4	41.1	37.5	55.0
	<i>Salix tetrasperma</i>	Salicaceae	2.3	23.7	12.5	12.8
Nareg	<i>Ailanthus excelsa</i>	Simaroubaceae	2.5	9.7	11.1	7.7
	<i>Ficus palmata</i>	Moraceae	2.5	8.4	5.6	5.5
	<i>Machilus duthiei</i>	Lauraceae	1.2	12.4	5.6	6.4
	<i>Myrica esculenta</i>	Myricaceae	6.2	9.4	5.6	7.0
	<i>Murraya koenigii</i>	Rutaceae	2.5	4.6	5.6	4.2
	<i>Pinus roxburghii</i>	Pinaceae	9.9	29.1	11.1	16.7
	<i>Punica granatum</i>	Lythraceae	3.7	3.6	11.1	6.1
	<i>Pyrus pashia</i>	Rosaceae	4.9	5.6	16.7	9.1
	<i>Quercus glauca</i>	Fagaceae	64.2	10.0	16.7	30.3
	<i>Quercus leucotrichophora</i>	Fagaceae	1.2	4.2	5.6	3.7
Chakla	<i>Zanthoxylum alatum</i>	Rutaceae	1.2	3.0	5.6	3.3
	<i>Ailanthus excelsa</i>	Simaroubaceae	2.5	4.5	8.3	5.1
	<i>Myrica esculenta</i>	Myricaceae	2.5	14.4	8.3	8.4
	<i>Olea glandulifera</i>	Oleaceae	1.3	10.3	8.3	6.6
	<i>Pinus roxburghii</i>	Pinaceae	2.5	45.2	16.7	21.5
	<i>Punica granatum</i>	Lythraceae	11.3	2.1	16.7	10.0
	<i>Quercus glauca</i>	Fagaceae	76.3	11.2	25.0	37.5
	<i>Quercus leucotrichophora</i>	Fagaceae	3.8	12.2	16.7	10.9
	<i>Debregeasia hypoleuca</i>	Urticaceae	1.1	6.3	5.9	4.4
	<i>Ehretia acuminata</i>	Boraginaceae	2.2	11.1	11.8	8.4
Tikri	<i>Ficus palmata</i>	Moraceae	1.1	3.5	5.9	3.5
	<i>Machilus duthiei</i>	Lauraceae	2.2	7.7	11.8	7.2
	<i>Manilkara kauki</i>	Sapotaceae	2.2	11.8	5.9	6.6

<i>Myrica esculenta</i>	Myricaceae	1.1	13.3	5.9	6.8
<i>Pinus roxburghii</i>	Pinaceae	5.6	17.8	5.9	9.8
<i>Prunus pudum</i>	Rosaceae	3.4	2.2	11.8	5.8
<i>Punica granatum</i>	Lythraceae	1.1	2.4	5.9	3.1
<i>Pyrus pashia</i>	Rosaceae	1.1	6.3	5.9	4.4
<i>Quercus glauca</i>	Fagaceae	76.4	15.1	17.6	36.4
<i>Toona ciliata</i>	Meliaceae	2.2	2.5	5.9	3.5

Table 3. Effect of diameter classes on growth and tree characteristics of *Quercus glauca* forests of Himachal Pradesh

Site	Diameter class (cm)	Density (Nha ⁻¹)	DBH (cm)	Height (m)	Crown width (m)	Crown length (m)	Basal area (m ² ha ⁻¹)	Volume (m ³ ha ⁻¹)
Shili	10-19.9	150	13.9	9.5	3.4	5.8	2.3	5.1
	20-29.9	125	24.2	20.5	7.2	14.4	5.7	27.6
	30-39.9	17	31.8	25.5	10.0	19.3	1.3	7.9
	40-49.9	17	41.4	28.5	12.0	19.0	2.2	15.0
	50-59.9	8	51.9	28.0	14.0	16.0	1.8	11.6
Oachghat	10-19.9	408.3	14.3	9.2	2.5	4.8	6.5	14.1
	20-29.9	333.3	24.7	14.5	3.9	8.2	16.0	54.3
	30-39.9	66.7	33.3	16.9	6.8	10.9	5.8	22.9
Deothal	10-19.9	200.0	12.7	8.0	3.3	5.3	2.5	4.7
	20-29.9	66.7	24.5	10.9	4.1	7.5	3.1	8.0
	30-39.9	16.7	37.6	16.5	6.0	12.0	1.8	7.1
	40-49.9	25.0	46.2	27.3	8.7	23.0	4.2	26.8
	50-59.9	8.3	52.0	30.0	15.0	27.0	1.8	12.4
Nareg	10-19.9	292	13.8	7.5	3.7	4.9	4.4	7.7
	20-29.9	83	22.8	13.5	5.4	9.2	3.4	10.8
	30-39.9	42	33.6	24.0	14.0	17.4	3.7	20.7
	40-49.9	17	45.4	27.5	18.5	20.5	2.7	17.3
Chakla	10-19.9	150	12.7	7.4	2.9	4.9	1.9	3.3
	20-29.9	158	24.2	10.7	3.8	7.3	7.3	18.3
	30-39.9	108	35.3	16.5	5.8	11.0	10.6	40.9
	40-49.9	42	43.1	21.0	8.8	13.8	6.1	29.9
	50-59.9	33	56.4	26.5	11.5	16.0	8.3	51.7
	60-69.9	0	0.0	0.0	0.0	0.0	0.0	0.0
	70-79.9	17	75.3	26.0	10.5	17.0	7.4	45.2
Tikri	10-19.9	208	14.3	6.9	2.1	4.1	3.3	5.4
	20-29.9	142	24.8	16.8	5.4	10.9	6.8	26.8
	30-39.9	117	34.5	16.2	5.8	10.5	10.9	41.4
	40-49.9	67	44.6	17.6	6.3	10.5	10.4	42.9
	50-59.9	25	54.1	23.3	7.7	16.0	5.7	31.4
	60-69.9	8	67.8	30.0	12.0	20.0	3.0	21.1

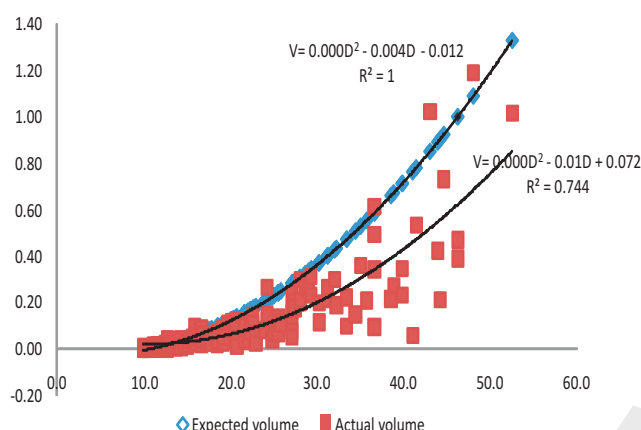
were found at Oachghat followed by Tikri and Deothal forest sites (Table 4). The minimum number of lopped trees was found at Shili. However, high intensity of lopping was observed at Deothal, Tikri and Oachghat with respective lopping intensity of 94.7%, 70.6% and 65%. Moderate lopping intensity was recorded at Nareg and Chakla with

respective lopping intensity of 46.2% and 45%. The lowest lopping intensity, 7.9%, was recorded at Shili forest.

Lopping intensity, which is an indicator of disturbance level, is influenced by protection, livelihood of the surrounding people and management of the forest. Shili, which is a mixed forest, was better protected by forest

Table 4. Effect of lopping on density, basal area and volume of *Quercus glauca* trees

Site	Density (N ha ⁻¹)			Basal area (m ² ha ⁻¹)		Volume(m ³ ha ⁻¹)	
	Unlopped	Lopped	Lopping intensity (%)	Unlopped	Lopped	Unlopped	Lopped
Shili	291.7	25.0	7.9	10.5	0.8	41.5	1.6
Oachghat	283.3	525.0	64.9	13.0	13.4	47.6	31.4
Deothal	16.7	300.0	94.7	3.3	8.1	20.6	19.4
Nareg	233.3	200.0	46.2	8.3	3.8	30.8	4.8
Chakla	275.0	233.3	45.9	22.4	10.7	100.7	16.2
Tikri	166.7	400.0	70.6	15.1	18.6	82.5	41.6

**Fig. 1.** Expected and actual volume of lopped *Quercus glauca* trees

department. Oachghat forest was under private ownership and mainly managed for fodder production. Though not effective, Nareg forest had protection by forest department. Livestock production in the surrounding area was high that weekend level of protection. Deothal, Chakla, and Tikri forests were open accessed communal forests that were used for grazing and lopping. In addition to distance to the village and size of the population, Maximum basal area and volume of lopped trees were recorded in Oachghat and Tikri. The large number of trees lopped in Oachghat and the bigger diameter trees lopped in Tikri magnified basal area and volume of lopped trees in these sites.

Based on data from unlopped trees volume equation of individual trees were developed. The best fit polynomial equation was $V = 0.00058D^2 - 0.004D - 0.012$ ($R^2 = 0.973$; where V = volume and D = diameter). Based on this equation, the expected volume of wood was analyzed and compared with the actual volume of wood that yielded a loss of 0.0724 m^3 volume of wood in every lopped tree (Fig. 1). By multiplying this value with the density of lopped trees in each site, loss in volume of wood per hectare was calculated. Thus, Oachghat forest had the highest loss of wood ($38 \text{ m}^3 \text{ ha}^{-1}$) followed by Tikri ($29.0 \text{ m}^3 \text{ ha}^{-1}$), Deothal

($21.7 \text{ m}^3 \text{ ha}^{-1}$), Chakla ($16.9 \text{ m}^3 \text{ ha}^{-1}$), Nareg ($14.5 \text{ m}^3 \text{ ha}^{-1}$), and Shili ($1.8 \text{ m}^3 \text{ ha}^{-1}$).

In general, human interference in the form of lopping, pollarding, and selective logging on *Quercus glauca* forests had an adverse effect on the structure and tree species composition of *Quercus glauca* forests. The hypothesis that lopping has a significant effect on growth and productivity of *Quercus glauca* trees hold true.

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Received 4 December, 2015; Accepted 17 May, 2016

Distribution of *Litsea floribunda* (Lauraceae), A Dioecious Tree Endemic to Western Ghats of India

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Abstract: Majority of *Litsea floribunda* populations comes between 41-60 cm girth classes. The species is frequently distributed in all the study sites, the higher density of the species found in Mullayyanagiri (47.67), lower in Kodachadri (20.00). Average importance value index of the species is 51.98 and the species basal area covers 1020.41 m² ha⁻¹. The abundance, frequency ratio of *Litsea floribunda* is >0.05 in all study sites, which indicated the clumped or contagious distribution pattern. The ratio of male trees are lower in number when compare to female trees, male trees need conservation measures for future regeneration of the species. The dominant companion tree species are Laural members such as *Cinnamomum*, *Neolitsea* and *Cryptocarya* sp.

Key Words: Altitude, Abundance, Dioecious plant, Endemic, Western Ghats

The Central Western Ghats spreads in Karnataka stretches about 550 kilometres which harbours 3900 species of flowering plants belongs to 199 families (Rao, 2012). Some of the arborescent genera having maximum endemic taxa are *Litsea*, *Cinnamomum*, *Actinodaphne* belongs to family Lauraceae. The genus *Litsea* belongs to family lauraceae is represented by more than 300 species in world (Mabberly, 2008); of them, 45 are reported from evergreen and semi-evergreen forests of the Western Ghats, India (Bhuniya *et al.*, 2010). *Litsea floribunda* (Blume) Gamble is a dioecious tree species endemic to India; commonly known as Sutnaga in Kannada. It is a medium sized tree with smooth, greyish brown bark, leaves simple, alternate, existing variation in leaf size, leaf blade sweet to taste smell slightly of crushed mango leaves. Inflorescence racemose with 6-8 flowers, dioecious, fruit ovoid berries seated on cupular perianth tube (Saldanha, 1996; Gamble and Fischer, 1998).

Understanding species diversity and distribution patterns is important for helping managers to evaluate the complexity and resources of these forests, trees from the major structural and functional basis of forest ecosystems and serve as indicators of changes at the landscape scale (Kumar *et al.*, 2006). Hence, the objective of present research paper is to address the distribution, diversity, male and female plant ratio, girth class distribution of *L. floribunda* in central Western Ghats areas of Karnataka, India.

MATERIAL AND METHODS

The study area is situated in Kemmannugundi (KNG), Mullayyanagiri (MLG) in Chikkamagaluru district, Kodachadri (KDC) in Shivamogga district and Madikeri

(MDK) in Kodagu district along the central Western Ghats regions of Karnataka state, India (Fig 1, Table 1). The vegetation of the sampling sites includes evergreen and semi evergreen forests. The wide range of ecological conditions and altitudinal variation resulted in diverse vegetations. In Kemmannugundi, Mullayyanagiri region the temperature varies between 10° to 32° C, in Kodachadri 15° to 35° C, in

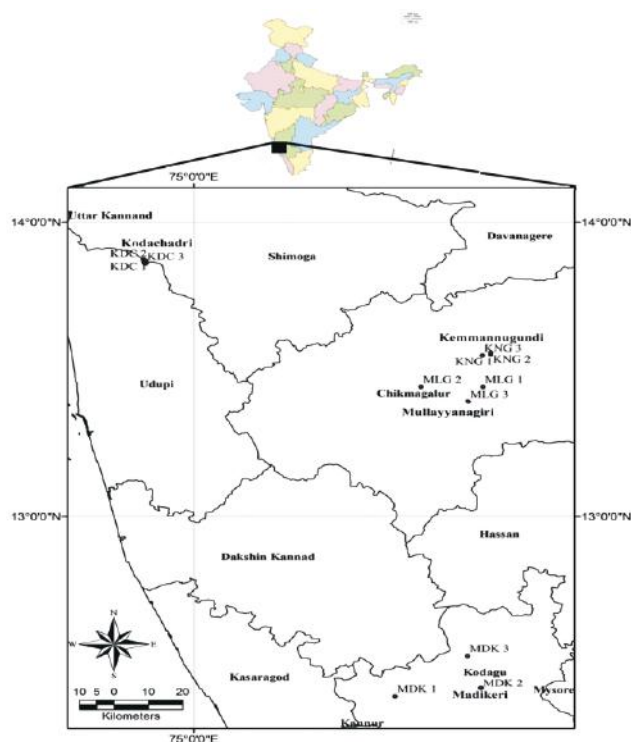


Fig. 1. Map showing the study area and study sites in three districts of central Western Ghats of Karnataka

Madikeri region 11° to 30° C across the different months in a year.

Stratified random sampling method was used to collect the tree data. A total of 12 belt transects of 250×4 m each were laid in the study area covering 3 transects in each study sites. The flowering season of *Litsea floribunda* is from July to January where regular weekly field survey were conducted throughout the flowering season in order to observe the variation in flowering (Fig. 2), differences in male and female flowers and their ratio, initiation of fruits. Based on the flowering characteristics the male and female trees were counted. All the companion tree species within transects were recorded and identified through standard manuals based on morphological characters. Girth was measured at breast height using a Freemans steel tape and the size class distribution of *L. floribunda* trees was calculated. Density, frequency, abundance, importance value index and basal area of the trees were calculated by following Mueller-Dambois and Ellenberg (1974). The importance value index was calculated by summing of relative density, frequency and relative dominance. Species diversity index was calculated by Shannon Wiener index, $H' = (-\sum ni/N) \ln ni/N$ where, ni/N , ni = importance value index of i^{th} species, N = sum of importance value index of all species. The species dominance index was calculated by using Simpson, $Cd = (-\sum ni/N)^2$ (Magurran, 2004). Spatial distribution was determined by following Whitford (1949), $WI = \text{abundance/frequency (A/F Ratio)}$. A value <0.025 would imply a regular distribution, values between 0.025-0.05 implicate a random distribution and a value >0.05 would mean a contagious distribution (Ndah *et al.*, 2013).

RESULTS AND DISCUSSION

Litsea floribunda is frequently distributed in all the study sites. Higher density observed in Mullayyanagiri (47.67) and lower in Kodachadri (20.00), of which Mullayyanagiri is highest elevated peak in Karnataka (Table 1). Density in Kemmannugundi and Madikeri was 35.67 and 33.00, respectively. Average density of the species is 33.58 showing moderate density in the study area. Importance

Table 1. Density, Importance value index (IVI), basal area, abundance frequency ratio (A/F) of *L. floribunda* in central Western Ghats of Karnataka

Sample sites	Density/Trans	IVI	Basal area (m ² ha ⁻¹)	A/F ratio
Kemmannugundi	33.67	44.46	885.27	4.60
Mullayyanagiri	47.67	60.00	1904.79	4.77
Kodachadri	20.00	37.71	393.38	2.33
Madikeri	33.00	68.67	898.19	3.52
Average	33.58	51.98	1020.41	3.86

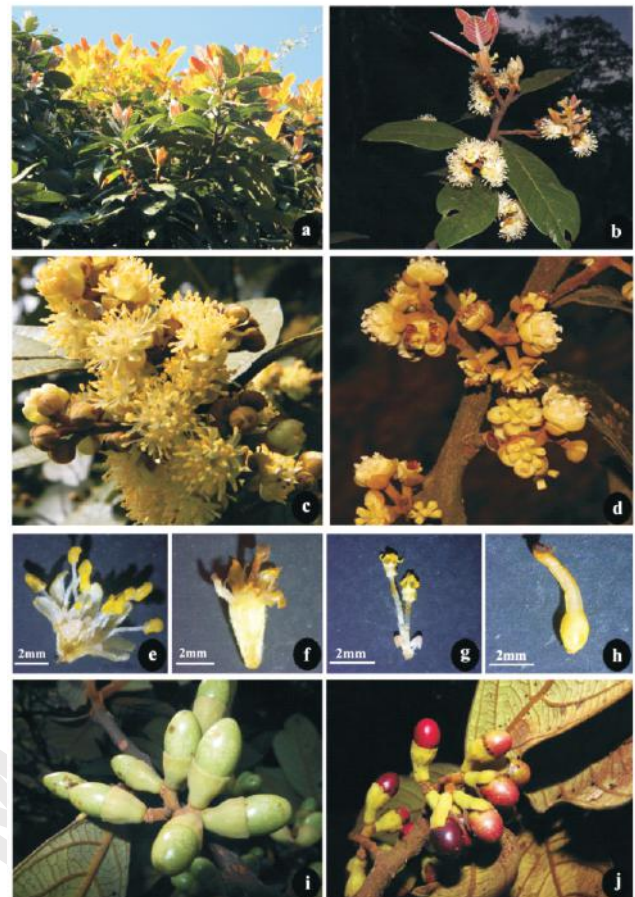


Fig. 2. *Litsea floribunda* (Blume) Gamble. a. Habit; b. Plant twig; c. Male inflorescence; d. Female inflorescence; e. Male flower; f. Female flower; g. Stamens; h. Ovary; i. Young fruits; j. Matured fruits.

value index ranged from 37.71 to 68.67, where the species showed highest in Madikeri study site (Table 1). *L. floribunda* species covers average of 1020.41 m²ha⁻¹ basal area where as higher in Mullayyanagiri, lower in Kodachadri. The higher basal area of the *Litsea* species may be due to the presence of adapted roots to absorb the nutrients for growth (Parthasarathy, 1999).

Abundance and frequency (A/F) ratio of *L. floribunda* is >0.05 in all regions showing a clumped or contagious pattern of distribution due to dioecious nature of tree. Clumping of individuals of the same species is often clearly related to gap formation and dispersal, pollination mechanism of the species. In one of the studies. *Cinnamomum* and *Neolitsea* showed clumped distribution belongs to same family (Upadhaya *et al.*, 2003). The majority of *L. floribunda* population showed their distribution in medium altitudinal height in hilly area for higher humidity, lower temperature regions and edaphic factors favours for distribution of *L. floribunda* (Acharya, 2011).

Male and female trees ratio: In all the study sites we observed the ratio of males to female trees. A total of 403 (293

females, 110 males) trees were found during flowering season (Fig. 3). The ratio of males to female is 1:2.6 in all the study sites, 1:1.5 in Kemmannugundi, 1:3.6 in Mullayyanagiri, 1:2.8 in Kodachadri, 1:3.3 in Madikeri. This showed that male trees are lower in number when compare to female trees in all the study sites. Male trees bears individually more flowers when compare to females. In some studies of dioecious species, *Litsea leefeana* in Australian tropical rain forests (House, 1992), the results of the study showed male tree population are more when compare to female. Conversely in our study on *Litsea floribunda* in central Western Ghats of Karnataka, showed male population are lower in number.

Size class distribution: The size class distribution was divided with a girth of 20-40, 41-60, 61-80 and >80 cm (Fig. 4). The data showed a bell shaped curve. Most of the trees (176)

girth ranged in 41-60 cm compare to other girths. The data envisages that number of seedlings and aged trees level are low, whereas, medium aged trees have higher basal area. Regeneration status of the trees is in moderate level.

Companion tree species of *Litsea floribunda*: A total of thirty tree species belong to sixteen families were suitable in all the study sites (Table 2). Most of the species belonged to the family Lauraceae (12 species) due to the preference of favourable environmental factors. It is evident that Lauraceae is the dominant family of the companion species in the Jaintia hills in north-east India (Upadhaya *et al.*, 2003). Plant diversity in montane wet forests of Southern Western Ghats, India accounting for 20% of individuals from Lauraceae family (Somasundaram and Vijayan, 2010). This showed that some Lauraceae members are distributed uniformly throughout the Western Ghats of India. Companion

Table 2. Frequency, density, abundance, importance value index (IVI) frequency ratio (A/F) of companion species of *Litsea floribunda* in central Western Ghats of Karnataka

Species	Family	Frequency (%)	Density / Trans	Abundance	IVI	A/F Ratio
<i>Actinodaphne hookeri</i>	Lauraceae	0.25	0.75	3.00	3.14	1.38
<i>Artocarpus heterophyllus</i>	Moraceae	0.08	0.08	1.00	0.83	1.38
<i>Callicarpa tomentosa</i>	Verbenaceae	0.50	1.08	2.17	5.74	0.50
<i>Cinnamomum verum</i>	Lauraceae	0.92	10.58	11.55	21.61	1.45
<i>Cryptocarya bourdillonii</i>	Lauraceae	0.58	2.58	4.43	8.40	0.87
<i>Ficus</i> sp.	Moraceae	0.17	0.33	2.00	1.88	1.38
<i>Garcinia gummi-gutta</i>	Clusiaceae	0.08	0.08	1.00	0.83	1.38
<i>Glochidion</i> sp.	Euphorbiaceae	0.33	1.67	5.00	5.05	1.73
<i>Lagerstroemia lanceolata</i>	Lythraceae	0.25	1.08	4.33	3.57	1.99
<i>Litsea ghatica</i>	Lauraceae	0.25	2.75	11.00	5.72	5.06
<i>Litsea glabrata</i>	Lauraceae	0.17	0.50	3.00	2.09	2.07
<i>Litsea laevigata</i>	Lauraceae	0.50	1.92	3.83	6.82	0.88
<i>Litsea mysorensis</i>	Lauraceae	0.58	1.25	2.14	6.68	0.42
<i>Litsea stocksii</i>	Lauraceae	0.25	1.75	7.00	4.43	3.22
<i>Litsea wightiana</i>	Lauraceae	0.33	0.83	2.50	3.97	0.86
<i>Macaranga peltata</i>	Euphorbiaceae	0.17	0.42	2.50	1.99	1.73
<i>Maesa indica</i>	Myrsinaceae	0.42	2.00	4.80	6.20	1.32
<i>Memecylon malabaricum</i>	Melastomataceae	0.17	0.17	1.00	1.66	0.69
<i>Neolitsea cassia</i>	Lauraceae	0.50	2.33	4.67	7.36	1.07
<i>Neolitsea</i> sp.	Lauraceae	0.25	2.08	8.33	4.86	3.83
<i>Neolitsea zeylanica</i>	Lauraceae	0.17	0.58	3.50	2.20	2.42
<i>Nothapodytes foetida</i>	Icacinaceae	0.25	0.92	3.67	3.36	1.69
<i>Nothopodia racemosa</i>	Anacardiaceae	0.08	0.08	1.00	0.83	1.38
<i>Ochlandra travancorica</i>	Poaceae	0.25	0.42	1.67	2.71	0.77
<i>Psychotria nigra</i>	Rubiaceae	0.17	0.17	1.00	1.66	0.69
<i>Symplocos</i> sp.	Symplocaceae	0.25	0.92	3.67	3.36	1.69
<i>Syzygium cumini</i>	Myrtaceae	0.67	2.67	4.00	9.23	0.69
<i>Terminalia paniculata</i>	Combretaceae	0.17	0.17	1.00	1.66	0.69
<i>Vernonia arborea</i>	Asteraceae	0.17	0.33	2.00	1.88	1.38

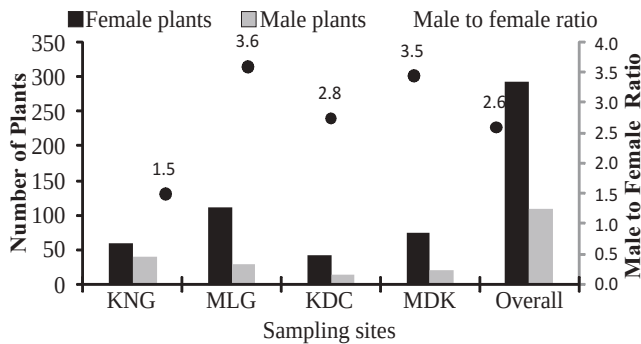


Fig. 3. Number and ratio of male and female trees of *L. floribunda* in central Western Ghats of Karnataka

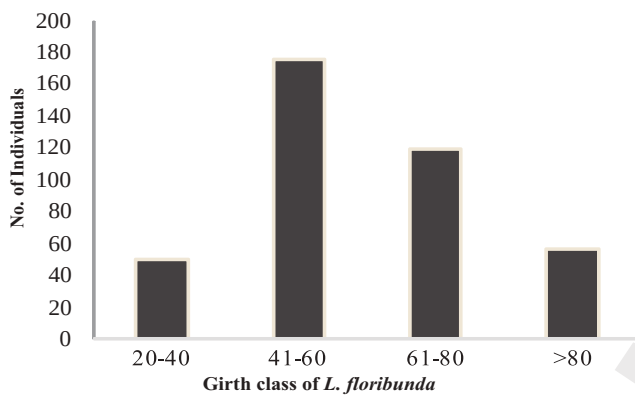


Fig. 4. Girth class distribution (cm) of *L. floribunda* in sampling sites of central Western Ghats of Karnataka

tree species distributed in central Western Ghats of three districts are concurrent to the study of vegetation of montane forest of Western Ghats (Brilliant *et al.*, 2012).

Cinnamomum population comprised higher frequency (0.92%) and minimum (0.08%) in *Artocarpous*, *Nothopegia* and *Garcinia* species. The density ranged between 0.08 to 10.58 across the different species (Table 2). Higher abundance is 11.55 in *Cinnamomum* and lower in *Terminalia*, *Memecylon* and *Garcinia* species. Importance value index ranged from 0.83 to 21.61 and abundance, frequency ratio of most of the Laural members is high, which indicates clumped distribution. It is evident by most seedlings were adopted to grow in conspecifics of mother trees (Giliba *et al.*, 2011; Deka *et al.*, 2012).

Shanon Weiner index has higher (2.32) in Kemmannugundi and lower (1.64) in Madikeri (Fig.5). Kemmannugundi is the most species rich habitat when compared with all study sites. The value of Simpson's index ranges between 0-1, maximum (0.84) in Madikeri and minimum (0.63) in Kodachadri (Fig. 5). The diversity of trees

of these study sites are resembles to the Sulimudi forests of Western Ghats, Kerala. The Shanon index of our study area is lower when compared with evergreen to semi evergreen forests of Sulimudi (2.64), Nilakkal (3.60) and Kakki (3.62) regions of Western Ghats (Magesh and Menon, 2011) and higher in Vagamon (2.09) forests (Brilliant *et al.*, 2012). Simpson Index is higher (0.84) compared to Urani (0.06) in Southern Western Ghats (Magesh and Menon, 2011) and in Vagamon region (0.36) (Brilliant *et al.*, 2012).

Rarefaction curve: Species area and species individual accumulation curve (Rarefaction curve) against the equal sized sampling sites in different vegetation types and different study sites showed a distinct difference in the richness, abundance (Jayakumar and Nair, 2013). Distribution of species in assemblages by comparing the steepness of the curve (Fig. 6) indicate high heterogeneity, one striking results of rarefaction from this graph was the Kemmannugundi has the highest curves. This implies that species diversity of all the individuals including *Litsea floribunda* was higher in this site per equal sized area. Madikeri has the most individuals per unit area, sampling of the all trees including *Litsea floribunda* is satisfactory because the rarefaction curve moves along with the same value and other than Madikeri region curves seems like

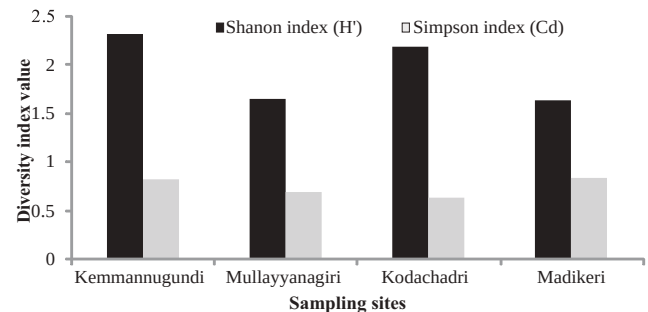


Fig. 5. Shanon and Simpson diversity index in sampling sites in central Western Ghats of Karnataka

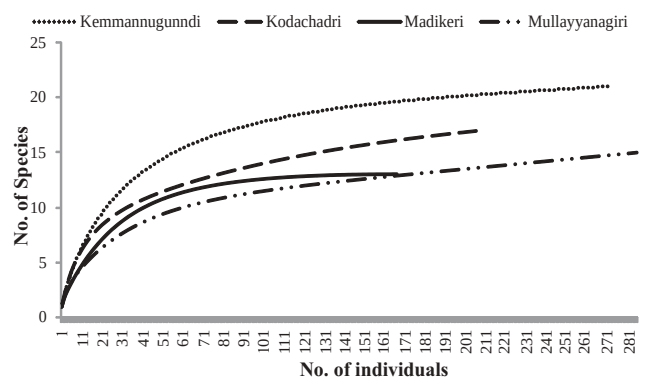


Fig. 6. Rarefaction curve of the species in sampling of all four study sites in central Western Ghats of Karnataka

growing level that indicates some more samplings are required for calculating the species richness.

This study revealed that all the four study sites in central Western Ghats had good species richness of *Litsea floribunda* and their associated species. The dominant companion species are *Cinnamomum*, *Neolitsea*, *Cryptocarya* and *Syzygium* species, laural members are the dominant in all the study sites. The data showed clumped distribution and seedlings are adopted to grow close to the mother trees. Ratio of male trees of *Litsea floribunda* were lower when female trees, which warrants proper management for conservation of these dioecious tree species.

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Effect of Chromium on *Lantana camara* L. Extract Treated Wood Species

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Abstract: The study conducted on chromium treatment on *Lantana camara* L. extract treated wood samples of *Pinus roxburghii* Sargent, *Celtis australis* L., and *Bombax ceiba* L. revealed an increase in chromium absorption, leaching and retention with the increase in chromic acid concentrations. Maximum was found at 10% chromic acid concentration and minimum at 5 per cent concentration. Among the treatments the highest chromium absorption, leaching and retention was recorded in control and lowest at 0.25 and 0.5% extract treatments. *Celtis australis* L. responded higher than other species and the minimum response was recorded in *Bombax ceiba* L.

Key Words: Chromic acid, Dimensional stability, Fixative, Preservation, Tree species

Wood is a three-dimensional biopolymer composite composed of an interconnected network of cellulose, hemicelluloses and lignin with minor amounts of extractives and inorganics. The hemicelluloses are the most hygroscopic component in the wood cell wall, but cellulose and lignin also contribute to hygroscopicity. The main problems associated with wood under outdoor use are its dimensional instability due to moisture sorption, breakdown of wood polymers by UV light, and its decay by micro-organisms.

Chemical modification of cell wall polymers is one of the effective methods to induce dimensional stability and biological resistance in wood (Rowell, 2005). The dimensional stability can be improved by bonding cell wall polymers with hydrophobic groups or bulking cell wall polymers with bonded chemicals (Kiguchi and Evans, 1998). Treatment of wood surfaces with aqueous solutions containing hexavalent chromium (Cr) compounds e.g., Chromium (VI) trioxide or CrO_3 , imparts many beneficial properties to wood which includes resistance to degradation, fixation of water soluble extractives in wood, reduction in swelling by water, impartation of a degree of decay resistance and improvement in durability of finishes on wood. Chromium trioxide imparts these properties in wood by bulking, as well as physico-chemical modification of the cell wall (Fiest, 1979). During the fixation of chromium in wood various reactions takes place between CrO_3 and the lignin/cellulose in wood. The fixation of CrO_3 derived compounds on wood have been explained as the combination of the various reactions, which indicates that Cr is fixed irreversibly to the lignin of wood as Cr (VI) and is weakly bound on the cellulose surface as Cr (III) of which

small amounts can be released in a water medium. Many beneficial properties can be improved in wood by treating with chemical containing hexavalent chromium (Cr^{+6}), particularly CrO_3 , therefore, the present study was carried out with the objective to study the role of chromium trioxide in extract fixation (Pizzi, 1981).

MATERIAL AND METHODS

The present investigation was conducted in the Department of Forest Products, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP), India. The wood samples of size $5\text{cm} \times 2.5\text{cm} \times 2.5\text{cm} \pm 0.25\text{cm} \times 0.15\text{cm} \times 0.15\text{cm}$ (longitudinal $\times$ radial $\times$ tangential) were prepared from air-dried woods of *Pinus roxburghii* Sargent, *Celtis australis* L. and *Bombax ceiba* L. These samples were properly planed and sanded for smoothness on surface and straight lines were drawn with lead pencil on all the planes of these samples for taking dimensions on the same point every time.

Preparation of extract and treatment: The tender twigs of *Lantana camara* L. along with leaves were collected shade dried and was grounded into fine powder. The powdered material was extracted with methanol in soxhlet apparatus on a boiling water bath. After complete extraction, methanol was completely distilled off from the extract and the residue was dried under vacuum. Stock solution of 10 per cent concentration was prepared by dissolving the vacuum dried extract in 5 per cent methanol. From the prepared 10 per cent stock solution, different concentrations of 0.00, 0.25, 0.5, 1.00, 1.50 and 2.00 per cent for dip treatment were prepared. The wood specimens of *Pinus roxburghii* Sargent, *Celtis australis* L., and *Bombax ceiba* L. were dried at $105 \pm 2^\circ\text{C}$ to

constant weights and dipped in the different concentrations (w/v) of *Lantana camara* L. extract solution for 72 hours. The samples meant for control were dipped in distilled water. The treated samples were then dried at $105 \pm 2^\circ\text{C}$ to constant weights. The differences in weights before and after treatment were taken as weight changes in the wood samples.

Chromium treatment: The *Lantana* extract treated wood specimens of *Pinus roxburghii* Sargent, *Celtis australis* L. and *Bombax ceiba* L. were oven dried at $105 \pm 2^\circ\text{C}$ to a constant weight. The triplicate specimens were dip treated in 5% and 10% chromium (VI) trioxide solution (chromic acid). The specimens were dip treated for 2 hours for each chromic acid concentration. The so treated wood specimens were air dried for four hours and then placed in a pre-heated oven at $135 \pm 2^\circ\text{C}$ for 30 minutes. Chemical pickup was determined from difference in oven dry weights before and after chromium treatment. The chromium treated wood samples were submerged in successive 150 ml portions of double distilled water at 25°C for 24 hours for chromium leaching estimation.

RESULTS AND DISCUSSION

The variation in oven dry weight of wood in different species is significant at 5 per cent level of significance (Table 1). The maximum variation in oven dry weight of wood has been recorded in *Celtis australis* L. and the lowest variation (0.058g) was observed in *Bombax ceiba* L. Among the treatments maximum variation in oven dry weight has been recorded in 1.50% concentration and the minimum variation in control. The values for control were negative, which indicated that the water soluble extractives may have leached out from untreated samples. While the treated samples gained weight because of absorption of the extract.

Chromium treatment: Maximum chromium absorption, chromium leaching and chromium retention was at 10 per cent chromic acid concentration and minimum at 5 per cent.

This is because the wood samples treated with higher concentration will allow more chromium to penetrate. Significant differences have been observed among species, treatments and chromium concentrations for chromium intake (Table 2). The maximum (0.645g) chromium absorption was in *Celtis australis* L. and minimum (0.167g) in *Bombax ceiba* L. Aggrawal *et al.*, (2003) have reported that in chromic acid treated *Bombax ceiba* L. wood, the samples regained almost the initial weight because chromic acid dries up at a faster rate and does not form any layer over the surface of wood like other water repellants. Among the treatments the absorption has shown an increase with the increase in concentration. The highest value (0.541g) has been observed in control and the lowest value (0.348g) at 0.25% concentration. The increase in chromium pick up both with the increase in concentration and dipping time for all the woods is because the samples treated for longer duration and with higher concentration, allow more chromium to penetrate into the wood samples.

The species, treatments and chromium concentrations for chromium leaching has shown significant differences (Table 3). The chromium leaching varied among different species due to the leaching properties that are influenced by the chemical composition through the quantity, nature and existence of the function groups needed for chemical reactions to take place, which ensure the binding of the preservative's agents. The leaching properties are also influenced by the structural composition through the pore-diffusive inhibition, which is caused by the number, size, and distribution of pores. The leachable hexavalent chromium appears to be mainly that part of chromic acid, which could not reach and react with reactive sites (mainly cellulose and lignin) because of higher concentration of chromic acid and or presence of extractives. The maximum (0.214g) chromium leaching has been found in *Celtis australis* L. and minimum (0.114g) in *Bombax ceiba* L. Among the treatments, the highest value (0.237g) for leaching has been observed in

Table 1. Variation in oven dry weight of treated and untreated wood (g)

Lantana concentrate (%) (T)	Species (S)			Mean
	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.	
0.2	0.086	0.090	0.029	0.068
0.5	0.117	0.058	0.060	0.078
1.0	0.121	0.096	0.086	0.101
1.5	0.122	0.090	0.116	0.109
2.0	0.085	0.081	0.146	0.104
Control	-0.097	-0.045	-0.088	-0.077
Mean	0.072	0.062	0.058	

CD (p=0.05)

Species (S) - 0.010 : Treatments (T) - 0.014 ; S x T-0.024

Table 2. Effect of chromium concentrations in its absorption in wood

Lantana concentrate (%)	Chromium concentrations (C)							Species (S)			
	5%							10%			
	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.	Mean	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.	Mean	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.
0.2	0.492	0.335	0.125	0.318	0.583	0.405	0.150	0.379	0.538	0.370	0.138
0.5	0.517	0.360	0.136	0.337	0.582	0.438	0.150	0.390	0.549	0.399	0.143
1.0	0.565	0.369	0.147	0.360	0.640	0.447	0.155	0.414	0.603	0.408	0.151
1.5	0.648	0.407	0.156	0.404	0.684	0.475	0.172	0.444	0.666	0.441	0.164
2.0	0.662	0.470	0.167	0.433	0.767	0.524	0.184	0.492	0.714	0.497	0.175
Control	0.743	0.550	0.207	0.500	0.852	0.643	0.252	0.583	0.798	0.597	0.230
Mean	0.604	0.415	0.156	0.392	0.685	0.489	0.177	0.450	0.645	0.452	0.167

CD (p=0.05) Species (S): 0.055; Treatments (T): 0.007; Concentrations ©: 0.004; T x S: 0.013; S x C: 0.007; T x C: 0.018; T x S x C : 0.018

Table 3. Effect of chromium concentrations in its leaching from wood (g)

Lantana concentrate (%) (T)	Chromium concentrations (C)							Species (S)			
	5%							10%			
	<i>Celtis australis</i> L.	<i>Pinus</i>	<i>Bombax ceiba</i> L.	Mean	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.	Mean	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.
0.2	0.131	0.146	0.068	0.115	0.208	0.166	0.104	0.159	0.169	0.156	0.086
0.5	0.151	0.165	0.103	0.139	0.225	0.175	0.104	0.168	0.188	0.170	0.103
1.0	0.164	0.167	0.117	0.150	0.251	0.177	0.127	0.185	0.208	0.172	0.122
1.5	0.179	0.192	0.134	0.168	0.245	0.170	0.149	0.188	0.212	0.181	0.142
2.0	0.197	0.159	0.117	0.157	0.258	0.208	0.082	0.182	0.227	0.183	0.099
Control	0.266	0.254	0.120	0.213	0.296	0.341	0.142	0.260	0.281	0.298	0.131
Mean	0.181	0.180	0.110	0.157	0.247	0.206	0.118	0.190	0.214	0.193	0.114

CD (p=0.05) Species (S): 0.016; Treatments (T): 0.023; Concentrations ©: 0.013; T x S: 0.040; S x C: 0.023; T x C: 0.032; T x S x C : 0.056

Table 4. Effect of chromium concentrations in its retention from wood (g)

Lantana concentrate (%) (T)	Chromium concentrations (C)					Species (S)				
	5%		10%			Mean		Mean		
	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.	Mean	<i>Celtis australis</i> L.	<i>Pinus roxburghii</i> Sargent	<i>Bombax ceiba</i> L.
0.2	0.362	0.189	0.058	0.374	0.238	0.046	0.219	0.368	0.214	0.052
0.5	0.366	0.195	0.033	0.357	0.263	0.046	0.222	0.362	0.229	0.039
1.0	0.401	0.202	0.029	0.389	0.270	0.028	0.229	0.395	0.236	0.029
1.5	0.468	0.214	0.022	0.438	0.305	0.023	0.255	0.453	0.260	0.023
2.0	0.466	0.323	0.051	0.509	0.320	0.079	0.303	0.487	0.321	0.065
Control	0.476	0.550	0.087	0.556	0.643	0.110	0.437	0.516	0.597	0.099
Mean	0.423	0.279	0.047	0.437	0.340	0.055	0.278	0.430	0.309	0.051
CD (p=0.05)	Species (S): 0.009; Treatments (T): 0.013; Concentrations ©: 0.008; T x S: 0.0230; S x C: 0.0133; T x C: 0.019; T x S x C: 0.032									

control and the lowest value (0.137g) at 0.25% concentration, which indicates that treatment may have some influence on the leaching of chromium.

The critical scrutiny of data on chromium retention showed significant difference among different species, treatments and chromium concentrations (Table 4). The maximum value (0.430g) has been observed in *Celtis australis* L. and minimum value (0.051g) in *Bombax ceiba* L. Among the treatments the retention shows an increasing trend with the increase in concentration. The highest (0.404g) retention has been recorded in control and lowest value (0.210g) in 0.50% concentration. This is because the chromic acid fixes by the formation of insoluble compounds or complexes and coordination of Cr to the hydroxyls in wood. Coetze (1980) has reported that higher retention level provide more water repellency to the wood, thus limiting leaching.

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Received 9 September, 2015; Accepted 16 December, 2015



Effect of Pre-sowing Seed Treatment on Germination and Seedling Growth of *Terminalia bellirica* (Gaertn.) Roxb.

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Abstract: The study describes the effects of depulping the fruits and soaking the seeds of *Terminalia bellirica* on seed germination and initial seedling growth in nursery condition including soaking in water, cow dung slurry for different durations and termite treatment. Due to the hard seed coat with thick pericarp having high content of phenolic compounds, *T. bellirica* seeds germinate irregularly taking longer time for nursery establishment. Germination parameters were significantly influenced by the pre-sowing treatments and the water soaking of the seeds for 24 hrs produced the highest germination. The pre-sowing treatments also influenced the initial seedling growth and the seedlings obtained after alternate drying and wetting of the seeds for five days recorded higher growth and biomass than untreated seeds.

Key Words: Bio-volume index, Biomass, Depulping, Germination, Pre-treatment, Seedling, *Terminalia bellirica*, Vigour index

Forests play key roles in the lives of people living in both mountains and lowland areas by supplying fresh water and oxygen as well as providing a diversity of valuable forest products for food and medicine. *Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae) is an ecologically and medicinally important deciduous canopy tree species having wide distribution throughout India, but with limited distribution and density in evergreen forests of the Western Ghats and is often associated with teak (*Tectona grandis*). Fruit of the species is one of the three ingredients of "Triphala", which is a popular Ayurvedic formulation (Kumar *et al.*, 2014) extensively used traditionally to recover from fever, cough, diarrhea, skin diseases, oral thrush (Elizabeth, 2005; Kumar *et al.*, 2010; Chaudhary *et al.*, 2012), hypertension, to reduce cardiac depression and the risk factors associated with the heart (Chaudhary *et al.*, 2012), remove toxins and other undesirable accumulations from the body, improve digestion and assimilation and acts as antioxidant. The fruits of *T. bellirica* contain very high level of total phenolic compounds (TPC) ($164.5 \text{ mg} \cdot \text{g}^{-1}$) (Bajpai *et al.*, 2005) including Glucoside (bellericanin) (Singh, 2006), Gallo-tannic acid, resins, oil (Nadkarni, 2002), Ellagic acid, gallic acid, lignans 7-hydroxy 3'4' flavone and anolignan B, tannins, ethyl gallate, phenyllembelin, β -sitosterol, mannitol, glucose, fructose and rhamnose (Anonymous, 2001; Saroya, 2011). Therefore, the fruits are being used as an antioxidant, antimicrobial (Alam *et al.*, 2011), antidiarrheal, anticancer, antidiabetic, antihypertensive and hepatoprotective agent (Motamarri *et al.*, 2012).

However, due to overexploitation (because of its multipurpose uses), tremendous population pressure, rural

poverty, absence of appropriate government policy, accelerated growth of synthetic drugs and inappropriate utilization of forest products, these vital plant resources are being declined. Besides these, natural regeneration through seeds is very poor, uncertain (Luna, 2005) and required long time to germinate, thus the State Forest Departments avoid the species in the afforestation and/or reforestation programs. This delay and irregularity in germination of seeds is believed to be due to the hard seed coat and thick fleshy pericarp of the fruits of the species (Shivanna *et al.*, 2007). The pericarp of the fruit contains very high level of phenolic compound (Bajpai *et al.*, 2005). Seed germination may be reduced by the presence of impermeable seed coats that restricts the availability of water, which can penetrate into seeds at their optimum concentration (Kumar *et al.*, 2015a, b). Fruit length was significantly and positively correlated with seed length. Seed diameter had significantly positive correlation with fruit weight and seed weight. Although commercial exploitation of *T. bellirica* is hampered by the shortage of superior plants, hard seed coat and heavy insect infestation of seedlings and primarily due to the difficulties experienced in propagating this species using the traditional method of grafting and the poor rooting ability of shoot cuttings. There are some studies which examined that positive correlation between *T. chebula* and *T. bellirica* seed germination and depulping of dry fruits by knife and soaking the seeds in cold water for various time periods (Hossain *et al.*, 2005a,b). Depulping of the *T. bellirica* fruits by rotting fleshy pulp in water would be one of the important options for depulping the dry fruits with knife or other mechanical scarifications but the technique and its subsequent effect on

seed germination have not been sufficiently investigated. Therefore, the present study has been designed to explore the enhanced seed germination as well as seedling growth performance of *T. bellirica* under different easily applicable pre-sowing treatments avoiding the laborious process of depulping the dry fruits of the species..

MATERIAL AND METHODS

The present investigation was carried out at College of Forestry, Kerala Agricultural University, Thrissur, Kerala during August 2013 to July 2014. The area experiences a warm and humid climate with distinct rainy season. Matured *Terminalia bellirica* fruits were collected from the College of Forestry campus. The average number of seeds per fruit and the number of seeds per kg were determined in 10 replications. The extracted seeds were cleaned and thoroughly hand mixed to improve the homogeneity during the sampling. The individual seed length, width, weight, volume, density and specific gravity were recorded from 100 randomly selected samples from the seed lot. Specific gravity was determined by specific gravity module (Schimadzu AU220). The pulp content of the seeds was also determined.

Seeds were subjected to pre-sowing treatments. The pre-treated seeds (100 x 3) were sown in polythene bags (11.43x15.24 cm, gauge 75 micron) containing the rooting medium soil, sand and FYM in the ratio 9:3:1 and daily germination counts were recorded. At the end of the trial, the percentage and peak value of germination and germination value (GV) of the species were calculated as suggested by Czabator (1962). Twelve seedlings per treatment were destructively sampled at monthly intervals till 180th day of sowing to record height, collar girth, tap root length, fresh and dry weight of root and shoot. Seedling quality indices were deduced from these observations. Quality index, which is a measure to assess the quality of seedling based on the height, stem diameter and dry biomass was also calculated using the following formula of Hatchell (1985). Bio-volume index, which is a non-destructive quick method to calculate the above-ground portion of the tree seedlings was also calculated using the formula.

Biovolume index= Plant height (cm) X Stem diameter (mm).

The vigour index (VI) of the seedlings was calculated using the formula (Abul-Baki and Anderson, 1973): $VI_1 = \text{Germination percentage} \times (\text{Shoot length} + \text{Root length or total seedling length})$, $VI_2 = \text{Germination percentage} \times \text{total dry weight}$.

The data generated were analysed using the IBM SPSS Statistics 20. The data on germination, seedling

growth, biomass, and quality indices were subjected to one way analysis of variance and the treatment means were compared with LSD (least significant difference).

RESULTS AND DISCUSSION

The seeds of *T. bellirica* were born in clusters at the terminal end; fruits collected were oval in shape. The light green fruits turned yellow and finally brown on ripening. With regards to individual seed attributes, length was 33.64, width 29.85, fresh weight 16.10g, the number of seeds per kilogram was 80.25, seed volume 20.80ml, and dry weight of seeds 4.50 were recorded. Our results are in conformity with similar studies in other tree species like *Pongamia pinnata* (Hooda *et al.*, 2009), *Grevillea robusta* (Murugesh, 2011), *Azadirachta indica* (Kaushik *et al.*, 2012), *Tectona grandis* (Jijeesh and Sudhakara, 2013), *Calophyllum inophyllum* (Ajeesh *et al.*, 2014) and *Thriphala* (Kumar *et al.*, 2014).

Influence of pre-sowing treatments on germination parameters

Germination parameters of the *T. bellirica* seeds were significantly influenced by pre-sowing treatments. The highest germination percentage (47.50), mean daily germination (0.84), peak value of germination (1.63) and germination value (0.52) were observed for the seeds with removed pulp and soaking in water for 24 hrs. The next highest germination was recorded for the seeds subjected to water soaking for 48 hrs. Although 14% seeds kept as control germinated. Only one per cent of acid scarified seeds (10% H_2SO_4 for 10 min.) germinated (Table 1). Generally, the pre-sowing treatments improve the germination (Kumar *et al.*, 2015a). The result of the present study were in the line of the findings of many other authors who mentioned that depulping of fruits and soaking of the seeds in cold water enhanced the seed germination of *T. bellirica* (Hossain *et al.*, 2005b).

Effect of seed pre-treatment on seedling performance:

Analysis of variance revealed significant difference in seedling height, collar girth, tap root length, fresh weight of roots, fresh weight of shoot, root biomass and shoot biomass over time. The growth attributes and biomass were influenced by the pre-sowing treatments. At the end of 180 days after planting, the highest growth attributes like collar girth (8.87mm), height (48.50cm) and the largest tap root (47.55cm) were obtained in cow dung slurry for 72 hrs. The lowest collar girth (6.38mm) and tap root (37.82cm) were recorded through water soaking for 24 hrs. The lowest height (37.87cm) was observed in acid scarified seeds (10% H_2SO_4 for 10 min.). With regards to fresh weight and dry weight of the seedlings, the highest fresh weight of root (18.86g) and

shoot (34.88g) and dry weight of root (6.24g) and shoot (11.96g) were recorded in cow dung slurry for 72 hrs. Seedling subjected to the lowest fresh weight root (8.84g), shoot fresh weight (21.55g) and dry root weight (2.86g) and dry shoot weight (7.226g) were recorded in control (Table 2).

In the present study, the initial nursery performance of the seedlings as measured by height, collar girth, tap root length and total biomass varied significantly due to pre-treatment over different periods. Seed traits such as seed size, seed coat thickness, shape and moisture content are already known to affect seed germination, dormancy and early seedling establishment (Suresh *et al.*, 2003; Singh and Saxena, 2009; Gunaga *et al.*, 2010; Jijeesh and Sudhakara, 2013; Ajeesh *et al.*, 2014; Kumar *et al.*, 2015a, b). The seeds treated with alternate drying and wetting for continuous five days (T5) reported better performance as compared to other pre-treatments.

The goal of forest tree nursery practices is to produce high quality seedlings with useful characters (Kumar *et al.*, 2015a, b). Evolving appropriate nursery management strategies to enhance productivity and thereby reducing the long nursery period has been the basic challenge. Among the variable silvicultural options, early tree nutrition practices have bagged considerable attention in the recent times in view of their long standing effect on tree growth and productivity. Seedling quality indices (vigour index I, vigour index II, and bio-volume and quality index) also varied among the pre-sowing treatments (Table 2). The vigour index I and II of the seedlings belonging to different seeds pre-treatments. At the end of 180 days after planting, the highest vigour index I (2613.51) was recorded in control, which was 4.04 times more compared to the least (646.89) value recorded for acid scarified seeds. Similarly, highest vigour index II (869.77)

was recorded in seeds water soaked for 24 hrs, which was 3.47 times more compared to the least (250.27) value recorded for acid scarified seeds (T8). However, the bio-volume index was highest (4658.43) in seedlings treated with cow dung slurry for 72 hrs (T6) and it was the lowest (2880.90) in acid scarification (10% H₂SO₄ for 10 min.) treatment.

The quality index also was the highest (1.15) for seedlings of T6 and the lowest quality index was (1.09) was recorded for alternate drying and wetting for continuous for five days (T5). The changes in the nutrient content and resave food can also influence the quality parameters. The increases in seed size a proportionate increment of quality indices observed in *C. inophyllum* (Kumar *et al.*, 2015b). Kumar (2016), also revealed that *Spondias pinnata* of bio-volume index was highest (545.32) in seedlings treated with alternate drying and wetting for continuous five days (T6) which was 3.33 times more compared to the lowest (163.28) in water soaking for 24 hrs (T2) at the end of the study. Hossain *et al.* (2013) reported the maximum vigor index for *T. chebula* (4105 four months after sowing the seeds) in depulped seeds soaking with water for 48 h. The results of the present study were also consistent with the findings of other authors. For instance, Shivanna *et al.* (2007) reported 51% - 60% germination of *T. bellirica* seeds when the mesocarp was depleting. A germination success of up to 50% was obtained when clean seeds (removing the dry pulp) were sown at BFRI (Shivanna *et al.*, 2007). The result of the present study was also supported by the findings of Hossain *et al.* (2005a,b) and mentioned that depulped seeds soaked in the cold water for 48 h gave the maximum germination and seedling growth for both the species *T. bellirica* and *T. chebula*. Usually depulping the fruits allows seed coat for water penetration which makes the seed.

Table 1. Response to various pretreatments of *Terminalia bellirica* seeds

Pre-treatment	Germination parameters			
	Germination percentage	Mean daily germination	Peak value of germination	Germination value
T1- Control	14.00	0.25	0.45	0.56
T2- Water soaking for 24 hrs	10.50	0.19	0.36	0.53
T3- Water soaking for 48 hrs	32.00	0.57	1.10	0.52
T4 - Removal of pulp and soaking in water for 24 hrs	47.50	0.84	1.63	0.52
T5 - Alternate drying and wetting for continuous five days	19.50	0.35	0.67	0.52
T6 - Cow dung slurry for 72 hrs	26.50	0.47	0.91	0.52
T7 - Water soaking for 72 hrs	15.50	0.28	0.53	0.53
T8 - Acid scarification (10% H ₂ SO ₄ for 10 min.)	1.00	0.02	0.05	0.38

Table 2. Growth attributes, biomass production and quality parameters of *Terminalia bellirica* as influenced by different pre-treatment at different monthly intervals

Days after planting	Treatments	Collar diameter (mm)	Shoot length (cm)	Tap root length (cm)	Fresh weight of root (g)	Root bio-mass (g)	Fresh weight of shoot (g)	Shoot bio-mass (g)	Vigour index I	Vigour index II	Bio-volume index	Quality index
30	T1	1.12	10.36	5.25	2.03	0.43	8.82	2.11	158.58	93.96	161.76	0.99
	T2	1.14	9.24	5.53	2.50	0.74	8.82	2.10	125.93	81.51	136.48	1.01
	T3	2.08	13.82	6.37	3.35	0.93	9.44	2.29	380.59	195.36	279.04	1.05
	T4	1.98	10.34	5.85	3.16	0.91	10.18	2.55	485.31	333.02	167.28	1.03
	T5	2.18	13.67	6.69	3.76	1.02	11.88	2.77	668.57	423.50	278.34	1.04
	T6	2.75	16.67	8.91	3.97	1.18	13.73	4.14	732.72	427.39	426.51	1.04
	T7	2.20	13.42	6.58	3.64	0.95	11.75	2.68	433.27	275.26	268.47	1.04
	T8	1.59	12.42	13.86	2.48	0.74	9.08	2.21	538.93	201.58	326.53	0.96
60	T1	1.71	15.94	15.59	4.43	1.05	11.37	2.88	683.06	269.10	502.43	1.01
	T2	1.63	14.25	17.39	5.61	1.35	13.58	3.03	648.85	306.07	451.06	1.01
	T3	2.63	21.11	19.46	6.07	1.73	12.22	2.98	581.66	199.95	856.43	1.06
	T4	3.36	17.15	18.32	5.64	1.43	14.94	3.14	362.08	166.54	608.24	1.02
	T5	2.91	19.98	12.48	5.44	1.37	16.68	4.13	275.86	153.43	648.45	1.04
	T6	3.31	22.07	13.35	7.03	1.85	18.94	4.88	666.91	391.55	781.84	1.06
	T7	2.94	20.41	19.32	5.91	1.71	15.84	3.91	1192.81	526.15	811.40	1.04
	T8	1.81	16.48	16.41	4.71	1.22	13.95	3.64	1084.29	498.08	542.28	1.01
90	T1	3.83	22.31	20.15	5.98	1.59	14.48	3.67	1274.16	482.10	947.21	1.04
	T2	3.71	17.88	28.62	5.77	1.45	14.35	3.51 ¹	1526.67	518.77	831.36	0.99
	T3	4.34	25.93	24.19	6.98	1.81	13.08	3.18	1436.21	426.85	1299.61	1.06
	T4	4.30	29.56	32.14	6.23	1.67	16.64	4.19	1336.88	396.72	1823.75	1.03
	T5	3.73	28.78	31.07	7.03	1.93	22.13	5.25	1226.93	493.23	1722.48	1.04
	T6	4.50	29.69	33.54	8.21	2.06	22.88	5.72	900.91	357.47	1877.40	1.04
	T7	3.89	26.07	25.81	5.92	1.57	17.20	4.20	527.45	190.83	1352.51	1.03
	T8	3.56	18.54	27.62	5.56	1.41	15.15	3.81	392.36	140.76	855.81	1.00
120	T1	4.36	34.55	22.96	6.18	1.65	16.35	5.39	584.84	183.00	1986.55	1.06
	T2	4.47	30.61	31.93	8.37	3.09	16.65	5.58	530.01	168.02	1914.85	1.12
	T3	5.18	28.37	36.53	7.66	2.03	16.85	5.75	1222.28	355.77	1841.43	1.04
	T4	5.09	31.73	36.11	6.99	2.99	16.99	5.90	2035.44	599.40	2153.00	1.10
	T5	5.13	33.94	34.75	9.71	3.22	24.83	8.28	2256.50	920.84	2331.11	1.08
	T6	6.19	34.87	38.76	14.38	4.01	25.48	8.62	2109.90	845.38	2567.37	1.11
	T7	5.98	33.87	35.54	7.84	2.47	17.90	5.55	1503.86	441.36	2350.47	1.07
	T8	5.84	25.27	32.47	6.05	1.59	16.16	5.15	1184.05	363.90	1458.74	1.02
150	T1	5.33	41.52	40.12	7.52	1.94	19.96	6.57	1768.53	474.60	3389.15	1.05
	T2	5.10	35.32	33.86	8.93	3.38	20.17	6.68	1418.23	482.89	2443.78	1.11
	T3	5.73	41.63	41.85	9.64	3.31	20.51	6.73	1196.51	341.39	3474.72	1.11
	T4	6.17	40.18	38.68	7.94	3.18	19.86	6.51	801.20	234.37	3169.13	1.10
	T5	6.57	41.94	40.71	10.23	3.69	28.60	9.70	702.51	274.33	3466.62	1.09

180	T6	7.40	44.73	42.46	16.38	5.10	31.38	10.86	1641.68	686.99	3899.42	1.13
	T7	6.63	39.76	39.21	9.49	3.28	24.28	8.10	2368.12	826.97	3140.25	1.09
	T8	6.39	33.45	35.26	7.54	1.94	22.85	7.74	2257.02	814.00	2298.01	1.04
	T1	6.97	44.74	42.36	8.84	2.86	21.55	7.22	2613.51	732.16	3897.44	1.09
	T2	6.38	39.40	37.82	9.53	3.52	22.96	7.68	2535.61	869.77	3041.96	1.11
	T3	7.34	44.46	43.71	10.31	3.85	23.76	7.94	2527.13	791.52	3919.45	1.12
	T4	6.72	43.83	42.21	8.94	3.22	21.61	7.22	1864.03	537.83	3771.28	1.10
	T5	7.94	46.64	45.93	12.30	4.05	31.68	10.59	1897.39	732.51	4316.84	1.09
	T6	8.87	48.50	47.55	18.86	6.24	34.88	11.96	1376.72	589.27	4658.43	1.15
	T7	6.94	41.81	40.82	11.28	3.92	28.35	9.30	837.05	327.73	3454.90	1.10
	T8	6.68	37.87	38.19	9.95	3.70	25.78	8.75	646.89	250.27	2880.90	1.10

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Received 26 November, 2015; Accepted 14 March, 2016

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Rose Petal Meal as a Potential Natural Carotenoid Source for Pigmentation and Growth of Freshwater Ornamental Fish, Koi Carp, *Cyprinus carpio* (Linnaeus)

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Abstract: An experimental study was conducted to assess the efficacy of experimental diets supplemented with natural carotenoid source i.e. rose petal meal (RPM) @ 1, 3, 5 and 7 % with respect to pigmentation, survival and growth in freshwater ornamental fish koi carp, *Cyprinus carpio* (Linnaeus) for 120 days. Five diets including one control (RPM0) without carotenoid source and four experimental supplemented pelleted diets with 1% (RPM1), 3% (RPM3), 5% (RPM5) and 7% (RPM 7) of powdered rose petals in control diet were prepared. At the termination of the experiment, colouration studies indicated significantly higher ($p \leq 0.05$) skin and muscle carotenoid ($\mu\text{g g}^{-1}$ wet weight basis) content in fish fed with RPM3 (3.04) followed by diet RPM5 (2.74), RPM1 (1.96), RPM7 (1.95) and RPM0 (1.24). RGB colour intensity based on digital photographs analysis was also found to be maximum in RPM3 followed by RPM1 & RPM7, RPM5 and RPM0. Fish growth in term of % NWG, PER and SGR was maximum in RPM1 (119.36 %, 0.79, 0.66) and minimum in RPM0 (71.74 %, 0.37, 0.45). Further, supplementation of RPM @ 1% (1.98) in koi carp diet also improved the FCR by 2.0 folds as compared to control (4.17). 100% survival of fish was recorded with diets supplemented with RPM1 and RPM3, while it was 90 % in RPM5 and RPM7 and 80 % in RPM0. During the experimental period, all the water quality parameters did not vary significantly in the treatments and remained in optimum range for fish growth. Hence, rose petal meal can be used up to 3 % in diet of ornamental koi (*C. carpio* L.) both as growth promoter and colour enhancer for enhanced commercial value.

Key Words: Carotenoids, Koi carp, Colour enhancement, Pigmentation, Rose petal meal

One of the most visual attractiveness of ornamental fishes is the beauty of colours they have, which determine the commercial value of these fishes and it further depends upon the intensity and pattern of pigmentation. Carotenoids are the main pigments, representing a class of over 600 natural lipid soluble pigments found in plants, algae, photosynthetic, some non-photosynthetic bacteria and animals, which are required for healthy growth, metabolism, photosynthesis and reproduction, besides colour development (Halten *et al.*, 1997). These carotenoids are widely distributed in nature occurring in plants and animals and are also produced by all photosynthetic active microorganisms. Fish contain various kinds of carotenoids, the proportion of which depends upon their physiological status and dietary conditions. Like other animals, *de novo* synthesis of carotenoids does not occur in fish (Goodwin, 1984). Hence, natural and supplementary feed are the only sources of these pigments in fish (Hata and Hata, 1972; Gupta *et al.*, 2007). Synthetic or natural carotenoids are required to be incorporated @ 50-400 mg kg⁻¹ in diet of indoor reared fish to develop colour similar to fish feeding on live food like algae, tubifex, zooplankton, etc. (Boonyaratapalin and Lovell, 1977) under outdoor conditions.

For supply of carotenoids, various synthetic as well as natural sources (animal and plant) are available and are

also being developed for use in aquaculture particularly for ornamental fishes. Synthetic processes results in production of only specific carotenoids like β -carotene or astaxanthin, whereas, natural sources are always a combination of several carotenoids like α -carotene, β -carotene, zeaxanthin, lutein, cryptoxanthin, etc. (Gupta *et al.*, 2007). Further synthetic carotenoids are expensive and their use in aquafeed is limited due to species specificity. Natural carotenoids of animal origin are mostly extracted from crustacean such as crayfish meal, shrimp meal, crab meal, etc. (Torrissen and Naevdal, 1984) and are rich source of astaxanthin. The major concerns with the production of animal based carotenoid are limited supply due to declining catches of crustaceans along with high expenses, which leads to increase in cost of carotenoid incorporated aquaculture diets. Plant based natural carotenoids are mainly derived from flowers, fruits, vegetables, algae, yeast, etc. (Tsushima and Matsuno, 1998; Chapman, 2000), which are not only inexpensive, but also a potential source of mixed carotenoids.

Few studies have demonstrated the individual effect of natural carotenoids on the growth, survival, colour development and antioxidation responses in ornamental fishes. Among natural flower pigment source, Rose (*Rosa indica*) is a woody perennial plant (family Rosaceae) and is

native to Asia. It is also a rich source of β -carotene (4.26 to 4.89 mg/100g) along with good amount of protein (17.7%) and lipid (5.25%). Few species of rose in the form of petal meal had been tried as a carotenoid source for various ornamental fish species (Mabellini *et al.*, 2011; Pailan *et al.*, 2012 a,b; Arulvasu *et al.*, 2013) with improvement in fish survival, growth and skin colouration.

In view of above discussion, the present study was designed to assess the efficacy of rose petal meal (*Rosa indica*) on colouration, survival and growth of ornamental Koi, *Cyprinus carpio* (Linn.).

MATERIAL AND METHODS

Fish conditioning and stocking: The experimental fish, koi carp, *Cyprinus carpio* L. were procured from ornamental fish culture unit of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The fishes were acclimatized under indoor conditions (for decolouration) for one month with control diet. Ten uniformly sized fishes (av. length 2-4 cm, av. weight 1-2 g) were stocked in glass aquaria, having 50 liters of water with continuous oxygen supply. The experiment (in triplicate) was carried out for 120 days.

Feed preparation, composition and fish feeding: Four experimental supplemented pelleted diets were prepared by adding carotenoid source i.e., rose petal meal (RPM) @ 1 % (RPM1), 3 % (RPM3), 5 % (RPM5) and 7 % (RPM7) in control diet (RPM0) by adjusting the level of other feed ingredients (Table 1). Rose flowers were procured from market, petals were separated from pods, dried under shade and grounded to prepare the meal. All the feed ingredients were powdered, mixed thoroughly and palletized. The proximate composition of feed ingredients and experimental diets (Table 2) was analyzed by following the methods of AOAC (2000). Carotenoid analysis of pigment source was done by following method of Cyanotech (2002). Pelleted feed was provided to the fish @ 5 % fish body weight twice a day. Amount of feed was adjusted at every sampling according to increase in fish weight.

Water quality parameters: Water quality parameters in terms of water temperature, pH, dissolved oxygen, total alkalinity, total hardness and ammonical nitrogen were measured at fortnightly intervals by following the methods of APHA (1991).

Growth parameters: Fish growth in terms of total body length and body weight was recorded at fortnightly intervals. At the end of the experiment per cent total body length gain (%TLG), Net weight gain (NWG), percent net weight gain (%NWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and condition factor

were calculated as follows

$$\%TLG = \frac{\text{Final total body length (cm)} - \text{initial body length (cm)}}{\text{initial total body length (cm)}} \times 100$$

$$NWG = \text{Average final body wt. (g)} - \text{Average initial body wt. (g)}$$

$$\%NWG = \frac{\text{Final body weight (g)} - \text{initial body weight (g)}}{\text{initial body weight (g)}} \times 100$$

$$SGR (\% \text{ increase in weight /day}) = \frac{\ln \text{ final body wt} - \ln \text{ initial body wt.}}{\text{Culture days}} \times 100$$

ln = Natural Logarithm

$$FCR = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}} \quad PER = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

$$\text{Condition factor} = \frac{\text{Body weight}}{(\text{Body length})^3} \times 100$$

Fish survival: Survival of the fish was calculated by subtracting the number of fishes harvested at the end of the experiment from the fish stocked at the initiation of the experiment.

Total carotenoid analysis in Fish muscle and skin:

Carotenoid analysis of fish muscle and skin was done by following method of Olson (1979) at initiation, middle and termination of the experiment.

Digital analysis of fish skin: The digital photographs of experimental fish were taken at monthly intervals with a Sony full HD camera (DSC-HX 300). Colour of the fish skin was measured from opercular region, base of dorsal fin and caudal peduncle area of at least three fishes from each replicate. While taking the photographs all camera conditions are kept constant.

RGB colouration scale: photographs were analyzed in adobe photoshop (Hancz *et al.*, 2003 and Tlustý, 2005) to compare these on the basis of RGB (Red, Green and Blue).

CIE colouration scale: To quantify the colour of the fish skin from digital photographs, CIE (Commission International de l'Eclairage) Lab is applied. Adobe Photoshop has a colour space called "Lab colour mode" which is based on CIE Lab. It consists of three parameters: L*, a* and b*. Where L* stands

Table 1. Composition (%) of experimental diets

Ingredients	Experimental diets				
	RPM0	RPM1	RPM3	RPM5	RPM7
Soybean meal*	25	25	24.5	24	24
Groundnut meal*	25	25	24.5	24	23
Mustard meal*	25	25	24	24	23
Rice bran*	20	19	19	18	18
Vegetable oil	02	02	02	02	02
Vitamin-mineral mixture	02	02	02	02	02
Salt	01	01	01	01	01
Rose petal meal (RPM)	-	1	3	5	7

for luminosity (lightness ranging from 0 for black to 100 for white), channel a* is the balance between red / green and channel b* describes the balance between yellow/blue.

RESULTS AND DISCUSSION

Water quality: The study revealed that during the experimental period, water quality parameters such as temperature ($29.27 \pm 0.60^\circ\text{C}$), pH (8.50 ± 0.10), dissolved oxygen ($6.85 \pm 0.28 \text{ mg l}^{-1}$), total alkalinity ($217.96 \pm 15.49 \text{ CaCO}_3 \text{ mg l}^{-1}$), total hardness ($271.78 \pm 9.35 \text{ CaCO}_3 \text{ mg l}^{-1}$) and ammonical-nitrogen ($0.203 \pm 0.02 \text{ mg l}^{-1}$) did not vary significantly in the treatments and were within the recommended range for ornamental fish culture (Wurts and Durborow, 1992; OATA, 2008 and Rinna *et al.*, 2013).

Fish survival : At the termination of the experiment, 100%

survival of fish was recorded with diets supplemented with RPM @ 1 and 3 %, while it was 90 % in diet containing RPM @ 5 and 7 % and 80 % in control (RPM0) (Table 3). The results revealed that carotenoid supplementation with rose petal meal improved fish survival significantly.

The results showed that different levels of pigment supplementation have positive effect on survival. Various studies on carotenoid incorporation resulted in improved fish survival. Pailan *et al.* (2012 b) also obtained 90-95 % survival of rosy barb with rose petal meal diet whereas, it was 85 % in control. Similar results were obtained by Arulvasu *et al.* (2013) in fry of *Xiphophorus helleri* fed with *Rosa rubiginosa*, where survival in control was lowest (76.6 %) in comparison to treatments having 200 mg kg^{-1} (90 %), 1000 mg kg^{-1} (93.3%) and 2000 mg kg^{-1} (96.6 %) of rose petal extract.

Table 2. Proximate composition (% DM basis) and gross energy (Kcal/g) of different feed ingredients and experimental diets and total carotenoid (mg/100g) content of rose petal meal and experimental diets

Ingredients/ feed	Crude protein	Ether extract	Crude fiber	Ash	NFE	Gross energy	Carotenoid content
Soybean meal*	39.37	1.46	9.20	6.13	43.82	4.14	-
Groundnut meal*	35.62	0.76	20.26	8.40	34.94	3.50	-
Mustard meal*	34.67	0.76	14.40	8.00	41.56	3.78	-
Rice bran*	21.94	1.46	10.86	7.00	58.72	3.77	-
Rose petal meal (RPM)	17.50	2.00	6.00	5.93	68.56	3.97	5.00
RPM0	31.93	1.04	13.20	7.60	46.46	3.79	-
RPM1	31.76	1.01	13.13	7.05	47.05	3.80	0.041
RPM3	31.89	1.06	12.85	6.98	47.22	3.83	0.110
RPM5	31.15	1.07	12.73	6.94	48.11	3.82	0.201
RPM7	30.85	1.09	12.60	6.91	48.55	3.83	0.272

* Solvent extracted

Table 3. Survival and growth parameters in different treatments

Parameters	Diets				
	RPM0	RPM1	RPM3	RPM5	RPM7
Survival (%)	80	100	100	90	90
Final total body length (cm)	6.24 ^{cd}	7.05 ^a	6.56 ^b	6.53 ^b	6.25 ^{cd}
% TLG	12.63	24.77	14.68	13.76	9.26
Final body weight (g)	3.83 ^{de}	4.87 ^a	4.60 ^{bc}	4.37 ^{cd}	3.90 ^{de}
NWG	1.60	2.65 (+65.63%)	2.40 (+50 %)	2.19 (+36.87 %)	1.66 (+3.75)
% NWG	71.74	119.36	109.09	100.45	76.14
FCR	4.17	1.98	2.18	2.84	3.45
PER	0.37	0.79	0.65	0.56	0.45
SGR	0.45	0.66	0.61	0.58	0.47
CF	1.57	1.38	1.62	1.56	1.57

TLG = Total length gain, NWG = Net weight gain, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR= Specific growth rate, CF = Condition factor

Values with same superscript in row do not differ significantly ($P \leq 0.05$)

Figures in parenthesis represent % change over control

Fish growth: Significantly higher ($p \leq 0.05$) fish growth in terms of %TLG, NWG and % NWG was observed in RPM1 (24.77 %, 2.65 g and 119.36 %) followed by RPM3, RPM5, RPM7 and RPM0. Likewise, PER and SGR was maximum in RPM1 (0.79, 0.66) and minimum in RPM0 (0.37, 0.45), whereas CF was maximum in RPM3 (1.62) and minimum in RPM 1 (1.38). The FCR was minimum in RPM1 (1.98) and maximum in RPM0 (4.17) (Table 3).

Improved fish growth has been found in pigment supplemented diets as carotenoids are known to play a positive role in the metabolism of fish (Tacon, 1981) and might improve nutrient utilization leading to improved growth (Amar *et al.*, 2001). In the present study also, it is clear that carotenoid do play a role in fish growth, as rose petal meal @ 1 % and 3 % results in positive effects on growth, in terms of increased weight gain and reduced FCR. Similar results were obtained by Joseph *et al.* (2011) in orange sword tail (*X. helleri*), where out of three levels (1, 3 and 5 %) of *Rosa indica*, 1 % level resulted in maximum daily weight gain. Pailan *et al.* (2012 a,b) reported highest absolute growth rate (g), % weight gain and lowest FCR (1.66) in dwarf gourami, *Colisa lalia* and rosy barb, *Puntius conchonius* with 6 and 4 % rose petal meal, respectively. Ramamoorthy *et al.* (2010) also showed enhanced growth of marine ornamental fish, *Amphiprion ocellaris* with natural pigment sources i.e. carrot, *Daucus carota*; marigold meal, *Tagetes erecta*; china rose petal, *Hibiscus rosasinensis* and rose petal, *Rosa chinensis* as compared to non-pigmented diet. Ezhil *et al.* (2008) and Sinha and Asimi (2007) also reported the positive effect of carotenoids from marigold and china rose petal on growth of sword tail (*X. helleri*) and gold fish (*Carassius auratus*), respectively. Marigold petal meal is being utilized successfully in inducing intense colouration pattern in ornamental fish species like tiger barb (Boonyarapatin and

Lovell, 1977), red sword tail (Ezhil *et al.*, 2008), gold fish (Kaur *et al.*, 2012).

Colour development : Highest carotenoid ($\mu\text{g g}^{-1}$ wet weight basis) content (3.04) was recorded in fish fed with diet RPM 3 followed by RPM5 (2.74), RPM1 (1.96), RPM7 (1.95) and RPM0 (1.24), respectively and was found to be 145.16, 120.96, 58.06 and 57.25 % higher than the fish fed on control diet (RPM0) (Table 4, Fig.1). Likewise digital parameters i.e. red, green and blue colouration on RGB and $L^*a^*b^*$ scale also showed corresponding results. As compared to initial skin colour, enhancement in colour was observed in terms of red, green and blue colouration in all the treatments. Values for red, green and blue colour were maximum in fish fed with diet RPM3 and minimum in fish fed with RPM0 (Table 4, Fig. 2). Similarly, among experimental diets (RPM1-RPM7), L^* (lightness) values were minimum and a^* and b^* were maximum in fish fed with diet RPM3).

Pigmentation of fish is generally affected by various factors including source, chemical structure and concentration of carotenoid, dietary lipids, fish species, feeding period and environmental conditions (Diler and Gokogulu, 2004; Lee *et al.*, 2010). Among natural pigment sources, many of the botanical additives have been used as cheap sources of pigmentation in different ornamental fish species (Ezhil *et al.*, 2008; Ramamoorthy *et al.*, 2010; Swain *et al.*, 2014). In present study, rose petal meal resulted in significantly increased carotenoid concentration at 3 % incorporation levels, showing direct relationship with concentration of carotenoids on deposition of carotenoids. Rose petal meal prepared from various rose varieties is also considered as a potential source of pigmentation at different levels. Pailan *et al.* (2012 a,b) obtained improved colour in dwarf gourami and rosy barb with rose petal meal supplemented diets by increasing RPM incorporation level from 1-6 and 1-4%, respectively. This was in accordance with

Table 4. Total carotenoid content ($\mu\text{g g}^{-1}$ wet weight basis), RGB and laboratory colouration in different treatments

Parameter	At initiation	At termination				
		Diets				
		RPM0	RPM1	RPM3	RPM5	RPM7
Total carotenoid	0.77 ^a	1.24 ^d ----	1.96 ^c (58.06)	3.04 ^a (145.16)	2.74 ^b (120.96)	1.95 ^c (57.25)
R (Red)	59 ^a	75.66 ^c	168.66 ^{ab}	174.66 ^a	160.00 ^b	168.66 ^{ab}
B (Blue)	51 ^a	66.66 ^e	153.33 ^b	165.00 ^a	134.00 ^{cd}	153.33 ^b
G (Green)	28 ^a	51.66 ^{de}	143.00 ^{ab}	159.66 ^a	137.66 ^{cd}	141.66 ^{ab}
L^* (luminosity)	22 ^a	31.00 ^{de}	65.66 ^c	62.00 ^c	67.66 ^a	63.00 ^c
a^* (balance between red / green)	1 ^a	1.89 ^{de}	5.66 ^a	4.66 ^{bc}	3.66 ^c	3.33 ^c
b^* (balance between yellow / blue)	16 ^a	15.00 ^{ab}	14.00 ^{ab}	17.00 ^a	9.33 ^{cd}	12.66 ^{bc}

Values with same superscript in row do not differ significantly ($P \leq 0.05$)

Figures in parenthesis represent % change over control

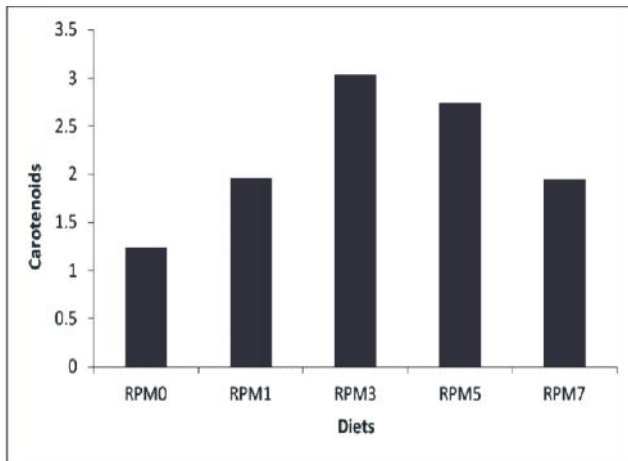


Fig. 1. Comparative total carotenoid content ($\mu\text{g g}^{-1}$ wet weight basis) in fish muscle and skin of koi carp *Cyprinus carpio* L. at the termination of the experiment

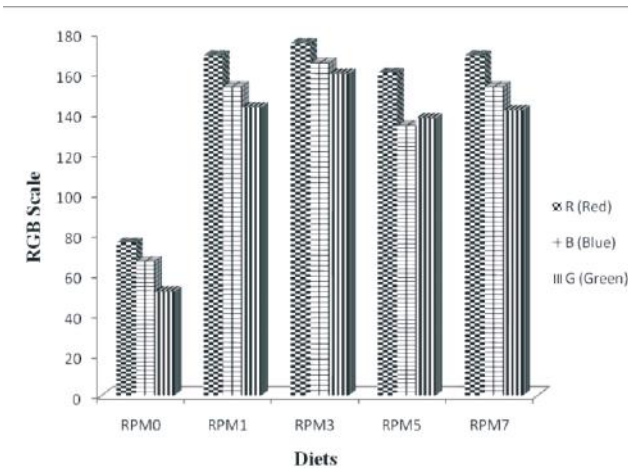


Fig. 2. Comparative RGB values of koi carp *Cyprinus carpio* L. at the termination of the experiment

the result of the present study, where maximum colour enhancement was achieved with RPM at incorporation level of 3 % as compared to both low (1 %) and high incorporation levels (5 and 7 %). Hence, an appropriate level of pigment source can be considered as one of the major factor, which determines optimum colour development in fish as well as shellfish.

The digital photographs showed maximum values for RGB along with decreased L^* and increased a^* and b^* values in RPM3. Similar results were observed by Hancz *et al.* (2003), where gold fish and koi carp were fed with paprika as a feed additive for colouration. Tlustý (2005) while feeding of American Lobster (*Homarus americanus*) also found the colouration values on RGB scale were proportional to amount of astaxanthin carotenoid in the diet. Sun *et al.* (2012) compared the effectiveness of synthetic and natural pigment

sources in colouration of Japanese ornamental koi carp (*C. carpio*). Synthetic carotenoid i.e. carophyll red @ 1.5 g kg^{-1} and natural pigment source i.e. *Spirulina platensis* @ 75 g kg^{-1} of diet resulted in similar colour improvement. This study clearly indicates effectiveness of natural carotenoids similar to synthetic sources in colour enhancement of koi.

It can be concluded from the experimental study that the rose petal meal has a positive effect on growth as well as colour enhancement of koi carp, *Cyprinus carpio* L. in indoor rearing conditions. Hence, rose petal meal can be supplemented in koi carp diet up to 3 % level both as colour enhancer and growth promoter.

ACKNOWLEDGEMENTS

Authors are thankful to Dean, College of Fisheries, GADVASU, Ludhiana, Punjab, for providing necessary research facilities to carry out the research work.

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Interactive Effect of Biofertilizers and Organic Potting Media on Growth and Flowering of *Calendula* (*Calendula officinalis* Linn.)

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Abstract: Studies were carried out to evaluate the interactive effect of biofertilizers and organic potting media on growth and flowering of *Calendula* (*Calendula officinalis* Linn.). Different media compositions comprising Cocopeat + Paddy Straw Compost (PSC), Cocopeat + Spent Mushroom Compost (SMC), Cocopeat + Vermicompost (VC) and Soil + Farm Yard Manure (FYM) were used in definite ratios (1:1) and seedlings were inoculated with biofertilizers such as *Azotobacter*, *Azospirillum* and PSB (Phosphate Solubilizing Bacteria). Maximum plant height (33.70 cm), plant spread (41.96 cm), number of branches/plant (29.13) and duration of flowering in days (125.00) were observed in Cocopeat + VC + PSB. However, early bud appearance (27.60 days), more number of flowers per plant (55.6) and maximum presentable life of a pot plant (82.4 days) were observed in Cocopeat + VC + Control (no inoculation), Cocopeat + VC + *Azospirillum* and Cocopeat + VC + *Azotobacter*, respectively. Bacterial count/density of *Azotobacter* and *Azospirillum* was maximum in Cocopeat + VC media composition counting 11.5×10^4 and 17.6×10^3 cfu/g, respectively. The maximum PSB count (1.20×10^4 cfu/g) was in Cocopeat + PSC.

Key Words: *Azotobacter*, *Azospirillum* and PSB, *Calendula*, Cocopeat + VC

Calendula (*Calendula officinalis* Linn.) commonly known as pot marigold is a herbaceous annual belonging to family Asteraceae and is believed to be native of Southern Europe. The quality production of potted ornamental plants can be obtained by the use of appropriate potting media capable of supplying essential nutrients besides providing good anchorage to plants in pots (Vendrame *et al.*, 2005). Nutrition is one of the important aspects in raising potted ornamental plants to obtain good quality flowers with higher yields per plant. Organic amendments are recognised as an effective mean to improve the structure, fertility, moisture holding capacity and crop yield of the soils and also to increase the crop yield. The use of biofertilizers and biostimulants is amongst the other viable and result oriented strategies to sustainable agricultural production systems (Ehteshami *et al.*, 2007). Organic farming is becoming more popular in India because people are now aware about the disastrous side effects caused by chemical farming on health and the environment and now prefer organically grown foods (Shakila and Anburani, 2008). Therefore, keeping in view the need and importance of biofertilizers and organic fertilizers, the present investigations were conducted with the objective of studying the effect of biofertilizers and organic media on growth and flowering of *calendula*.

MATERIAL AND METHODS

The present investigation was carried out in Punjab Agricultural University during October 2012 to April 2013. The experiment was in a factorial randomized block design with 4 media combination treatments, replicated thrice with 9 plants per replication. Four different media compositions

were selected and mixed in equal ratio. The growing media mixtures were filled in 8-inch earthen pots. Proper care was taken to uniformly fill all pots by tapping to maintain equal compaction levels. The one month old seedlings (4 inches) of *calendula*, of uniform size and age, were transplanted (one seedling per pot). Before transplantation of seedlings into pots, seedlings were given root dip treatment with biofertilizers (*Azotobacter*, *Azospirillum* and Phosphate Solubilizing Bacteria (PSB)) for 30 minutes and then transplanted. The media compositions were Cocopeat + Paddy Straw Compost (PSC), Cocopeat + Spent Mushroom compost (SMC), Cocopeat + Vermicompost (VC) and Soil + Farm Yard Manure (FYM), all in 1:1 ratio. Hence four sub-treatments were control (no inoculation), *Azotobacter* inoculated media, *Azospirillum* inoculated media and PSB inoculated media.

The pots were irrigated once a week during the months of November to February and twice a week during March onward. The mean temperature during the course of the experiment varied from 5 to 34°C. Dried and shriveled buds/flowers were periodically removed to encourage more bud initiation for longer duration of flowering with quality blooms. The air-dried media samples were used for specific determination of maximum water-holding capacity, bulk density (Mg m^{-3}) using the soil core method, organic carbon (C; Nelson and Sommers, 1982), and nitrogen (N, Subbiah and Asija, 1956). The maximum water-holding capacity (WHC) was determined with the help of Keen's box (steel box of 5.0 cm i.d. and 4 cm high with perforated bottom and a filter paper disc fixed with a steel ring at the bottom end) for the soil samples drawn at harvest.

The different treatments and basic characteristics of different growing media are presented in Table 1 and 2.

The observations such as plant height (cm), plant spread (cm), number of branches /plant, number of days to bud emergence, number of flowers and flowering duration (days) were recorded from the onset of reproductive phase until 50% withering of flowers. Bacterial count of *Azotobacter*, *Azospirillum* and PSB was also calculated with respect to different potting media compositions at the time of harvesting. The count of bacteria per gram of potting media was taken at monthly interval. The samples were taken from all the treated plots and were analyzed for their microbial population after a fixed interval of one month. The media samples were collected from near the roots of the plants at a depth of 0-15 cm. The isolation of bacteria from the media samples was done by using three media; Jensen's medium (*Azotobacter* isolation), Dobereiner's medium (*Azospirillum* isolation) and Pikovskaya medium (PSB isolation) following pour plate method for *Azospirillum* and streak plate method for *Azotobacter* and PSB.

Table 1. Basic characteristics of different growing media

Media	N (%)	C (%)	P (%)	K (%)	pH	EC (dSm ⁻¹)
Cocopeat + PSC	0.68	0.79	0.14	0.35	8.26	0.87
Cocopeat + SMC	0.96	1.26	0.13	0.23	7.15	0.38
Cocopeat + VC	1.04	2.08	0.13	0.34	6.96	1.64
Soil + FYM	0.20	1.32	0.14	0.25	7.47	0.39

RESULTS AND DISCUSSION

Vegetative attributes: The maximum (33.70 cm) plant height was obtained in Calendula rooted seedlings were inoculated with PSB and raised in media mixture comprising cocopeat+VC followed by cocopeat+VC+*Azospirillum*, cocopeat+VC+*Azotobacter* and cocopeat+VC, which were at par with each other. The minimum plant height i.e. 22.10cm was obtained in Cocopeat + PSC + *Azospirillum*, which was at par with cocopeat+PSC and Soil+FYM+*Azospirillum*. The present investigation finds the support of Tantawy *et al.* (2009) who recorded the beneficial effect of PSB when seedlings of tomato were inoculated with culture of PSB. The significant improvement in the height of plants inoculated with PSB is due to the fact that these biofertilizers help to solubilize the unavailable form of P into available form thus improved the uptake of P which in turn improved the plant height (Patel *et al.*, 1998). Maximum plant spread of 41.96 cm was obtained when seedlings were treated with T₁₂ followed by T₁₁, T₁₀ and T₉) recording 38.54, 38.49 and 38.09 cm plant spread, respectively which were at par with each other. The minimum plant spread of 25.61 cm was obtained in T₁ (Table 2). Maximum numbers of branches/plant i.e. 29.13 were obtained in T₁₂ which was at par with T₁₀. The minimum number of branches/plant was in T₁ (14.80), which was at par with T₂, T₃, T₄ and T₁₅ (Table 2).

Flowering attributes: The maximum number of flowers per

Table 2. Interactive effect of biofertilizers and organic potting media on vegetative attributes of *Calendula officinalis*

Treatments	Plant height (cm)	Plant spread (cm)	Number of branches /plant	Number of flowers/ plant	Flowering duration (days)	Presentable life of a pot plant (days)
T ₁ Cocopeat + PSC	23.20	25.61	14.80	17.50	90.2	46.2
T ₂ Cocopeat + PSC + <i>Azotobacter</i>	26.30	30.06	15.83	20.20	85.0	45.2
T ₃ Cocopeat + PSC + <i>Azospirillum</i>	22.10	28.10	15.13	22.30	89.8	48.4
T ₄ Cocopeat + PSC + PSB	23.70	30.33	16.30	26.50	87.0	49.4
T ₅ Cocopeat + SMC	24.60	31.39	20.38	30.10	106.0	56.2
T ₆ Cocopeat + SMC + <i>Azotobacter</i>	24.20	30.80	21.33	35.40	109.0	64.4
T ₇ Cocopeat + SMC + <i>Azospirillum</i>	29.70	32.10	22.18	39.10	111.0	56.8
T ₈ Cocopeat + SMC + PSB	26.50	33.99	22.73	38.70	114.0	61.4
T ₉ Cocopeat + VC	30.40	38.09	25.30	49.80	122.0	80.0
T ₁₀ Cocopeat + VC+ <i>Azotobacter</i>	31.50	38.49	28.55	45.90	124.0	82.4
T ₁₁ Cocopeat + VC + <i>Azospirillum</i>	31.80	38.54	26.80	55.60	124.0	78.0
T ₁₂ Cocopeat + VC + PSB	33.70	41.96	29.13	54.20	125.0	78.4
T ₁₃ Soil + FYM	24.80	31.19	19.08	32.50	114.0	70.6
T ₁₄ Soil + FYM+ <i>Azotobacter</i>	26.00	31.00	18.83	29.60	117.0	69.2
T ₁₅ Soil + FYM + <i>Azospirillum</i>	22.30	28.83	15.90	25.50	117.0	71.0
T ₁₆ Soil + FYM + PSB	28.10	34.45	18.93	28.10	113.0	73.0
CD (p=0.05)	1.79	1.85	1.56	0.17	2.11	4.29

plant i.e. were recorded in T_{11} (55.60) followed by T_{12} and T_9 . However, minimum number of flowers per plant were observed in T_1 (17.50). Relatively high nitrogen content of Cocopeat + VC during initial stages might have resulted in better vegetative growth of plants leading to production of more flowers per plant at later stage. Increase in flower production with increasing nitrogen content has been reported by Vijaykumar *et al.* (1998).

Maximum duration of flowering was observed in treatment T_{12} (125.00 days) which was to be at par with T_{11} and T_{10} . The minimum duration of flowering was observed in treatment T_2 (85.00 days) which was at par with T_4 (87.00 days). The increase in days to flowering duration could be attributed to conducive conditions in the substrate and higher nutrient uptake and utilization in plants grown in these substrates (Dutt *et al.*, 2002). Maximum presentable life of a pot plant was observed in T_{10} (82.40 day), which was found to be at par with treatments T_9 and T_{12} . However, minimum presentable life of a pot plant i.e. was observed in treatment T_2 (45.20 days), which was at par with T_1 , T_3 and T_4 . High nitrogen content of VC might have resulted in better vegetative growth of plants leading to more number of days to presentable life of pot plant

Soil physical attributes: The maximum water-holding capacity (WHC) greatly differed among the treatments (Table 3). The media composition T_5 recorded 295.39% WHC, whereas, the media T_{13} recorded the lowest value i.e. 53.60%. The maximum water holding capacity (%) for different organic potting media has been compared in Figure 1. The difference in WHC among the media compositions could be due to diversity in the total porosity and pore-size distribution. The lightweight, fibrous cocopeat adds greater porosity and helps hold greater amounts of water. The lowest (0.02 Mg m⁻³) bulk density was in Cocopeat + VC and the greatest value of bulk density for Soil + FYM (Table 3). The bulk density for different organic potting media has been compared in Figure 2. Being fibrous, the cocopeat fiber evenly spread in the soil, and because of its light weight it reduces the bulk density with simultaneous increment in total porosity, which affects the WHC (Awang *et al.*, 2009).

Count of bacteria per gram of potting media: The organic potting media without inoculation showed some bacterial densities but were less than the inoculated ones. When roots of rooted seedlings of *Calendula officinalis* were treated with *Azotobacter*, Cocopeat + VC organic potting media showed maximum bacterial density of 11.50×10^4 cfu/g followed by Cocopeat + SMC (6.75×10^4 cfu/g), Cocopeat + PSC (6.50×10^4 cfu/g) and Soil + FYM (5.12×10^4 cfu/g), which was higher in the inoculated plants than control. Similar trend was

Table 3. Water-holding capacity, soil bulk density and total porosity of different media

Media composition	Maximum water holding capacity (%)	Bulk density (Mg/m ³)	Total porosity (%)
Cocopeat + PSC (1:1)	274.54	0.10	94.1
Cocopeat + SMC (1:1)	295.39	0.07	96.3
Cocopeat + VC (1:1)	231.09	0.02	98.8
Soil + FYM (1:1)	53.60	0.25	85.9

followed when the roots were treated with *Azospirillum*. The maximum density of 17.60×10^3 cfu/g was in organic potting media Cocopeat + VC followed by Cocopeat + PSC (17.47×10^3 cfu/g), Cocopeat + SMC (16.60×10^3 cfu/g) and Soil + FYM (7.07×10^3 cfu/g). It is well known that *Azospirillum* and *Azotobacter* not only provide nitrogen but also produce a variety of growth promoting substances. The bacterial density increases from sowing to 60 DAT and 120 DAT to harvesting but density decreases from 60-90 DAT. This trend was followed in *Azotobacter* and *Azospirillum* bacteria. The decrease in bacterial count or density may be due to decrease in temperature during January (60-90 DAT). In case of inoculation of roots of rooted seedlings with PSB strain of bacteria, maximum density (1.20×10^4 cfu/g) was observed in Cocopeat + PSC organic potting media followed by Soil + FYM (0.80×10^4 cfu/g), Cocopeat + VC (0.50×10^4 cfu/g) and Cocopeat + SMV (0.36×10^4 cfu/g). This bacterial count was recorded to be higher as compared to the uninoculated one. Higher densities of phosphorus solubilizing bacteria could be attributed due to its capacity to bring insoluble phosphorus in soil into soluble form by producing organic acids.

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Table 4. *Azotobacter*, *Azospirillum* and PSB count per gram of various organic potting media

Organic Potting Media	Treatments	17 th Oct. (Sowing)	18 th Nov. (30 DAT)	18 th Dec. (60 DAT)	17 th Jan. (90 DAT)	16 th Feb. (120DAT)	18 th March (harvesting)
<i>Azotobacter</i> count per gram of various organic potting media. <i>Azotobacter</i> count (cfu/g)							
Cocopeat + PSC	Control	3.10 × 10 ⁴	3.10 × 10 ⁴	3.70 × 10 ⁴	3.05 × 10 ⁴	4.50 × 10 ⁴	5.20 × 10 ⁴
	Inoculated	----	4.04 × 10 ⁴	5.10 × 10 ⁴	3.80 × 10 ⁴	4.70 × 10 ⁴	6.50 × 10 ⁴
Cocopeat + SMC	Control	3.50 × 10 ⁴	3.85 × 10 ⁴	4.05 × 10 ⁴	1.20 × 10 ⁴	3.00 × 10 ⁴	4.90 × 10 ⁴
	Inoculated	----	4.00 × 10 ⁴	4.60 × 10 ⁴	1.50 × 10 ⁴	3.15 × 10 ⁴	6.75 × 10 ⁴
Cocopeat + VC	Control	4.50 × 10 ⁴	4.57 × 10 ⁴	4.80 × 10 ⁴	2.70 × 10 ⁴	4.19 × 10 ⁴	4.50 × 10 ⁴
	Inoculated	----	4.90 × 10 ⁴	5.10 × 10 ⁴	3.10 × 10 ⁴	5.40 × 10 ⁴	11.50 × 10 ⁴
Soil + FYM	Control	0.20 × 10 ⁴	1.60 × 10 ⁴	1.80 × 10 ⁴	0.90 × 10 ⁴	2.40 × 10 ⁴	2.85 × 10 ⁴
	inoculated	----	2.10 × 10 ⁴	2.20 × 10 ⁴	1.25 × 10 ⁴	3.10 × 10 ⁴	5.12 × 10 ⁴
<i>Azospirillum</i> count per gram of various organic potting media. <i>Azospirillum</i> count (cfu/g)							
Cocopeat + PSC	Control	3.70 × 10 ³	4.37 × 10 ³	5.00 × 10 ³	2.65 × 10 ³	7.05 × 10 ³	12.50 × 10 ³
	Inoculated	----	5.75 × 10 ³	11.90 × 10 ³	3.08 × 10 ³	9.50 × 10 ³	17.47 × 10 ³
Cocopeat + SMC	Control	1.75 × 10 ³	2.00 × 10 ³	3.30 × 10 ³	4.17 × 10 ³	9.45 × 10 ³	15.50 × 10 ³
	Inoculated	----	2.50 × 10 ³	4.50 × 10 ³	4.85 × 10 ³	12.00 × 10 ³	16.60 × 10 ³
Cocopeat + VC	Control	2.37 × 10 ³	2.47 × 10 ³	3.70 × 10 ³	2.15 × 10 ³	9.30 × 10 ³	16.20 × 10 ³
	Inoculated	----	3.77 × 10 ³	5.20 × 10 ³	7.70 × 10 ³	13.70 × 10 ³	17.60 × 10 ³
Soil + FYM	Control	0.20 × 10 ³	1.50 × 10 ³	1.75 × 10 ³	0.90 × 10 ³	3.60 × 10 ³	5.60 × 10 ³
	Inoculated	----	2.10 × 10 ³	2.50 × 10 ³	1.50 × 10 ³	5.77 × 10 ³	7.07 × 10 ³
Phosphorus Solubilizing Bacteria (PSB) count per gram of various organic potting media. PSB count (cfu/g)							
Cocopeat + PSC	Control	0.19 × 10 ⁴	0.21 × 10 ⁴	0.10 × 10 ⁴	0.13 × 10 ⁴	0.17 × 10 ⁴	0.20 × 10 ⁴
	Inoculated	----	1.10 × 10 ⁴	0.70 × 10 ⁴	0.90 × 10 ⁴	1.00 × 10 ⁴	1.20 × 10 ⁴
Cocopeat + SMC	Control	0.23 × 10 ⁴	0.26 × 10 ⁴	0.29 × 10 ⁴	0.14 × 10 ⁴	0.25 × 10 ⁴	0.20 × 10 ⁴
	Inoculated	----	0.30 × 10 ⁴	0.38 × 10 ⁴	0.25 × 10 ⁴	0.29 × 10 ⁴	0.36 × 10 ⁴
Cocopeat + VC	Control	0.20 × 10 ⁴	0.20 × 10 ⁴	0.30 × 10 ⁴	0.13 × 10 ⁴	0.30 × 10 ⁴	0.36 × 10 ⁴
	Inoculated	----	0.70 × 10 ⁴	0.50 × 10 ⁴	0.45 × 10 ⁴	0.52 × 10 ⁴	0.80 × 10 ⁴
Soil + FYM	Inoculated	----	0.30 × 10 ⁴	0.45 × 10 ⁴	0.22 × 10 ⁴	0.45 × 10 ⁴	0.50 × 10 ⁴
	Control	0.10 × 10 ⁴	0.12 × 10 ⁴	0.25 × 10 ⁴	0.24 × 10 ⁴	0.22 × 10 ⁴	0.29 × 10 ⁴

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Influence of Substitution of Dietary Fish Oil with Different Vegetable Oils on Growth Performance, Body and Fatty Acid Composition of Juvenile Common Carp, *Cyprinus carpio* (Linn.)

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Abstract: Three different vegetable oils (canola, soybean and sunflower oil) were used in different substitution ratios (25%, 50%, 75% and 100%) with fish oil to investigate the effects of replacing dietary fish oil with different vegetable oil sources on growth performance, muscle proximate and fatty acid composition of juvenile *Cyprinus carpio*. Significant differences were not detected during 60 days feeding trial in net weight gain, specific growth rate and the muscle proximate composition (crude protein, crude lipid, moisture and ash) of common carp. However, significant differences among fish fed control diet versus other treatment diets were determined in the tissue fatty acid composition. The dietary fatty acid profile reflected the fatty acid composition of fish. Vegetable oil inclusion significantly reduced eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations in fish muscle. The ratios of n-3 to n-6 fatty acid also decreased with increasing vegetable oil percentages in all the diets.

Key Words: Common carp, Fish oil replacement, Fatty acids, Vegetable oils

Fish oil is used as the main lipid source in fish diets. Fat soluble vitamins and more importantly fish oil has a high level of n-3 highly unsaturated fatty acids (n-3 HUFA), particularly, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have high health value for fish and human beings. Aquaculture largely depends upon capture fisheries for fish oil used in aquafeeds. Since aquaculture is expanding and capture fisheries is contracting, the supply of fish oil is becoming limited and hence more expensive. It has created pressure on the aqua-feed manufacturers to replace these ingredients with some sustainable alternatives (Pickova and Morkore, 2007). Vegetable oils (VOs) are rich in C18 polyunsaturated fatty acids (C18 PUFA) but lack the n-3 HUFAs such as EPA and DHA which are abundant in FOs (Turchini *et al.*, 2009; Asdari *et al.*, 2011). Some freshwater fish can elongate and desaturate FAs with 18 carbons, specifically linolenic acid to PUFA with 20-22 carbons of the n-3 series (Sargent *et al.*, 2002). This ability to synthesize EPA and DHA from linolenic acid allows the formulation of diets containing less expensive plant oils. Thus, it is important to study the impact of vegetable oils on the growth performance and, more importantly, on the fatty acid composition of the edible part of the fish fed these oils. Realizing the need for the use of vegetable oils as fish feed ingredients, some work in this direction has been done quite recently. The fish species which have been studied include rainbow trout (Masiha *et al.*, 2013; Yildiz *et al.*, 2013); brown trout (Arslan *et al.*, 2012); African catfish (Bababola *et al.*, 2011), etc. There is scanty information about such study on carps, which are important

freshwater food fishes and form about 86% of the Asian aquaculture, which contributes more than 85% to world fish production. Carps are an important freshwater fish species for aquaculture all over the world with an annual global production of 3.4 million tons (FAO, 2012). The present study was therefore, conducted to identify a vegetable oil which is capable of completely or partially replacing fish oil without compromising with nutritional quality and fatty acid composition of *Cyprinus carpio* (Linn.).

MATERIAL AND METHODS

Thirteen isonitrogenous, isoenergetic and isolipidic experimental diets were formulated. The lipids used in diets were fish oil (FO), soybean oil (SBO), canola oil (CO) and sunflower oil (SFO). The first diet contained only fish oil (FO) as the primary lipid source and was named the FO diet. The fish oil was partially or totally replaced by SBO, CO and SFO in other diets containing; 25% (25 SBO, 25 CO and 25 SFO), 50% (50 SBO, 50 CO and 50 SFO), 75% (75 SBO, 75 CO and 75 SFO), and 100% (100 SBO, 100 CO and 100 SFO) alternative lipid sources. The dietary ingredients and proximate compositions of different experimental diets are given in Table 1.

Experimental fish, feeding and sampling: The common carp (*Cyprinus carpio* Linn.) fingerlings were randomly selected for the experiment and acclimatized for one week to laboratory conditions in 100 litre plastic tubs. The experiment was run in triplicates for 60 days in plastic tubs of 34 litres capacity fitted with complete aeration and filtration system, with 12 fishes in each of it. Fish were fed twice daily on

Table 1. Formulation and proximate composition of experimental diets

Ingredients (%)	FO	25CO	50CO	75CO	100CO	25SBO	50SBO	75SBO	100SBO	25SFO	50SFO	75SFO	100SFO
Fish oil	20	15	10	5	20	15	10	5	20	15	20	15	10
Canola oil	-	5	10	15	-	-	-	-	-	-	-	-	-
Soybean oil	-	-	-	-	-	5	10	15	20	-	-	-	-
Sunflower oil	-	-	-	-	-	-	-	-	-	5	10	15	20
Soybean meal	20	20	20	20	20	20	20	20	20	20	20	20	20
Groundnut oil cake	20	20	20	20	20	20	20	20	20	20	20	20	20
Mustard oil cake	20	20	20	20	20	20	20	20	20	20	20	20	20
Wheat flour	5	5	5	5	5	5	5	5	5	5	5	5	5
Rice bran	5	5	5	5	5	5	5	5	5	5	5	5	5
Corn starch	5	5	5	5	5	5	5	5	5	5	5	5	5
Vitamin & mineral mixture	1	1	1	1	1	1	1	1	1	1	1	1	1
Molasses	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Iodized salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (%)													
Moisture	5.21±0.08	5.15±0.11	5.11±0.08	5.05±0.15	5.13±0.06	5.05±0.05	5.15±0.12	5.01±0.12	5.06±0.10	5.18±0.12	5.25±0.10	5.08±0.08	5.18±0.12
Crude protein	26.83±0.58	26.83±1.05	25.95±0.77	25.66±0.77	26.54±0.77	26.83±1.05	26.83±1.27	26.54±1.27	26.83±0.58	26.83±0.30	26.53±0.78	27.12±0.50	26.54±0.98
Total lipid	19.93±0.13	20.10±0.17	20.06±0.13	20.23±0.08	20.30±0.05	20.33±0.08	20.03±0.12	19.93±0.24	320.20±0.05	19.80±0.23	20.20±0.11	20.06±0.13	20.30±0.05
Ash	8.43±0.07	8.28±0.23	8.43±0.07	8.30±0.07	8.43±0.07	8.33±0.06	8.46±0.13	8.16±0.14	8.58±0.08	8.25±0.18	8.43±0.07	8.22±0.15	8.43±0.07
Carbohydrate	39.58±0.80	39.47±1.21	40.42±0.75	40.75±0.67	39.59±0.84	39.45±1.13	39.52±1.24	40.34±1.22	39.31±0.55	39.93±0.35	39.58±0.83	39.50±0.50	39.54±1.40

Note: FO: Fish oil; CO: Canola oil; SBO: Soybean oil; SFO: Sunflower oil; Values are means ± S.E; Values with no superscript in a row do not differ significantly at $p \leq 0.05$

experimental diets @ 5% fish biomass. At the end of experiment each fish was scaled, finned, headed and gutted. The fish samples were then cleaned with tap water and the muscles were taken from whole fish body. Bones were removed and the boneless muscles thoroughly mixed together to form a composite or representative sample of edible portion of the fish. The whole procedure was carried on ice and completed within 10 minutes. The composite sample was packed in clean labeled Ziploc polythene bags and stored at -25 °C for down processes.

Survival and growth performance: Survival (%) was calculated by comparing the live carp recovered at the end of the experiment with the total carp stocked at the start of the experiment. Growth was estimated in terms of net weight gain (NWG) and specific growth rate (SGR) by using the following formulae:

$$\text{NWG (g)} = \text{Final body weight (g)} - \text{Initial body weight (g)}$$

$$\text{ADWG (\%W/d)} = \frac{\text{Average final body weight} - \text{Average initial body weight}}{\text{Period of culture (days)}}$$

$$\text{SGR (\%W/d)} = \frac{L_n \text{ final body weight} - L_n \text{ initial body weight}}{\text{Period of culture (days)}} \times 100$$

Biochemical composition analysis: Proximate analysis of experimental diets and fish flesh (prepared as composite) was conducted using standard procedures (AOAC, 2000). Percentage moisture was determined by drying 2g sample at 100±2°C to constant weight, crude proteins (CP) was measured by Kjeldhal method, total lipid content by solvent extraction method, ash by incineration in a muffle furnace. Carbohydrate content was calculated by difference (FAO, 2004):

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ crude proteins} + \% \text{ total lipids} + \% \text{ ash})$$

Fatty acid analysis was carried out by Gas Chromatography (AOAC, 2000) using M/s Nucon Engineers AIMIL Gas Chromatograph (solid state) model Nucon series 5700/5765 equipped with flame ionization detector.

RESULTS AND DISCUSSION

Growth performance and survival: The NWG (1.18-1.33g) and ADWG (0.020-0.024%) of fish were not significantly different among different treatments. At the end of experiment, survival was 100% in all the treatments. SGR did not show significant differences among all the treatment groups (Table 2).

Proximate and fatty acid composition: No significant differences were observed among the different treatments in

moisture, crude protein, total lipid, ash and carbohydrate content. Crude protein and total lipids varied from 14.28-15.14% to 3.78-3.95%, respectively between different dietary treatments compared to the initial values, which were 12.91 and 2.68%, respectively (Table 3). The fatty acid composition of the diet was mirrored in muscle total lipid of fish. There was a significant difference in the concentration of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and PUFAs in the muscle of *Cyprinus carpio* fed on different experimental diets. In different treatments, palmitic acid and stearic acid were the predominant SFAs. Total SFA was highest in fish fed the 100% SFO and was lowest in fish fed 25% CO. Levels of MUFA ranged from 9.94% (FO) to 48.98% (100% CO) and were observed to be significantly highest in fish fed the 100% CO diet. The highest total n-6 fatty acids were in fish fed 100% SFO and lowest in fish fed 75% SBO. However, the total n-3 fatty acids in fish fed 100% FO were highest, but lowest in fish fed 100% SFO. The level of EPA and DHA in the control (FO) was significantly higher as compared to 100% SFO treatment. The ratios of n-3 to n-6 fatty acid decreased with increasing vegetable oil percentages in all the diets. Therefore, fish fed the 100% FO diet had the highest ratios of n-3 to n-6 fatty acid (Fig. 1). The SFA/PUFA ratio, SFA/UFA ratio and 18:1 n-9/n-3 ratio were highest in the 25 SBO, 75 SBO and 100 CO treatments, respectively.

The results of the present study suggest that canola, soybean and sunflower oils can be used to replace fish oil without adverse effects on growth performance of common carp fingerlings, as reported in other studies (Martino *et al.*, 2002; Regost *et al.*, 2003; Turchini *et al.*, 2003; Karayucel and Dernekbası, 2010). However, total substitution of fish oil by either soybean or linseed oil significantly reduced growth performance of gilthead sea bream (Montero *et al.*, 2008) and fish oil by coconut oil did not affect the growth performance in milkfish (Alava, 1998).

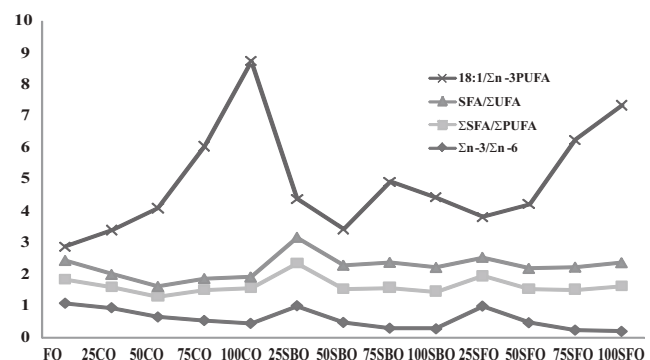


Fig. 1. n-3/n-6 ratio, SFA/PUFA ratio, SFA/UFA ratio and 18:1 n-9/n-3 ratio of *Cyprinus carpio* (Linn.) fed different experimental diets

Table 2. Fatty acid composition (%) of experimental diets

Treatment/FA	FO	25CO	50CO	75CO	100CO	25SBO	50SBO	75SBO	100SBO	25SFO	50SFO	75SFO	100SFO
10:0	3.89 ^a	3.52 ^a	2.54 ^b	1.28 ^c	0.59 ^d	3.70 ^a	2.60 ^b	0.83 ^d	0.27 ^e	3.42 ^a	2.32 ^b	1.09 ^c	0.36 ^e
11:0	1.66 ^a	0.89 ^b	0.86 ^b	0.45 ^c	0.11 ^d	1.27 ^{ab}	0.96 ^b	0.53 ^c	0.25 ^{cd}	1.21 ^{ab}	0.84 ^b	0.35 ^{cd}	0.00
14:0	3.92 ^a	3.78 ^a	3.56 ^a	3.38 ^a	3.29 ^{ab}	3.29 ^{ab}	2.70 ^b	2.08 ^c	1.41 ^{de}	2.96 ^b	1.85 ^c	0.93 ^e	0.50 ^f
16:0	17.5 ^a	14.33 ^c	11.48 ^{de}	7.96 ^f	4.56 ^g	16.57 ^b	14.63 ^c	12.72 ^d	10.55 ^e	16.02 ^b	13.56 ^{cd}	11.09 ^{de}	8.59 ^f
18:0	12.31 ^a	9.13 ^c	7.21 ^d	4.25 ^e	1.38 ^e	10.03 ^b	7.77 ^d	5.46 ^e	3.21 ^e	9.71 ^{bc}	7.13 ^d	4.48 ^e	1.98 ^f
ΣSFA ^a	39.28 ^a	31.65 ^c	25.65 ^e	17.32 ^f	9.93 ^h	34.86 ^b	28.66 ^d	21.62 ^{ef}	15.69 ^f	33.32 ^b	25.70 ^e	17.94 ^f	11.43 ^g
18:1 n-9	25.84 ^f	34.92 ^d	42.26 ^c	52.35 ^b	58.53 ^a	27.13 ^f	26.21 ^f	25.39 ^f	24.66 ^f	29.04 ^e	30.24 ^e	31.48 ^{de}	32.43 ^{de}
Σ MUFA ^b	25.84 ^{gh}	34.92 ^d	42.26 ^c	52.35 ^b	58.53 ^a	27.13 ^g	26.21 ^g	25.39 ^{gh}	24.66 ^h	29.04 ^f	30.24 ^f	31.48 ^e	32.43 ^e
18:3 n-3	12.40 ^a	11.65 ^{ab}	11.13 ^b	10.45 ^c	9.26 ^d	11.06 ^b	9.88 ^{cd}	8.62 ^e	7.31 ^f	10.60 ^c	8.82 ^e	7.02 ^f	5.13 ^g
20:5 n-3	2.46 ^a	1.84 ^b	1.24 ^c	0.60 ^d	0.11 ^e	1.80 ^b	1.28 ^c	0.53 ^d	0.13 ^e	1.73 ^b	0.95 ^c	0.48 ^d	0.12 ^e
22:6 n-3	3.04 ^a	2.21 ^b	1.51 ^c	0.74 ^d	0.23 ^e	2.28 ^b	1.38 ^c	0.69 ^d	0.27 ^e	2.08 ^b	1.36 ^c	0.63 ^d	0.26 ^e
Σn-3PUFA ^c	17.9 ^a	15.70 ^b	13.88 ^c	11.79 ^d	9.60 ^f	15.14 ^b	12.54 ^c	9.84 ^d	7.71 ^e	14.41 ^{bc}	11.13 ^{de}	8.13 ^g	5.51 ^h
18:2 n-6	9.51 ^f	11.85 ^k	14.20 ^j	16.53 ^j	18.08 ^h	19.91 ^h	30.32 ^f	40.71 ^d	48.13 ^a	18.94 ^h	28.40 ^g	37.85 ^e	45.23 ^b
20:4 n-6	5.75 ^a	5.04 ^b	3.22 ^c	1.63 ^d	0.36 ^f	5.88 ^a	3.02 ^c	1.58 ^d	0.51 ^e	5.06 ^b	3.412 ^c	1.50 ^d	0.38 ^e
Σn-6PUFA ^d	15.26 ^h	16.89 ^{gh}	17.42 ^g	18.16 ^g	18.44 ^g	25.79 ^f	33.34 ^e	42.29 ^c	48.64 ^a	24.00 ^f	31.81 ^e	39.35 ^d	45.61 ^b
ΣPUFA ^e	33.16 ^f	32.59 ^f	31.30 ^f	29.95 ^f	28.04 ^f	40.93 ^d	45.88 ^c	52.13 ^b	56.35 ^a	38.41 ^e	42.94 ^d	47.48 ^c	51.12 ^b
Σ UFA ^f	59.00 ^g	67.51 ^f	73.56 ^e	82.30 ^{bc}	86.57 ^a	68.06 ^f	72.09 ^e	77.52 ^d	81.01 ^c	67.45 ^f	73.18 ^e	78.96 ^d	83.55 ^b
n3/n6	1.17 ^a	0.92 ^{ab}	0.80 ^b	0.65 ^c	0.52 ^d	0.58 ^{cd}	0.37 ^e	0.23 ^f	0.15 ^g	0.60 ^{cd}	0.35 ^e	0.20 ^f	0.12 ^g
SFA/PUFA	1.18 ^a	0.97 ^a	0.82 ^b	0.58 ^c	0.35 ^d	0.85 ^b	0.62 ^c	0.41 ^{cd}	0.27 ^e	0.86 ^b	0.60 ^c	0.37 ^d	0.22 ^e
SFA/UFA	0.66 ^a	0.47 ^b	0.35 ^c	0.21 ^d	0.11 ^e	0.51 ^b	0.40 ^c	0.27 ^d	0.19 ^e	0.50 ^b	0.35 ^c	0.22 ^d	0.13 ^e
18:1 n-9/n-3	1.44 ^g	2.22 ^e	3.04 ^d	4.44 ^b	6.09 ^a	1.80 ^f	2.09 ^e	2.58 ^{de}	3.20 ^d	2.01 ^e	2.71 ^{de}	3.87 ^c	5.88 ^a

Note: FA: Fatty acid; FO: Fish oil; CO: Canola oil; SBO: Soybean oil; SFO: Sunflower oil; ΣSFA includes Capric acid (10:0), Undecylic acid (11:0), Myristic acid (14:0), Palmitic acid (16:0) and Stearic acid (18:0); ΣMUFA includes Oleic acid (18:1n-9); Σn-3 PUFA includes Linolenic acid (18:3n-3), Eicosapentaenoic acid (20:5n-3) and Docosahexaenoic acid (22:6n-3); Σn-6 PUFA includes Linoleic acid (18:2n-6) and Arachidonic acid (20:4n-6); ΣPUFA includes Σn-3 PUFA and Σn-6 PUFA; ΣUFA includes ΣPUFA and ΣMUFA; Values are means ± S.E; Values with different superscript in a row differ significantly at p ≤ 0.05

Likewise, no reduction in growth with high fish oil replacement by vegetable oils in diet of Atlantic salmon was reported and provided minimum EPA + DHA level (Turchini *et al.*, 2009). Further, vegetable oils have been reported to improve the growth of some freshwater fishes (Ng *et al.*, 2003). It has also been shown that high levels of dietary n-3 HUFA decreased the growth performance of Atlantic salmon (*Salmo salar*) (Menoyo *et al.*, 2003). Muscle proximate composition in different trials was also not significantly

affected by addition of vegetable oils in experimental diets. The considerable differences were recorded in the fatty acid composition of fish fed different experimental diets. Studies show that replacing fish oil with vegetable oils consistently results in major changes in fatty acid profile of fish (Turchini *et al.*, 2003). Major consequences of these effects can be altered lipid content and fatty acid composition of the flesh, both of which can alter the nutritional quality of the fish as food for the human consumption (Sheehan *et al.*, 1996;

Table 3. Growth performance of *Cyprinus carpio* (Linn.) fed different experimental diets

Parameters	NWG (g)	ADWG (%W/d)	SGR (%W/day)	Survival (%)
FO	1.20±0.02	0.021±0.001	0.20±0.01	100±0.00
25 CO	1.23±0.02	0.022±0.001	0.19±0.01	100±0.00
50 CO	1.25±0.02	0.022±0.001	0.21±0.00	100±0.00
75 CO	1.18±0.02	0.021±0.000	0.22±0.01	100±0.00
100 CO	1.21±0.03	0.022±0.001	0.19±0.01	100±0.00
25 SBO	1.26±0.02	0.024±0.001	0.21±0.00	100±0.00
50 SBO	1.25±0.03	0.024±0.000	0.20±0.00	100±0.00
75 SBO	1.26±0.03	0.024±0.000	0.22±0.01	100±0.00
100 SBO	1.21±0.02	0.023±0.001	0.20±0.00	100±0.00
25 SFO	1.26±0.06	0.020±0.000	0.19±0.01	100±0.00
50 SFO	1.37±0.12	0.023±0.001	0.21±0.00	100±0.00
75 SFO	1.28±0.06	0.021±0.000	0.23±0.02	100±0.00
100 SFO	1.33±0.09	0.022±0.001	0.22±0.01	100±0.00

Note: FO: Fish oil; CO: Canola oil; SBO: Soybean oil; SFO: Sunflower oil; NWG: Net weight gain (g); ADWG: Average daily weight gain (%W/d); SGR: Specific growth rate (%W/d); Survival (%); Values are means ± S.E; Values with no superscript in a row do not differ significantly at $p \leq 0.05$

Table 4. Proximate composition (%) of *Cyprinus carpio* (Linn.) fed different experimental diets

Parameters Treatments	Moisture	Crude protein	Total lipid	Ash	Carbohydrate
Initial (Zero day)	76.53±0.06	12.91±0.20	2.68±0.04	1.53±0.04	6.34±0.33
After 60 days					
FO	75.45±0.52	14.58±0.50	3.78±0.02	1.43±0.04	5.53±0.40
25 CO	73.88±0.25	14.87±0.50	3.92±0.08	1.40±0.07	5.92±0.64
50 CO	73.90±0.28	14.58±0.77	3.90±0.07	1.36±0.01	6.25±0.92
75 CO	74.20±0.76	14.95±0.58	3.94±0.07	1.48±0.09	5.41±0.68
100 CO	74.58±0.70	14.87±0.50	3.87±0.07	1.48±0.03	5.18±0.94
25 SBO	73.25±0.05	14.87±0.50	3.85±0.07	1.46±0.04	6.07±0.96
50 SBO	73.73±0.36	15.16±0.30	3.86±0.06	1.50±0.01	5.74±0.31
75 SBO	73.68±0.09	15.44±0.57	3.94±0.07	1.56±0.01	5.43±0.56
100 SBO	74.11±0.31	14.87±0.50	3.90±0.07	1.51±0.01	5.57±0.59
25 SFO	73.88±0.25	14.58±0.30	3.90±0.06	1.45±0.03	6.18±0.41
50 SFO	73.88±0.25	14.28±0.58	3.90±0.07	1.43±0.06	5.62±0.80
75 SFO	73.96±0.19	14.87±0.50	3.87±0.07	1.48±0.06	5.80±0.70
100 SFO	73.91±0.28	14.95±0.58	3.95±0.07	1.53±0.01	5.64±0.83

See Table 2 for treatment details

Table 5. Fatty acid composition of *Cyprinus carpio* (Linn.) fed different experimental diets

Treatment	FO	25CO	50CO	75CO	100CO	25SBO	50SBO	75SBO	100SBO	25SFO	50SFO	75SFO	100SFO
10:0	4.42 ^b	2.62 ^a	2.75 ^d	3.30 ^c	2.36 ^d	5.41 ^a	4.89 ^{ab}	4.41 ^b	5.83 ^a	2.56 ^d	3.08 ^c	3.06 ^c	3.10 ^c
11:0	2.40 ^a	1.47 ^c	1.42 ^c	2.11 ^{ab}	1.14 ^d	1.91 ^b	1.20 ^d	1.50 ^c	2.40 ^a	1.86 ^b	1.77 ^b	1.29 ^d	1.92 ^b
14:0	-	-	0.39 ^d	0.50 ^c	0.74 ^b	0.60 ^{bc}	0.92 ^a	1.09 ^a	0.32 ^e	0.39 ^f	0.31 ^e	0.40 ^d	0.41 ^d
16:0	9.50 ^f	12.39 ^d	14.86 ^{bc}	15.52 ^b	14.22 ^c	11.81 ^d	9.25 ^f	10.17 ^e	9.58 ^e	8.04 ^b	14.80 ^{bc}	15.91 ^b	17.53 ^a
18:0	15.96 ^d	6.75 ^f	4.01 ^g	4.99 ^{fg}	5.84 ^f	22.73 ^a	17.43 ^c	18.91 ^b	18.62 ^{bc}	14.38 ^e	14.24 ^b	17.76 ^c	19.88 ^b
ΣSFA ^a	32.38 ^c	23.25 ^f	23.44 ^f	25.94 ^e	24.30 ^{ef}	42.13 ^a	33.71 ^c	35.86 ^c	36.66 ^b	27.17 ^d	34.21 ^b	37.76 ^b	42.85 ^a
18:1 n-9	9.94 ^f	23.97 ^e	37.01 ^c	43.06 ^b	48.98 ^a	19.25 ^g	12.08 ^f	17.23 ^{gh}	16.34 ^h	18.16 ^g	21.48 ^f	24.43 ^e	27.07 ^d
Σ MUFA ^b	9.94 ^f	23.97 ^e	37.01 ^c	43.06 ^b	48.98 ^a	19.25 ^g	12.08 ^f	17.23 ^{gh}	16.34 ^h	18.16 ^g	21.48 ^f	24.43 ^e	27.07 ^d
18:3 n-3	18.37 ^a	13.78 ^b	13.00 ^c	9.17 ^e	6.63 ^f	13.01 ^c	9.31 ^e	5.44 ^g	7.00 ^f	11.81 ^d	8.96 ^e	5.04 ^g	5.08 ^g
20:5 n-3	1.78 ^a	1.78 ^a	0.98 ^b	0.50 ^d	0.25 ^f	1.04 ^b	0.28 ^f	0.38 ^e	0.03 ^h	0.89 ^b	0.60 ^c	0.40 ^e	0.11 ^g
22:6 n-3	2.34 ^a	1.74 ^b	0.95 ^c	0.63 ^d	0.31 ^e	1.76 ^b	1.00 ^c	0.58 ^d	0.35 ^e	1.66 ^b	0.98 ^c	0.62 ^d	0.24 ^f
Σn-3PUFA ^c	22.50 ^a	17.12 ^b	14.95 ^c	9.67 ^e	6.88 ^f	15.81 ^c	10.60 ^e	6.76 ^f	7.39 ^f	14.37 ^d	10.54 ^e	6.07 ^{df}	5.44 ^g
18:2 n-6	8.20 ^{bc}	8.71 ^b	11.48 ^a	8.94 ^b	8.41 ^{bc}	6.37 ^d	9.39 ^b	6.01 ^d	6.26 ^d	7.72 ^c	9.30 ^b	8.91 ^b	7.61 ^c
20:4 n-6	12.16 ^c	8.99 ^e	9.52 ^e	7.88 ^f	6.07 ^g	9.13 ^e	11.97 ^d	15.13 ^c	19.59 ^a	6.40 ^{fg}	12.03 ^d	15.04 ^c	17.00 ^b
Σn-6PUFA ^d	20.36 ^c	17.71 ^b	21.00 ^c	17.16 ^d	14.48 ^f	15.50 ^e	21.36 ^c	12.14 ^b	23.85 ^{ab}	14.12 ^f	21.34 ^c	23.29 ^b	24.60 ^a
ΣPUFA ^e	42.86 ^a	34.83 ^b	35.95 ^b	26.83 ^e	21.37 ^f	31.31 ^{cd}	31.96 ^c	27.90 ^e	31.24 ^{cd}	28.47 ^{de}	31.88 ^c	29.36 ^{fg}	30.04 ^d
Σ UFA ^f	52.80 ^c	57.07 ^b	72.96 ^a	69.90 ^a	70.36 ^a	50.56 ^d	44.04 ^f	45.13 ^f	47.58 ^e	46.63 ^e	53.38 ^c	53.79 ^b	57.11 ^b
n3/n6	1.10 ^a	0.96 ^a	0.67 ^c	0.56 ^d	0.47 ^e	1.02 ^a	0.50 ^{de}	0.32 ^f	0.30 ^f	1.01 ^a	0.49 ^{de}	0.26 ^c	0.22 ^g
SFA/PUFA	0.75 ^e	0.66 ^e	0.65 ^e	0.96 ^d	1.13 ^c	1.34 ^a	1.05 ^d	1.28 ^b	1.18 ^c	0.95 ^d	1.07 ^d	1.28 ^c	1.42 ^b
SFA/UFA	0.61 ^d	0.40 ^e	0.32 ^e	0.37 ^e	0.34 ^e	0.83 ^a	0.76 ^b	0.80 ^a	0.76 ^b	0.60 ^d	0.65 ^d	0.71 ^{bc}	0.75 ^b
18:1 n-9/n-3	0.44 ^f	1.40 ^e	2.47 ^d	4.18 ^{bc}	6.81 ^a	1.21 ^e	1.14 ^e	2.54 ^d	2.21 ^d	1.26 ^c	2.03 ^e	4.02	4.97

Note: Values with no superscript in a row do not differ significantly at $p \leq 0.05$

Johansen and Jobling, 1998; Torstensen *et al*, 2000; Rosenlund *et al*, 2001; Bell *et al*, 2001, 2002).

The substitution of fish oil with different vegetable oils in the common carp diet have been possible without any negative effects on the growth performance and muscle proximate composition. However, the reflection of the dietary oil source on the muscle fatty acid composition of the fish could be a potential drawback for vegetable oil substitution from a human nutritional point of view, given the decreased in levels of EPA and DHA in fish fed the vegetable oil diets. Further investigation into the benefits of other vegetable oils or indeed a blend of various vegetable oils is required in order to reduce usage of traditionally used fish oils. Another method could be by re-feeding with 100% fish oil diets to recover n-3 HUFA levels in fish previously fed mixed vegetable oils.

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Received 20 October, 2015; Accepted 5 April, 2016



Degradation Status of Amarogentin on Storage in Different Solvents

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Abstract: *Swertia chirayita* is an important medicinal plant which is known for its bitter principles. The bitterness in *S. chirayita* is due to presence of two secoiridoid glycosides amarogentin and amaroswerin. Stability of amarogentin (a bitter compound) in different solvent systems was determined by using HPLC technique to identify the best solvent systems for its storage purpose. Pure amarogentin (isolated from *Swertia chirayita*) was stored in methanol, methanol-water mixtures (75:25, 50:50, and 25:75) and water for six months. The initial value of amarogentin content (99.87%) recorded at the time of storage, reduced to 97.21% in pure methanol, 27.81% in 75:25 mixture of methanol: water, 18.35% in 50:50 mixture of methanol: water, 21.46% in 25:75 mixture of methanol: water and 45.83% in pure water after six months of storage. The stored samples were also qualitatively monitored through TLC using chloroform: methanol: water (65:25:10) as the mobile phase and fast red B salt solution as the detection reagent. Additional spots detectable with fast red B salt solution started appearing after four and six months of storage. Additional peaks were also observed in the HPLC chromatograms.

Key Words: Amarogentin stability, HPLC and TLC, *Swertia chirayita*

Swertia is an important genus of family Gentianaceae (Order: Gentianales), which is chiefly distributed in the mountainous regions of Tropical Asia, Europe, America and Africa. Out of 40 species of *Swertia* reported in India (Anonymous, 1976), 32 species occur in the Indian Himalayan Region (IHR) with 16 species restricted to northwest Himalaya (Garg, 1987). Although several *Swertia* species have been described in different traditional systems of medicines useful in the treatment of various ailments, *Swertia chirayita* occupies the most prominent position as regards its therapeutic properties (Joshi and Dhawan, 2005). *Swertia chirayita* is reported to occur at altitude of 1200-3000m from Kashmir to Bhutan and Khasi hills at 1200-1500m. Crude drug supplies of *S. chirayita* still depend on wild sources which are becoming critical on account of over harvesting leading to its categorization under 'Critically Endangered' category (Anonymous, 1997; Shreshta and Joshi, 1992; Rai *et al.*, 2000; Badola and Pal, 2002).

Swertia chirayita holds a place of pride in the Indian system of medicine since ages and its medicinal usage is reported in Indian, British and American pharmacopoeias (Anonymous, 1976). The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin (most bitter compound isolated), swerchirin and swertiamarin (Nadkarni, 1998; Keil *et al.*, 2000). Amarogentin (bioactive compound) is a secoiridoid glycoside and is the most acerbic substance and tastes bitter even at a dilution of 1:58,000,000 (Arino *et al.*, 1997).

The bioactive compounds in different medicinal plants differ from each other in having different types of chemical structures leading towards their different degree of stabilities.

These compounds oftenly undergo degradation during drying, storage and processing due to enzymatic and microbiological reactions, moisture, light, heat which affect their concentration significantly. Knowledge of methods and conditions of storage of bioactive contents of such a highly medicinal important plant are very important in order to maintain the quality of a given product after harvest/extraction/isolation. Though no specific studies on stability of amarogentin (a secoiridoid types of bitter compound) on storage in different solvents are available, therefore a study was conducted to determine stability of amarogentin in different solvent systems to ensure the most probable solvent system that would meet quality preservation of amarogentin in liquid form for common application.

MATERIAL AND METHODS

The present study was carried out at Dr. Y. S. Parmar University of Horticulture and Forestry, Solan, H.P. The pure amarogentin used during the present study was isolated from the field grown plants of *Swertia chirayita* cultivated at medicinal and aromatic plants research farm, Shilly (Altitude 1550m amsl, latitude- N 30° 54' 30" and longitude E 77° 07' 30").

Extraction and isolation of amarogentin: The air dried, powdered stem part of *Swertia chirayita* was initially defatted with petroleum ether (40-60°C) and then bitter compounds were extracted with methyl alcohol through refluxion. The dried methanolic extract was then mixed with water and less polar compounds were removed by repeated extraction with chloroform through separating funnel. The aqueous layer containing bitter compounds was then extracted with n-butyl

alcohol. The n-butyl alcohol extract containing bitter compounds was dried in rotary vacuum evaporator. The separation of residue containing bitter compounds into individual bitter compounds was done through column chromatography using silica gel (60-120 mesh) as adsorbent and CHCl_3 : MeOH (95:5-85:15) as solvent system. The different fractions obtained from column were monitored through TLC by using lower layer of chloroform: methanol: water (65:20:10) as solvent system and fast red-B salt solution as detection reagent. The pooled fractions containing Amarogentin were concentrated, dried under vacuum and further purified by crystallization by dissolving in minimum amount of methanol and ethyl acetate. The authenticity of purified amarogentin was ascertained through comparison with standard amarogentin by TLC and HPLC. This isolated amarogentin was used for conducting the experiment.

Solvents and storage durations: The amarogentin was stored in five different solvent mixtures viz. methanol, methanol: water (75:25), methanol: water (50:50), methanol: water (25:75), water. The period of storage was upto six months and status of amarogentin was tested every two months through TLC and HPLC. Storage at each duration was replicated three times. The analysis of variance using CRD factorial design was applied on the data for comparison.

HPLC analysis: Analytical method for monitoring of amarogentin was standardized on binary Waters HPLC unit using Waters HPLC pump 515 with Waters spherisorb 5 μm ODS 2 analytical column (4.6 x 250mm) and dual λ absorbance detector 2487. Twenty microlitre of samples were injected and run at flow rate of 1.0ml/minute using methanol: water (45:55) as the mobile phase and monitoring was done at 235nm. The calibration curve was linear having correlation coefficient 0.9978 for three concentrations (50 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$ & 200 $\mu\text{g/ml}$) replicated four times.

Percentage of amarogentin in samples was calculated by the following formula:

$$\text{Bitter content \%} = \frac{\text{Test area}}{\text{Standard area}} \times \frac{\text{Wt. of standard}}{\text{Standard dilution}} \times \frac{\text{Test dilution}}{\text{Test weight}} \times 100$$

RESULTS AND DISCUSSION

The observations on the effect of the storage durations (0-6 months) under different solvent systems were recorded for variation in amarogentin content (%) and are presented in Table 1 and Table 2. The analysis of stored samples showed appreciable decrease in amarogentin content in methanol: water mixtures as well as in water. The initial value of amarogentin content (99.87%) recorded at the time of storage, decreased to 97.21% in pure methanol, 27.81% in methanol: water (75:25) mixture, 18.35% in methanol: water (50:50) mixture, 21.46% in methanol: water (25:75) mixture and 45.83% in pure water after six months of storage. Among different solvent systems taken for storage of amarogentin, the mean value of amarogentin after six months storage was found maximum (98.58%) in methanol and minimum (45.23%) in methanol: water (50:50%) mixtures. The mean value of amarogentin content (99.87%) in different solvent systems recorded at the time of storage decreased to the 42.13 % after six months of storage. Storage durations had significant effect on per cent decrease in amarogentin content. Among different solvent systems, the average per cent decrease in amarogentin content was 2.66% in pure methanol, 72.06% in methanol: water (75:25) mixture, 81.52 % in methanol: water (50:50) mixture, 78.41% in methanol: water (25:75) mixture and 54.04% in pure water after six months of storage (Table 2). The average % loss in amarogentin among different storage durations varied from 38.48 % to 57.74% (Table 2). The maximum % loss in amarogentin was after six month of storage.

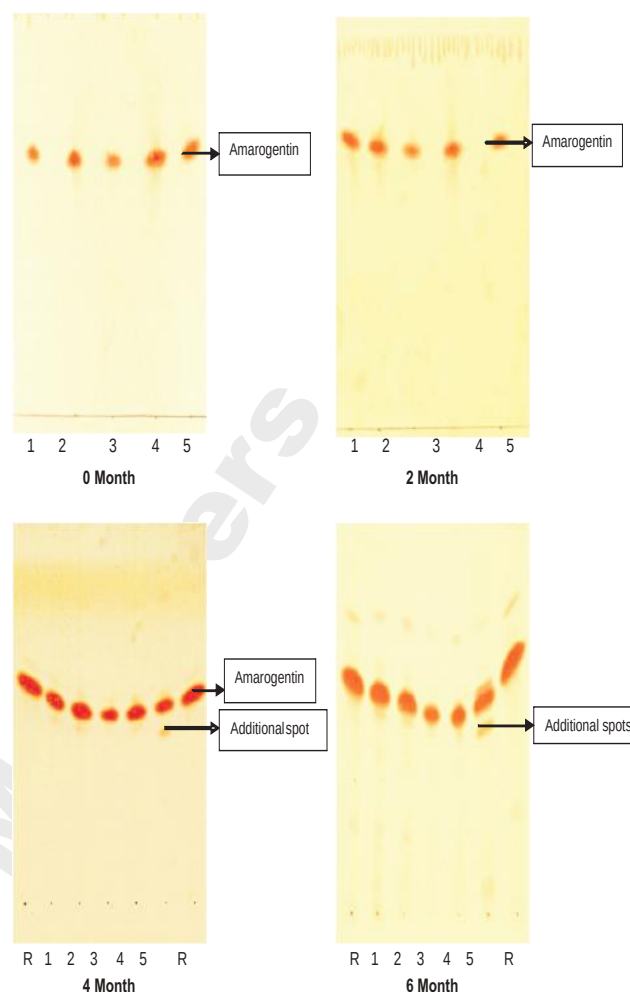
Table 1. Effect of different storage durations under different solvents on the stability of amarogentin

Solvents	Storage durations				
	0 Month	2 month	4 month	6 month	Mean
Methanol	99.87 (88.09)	99.04 (84.46)	98.20 (82.31)	97.21 (80.47)	98.58 (83.83)
Methanol: Water (75:25%)	99.87 (88.09)	46.45 (42.96)	28.30 (32.14)	27.81 (31.83)	50.61 (48.76)
Methanol: Water (50:50%)	99.87 (88.09)	40.22 (39.36)	22.50 (28.31)	18.35 (25.36)	45.23 (45.28)
Methanol: Water (25:75%)	99.87 (88.09)	47.65 (43.65)	25.05 (30.04)	21.46 (27.59)	48.51 (47.34)
Water	99.87 (88.09)	73.61 (59.10)	52.96 (46.70)	45.83 (42.61)	68.07 (59.12)
Mean	99.87 (88.09)	61.39 (53.91)	45.40 (43.90)	42.13 (41.57)	

*Figures in are transformed value using arc sine transformation

	CD (p=0.05)	SE ($\pm$)
Storage durations	0.636	0.314
Solvent systems	0.711	0.352
Storage durations x Solvent systems	1.003	0.497

TLC profile of amarogentin showed additional spots with fast red B salt solution after four and six months of storage indicating degradation of amarogentin content (Fig.1). Out of five solvent/solvent mixture additional spots started appearing after two and six months in amarogentin sample stored in pure water only. Appearance of number of additional spots started increasing with increased in storage durations. Additional peak was also visible in the HPLC chromatograms of amarogentin in solvent mixtures methanol-water in the ratio of 75:25%, 50:50% and 25:75% after six months of storage plates (Fig. 3, 4, 5). The structure of bitter secoiridoid amarogentin has lactone ring, ester group and phenolic hydroxyl groups. In presence of water under long storage both lactone ring and ester group undergoes hydrolysis, thus it could be foremost reason for maximum degradation of amarogentin after prolonged storage in solvent mixture with water. However, the exact degradation mechanism can be known only after isolation of the new compounds formed during storage. Such studies on stability of secoiridoids in genus *Swertia* particularly *S. chirayita* has not been done earlier however, similar studies have been made to some extent in other medicinal plants. Our studies are in confirmation with the related studies conducted on degradation of bioactive compounds in *Valeriana officinalis* by Wills and Shohet (2009). They analyzed dried valerian (*Valeriana officinalis*) root powder at 5, 14 and 30°C under low, moderate and high humidity conditions for 6 months and observed that the concentration of valerenic acid significantly decreased over time. Similar findings has been found on degradation of valepotriates by Gupta *et al.* (2011). TLC and HPLC techniques were also found effective in detecting degradation in Stevioside-A and Rebaudioside-A in carbonated phosphoric citric acidified beverages after two months of storage (Chang and Cook, 1983). Storage durations (24, 48, and 72 h at 80 °C) had also



Amarogentin stored in Solvents/ Solvent mixture:

1. Methanol 2. Methanol: water (75:25) 3. Methanol: water (50:50)
4. Methanol: water (25:75) 5. Water
R. Amarogentin (RefSpraying reagent- Fast Red B salt solution)
TLC Mobile phase- chloroform: methanol: water (65:25:10)

Fig. 1. TLC fingerprint profile of amarogentin stored in different solvents

Table 2. Percent decrease in amarogentin content on storage in different solvents

Solvents	Storage durations			
	2 month	4 month	6 month	mean
Methanol	0.83 (5.13)	1.67 (7.41)	2.66 (9.30)	1.72 (7.28)
Methanol: Water (75:25%)	53.42 (46.95)	71.57 (57.76)	72.06 (58.07)	65.68 (54.26)
Methanol: Water (50:50%)	59.65 (50.55)	77.37 (61.58)	81.52 (64.51)	72.84 (58.88)
Methanol: Water (25:75%)	52.22 (46.25)	74.82 (59.86)	78.41 (62.29)	68.48 (56.13)
Water	26.26 (30.81)	46.91 (43.21)	54.04 (47.30)	42.40 (40.44)
Mean	38.48 (35.94)	54.47 (45.96)	57.74 (48.29)	

*Values in Parentheses are transformed value using Arc sine transformation

	CD (p=0.05)	SE (±)
Storage durations	0.629	0.217
Solvent systems	0.813	0.820
Storage durations x Solvent systems	1.407	0.485

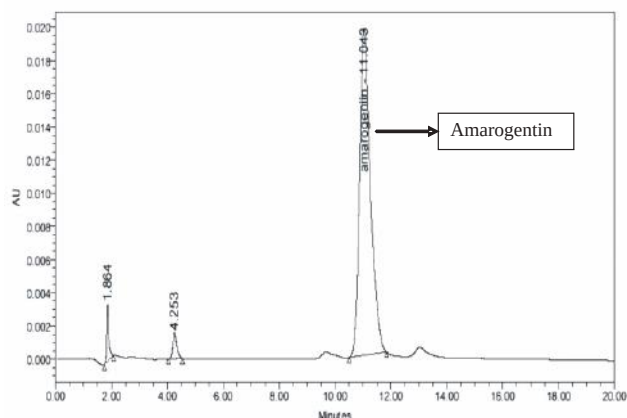


Fig. 2. HPLC chromatogram of stored samples of amarogentin at 0 month

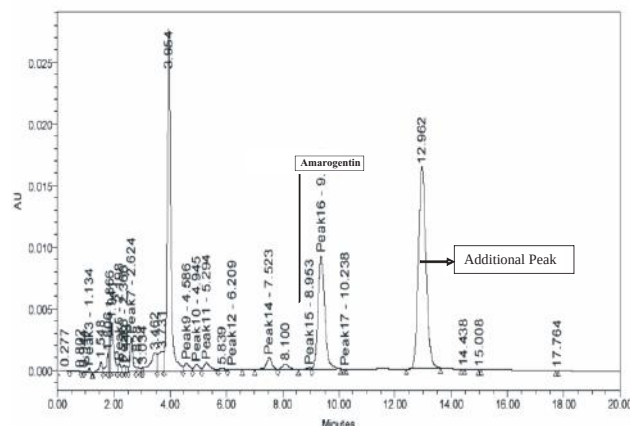


Fig. 3. HPLC chromatograms of stored samples of amarogentin in methanol: water (75:25) after 6 months of storage

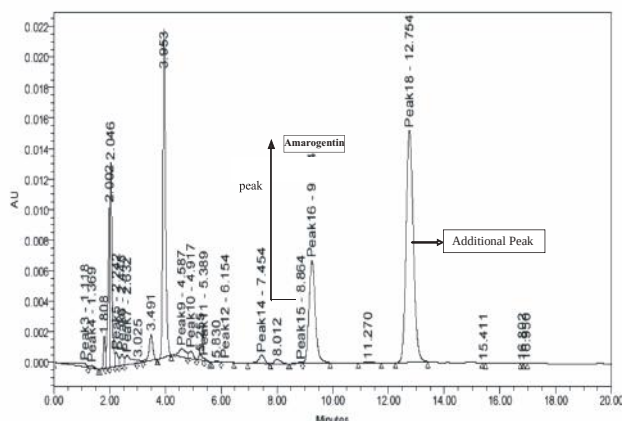


Fig. 4. HPLC chromatograms of amarogentin stored in methanol: water (50:50) after 6 months of storage

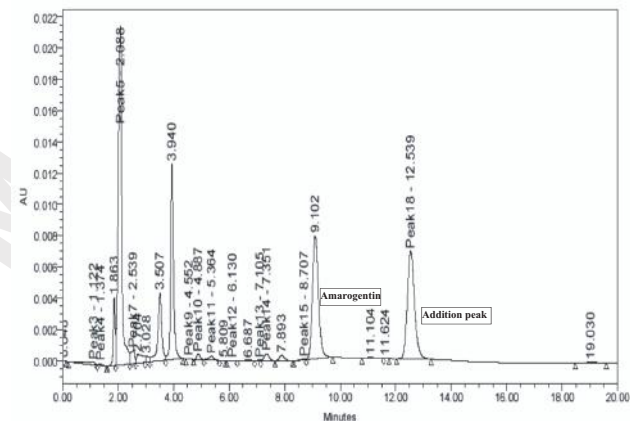


Fig. 5. HPLC chromatograms of amarogentin stored in methanol: water (25:75) after 6 months storage

significant effects on degradation of contents of stevioside A and rebaudioside A (bioactive compounds in *Stevia rebaudiana*). The contents of both sweeteners were reduced to 70% and stevioside A was less stable than rebaudioside A (Rieck *et al.*, 2010).

Quantitative and qualitative analysis of loss of amarogentin content on storage in different solvents was carried out by HPLC and TLC techniques, respectively. The present results indicate that the minimum loss of amarogentin was observed in methanol solvent. Therefore, pure methanol shows promise as an appropriate solvent for storage of amarogentin (an bioactive chemical of *Swertia chirayita*).

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Received 20 October, 2015; Accepted 19 April, 2016

Oh y for Members



Influence of Plant Growth Promoting Rhizobacteria (PGPR) on Growth and Yield of Strawberry (*Fragaria x ananassa* Duch.) cv. Chandler

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Abstract: The effect of five strains of plant growth promoting rhizobacteria (PGPR) (*Bacillus licheniformis* CKA 1, *Bacillus subtilis* CB 8 A, *Bacillus* sp. RG1, *Bacillus* sp. S₁ and *Bacillus* sp. S₂) was observed on plant growth and yield of strawberry. The treatments resulted in increased plant growth and yield as compared to control. The maximum plant height and spread were recorded from *Bacillus licheniformis* CKA 1, whereas, leaf area and plant biomass were maximum in *Bacillus* sp. S₂ treated plants when these strains were applied through root dip + foliar application methods. The number of crowns and the number of runners per plant were highest in plants treated with *Bacillus* sp. RG1 and *Bacillus* sp. S₁, respectively. Application of *Bacillus* sp. S₂ through root dip + foliar application method resulted in earliest flowering, harvesting and maximum flowering duration. The harvesting duration was longest in *Bacillus subtilis* CB 8A treated plants. The number of fruits and yield per plant were maximum in *Bacillus* sp. S₂ treated plants applied as root dip + foliar application method. The study revealed beneficial effect of plant growth promoting rhizobacteria (PGPR) on plant growth and yield of strawberry.

Key Words: *Bacillus* sp., Growth, Plant Growth Promoting Rhizobacteria, Strawberry, Yield

Strawberry (*Fragaria x ananassa* Duch.) is known as one of the most attractive, delicious and refreshing fruit of the world and occupies a significant place in fruit growing. The modern strawberry cultivation that warrants high yield and quality requires extensive use of chemical fertilizers, which are costly and create environmental hazards. Thus, the use of plant growth promoting rhizobacteria is becoming a resurgence of interest in sustainable and organic cultural practices (Esitken *et al.*, 2005). Plant growth promoting rhizobacteria improve plant growth and yield by means of producing plant growth regulators (auxin, gibberellins, cytokinins etc.), solubilizing organic phosphate or mineralizing organic phosphate or other nutrients, fixing atmospheric nitrogen, facilitating the uptake of nutrients and preventing deleterious effects of chemical fertilizers on soil. Various research workers have found that plant growth promoting rhizobacteria could stimulate growth and increase yield in apple, sweet cherry, citrus, raspberry, high bush blueberry, mulberry and apricot (Pirlak and Kose, 2009).

The objective of this study was to observe the growth promoting effect of root and foliar application of plant growth promoting rhizobacteria on growth and yield of strawberry cultivar 'Chandler'.

MATERIAL AND METHODS

The present investigation was carried out at Model Farm of Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, (HP), India during 2012-13, where five plant

growth promoting rhizobacteria viz., S₁- *Bacillus licheniformis* CKA 1, S₂- *Bacillus subtilis* CB 8 A, S₃- *Bacillus* sp. RG1, S₄- *Bacillus* sp. S₁ and S₅- *Bacillus* sp. S₂ were applied as root dip, foliar application and root dip + foliar application methods. The resultant sixteen treatments were evaluated in randomized block design with three replications. Experiment included 48 beds (2x2 m) in which strawberry cultivar 'Chandler' runners were planted at a spacing of 50 x 25 cm. In root dip method, the roots of strawberry runners were dipped in bacterial suspensions (10⁹ CFU/ml) for 45-60 minutes before planting while foliar application was given 20 days before expected flowering.

The plant height (cm), spread (cm), number of crowns per plant, number of runners per plant, date of first flowering, duration of flowering, number of fruits per plant, date of first harvesting, duration of harvesting and yield per plant (g) were recorded as per standard practices. The leaf area (cm²) was measured by leaf area meter (Licor-Model 3100) and the plant biomass (g) was determined by taking total dry weight of shoots and roots of plants at the end of the experiment.

RESULTS AND DISCUSSION

All the treatments had better response over control (sterile water application). The root + foliar application of rhizobacteria showed best results with respect to plant growth parameters as compared to independent treatment of root and foliar methods of application. The maximum plant

height (29.50 cm) and plant spread (31.03 cm) were recorded in S_1 + root dip method + foliar application, whereas, maximum leaf area (130.67 cm²) was obtained in S_5 + root dip method + foliar application followed by T_3 (129.90 cm²). The number of crowns was significantly more in T_9 (4.62), which was at par with T_{15} (4.44). The maximum number of runners (48.43) was observed in T_{12} (S_4 + root dip method + foliar application) followed by T_3 (40.00) showing significant improvement over control (22.25). Plant biomass on dry weight basis (7.62 g) was significantly maximum in S_5 + root dip method + foliar application than in other treatments (Table 1).

The results on plant growth promoting effects of PGPR in strawberry were supported by the findings of Pirlak and Kose (2010) who also reported that the root+ foliar application of PGPR strains (*Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3) significantly increased the plant height, number of runner per plant, leaf area and the ratio of usable runner in strawberry cultivar Selva. Esitken *et al.* (2010) also found that the inoculation of M-3 and foliar application of OSU-142 and BA-8 bacteria stimulated plant

growth in strawberry, while Pedraza *et al.* (2010) reported increased dry weight of root and shoot in strawberry cultivars 'Milsei', 'Selva' and 'Camarosa' with root inoculation of *Azospirillum brasilense* (strains REC 3, RLC 1 and PEC 5). The findings are also in line with the reports of Yadav *et al.* (2010) and Karlidag *et al.* (2013) as they reported increased plant growth in strawberry with the application of plant growth promoting rhizobacteria.

The flowering (16th February, 2013) and harvesting (3rd April, 2013) were earliest in T_{15} , whereas, they were late in T_5 (14th March and 9th April, respectively). In flowering, T_3 (S_1 + root dip method + foliar application) attained the second position (22nd February, 2013), while S_2 + root dip method + foliar application did likewise (4th April, 2013) in harvesting (Table 2). This study shows that the flowering and harvesting was earliest in root + foliar application of plant growth promoting rhizobacteria (PGPR). The duration of flowering was longest in T_{15} (78.33 days) followed by T_3 (72.33 days) and minimum in T_2 (56.67 days), whereas, the duration of harvesting was longest in T_6 (44.67 days), which was statistically at par with T_9 (43.00 days). The number of fruits

Table 1. Effect of plant growth promoting rhizobacteria on growth parameters of strawberry cultivar Chandler

Treatments		Plant height (cm)	Plant spread (cm)	Leaf area (cm ²)	Number of crowns plant ⁻¹	Number of runners plant ⁻¹	Plant biomass on dry wt. basis (g plant ⁻¹)
T_1	S_1 + Root dip methods	24.03	26.78	125.07	3.78	35.58	5.46
T_2	S_1 + Foliar application	25.52	25.97	115.52	3.50	31.75	4.85
T_3	S_1 + Root dip method + Foliar application	29.50	31.03	129.90	4.18	40.00	5.57
T_4	S_2 + Root dip methods	23.33	27.90	109.83	3.73	30.92	5.47
T_5	S_2 + Foliar application	25.57	26.93	108.97	3.44	28.09	4.50
T_6	S_2 + Root dip method + Foliar application	25.96	29.92	110.27	4.00	32.54	5.55
T_7	S_3 + Root dip methods	22.87	25.06	110.12	4.38	36.43	5.40
T_8	S_3 + Foliar application	23.42	24.34	107.85	4.08	28.17	5.00
T_9	S_3 + Root dip method + Foliar application	25.70	28.18	125.05	4.62	38.79	6.39
T_{10}	S_4 + Root dip methods	23.17	26.34	111.18	3.87	38.09	6.35
T_{11}	S_4 + Foliar application	26.15	25.95	107.78	3.82	28.83	6.25
T_{12}	S_4 + Root dip method + Foliar application	26.63	27.40	116.55	4.08	48.43	6.37
T_{13}	S_5 + Root dip methods	22.92	24.13	121.89	4.07	31.58	5.70
T_{14}	S_5 + Foliar application	23.85	23.37	120.48	3.85	28.67	4.40
T_{15}	S_5 + Root dip method + Foliar application	27.48	28.45	130.67	4.44	36.40	7.62
T_{16}	Control (Sterile water application)	22.20	23.05	104.70	3.33	22.25	3.88
CD (p=0.05)		2.19	1.92	2.96	0.51	2.84	1.16

Table 2. Effect of plant growth promoting rhizobacteria on yield parameters of strawberry cultivar Chandler

Treatments*	Dates of first	Duration of	Dates of first	Duration of fruit	Number of	Yield per plant
T ₁	Feb. 25	69.00	Apr. 5	34.00	14.82	192.15
T ₂	Mar. 9	56.67	Apr. 8	37.33	16.10	229.50
T ₃	Feb. 22	72.33	Apr. 5	42.00	16.73	234.21
T ₄	Mar. 8	58.00	Apr. 7	35.67	14.53	186.73
T ₅	Mar. 14	57.00	Apr. 9	38.33	16.40	214.72
T ₆	Feb. 26	68.00	Apr. 4	44.67	16.60	223.48
T ₇	Mar. 1	61.67	Apr. 7	34.33	15.27	185.07
T ₈	Mar. 4	60.67	Apr. 8	38.00	16.93	211.94
T ₉	Feb. 27	64.00	Apr. 6	43.00	17.27	214.09
T ₁₀	Mar. 6	65.33	Apr. 8	32.83	14.53	182.31
T ₁₁	Mar. 7	60.67	Apr. 8	34.00	15.47	208.94
T ₁₂	Mar. 2	66.00	Apr. 5	43.87	15.80	210.38
T ₁₃	Feb. 24	70.33	Apr. 5	37.00	16.53	222.03
T ₁₄	Mar. 5	62.33	Apr. 7	40.00	17.33	251.89
T ₁₅	Feb. 16	78.33	Apr. 3	42.33	18.07	253.64
T ₁₆	Mar. 6	71.33	Apr. 8	32.00	13.20	146.43
CD (p=0.05)	-	1.96	-	1.72	2.08	1.93

*Details in table 1

and yield per plant were highest (18.07 and 253.64 g, respectively) in T₁₅ which were statistically at par with T₁₄ (17.33 and 251.89 g, respectively).

The results further revealed that the plant growth promoting rhizobacteria treatments resulted in earliest flowering and harvesting of fruits. This helped in extending the flowering and harvesting durations thereby enhancing yield. The results are in conformity with the work of Pirlak and Kose (2009) who reported increased number of fruits and yield per plant with root + foliar application of plant growth promoting rhizobacteria in strawberry cultivar Selva. Tripathi *et al.* (2014) also studied the effect of *Azotobacter* and PSB on flowering and yield of strawberry cv. Chandler and recorded significantly increased number of flowers, fruit set per plant, early flowering, maximum duration of harvesting and yield per plant with combined application of *Azotobacter* and phosphate solubilizing bacteria (each 6 kg ha⁻¹). The increased fruit yield with the use plant growth promoting rhizobacteria in strawberry was also reported by Ipek *et al.* (2014).

It can be concluded that the plant growth promoting rhizobacteria (PGPR) exhibit beneficial effects on growth and

yield of strawberry. They have a great potential for current strawberry production systems and can be utilized for sustainable and ecological fruit production which will help in eliminating the use of chemical fertilizers to a great extent.

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Received 11 September, 2015; Accepted 27 March, 2016



Occupational Health Hazards among Dyeing Workers in Karur Region of Tamilnadu, India

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Abstract: The changes in blood by means of haematological parameters viz., Total Leucocyte Count (TLC), Differential Count (DC) of polymorphs, lymphocytes, eosinophil and basophil, Erythrocyte sedimentation rate (ESR) and haemoglobin (Hb) concentration were monitored and significant changes in various haematological parameters were observed. The observed haematological parameters of exposed dyeing workers exhibited a significant deviation from the standards, which indicates that exposure to dyeing chemicals makes body more vulnerable to dermatitis, anemia, eosinophilia and tuberculosis infections.

Key Words: Dyeing workers, Haematological parameters, Occupational Disease

Workers involved textile dyeing and printing operations are exposed to the health hazardous chemicals, which include bleaching agents, various dyes and color pigments (Huter, 1978). The health hazards among dyeing workers in general include acute poisoning, allergies and chemical burns by inhalation, ingestion or skin contact with constituents of dyeing process liquids, or poisonous gases released during the dyeing process (e.g. H₂S gas) (Sargunar and Ravikrishnan, 2012). Toxic effects of dyes, in particular Azo-dyes, which cause asthma, contact dermatitis to the workers of textile industry, have already been reported (Soyinka *et al.*, 2007; Singhi *et al.*, 2005). The association between employment-exposure to textile industry and cancer have also been detected for bladder, nasal, esophagus, stomach, colon, rectal, nasopharyngeal and lung cancers (Vutukuru, 2005; Lyon *et al.*, 2007). However, its influence on haematological parameters of workers who inherently use their bare hands to dye the knit materials without the use of protective clothing, has hardly been reported. Haematological factors have often been used to determine the effects of various pollutants on human health. Hence, in the present study the haematological parameters including total leucocyte count (TLC), differential count (DC) of polymorphs, lymphocytes, eosinophil and basophil, erythrocyte sedimentation rate and hemoglobin concentration in four types of cancer causing conditions viz., tuberculosis, dermatitis, anemia and eosinophilia were recorded to assess the toxic effects of textile dyes on allergic manifestations and hypersensitivity among dyeing workers.

MATERIAL AND METHODS

The data related to age, salary, smoking habit, job

duration, exposure time per day and history of people infected with any disease or allergies working in textile dyeing processes as well as control subjects were initially observed. Randomly, ten healthy workers, exposed to fume of dyes, having direct contact with dyeing chemicals without the use of protective clothing, in their dyeing units of Karur region of Tamilnadu state, India, were included in this study. Capillary and venous blood samples were collected randomly into lithium heparin specimen tubes, with the help of sterilized disposable syringes. After thorough mixing, the samples were transferred to laboratory under maximum possible precautionary measures. These blood samples were used for haematological studies.

Estimation of haemoglobin (Hb) was done using Haemometer. Total leucocyte count (TLC) was done using Haemocytometer. Differential count was done with help of Leishman's stain. A/G ratio was determined using Biuret reagent. Erythrocyte sedimentation rate (ESR) was determined by the Westergren's method. Staining of tuberculosis bacilli was carried out following the Ziehl-Neilsen method (Dacie and Lewis, 1991).

RESULTS AND DISCUSSION

In the present study, significant changes in various haematological parameters were observed in exposed workers under four different diseases viz. tuberculosis, eosinophilia, dermatitis and anaemia (Table 1-4). The observed haematological parameters of exposed dyeing workers from ten different places of Karur district of Tamilnadu State, India, exhibited a significant deviation from the standards under each of the four disease testing conditions.

Table 1. Comparison of various haematological parameters of dyeing workers with standard under tuberculosis condition

Blood parameters	Standard value	Blood samples collected from dyeing workers									
		BS1	BS2	BS3	BS4	BS5	BS6	BS7	BS8	BS9	BS10
TC (number of cells/mm ³)	4000-10,000	8500	9800	6300	5400	7800	10000	9600	6800	9500	6400
DC (%)	P	40-60	60	57	55	56	60	54	56	60	53
	L	20-40	28	31	35	31	26	32	35	32	32
	E	upto 6	6	6	7	7	9	7	6	3	6
	M	2-10	4	4	2	3	3	5	2	4	7
	B	upto 2	2	2	1	2	2	2	1	1	2
ESR (mm/hr)	5-20	6	5	7	15	8	9	7	14	16	18
Hb (grams %)	Cyst	7	8	9	8	15	14	10	15	13	16
A/G Ratio	1.8:1-2.5:1	1.664:1	1.662:1	1.661:1	1.662:1	1.5:1	1.664:1	1.661:1	1.5:2	1.661:1	1.664:1

Under tuberculosis disease testing conditions, there were substantial decreases in Hb counts except in two blood samples (Table 1). A significant decrease observed in Hb level may be due to decreased synthesis of Hb in bone marrow, over hydration, malnutrition, pregnancy and liver disease (Liaqat *et al.*, 2009; González-Alonso *et al.*, 2006). Deviation in the protein content is due to over intake or over hydration. Erythrocytes and Hb have been postulated to function as O₂ sensors and controllers of local blood flow and O₂ delivery (Pradip *et al.*, 2005). Acid-Fast Bacillus test revealed that all the ten blood samples were infected by *Mycobacterium tuberculosis* (Table 1).

Significant increase in differential leukocyte counts was observed in dermatitis condition (Table 2). Lymphocytes are the second numerous class of leukocytes, accounting for 20-40% of the total leukocytes. Changes observed in lymphocytes count at few instances, may be due to altered immune responses/reactions resulting in lymphocytosis in the exposed workers (Goyarts *et al.*, 2006; Jude *et al.*, 2002). Lymphocytes percentage can be increased by lymphatic leukemia and thyrotoxicosis, whereas syphilis and any other in toxications can reduce the same (Table 2).

However, in the Anemia condition, increases in Eosinophil count as well as a decrease in Hb counts were observed (Table 3). Eosinophil will be increased by any radiation, allergic condition or parasitic infection. Monocytes' leukemia will be increased by intake high amount of lipids and a protozoan infection. Albumin/Globulin (A/G) ratio in the anemia condition was lesser than the standards. The decreased values of A/G ratio were due to the decrease in haemoglobin and due to liver and kidney diseases, inflammation, pregnancy and malignancy whereas the

increased values of leucocyte counts and ESR were due to the dehydration. Erythrocytes itself functions as an O₂ sensor, contributing to the control of O₂ delivery by releasing the vasodilators ATP and S-nitrosohaemoglobin with the offloading of O₂ from the hemoglobin molecule (Gleich, 2000). Elucidating the role of erythrocytes number on the control of blood flow could provide new insights into the regulation of vascular tone in patients suffering from anemia and polycythaemia (Gleich, 2000). In present investigation, no significant change in erythrocytes was recorded in any of the comparisons. There are various compensatory mechanisms in the body, which work on stress conditions to keep total erythrocytes counts in normal range. No significant change, closer values towards low range, in ESR was observed indicating the insensitivity of this parameter on exposure to textile dyeing chemicals, hence can be excluded while planning the future study in this respect (Table 3).

Under eosinophilia conditions, an increase in eosinophil and a decrease in basophil count were observed (Table 4). Basophile can be increased by polycythemia (increased white blood cell, WBC), chicken pox, small pox and all intoxication, whereas decreased by pregnancy and hypothyroidism. Eosinophils, characterized by their large granules and bi-lobed nucleus destroy bacteria intracellularly to any great extent. It is showing that dyeing workers may be experiencing hypersensitivity or allergic diseases. Since raised level of eosinophils has been associated with parasitic infections and allergic responses (El-Banna *et al.*, 2006; Nassenstein *et al.*, 2003). Significantly higher number of eosinophils has been reported in patients positive for NGF (nerve growth factor), which plays an important role in allergies and lung respiratory diseases (González-Alonso *et al.*, 2006).

Table 2. Comparison of various haematological parameters of dyeing workers with standard under anaemia condition

Blood parameters	Standard value	Blood Samples collected from dyeing workers									
		BS1	BS2	BS3	BS4	BS5	BS6	BS7	BS8	BS9	BS10
TC (number of cells/mm ³)	4000-10,000	6800	9500	9800	9900	8300	10200	7400	7500	5200	9700
DC (%)	P 40-60 L 20-40 E upto 6 M 2-10 B upto 2	67 21 6 4 2	60 27 7 4 2	70 21 6 2 1	60 29 6 4 1	71 20 6 2 1	66 23 7 2 2	68 22 6 2 2	62 25 7 4 2	61 28 8 2 1	65 23 7 4 1
ESR (mm/hr)	5-20	9	8	10	12	7	15	12	18	20	17
Acid-Fast Bacillus test	Blue – normal Red-	Red coloured Bacilli	Red coloured Bacilli	Red coloured Bacilli	Red coloured Bacilli	Red coloured Bacilli	Red coloured Bacilli	Red coloured Bacilli	Red coloured Bacilli	Red coloured Bacilli	Red coloured Bacilli
Hb (gram %)	12-14	8	5.4	7	6	12	9	14	9.4	6	7

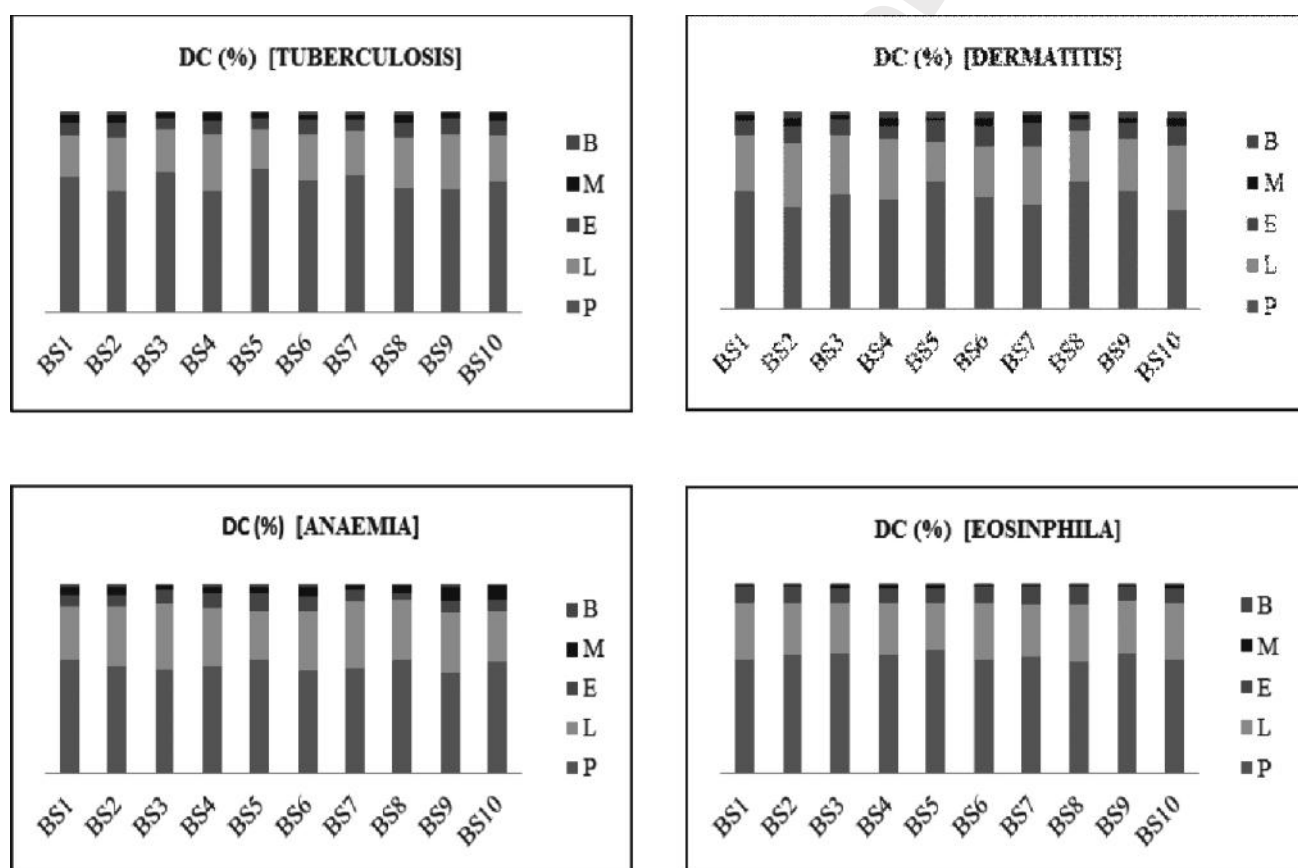
Table 4. Comparison of various haematological parameters of dyeing workers with standard under eosinophilia condition

Blood parameters	Standard value	Blood Samples collected from dyeing workers									
		BS1	BS2	BS3	BS4	BS5	BS6	BS7	BS8	BS9	BS10
TC (number of cells/mm ³)	4000-10,000	7200	8400	8600	8800	9000	9600	9800	9900	10000	9000
DC (%)	P 40-60 L 20-40 E upto 6 M 2-10 B upto 2	60 28 7 3 2	52 32 9 4 3	58 30 8 2 2	56 30 7 4 3	65 20 10 2 3	57 25 11 4 3	53 29 12 4 2	65 25 6 2 2	60 26 8 3 3	50 33 10 4 3
ESR (mm/hr)	5-20	5	6	8	9	11	14	5	10	8	6
Motion	Ova Cyst	Guinea worm NIL	Hook worm (Hb) NIL	Strongyloides stercoralis NIL	Strongyloides stercoralis NIL	Hook worm NIL	Round worm NIL	Tape worm NIL	Thread worm NIL	Round Worm NIL	Strongyloides stercoralis NIL

Table 3. Comparison of various haematological parameters of dyeing workers with standard under dermatitis condition

Blood parameters		Standard value	Blood Samples collected from dyeing workers									
			BS1	BS2	BS3	BS4	BS5	BS6	BS7	BS8	BS9	BS10
TC (number of cells/mm ³)		4000-10,000	8800	9000	9200	8900	6500	9900	10000	9700	9900	8200
DC (%)	P	40-60	60	62	63	62	65	60	61	59	63	60
	L	20-40	30	28	27	28	25	30	28	30	28	30
	E	upto 6	8	8	7	7	7	8	9	9	7	7
	M	2-10	1	1	2	2	2	1	1	1	1	2
	B	upto 2	1	1	1	1	1	1	1	1	1	1
ESR (mm/hr)		5-20	6	7	9	10	12	15	9	17	10	16

TLC – Total Leucocyte Count, DC – Differential Count, P – Polymorphs, L – Lymphocytes, E – Eosinophil, B – Basophil, ESR – Erythrocyte sedimentation rate, Hb – Haemoglobin, A/G ratio – Albumin/Globulin ratio

**Fig. 1.** Differential counts of blood samples under four different disease conditions

In conclusion, the results obtained in the present study indicate that exposure to dyeing chemicals makes body more vulnerable to dermatitis, anemia, eosinophilia and tuberculosis infections. Significant haematological changes observed in this study are needed to be elucidated so that the post effects of toxicant contamination may be monitored and eliminated. This will not only help in the protection of the surrounding environment, but also in the prevention of malignant diseases among the dyeing workers in the

factories as well as those residing in and around the dyeing units.

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Protective Clothing for the Pesticide Applicators

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Abstract: Seven protective garments beak shaped mask, cap with mask, hood mask, scarf mask, starched kurta, jacket and *pyjama* of water proof fabric were designed to protect pesticide applicators from the health hazards. The level of acceptability and satisfaction of the functional features of these protective garments were assessed separately. All the respondents were highly satisfied with all the features of the beak shaped mask, hood mask and kurta. The respondents were satisfied with the functional features of cap with mask but were not satisfied with the protection provided by scarf mask. Waterproof garments provided protection from pesticide but were not comfortable to wear during summers.

Key Words: Applicator, Pesticides, Problems, Protective garments, Satisfaction

Crop protection from different pests plays a vital role in sustaining the productivity and production in today's agriculture. The use of plant protection chemicals for this purpose is not a new phenomenon. However, the widespread use of plant protection chemicals came to limelight during the transition phase from subsistence farming to modern farming when high yielding varieties of crops were introduced. In the present situation, with the burgeoning population and very little scope for expanding the area under agriculture, the crops will have to be protected from the ravages of the pests and diseases.

The introduction of synthetic insecticides – organophosphate insecticides in the 1960s, carbamates in 1970s and pyrethroids in 1980s and the introduction of herbicides and fungicides in the 1970s–1980s contributed greatly to pest control and crop productivity (Aktar *et al.*, 2009). Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not the case, so the controversy of use and abuse of pesticides has surfaced. The rampant use of these chemicals, under the adage, “if little is good, a lot more will be better” has played havoc with human and other life forms.

The importance of education and training of workers as a major vehicle to ensure a safe use of pesticides is being increasingly recognized. The increased use of pesticides in agriculture needs proper skill and management quality of the highest order to avoid potential threat of exposure to applicators as well as to the others. Occupational hazards due to pesticide may occur to applicators during the process of various field operations starting from land preparation to the post harvest stage. Certain pesticides are known to elicit their adverse effects by mimicking or antagonizing natural hormones in the body and it has been postulated that their long-term, low-dose exposure is

increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Brouwer *et al.*, 1999, Crisp *et al.*, 1998; Hurley *et al.*, 1998). To combat with these problems, a study was undertaken to develop special protective clothing for the pesticide applicators to protect them from the exposure and to further analyse the acceptability of the same in three villages of Ludhiana.

Seven protective garments were designed to protect applicators from the health hazards. The designed garments included beak shaped mask, cap with mask, hood mask, scarf mask, starched kurta, jacket and *pyjama* of water proof fabric. Beak shaped mask, cap with mask, and scarf mask were developed and stitched using fine cotton fabric, whereas, hood mask was designed and stitched using knitted cotton and water proof fabric was used for developing jacket and *pyjama*. The acceptability of the designed protective garments was evaluated on the selected pesticide applicators.

MATERIAL AND METHODS

A sample of ninety respondents, thirty each from three village Malerkotla (Ml), Mansuran (Mn) and Samrala (Sm) was selected to get the required information. The pesticide applicators were personally contacted and requested to wear the designed protective clothing during pesticide application for a period of one month (June). They were asked to provide independent opinions regarding the desired information needed for this study. After a period of one month, the respondents were contacted personally to get the required information about the acceptability of the designed protective clothing. It was observed that manual pump was used to spray the pesticide.

Suitability of every functional feature incorporated

in a garment/accessory was assessed on 3 point continuum scale i.e. highly satisfied (H.S.), satisfied (S.) and not satisfied (N.S.) and was given weightage 3, 2, 1 respectively. The frequency was multiplied with weightage. The scores were added and then were divided with number of respondents (N). Weighted mean score (WMS) of all the characteristics of garments were added and then were divided by the number of characteristics. Any functional feature having WMS between 1-1.66 was rated as not satisfied, 1.67-2.33 was rated as satisfied and scoring 2.34-3.00 was rated as highly satisfied. The attribute scoring

maximum WMS was given first rank, where as the attribute scoring least WMS was given last rank.

RESULTS AND DISCUSSION

Protective masks: The respondents were highly satisfied with thirty two features of the four masks (Table 1). They gave first rank to fourteen different features. These attributes are easy to don, easy to doff, comfort of the cotton fabric attached to it for breathing, and the comfort of elastic at sides of the mask during wear in case of beak shaped mask; protection of their head from pesticide vapours, ease to don and doff,

Table 1. Distribution of respondents according to their satisfaction level for the features of beak shaped mask, cap with mask, hood mask and scarf mask

Characteristics	Weighted score			W.M.S.	Rank
	MI	Mn	Sm		
Beak shaped mask					
Protects from pesticide inhalation	90	87	84	2.90	XV
Easy to don	90	90	90	3.00	I
Easy to doff	90	90	90	3.00	I
Cotton fabric					
Comfortable for breathing	90	90	90	3.00	I
Elastic at sides of mask					
Comfortable during wear	90	90	90	3.00	I
Cap with mask					
Cap					
Protects head from pesticide vapors	90	90	90	3.00	I
Mask of fine cotton fabric					
Protects from pesticide inhalation	63	54	66	2.03	XXXIII
Elastic of mask is comfortable	84	81	84	2.77	XX
Overall shape and size of mask					
Height of mask	84	87	84	2.83	XVII
Width of mask	78	81	72	2.57	XXIX
Comfortable	84	81	81	2.73	XXIV
Easy to don	90	90	90	3.00	I
Easy to doff	90	90	90	3.00	I
Studs at sides of mask to attach it to the mask					
Comfortable	90	90	90	3.00	I
Hood mask					
Hood cotton hosiery fabric					
Protects head from pesticide vapors	87	87	84	2.87	XVI
Easy to don	90	90	90	3.00	I
Easy to doff	84	81	84	2.77	XX

Comfortable	84	87	78	2.77	XX
Mask of fine cotton fabric					
Protects from pesticide inhalation	78	75	78	2.57	XXIX
Height of mask	90	90	90	3.00	I
Width of mask	90	90	90	3.00	I
Elastic at the nose portion in the mask					
Comfortable	84	75	78	2.63	XXVI
Strings at neck portion for tying					
Comfortable	90	90	90	3.00	I
Scarf mask					
Head cover of double layered voile fabric					
Protects head from pesticide vapors	36	36	39	1.23	XXXVII
Protects neck from pesticide vapors	81	75	84	2.67	XXV
Mask of double layer voile fabric					
Protects from pesticide inhalation	48	51	51	1.67	XXXIV
Elastic at nose portion in the mask					
Keep mask in position	45	51	48	1.60	XXXV
Comfortable	84	87	78	2.77	XX
Elastic at forehead					
Comfortable	78	75	84	2.63	XXVI
Width of elastic	45	51	48	1.60	XXXV
Shape of mask					
Height of mask	90	90	90	3.00	I
Width of mask	90	90	90	3.00	I
Size of head cover					
Satisfied	84	75	78	2.63	XXVI
Strap for knotting					
Easy to tie knot	84	29	84	2.83	XVII
Easy to open knot	72	72	84	2.53	XXIX
Overall scarf mask					
Easy to don	84	75	78	2.83	XVII
Easy to doff	84	75	78	2.53	XXIX

comfort of studs at sides of mask to attach it to the mask in regards of cap with mask; easy to don, comfort of the strings at neck portion for tying, width of the mask made from fine cotton fabric and the height of the mask in respect of hood mask; and adequate height and width of the scarf mask. All these attributes were given weighted mean score of 3.00.

These features were followed by protection from pesticide inhalation of the beak shaped mask with WMS of 2.90 (Rank 15), protection of the head from pesticide vapors

of the hood mask with WMS of 2.87 (Rank 16), height of cap with mask, ease in tying knot of scarf mask, ease in donning scarf mask, comfort of elastic of the cap with mask, ease in doffing and comfort of hood mask, comfort of elastic of scarf mask, comfort of overall shape and size of cap with mask, protection of neck from pesticide vapors of scarf mask, comfort of elastic at nose portion of the hood mask, comfort of elastic at forehead of scarf mask, satisfaction from size of the head cover of scarf mask, width of cap with mask, protection

Table 2. Distribution of respondents according to their satisfaction level for the features of shirt (*kurta*), jacket and *pyjama* of water proof garment

Characteristics	Weighted score			W.M.S.	Rank
	MI	Mn	Sm		
Shirt (kurta)					
Chinese collar					
Protects neck from pesticide vapors	84	84	84	2.80	VIII
Elasticized cuffs					
Protects arms from pesticide vapors	84	87	84	2.83	I
Size of elasticized cuff					
Comfortable	84	87	84	2.83	I
Jacket of water proof garment					
Front opening with zip					
Easy to operate	90	87	87	2.93	I
Polo collar					
Protects neck from pesticide vapors	90	84	87	2.90	VI
Comfortable	60	57	48	1.83	XI
Full sleeves with elastic at wrist level					
Protects arms from pesticide vapors	90	84	87	2.90	VI
Comfortable during wear					
Less than 1 hour	30	30	30	1.00	XII
More than 1 hour	-	-	-	-	
Pyjama of water proof garment					
Elastic at bottom					
Easy to don	90	87	87	2.93	I
Easy to doff	90	87	87	2.93	I
Comfortable	81	87	87	2.83	VIII
Elastic at waist level					
Comfortable	81	87	87	2.83	VIII
Comfort during wear					
Less than 1 hour	30	30	30	1.00	XII

from pesticide inhalation from fine cotton fabric of hood mask, ease in opening knot and doffing of scarf mask.

The respondents were satisfied with two features of two different masks. Rank thirty three was given to the protection the mask (cap with mask) of fine cotton fabric provided with WMS of 2.03. Thirty fourth rank was given to the protection from pesticide inhalation by scarf mask with WMS of 1.67.

The respondents were not satisfied with three features of scarf mask only. The scarf mask had elastic at

nose portion in the mask to keep it in position. The respondents gave thirty fifth rank with WMS of 1.60 to keeping mask in position and width of elastic at the forehead. The respondents were not satisfied with protection from scarf mask as the head cover of double layered voile fabric was not able to protect head from pesticide vapours and gave least rank (37th with WMS of 1.23).

Protective garments: The respondents were highly satisfied with ten attributes of the developed garments i.e. the operation of the zipper in the front opening of the jacket

and ease in donning and doffing of the pyjama and rank I was given with weighted mean score of 3.00. It was followed by the protection given to the neck from pesticide vapours by the polo collar of the jacket of the water proof fabric, the protection given to the arms from pesticide vapours with full sleeves jacket with elastic (weighted mean score 2.90) and both these aspects were ranked IV. The protection to the arms from pesticide vapors from elasticized cuffs, the comfort from the size of the elasticized cuffs of the *kurta*, comfort of the elastic at bottom and waist level of the pyjama (weighted mean score 2.83) were given rank six. The protection of the neck of the respondents from pesticide vapours with Chinese collar of the *kurta* was given rank X with weighted mean score of 2.80.

Though the jacket and *pyjama* made from water proof fabric was repellant to pesticides, but the most undesirable feature was that it was not breathable and was

uncomfortable to wear in hot weather. This feature was given last rank with WMS of 1.00.

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Food Safety issues and Related Standards: Awareness and Knowledge of Home Food Makers in Ludhiana

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Abstract: The results revealed that the mean awareness mean awareness score of all the respondents came out to be 9.25 out of a maximum of 15, indicating that the respondents were considerably aware (61.7%) about the food safety issues. The High mean awareness scores were in respondents >45 years, post graduates, respondents with annual family income of more than Rs. 5 lacs. Respondents belonging to Urban High Income category were having the highest awareness score(72.5%) while the Rural High Income category were least aware (52.1%) of food safety issues. An enquiry about the knowledge of various food safety standards prevalent revealed highest awareness for ISI standards, followed by Agmark standard, FPO and MPO. Only 14.17% of the respondents were aware about HACCP. Further it was seen that the urban respondents(25.83%) were having better awareness about the food standards as compared to the rural respondents(2.50%). A significant negative correlation was found between food safety awareness with the incidence of food borne diseases.

Key Words: Awareness scores, Food borne diseases, Food safety awareness, Food standards

The FAO/WHO International Conference on Nutrition (Rome, 1992), declared that "...access to nutritionally adequate and safe food is a right of each individual". Seen from this perspective, food safety must be given higher priority by governments, industry and consumers themselves (WHO, 2000). Food can be mishandled at many places during food preparation, handling and storage and several studies indicate that consumers have inadequate knowledge about procedures needed to prevent food borne illnesses at home (Medeiros *et al.*, 2001; Meer and Misner, 2000). Most cases of food borne illnesses are preventable if food protection principles are followed from production to consumption. Since, it is currently impossible for food producers to ensure a pathogen free food supply, the home food preparers is a critical link in the chain to prevent food borne illnesses. Food handlers at all ages seem to think that they know how to handle food safely, but their self-reported food handling behaviors do not support this confidence (Gettings and Kiernan, 2001). The most challenging areas to achieve consumer food safety include poor hand washing practices, cross- contamination, and improper storage of food items. Common mistakes identified include serving contaminated raw food, cooking/heating food inadequately, infected persons handling implicated food and poor hygiene practices (WHO, 2000). Home food safety can be controlled through the education of the consumer. There is a need for continued efforts toward educating consumers on the hazards of improper food handling. There is a need for educational programs for improving food safety knowledge and for controlling food borne diseases. A positive attitude toward foodborne diseases control and preventive measures

was reported by the great majority of food handlers and is more likely to be achieved by those who had attended education courses. (Angelillo *et al.*, 2000)

The study aims to assess the awareness of the consumers regarding food safety and attempts to find a correlation between awareness and the incidence of food borne diseases. In case of developing countries like India where the rate of literacy is low and more emphasis is being laid on food security rather than food safety. This further leads to a low awareness level regarding food safety as a result the incidence of food borne diseases is on a rise in these countries. So there is a need to emphasize the importance of food safety awareness in the reduction of incidence of food borne diseases and the present study is an attempt in this direction.

MATERIAL AND METHODS

The present study was undertaken to explore food safety awareness and knowledge regarding food safety standards among rural and urban home food preparers. The methodology used for the study included the survey of the rural and urban home food preparers in Ludhiana District of Punjab (India). For achieving the objectives of the study, 120 home food preparers each from rural and urban households in Ludhiana district were included in the sample, thus making total sample of 240. For collecting information pre structured non disguised questionnaire was prepared covering all the aspects of food safety. Inventory for food safety awareness was prepared from available secondary sources as well as expert advice. Questions were framed to elicit the information on food safety awareness of homemakers and their

knowledge regarding food safety standards.

The questionnaire was pre-tested by undertaking a pilot study involving five rural home food preparers and five urban home food preparers. On the basis of the feedback received from the pilot survey, the questionnaire was finalized by incorporating the changes. Data was collected by personally administering the questionnaire to the rural and urban home food preparers on the food safety awareness. For finding out the awareness of the respondents, they were asked 15 basic questions pertaining to the food safety. Depending upon the correctness of the responses, the respondents were given awareness score out of 15. Correlation was used to find out relationship between awareness score and disease incidence score.

RESULTS AND DISCUSSION

The respondents were considerably aware about the food safety issues with 61.7% awareness. As evident from the table, the mean awareness score of the respondents, aged more than 45 years came out to be the highest (67.0%) among the various age categories. The awareness of the respondents aged less than 30 years came to 62.2% while the lowest awareness of 59.9% was observed for the respondents aged 30-45 years. There was no significant difference in the mean awareness of respondents on the basis of age (Table 1) Gettings and Kiernan (2001) observed that food handlers at all ages think that they know how to handle food safely, but their self-reported food handling behaviors do not support this confidence. The highest mean awareness score was 12.60 in post graduates followed by the graduated. There was a significant difference in the mean awareness score obtained by the various categories segregated on the basis of the educational qualification ($p < 0.01$). Riordan *et al.* (2002) observed that 64 percent of Irish expressed concern for the safety of food. The respondents from the service class and business class were having mean awareness score of 10.16 and 8.88 with significant difference ($p < 0.01$). Higher mean

Table 1. Food safety awareness score

Category	Mean score	N	F-value
Total	9.25 ± 2.01	240	-
Age category			
< 30 Years	9.33 ± 2.00	123	2.47
30-45 Years	8.99 ± 1.99	98	
> 45 Years	10.05 ± 1.98	19	
Educational qualification			
Matric and Below	7.35 ± 0.75	49	375.13**
10+2	8.15 ± 0.79	82	
Graduate	9.77 ± 0.83	64	
Post Graduate	12.60 ± 1.03	45	
Occupation			
Business	8.88 ± 1.83	171	21.49**
Service	10.16 ± 2.15	69	
Annual family income			
< Rs. 2 Lacs	9.28 ± 1.72	25	3.39*
Rs. 2-5 Lacs	9.06 ± 1.98	168	
> Rs. 5 Lacs	9.91 ± 2.16	47	
Family size			
Four or Less	9.41 ± 1.99	127	1.03
5-8	9.02 ± 2.07	95	
More than 8	9.33 ± 1.82	18	
Background			
Rural lower income	8.00 ± 1.01	60	48.70**
Rural higher income	7.82 ± 1.09	60	
Urban higher income	10.88 ± 1.73	40	
Urban higher income	10.52 ± 1.96	40	
Urban lower income	10.38 ± 1.84	40	

**significant at 1% level; *significant at 5% level

awareness score in case of service class as compared to that of business class can be attributed to the usual trend of obtaining higher education in service class as compared to the business class. Further, it can be seen from the table that the respondents having annual family income of more than Rs. 5,00,000 were having relatively higher mean awareness score of 9.91 as compared to the other groups, segregated

Table 2. Background wise distribution of respondents according to knowledge about food standards

Food Standard	ISI	Agmark	FPO	MPO	HACCP
Total (n=240)	225 (93.75)	206 (85.83)	80 (33.33)	78 (32.50)	34 (14.17)
Rural Area (n=120)	107 (89.17)	93 (77.50)	23 (19.17)	23 (19.17)	3 (2.50)
Rural Lower Income (n=60)	50(83.33)	42(70)	5(8.3)	5(8.3)	-
Rural Higher Income (n=60)	57(95)	51(85)	18(30)	18(30)	3(5)
Urban Area (n=120)	118 (98.33)	113 (94.17)	57 (47.50)	55 (45.83)	31 (25.83)
Urban Higher Income (n=40)	40(100)	39(97.5)	21(52.5)	21(52.5)	16(40)
Urban Medium Income (n=40)	40(100)	38(95)	20(50)	20(50)	13(32.5)
Urban Lower Income (n=40)	38(95)	36(90)	16(40)	14(35)	2(5)

Figure in parentheses represent percentage

on the basis of annual family income. The mean awareness scores on the basis of annual family income were significantly different ($p < 0.05$) being maximum in > 5 lacs category whereas lowest in 2-5 lacs category. Whereas the family size didn't had any effect on the awareness scores of the respondents. According to Patil *et al.* (2005) high-income individuals reported greater consumption of raw foods, less knowledge of hygiene, and poorer cross-contamination practices. There was a significant difference among the mean awareness score of various categories in rural and urban settings on basis of their income. The respondents belonging to urban high income category were having the maximum awareness score of 10.88 followed by urban medium income category (10.52). The respondents from the rural areas were having relatively lower mean awareness 8.00 in case of the Rural Lower Income category while a mean awareness score of 7.82 for the respondents belonging to Rural high income.

Knowledge about food standards: The highest awareness was seen for ISI standards, followed by an awareness regarding Agmark standard, FPO and MPO. The respondents were having very little awareness about Hazard Analysis Critical Control Point (HACCP) as only 14.17 percent of the total respondents were aware about this food standard. The urban respondents (25.83) were having better awareness about the food standards as compared to the rural respondents (2.50). Marais *et al.* (2006) reported that only 6% of managers reported awareness of HACCP being mandatory in South Africa. The rural households were having negligible knowledge about HACCP. None of the respondent in the rural lower income category was aware about HACCP, while only five percent of the respondents in the rural higher income category had heard about HACCP. Knowledge of the rural respondents about ISI, Agmark was more as compared to their knowledge about FPO and MPO. Further, it can be seen that almost all the respondents (100-90%) from urban respondents were aware about ISI and Agmark and fifty percent of the respondents from the urban high and medium category about FPO and MPO. The awareness about HACCP was more in the urban higher (40) and middle (32.5) income categories as compared to urban lower income categories (5).

Food safety awareness and incidence of diseases: Strong negative correlation was observed between food safety practices and incidence of food borne diseases with coefficient of -0.744 ($p < 0.01$). There was significant negative correlation between food safety awareness and incidence of diseases in case of all categories based on background, age, occupation and annual family income. (Meer and Misner, 2000; Redmond and Griffith, 2003) concluded that consumers have inadequate knowledge about procedures

Table 3. Overall Correlation Results (n=240)

Parameter	Awareness and disease incidence
Total	-0.744**
Background	
Rural lower income	-0.531**
Rural higher income	-0.505**
Urban higher income	-0.866**
Urban medium income	-0.686**
Urban lower income	-0.693**
Age	
< 30 Years	-0.754**
30-45 Years	-0.702**
> 45 Years	-0.833**
Educational qualification	
Matric and below	-0.301*
10+2	-0.568**
Graduate	-0.627**
Post Graduate	-0.279
Occupation	
Business	-0.744**
Service	-0.713**
Annual family income	
< Rs. 2 Lacs	-0.807**
Rs. 2-5 Lacs	-0.688**
> Rs. 5 Lacs	-0.870**
Family size	
Four or less	-0.759**
5-8	-0.754
More than 8	-0.541*

**significant at 1% level; *significant at 5% level

needed to prevent food borne illnesses at home. In another study conducted by Muinde and Kuria, 2005 observed that vendors of street foods in Kenya were not aware of hygienic and sanitary practices.

Higher educational qualification of the respondents translated into better food safety awareness. The respondents from high and middle income category from the urban sector were having better awareness regarding food safety issues and food safety standards as compared to the rural counterparts and the level of food safety awareness was negatively correlated with incidence of food borne diseases.

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Received 21 September, 2015; Accepted 10 February, 2016



Interrelationship among Dietary Diversity, Socioeconomic Factors and Food Security in Rural Farm Households

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Abstract: A total of 90 farm households from three land holding categories viz. small (1-2 hectares), semi-medium (2-4 hectares) and medium (4-10 hectares) were selected to determine the interrelationship among diet diversity, socio-economic factors and food security. Vitamins A, B₁, C and calcium had Nutrient Adequacy Ratio (NAR) more than 100%, while only Vitamin B₁₂ had NAR% less than 50%, other six nutrients namely energy, protein, vitamin B₂, B₃, folacin and iron had NAR between 51-75%. The increase in expenditure on food, resulted in significant increase in Dietary Diversity Score (DDS) but household size showed negative but significant association with DDS. The education of respondents and total expenditure on food resulted in a significant increase in Food Variety Score (FVS), but there was significant decrease in FVS with the increase in household size.

Key Words: Dietary diversity score, Food security, Food variety score, Mean adequacy ratio, Nutrient adequacy ratio

Dietary diversity has long been recognized by nutritionists as a key element of high quality diets and is defined as the number of different foods or food groups consumed over a given reference period. The nutrients essential for meeting nutritional requirements are not all usually found in a single food item; they are however present in a diet composed of a number of foods. Increasing the variety of foods across and within food groups is recommended by most dietary guidelines internationally (FAO, 1996), because it is thought to ensure adequate intake of essential nutrients and thus, to promote good health.

Lack of dietary diversity is particularly a severe problem among poor populations in the developing world, because their diets are predominantly based on starchy staples and often include little or no animal products and few fresh fruits and vegetables. These plant based diets tend to be low in a number of micronutrients, and the micronutrients they contain are often in a form that is not easily absorbed. The rationale for emphasizing dietary diversity in developing countries stems mainly from a concern related to nutrient deficiency and the recognition of the importance of increasing food and food group variety to ensure nutrient adequacy. Therefore, dietary diversity is promoted to enhance the chances of achieving an adequate diet, lessen the risks of developing a deficiency or excess of any one nutrient, ensure an appropriate balance of micronutrients as well as energy from fat, and reduce the likelihood of exposure to excessive amounts of contaminants. Access to an adequate supply of food is the most basic of human needs

and rights. Hungry people cannot work and hungry children cannot learn leading to deprivation of nutrient adequacy and ultimately food insecurity (Sodhi, 2000).

Household food security is the result of interaction of broad spectrum of host and ecological factors namely per capita income, education, family size, male and female participation in income generating activities in addition to their regular job and time spent on food preparation (Bains and Mann, 1997). Hence, dietary diversification is a recommended approach to alleviate nutritional problems resulting from food insecurity and inadequate intake of micronutrients.

Consuming a wide variety of foods among and within food groups is therefore, a recommended strategy to help ensure adequate intake of micronutrients. It is thus, important to develop simple methods and simple indicators to be used as proxies for measuring overall dietary quality in different contexts. The state of Punjab is considered to be the grain bowl of India, where green revolution has brought about remarkable changes in wheat and rice production. As a result, income and per capita food supplies have also improved in rural farm community in spite of population increase, but still the nutrient intake is not adequate. The study determined the interrelationship among diet diversity, socio-economic factors and food security in this community.

MATERIAL AND METHODS

A total of 90 farm families, 31, 30 and 29 each from three categories based on their operational land holdings viz.

small (1-2 hectares), semi-medium (2-4 hectares) and medium (4-10 hectares) from village Lalton, district Ludhiana, Punjab, India were selected to collect the socio-economic and dietary information. A questionnaire to collect socio-economic and dietary information was developed. The socio-economic survey included family size and type, land holding, family income and educational level.

Food items were categorized into nine food groups. Expenditure on each food item was estimated using information on the market prices of food items purchased by the families per month. The information regarding the quantities of foods consumed by the female respondents of the household in the age group of 18-45 years was collected by '24-hour recall method' for three consecutive days. The food intake was categorized under 9 food groups comprising of cereals, pulses, green leafy vegetables, roots and tubers, other vegetables, fruits, milk and milk products, fats and oils, sugars and jaggery. Nutrient intake was calculated using MSU Nutriguide Computer Programme (Song *et al.*, 1992).

Food Variety Score (FVS) represents the number of food items consumed over a period of seven days. A list of 49 food items commonly consumed by the studied community was prepared. One point was given for each food category eaten either once or at any frequency throughout the week and each food category was scored only once. Points were added and the resultant score represented FVS of the respondent. Average FVS for three categories was calculated separately by dividing the sum of FVS with total number of respondents (Savage *et al.*, 1997).

Dietary diversity score (DDS) represents the number of different food groups consumed over a given reference period and calculated using a set of 12 food groups. The choice of 12 food groups was based on outcomes of Food and Nutrition Technical Assistance (FANTA) project (Swindale and Bilinsky, 2006).

Information on respondent's food consumption was collected using the previous 24-hours as a reference period (24-hour recall) as longer reference periods result in less accurate information due to imperfect recall. Food groups consumed by the females in the home, or prepared in the home for consumption by females outside the home were included. One code was given for food group consumed during the previous 24-hour and '0' code was given for food group not consumed. DDS was calculated by summing the number of different food groups consumed. Average DDS for three categories was calculated separately by dividing the sum of DDS with total number of respondents. To set DDS targets, the dietary diversity patterns of wealthier households which belonged to medium (4-10 hectare) land holding category were used. The average DDS in the richest 33 % of

the subjects served as a guide for setting the target level of DDS for the purpose of performance monitoring.

The nutrient adequacy ratios (NAR %) were calculated for each of 9 micronutrients (vitamin A, B₁, B₂, B₃, B₁₂, C, iron, calcium and folate) and energy and protein. NAR was calculated as the intake of a nutrient divided by the recommended dietary allowance (RDA) for that nutrient (ICMR, 2010). Mean nutrient adequacy ratio (MAR %), the mean ratio of intake to recommended intake (each truncated at 100 %) of energy, protein and nine micronutrients was calculated as a measure of the adequacy of the overall diet. For both NAR and MAR, a value of 100 % is the ideal since it means that the intake is same as the requirement (Torheim *et al.*, 2004).

$$\text{NAR \%} = (\text{Intake of nutrient} / \text{Recommended intake of nutrient}) \times 100$$

$$\text{MAR \%} = \text{Sum of each NAR (truncated at 100 \%)} / \text{No. of nutrients}$$

Nutrient adequacy ratios (NAR %) were categorized using a classification given by Jood *et al.* (1999).

Analysis of variance was employed to assess the difference of expenditure, nutrient intake, food variety score (FVS), dietary diversity score (DDS), nutrient adequacy ratio (NAR) and mean adequacy ratio (MAR) in the three groups. The values for critical difference (CD ($p=0.05$)) were calculated where variance ratio was significant. Multiple regression was employed to measure the effect of socio-economic factors on dietary diversity score (DDS), food variety score (FVS) and mean nutrient adequacy ratio (MAR). Coefficients of correlation were derived between food intake, socio-economic factors, dietary diversity indicators (DDS and FVS) and food security indicators (MAR and NAR) using (SAS 9.2 Software 2009).

RESULTS AND DISCUSSION

The prime occupation of households was agriculture a significantly higher agricultural income was observed in Group III when compared to Group I and II. 73.5% of the farm households were engaged in agriculture alone, while 17.8, 7.4 and 1.4% had additional occupation of dairy, service and business. The results highlighted that dairy was an important contributor to economic status of farm households. A significantly higher total and per capita income was observed in Group II when compared to Group I and Group III (Table 1).

The significantly higher expenditure on cereals was observed in Group II when compared to Group I. Group II also spent more on pulses in comparison to Group I and Group III, however, a significant difference was observed only between group II and III. Similar to cereals and pulses, the expenditure

on vegetable group was significantly higher in Group II when compared to Group III. Except fruits, expenditure on milk and milk products was also maximum in Group II and was significantly higher when compared to Group I. Expenditure on fats and oils was also highest in Group II and was significantly higher than Group III. Contrary to this, the households in Group I spent significantly more on sugars than Group III. As household income increases, the total food consumption also increases, but the share of the food consumption in total expenditure decreases. The share of the total food consumption in total household's expenditure was 38.0 % in rural areas and 25.8% in urban areas as reported by Akbay (2006).

The household size between 5-7 members was among majority of the Group I farm households (55%) while 39% had family size of 4 and less members. Group III farm households had equal percentage i.e. 41% of households in two family size categories i.e. 4 and less and between 5-7 members. Majority of Group II farm households (47%) had small family size (4 and less). The lesser percentage Group I, II and III farm households had family size larger than 8 members. The educational status of the respondents who were home makers revealed that 32 and 33% of them in Group I and Group II households studied up to 10th grade. Percentage of graduates was higher in Group III followed by Group II. The undergraduate percentage was only 16, 17 and 10% in the three groups. On an average, 22 and 18% homemakers were intermediate and graduates, while only 5% were postgraduates. On the other hand, 23 and 14%

spouses of homemakers were intermediate and graduates, respectively.

Out of 11 NAR% calculated, four of the nutrients namely Vitamin A, B₁, C and calcium had NAR more than 100%, while only Vitamin B₁₂ had NAR less than 50%, other six nutrients had NAR ranged between 51-75%. MAR for Group I, II and III was 76.2, 83.6 and 80.8%, respectively (Table 4). Overall adequacy was comparable between the three groups, however, Group I had significantly lower MAR as compared to Group II (Table 2).

Average dietary diversity score was 8.8 and used as a reference against which the dietary diversity score of the subjects in the three groups. The dietary diversity score of the subjects in Group I, II and III was 7.4, 8.1 and 8.4, respectively, being significantly ($p \leq 0.05$) in Group III than Group I (Table 2). Though the dietary diversity score was inadequate in all the groups, the subjects in Group III had dietary diversity score nearest to the reference value (8.8). The value of DDS was 84.1, 92.0 and 95.4% of the reference value in the three groups. The highest score in Group III can be attributed to higher incomes leading to more diversified diets (Table 2). Ramachandran (2007) reported that Indian diets continue to be mainly cereal based, lack diversity and are monotonous. Kennedy *et al.* (2010) found that increasing dietary diversity is associated with increased household food access as well as individual probability of adequate nutrient intake.

A significantly lower food variety score was observed in group I (18.9) when compared with group III

Table 1. Economic Status and percent expenditure of small (Group I), semi-medium (Group II) and medium (Group III) farm families

Occupation	Group I n=31	Group II n=30	Group III n=29	Overall N=90
Agriculture	12,451 ^c (69.2)	14,860 ^b (79.3)	20,275 ^{bc} (72.0)	15,862 (73.3)
Dairy	2,854 (15.8)	3,783 (20.2)	4,844 (17.2)	3,824 (17.7)
Service	2293 ^a (12.7)	100 ^{ab} (0.5)	2,534 ^b (9.0)	1,642 (7.6)
Business	419 (2.3)	0 (0)	517 (1.8)	312 (1.4)
Total income	18,017 ^c	18,743 ^b	28,170 ^{bc}	21,640
Per capita income	3,866 ^c	4,137 ^b	5,556 ^{bc}	4,519
Percent expenditure on food				
Cereals	3.33 ^a	4.25 ^a	3.64	3.74
Pulses	1.04	1.09 ^b	0.76 ^b	0.96
Vegetables	2.53	3.09 ^b	2.26 ^b	2.63
Fruits	1.23 ^c	1.51	2.40 ^c	1.71
Milk and milk products	8.37 ^a	12.54 ^a	10.09	10.33
Fats and edible oils	1.22	1.33 ^b	0.95 ^b	1.17
Sugars	0.65 ^c	0.62	0.43 ^c	0.57
Total	18.37 ^a	24.43 ^a	20.53	21.11

^{a,b,c} Significant difference between I and II, respectively
Values in parenthesis are percent of total income

Table 2. Mean adequacy ratio (MAR), nutrient adequacy ratios (NARs), food variety score (FVS) and dietary diversity score (DDS) of adult women belonging to small, semi-medium and medium farm households

	Group I n=44 Mean $\pm$ S.E.	Group II n=46 Mean $\pm$ S.E.	Group III n=43 Mean $\pm$ S.E.	Overall N=133 Mean $\pm$ S.E.
NAR %				
Energy	78.8	83.7	81.6	81.4
Protein	90.8 ^a	101.4 ^a	95.0	95.7
Vitamin A	138.1 ^c	129.1 ^b	214.4 ^{bc}	160.5
Vitamin B1	131.0	143.0	175.1	149.7
Vitamin B2	76.8 ^{ac}	95.4 ^a	97.6 ^c	89.93
Vitamin B3	67.4 ^c	67.1 ^b	59.2 ^{bc}	64.6
Vitamin B12	45.4 ^a	67.3 ^a	56.2	56.3
Folacin	74.3	82.3 $\pm$ 3.9	81.1 $\pm$ 7.9	79.2 $\pm$ 4.9
Vitamin C	159.3 ^c	198.9 ^b	301.06 ^{bc}	219.7
Iron	70.9	67.1	64.4	67.5
Calcium	185.7	230.7	236.5	217.6
MAR (%)	76.2 ^a	83.6 ^a	80.8	80.2
DDS	7.4 ^c	8.1	8.4 ^c	7.9
FVS	18.9 ^c	20.3	21.7 ^c	20.3

^{a,b,c} Significant difference between I and II, II and III, I and III, respectively

(21.7). As per classification by Savige *et al.* (1997), dietary adequacy categorized as 'good' was observed in 11.6 % of women belonging to Group III. Dietary adequacy categorized as 'fair' was highest in Group III (65.1%) followed by Group I and Group I. FVS considered as 'poor' was highest in Group I (56.8%) followed by Group II and I. FVS regarded as 'very poor' was observed only in Group I (11.4%). Torheim *et al.* (2004) reported FVS of 23 out of total score of 76 among women from rural Mali. The results showed that the overall dietary quality of women was poor.

A positive and significant relationship between DDS and FVS; between DDS and MAR and between FVS and MAR showed that all the three can be used effectively as indicators of food security. However, DDS and FVS being simple can be substituted for the more complex and time consuming MAR method in large sample studies. Guthrie and Scheer (1981) also suggested that dietary score can substitute more complex dietary analysis. FVS and DDS had a positive correlation with MAR among 15-45 years old rural Mali women as reported by Torheim *et al.* (2004).

Among the socio-economic variables, total income of the households showed a positive and significant correlation with DDS and FVS of the subjects. The income from agriculture also showed a positive correlation with DDS and FVS indicating that diversity in diet can be achieved through improved incomes. Total land owned by the households was positively and significantly correlated with DDS, FVS and MAR%. Operational land holding of the

households also showed a positive correlation with DDS, FVS and MAR indicating that women belonging to households with larger land holdings and higher incomes had more diversity in their diets. Income from dairy showed a positive correlation with FVS and MAR of the subjects. Education of the respondent was positively and significantly correlated with DDS whereas, education of the spouse did not show any significant correlation (Table 3). Musebe and Kumar (2002) reported that undernourished population was highest among the landless and households with illiterate heads. Agrahar-Murugkar and Pal (2005) reported that farming played a significant role in the consumption of cereals and fruits.

Shariff and Khor (2008) reported that land ownership per se may not be an important predictor of food security but its utilization may be protective against household food security. Thiele *et al.* (2003) reported that high diet quality in terms of consumption of vitamins, minerals and trace elements positively associated with educational level. On the other hand, Ahn *et al.* (2006) found that among less educated, the diet variety depends more on income than it does among higher educated.

Among the dietary factors, cereals were positively correlated with DDS, FVS and MAR. Among the vegetable group, green leafy vegetables were significantly correlated with MAR, while other vegetables were significantly correlated with DDS and MAR. Fruits, milk and milk showed significant and positive correlation with DDS, FVS

Table 3. Relationship of socio-economic factors with dietary diversity and food security indicators

Variables	DDS	FVS	MAR%
Socio-economic factors			
Total land owned	0.38***	0.39***	0.31***
Operational land	0.34***	0.39***	0.27**
Total income	0.32***	0.35***	NS
Income from agriculture	0.32***	0.35***	NS
Income from dairy	NS	0.19**	0.27**
Education of respondent	0.21**	NS	NS
Food intake			
Cereals	0.28***	0.32***	0.22**
Green leafy vegetables	0.13	0.13	0.24**
Roots & tubers	0.01	0.08	0.16
Other vegetables	0.26**	0.14	0.19**
Fruits	0.35***	0.28***	0.27**
Milk and milk products	0.32***	0.26**	0.27**
Nutrient intake			
Energy	NS	NS	0.45***
Carbohydrates	NS	NS	0.29***
Protein	NS	0.18**	0.69***
Fat	NS	NS	0.22**
Vitamin A	0.23**	0.25**	0.29***
Vitamin B ₂	NS	NS	0.53***
Vitamin B ₃	NS	NS	0.53***
Vitamin B ₁₂	0.27**	NS	0.43***
Folacin	NS	NS	0.43***
Vitamin C	0.28***	NS	0.26**
Iron	NS	NS	0.45***
Calcium	0.32***	0.29***	0.39***
Zinc	NS	NS	0.56***

Values are correlation coefficients (r)

DDS: Dietary Diversity Score; FVS: Food Variety Score; MAR: Mean Adequacy Ratio; **, *** Significant at 5 %; 1%, respectively; NS: Non significant

MAR%. Torheim *et al.* (2004) observed that most important factor explaining MAR were the milk and vegetables consumed by the women. MAR showed a significantly positive correlation with all the nutrients as expected. The intake of foods like vitamin A and calcium intake was significantly correlated with both DDS and FVS, while protein was significantly correlated with FVS while Vitamin B₁₂ and C showed positive correlation with DDS. Spigelski (2004) reported a significantly positive correlation between dietary diversity scores and intakes of calcium, iron, zinc, vitamin A, vitamin C, thiamine, riboflavin, vitamin B₆. Acham *et al.* (2011) reported a strong significantly positive relationship between the nutrient intakes of calcium, iron, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B₆, folate and vitamin B₁₂, vitamin C and MAR with the DDS.

Multiple regression analysis revealed that with the increase in expenditure on food, there was a significant increase in DDS, whereas, the household size was negatively but significantly associated with DDS. The education of respondents and total expenditure on food resulted in a significant increase in FVS whereas; with the increase in household size there was significant decrease in FVS. A significant increase in MAR was also observed with the increase in size of land holding, total income, income from agriculture and dairy. Increase in income from service also significantly increased MAR (Table 4). Hatleiy *et al.* (1999) reported that dietary diversity increases with socio-economic status both in urban and rural areas and irrespective of the diversity indicator used (FVS or DDS). Faber *et al.* (2004)

Table 4. Ordinary least square estimates of dietary diversity score (DDS), food variety score (FVS), mean adequacy ratio (MAR) and socio-economic factors

Variable	Regression Coefficient		
	DDS	FVS	MAR
	7.52 (4.1*10 ⁻¹⁹)	20.39 (3.51*10 ⁻²²)	84.28 (2.98*10 ⁻³⁸)
Total income	3.88*10 ⁻⁵ (4.1*10 ⁻¹⁹)	0.0002 (0.20)	0.001 (0.01)**
Income from agriculture	1.29*10 ⁻⁵ (0.81)	9.72*10 ⁻⁶ (0.94)	0.001 (0.04)**
Income from service	-4.9*10 ⁻⁵ (0.35)	-0.0001 (0.42)	0.004 (0.12)*
Income from business	3.87*10 ⁻⁵ (0.61)	4.27*10 ⁻⁵ (0.81)	0.0003 (0.51)
Income from dairy	-1.1*10 ⁻⁵ (0.87)	-4.5*10 ⁻⁶ (0.98)	0.001 (0.01)**
Education of respondent	0.034 (0.23)	0.02 (0.09)*	-0.12 (0.43)
Education of spouse	0.032 (0.31)	-0.12 (0.75)	0.03 (0.87)
Total expenditure on food	0.03 (0.11)*	0.08 (0.10)*	0.04 (0.73)
Size of land holding	0.14 (0.25)	-0.11 (0.70)	1.39 (0.03)**
Operational land holding	-0.12 (0.28)	0.10 (0.69)	-0.33 (0.57)
Household size	-0.13 (0.06)*	-0.34 (0.04)*	0.57 (0.13)*
R ²	0.27(0.006)***	0.32(0.001)***	0.23 (0.03)**

R² – Coefficient of multiple determinations

Values in parenthesis are p-value

*, **, *** Significant at 10 %, 5 %, 1%, respectively

reported that compared to the households with a DDS > 4, households with a DDS ≤ 4 experienced more food shortages and had a higher household food insecurity and access scale (HFIAS). An inverse relationship between HFIAS and dietary diversity was observed.

The study concluded that there is a strong interrelationship observed between DDS, FVS and MAR indicating that all three can be used effectively as indicators of food security. However, DDS and FVS being simple can be substituted for the more complex and time consuming MAR method in large sample studies. Total income, income from both agricultural and dairy, total land holdings and educational level of the female members of the households was crucial for dietary diversity. Vitamin A, B₁₂, C and calcium intake were found to be dependent on dietary diversity. Therefore, dietary diversity is essential to achieve food security.

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Study of Body Perception and Overweight Prevalence among Punjabi College Girls

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Abstract: The prevalence of overweight and obesity was higher in urban college girls (35%) in comparison to rural girls (14.5%). The perception of normal girls towards their body weights was better in rural group in comparison to urban group. 27% of obese girls both in urban and rural groups under reported their weights. Based on the results of the study, it is recommended that nutrition knowledge should be given to the college students especially girls so that, they can achieve optimum body composition and desirable body weights despite their heredity and family history of obesity.

Key Words: Obesity, Overweight, Rural college girls, Urban college girls

The prevalence of obesity is increasing worldwide at an alarming rate in both developing and developed countries. It has become a serious epidemic health problem, estimated to be the fifth leading cause of mortality at global level (James *et al.*, 2004). One of the major causes of obesity is the changes in the diet in terms of quantity and quality which has become more westernized (Antonio and Chiara, 2005). College students are highly exposed to unhealthy eating habits, leading to body weight gain. Moreover, decreased activity of daily living, increasing sedentary behavior and decreased work related physical activity contribute to decreased energy expenditure, which seem to be more important in contributing to the problem of obesity in youngsters.

Body perception or image is a multi-dimensional construct that involves internal biological and psychological factors as well as external cultural and social factors especially for young adults (Sira and Ballard, 2011). Learning to accept oneself, irrespective of body type or imperfections is a mentally healthy concept. However, the young adults who perceive their weight as normal, when in actuality they are overweight or obese, are in danger if they are not aware of health implications of chronic diseases. Though obesity is a multi-dimensional disorder but the determinants vary in urban and rural college girls. Therefore, this study aimed to assess the prevalence of overweight and obesity among urban and rural college girls and to determine body perception and satisfaction among these college girls.

MATERIAL AND METHODS

The college of Home Science, PAU, Ludhiana representing for urban college girls with undergraduate student strength of 273 girls and Guru Nanak National

College for women, Nakodar, representative of rural girls with undergraduate student strength of 379 had been chosen to study prevalence of obesity among college girls. The body mass index (BMI) was a criterion for the assessment of obesity. Thirty normal ($BMI: 20-22.9 \text{ kg m}^{-2}$) and thirty obese ($BMI \geq 23 \text{ kg m}^{-2}$) girls each from the urban and rural college were chosen randomly, thus making a total sample size of 120 subjects to obtain the required information on body perception and satisfaction. The anthropometric measurements viz. height, weight, waist circumference, hip circumference, triceps, biceps, sub-scapular and supra-iliac skinfold thickness measurements were taken using standard methods given by Jelliffe (1966). The derived anthropometric measurements such as BMI, waist hip ratio, wrist height ratio and body fat were calculated using basic measurements. Information regarding the body perception and satisfaction of the subjects was recorded using a questionnaire. Means, standard deviations and percentages were computed.

RESULTS AND DISCUSSION

No difference was observed in height of urban and rural girls. Similarly, the height did not vary significantly for the girls of different economic groups. There was a wide variation in the weights, the mean values of 56.07 and 50.90 kg for urban and rural girls, respectively. Economic status did not show any effect on the weight of both urban and rural girls. The mean BMI of urban and rural girls was 22.21 and 20.18 kg m^{-2} , respectively. BMI was significantly ($p \leq 0.01$) higher among urban girls while no significant difference in three income categories.

The percentage of pre-obese girls in urban and rural categories were 24 and 10 per cent, respectively (Fig. 1). The

percentage of girls in obese class I were 10 and 4 percent in urban and rural categories, respectively. The prevalence of overweight and obesity was higher in urban college girls when compared to rural girls. On the other hand, the percentage of underweight girls was much higher in rural category (35.5%) as compared to urban category (19.5%). Overall, obesity prevalence was 35% and 14.5% in urban and rural girls, respectively. The results further revealed that the incidence of obesity among urban and rural girls from uppermost income group was not significantly different. A study conducted in Hyderabad, India by Bhongir *et al.* (2011) revealed that BMI was significantly higher in urban students (31.9%) when compared to rural girls (3.5%). The incidence of overweight / obesity among Punjabi college girls was higher than their counterparts from Hyderabad.

Basic and derived anthropometric measurements

The average height of urban normal and obese girls was 1.59 and 1.58 m and of rural normal and obese girls was 1.58 and 1.59 m, respectively. The differences were non significant in the body weights of urban and rural girls.

The mean biceps, triceps, sub-scapular and supra-iliac skinfold measurements of normal girls in urban group were 13.04, 11.22, 11.95 and 9.85 cm respectively. The corresponding values in rural group were 13.15, 10.46, 10.13 and 10.04 cm. No significant difference in skinfold measurements of urban and rural groups however, a highly significant difference was observed in the four skinfold measurements of normal and obese girls.

The average waist, hip and wrist circumference of normal girls in urban group were 72.68, 98.01 and 16.19 cm, respectively, while the corresponding values in rural group were 70.32, 97.22 and 16.40 cm. Similarly, the average values for three circumferences in obese girls in urban obese group were 84.26, 107.69 and 17.36 cm, respectively and the corresponding values in rural group were 84.15, 111.12 and 17.80 cm. The statistical analysis showed a non significant difference in three circumferences of urban and rural girls, however, a highly significant ($p \leq 0.01$) values were observed in case of obese subjects when compared to normal subjects. Babitha and Kusuma (2009) reported that the waist circumference of obese and non-obese Rajasthani women were 83.2 and 67.7 cm, respectively. The results of the present study were close to the reported study.

The average body mass index (BMI) of the normal urban and rural girls was 21.62 and 21.20 kg m⁻², respectively. The corresponding values for obese urban and rural girls were 27.97 and 27.44 kg m⁻². No significant difference was found between the BMI of urban and rural groups, however 29.4% higher BMI was observed in obese girls when it was compared to the BMI of normal girls.

Table 1. Height, weight and Body Mass Index (BMI) of girls (18-22y) from three income categories studying in the selected urban and rural areas

	Group I (<Rs25000)				Group II (Rs 25000-50000)				Group III (>Rs 50000)				All Income Groups	
	Urban (n= 53)	Rural (n= 313)	t-value	Urban (n= 138)	Rural ((n=62)	t-value	Urban (n= 83)	Rural (n=4)	t-value	Urban (n=274)	Rural (n=379)	t-value		
Height, m														
Mean	1.59	1.59	NS	1.59	1.61	NS	1.58	1.57	NS	1.59	1.59	NS		
Weight, Kg														
Mean	54.04	50.53	3.12**	56.72	52.65	2.34*	57.90	52.35	NS	56.07	50.90	6.35**		
BMI, kg/m ²														
Mean	21.37	20.13	2.82**	22.41	20.33	3.32**	23.14	21.27	NS	22.21	20.18	6.40**		

NS Non significant; *Significant at 10%; **Significant at 5%; ***Significant at 1%

Kuriyan *et al.* (2007) reported that BMI of normal and overweight young women from Bangalore was 20.7 and 26.6 kg/m², respectively. The average waist/hip ratio of normal urban and rural girls was 0.74 and 0.73 while of obese urban and rural girls, it was 0.78 and 0.76. There was no significant difference between waist/hip ratio of urban and rural girls, however, a highly significant ($p \leq 0.01$) difference was observed in this ratio for normal and obese girls. A cut-off point of 0.8 for waist and hip ratio has been taken as reference value (Ghafoorunissa and Krishnamurthy, 2000). All the girls had waist/hip ratio below the cut-off value. The optimum waist/hip ratio of the obese girls was attributed to

higher values for both waist and hip circumference when compared to normal girls. Wrist/height ratio is considered as an indicator of body frame. The average wrist/height ratio of normal urban and rural girls was 9.84 and 9.63 while it was 9.22 and 8.96 for obese urban and rural girls, respectively. A highly significant ($P \leq 0.01$) difference was also found in the wrist/height ratio of normal and obese girls. As per classification of body frame given by Grant (1980), all the girls were found to be in the category of large body frame.

Body Perception and Satisfaction

Among urban group, 17% of girls with normal BMI perceived themselves overweight while 3% perceived

Table 2. Classification of urban and rural girls belonging to different income groups on the basis of their Body Mass

Classification	Group I (Rs< 25000)	Group II (Rs 25000- 50000)	Group III (Rs>50000)
Urban			
Obese	24(28)	53(38)	19 (39.5)
Normal	43 (49)	59(43)	22 (46)
Underweight	20(23)	26(19)	7(14.5)
Total	87(100)	138(100)	48(100)
Rural			
Obese	46(14.5)	9(14.5)	1 (25)
Normal	156(50)	31(50)	1 (25)
Underweight	111(35.5)	22(35.5)	2(50)
Total	313(100)	62(100)	4(100)

Figures in parenthesis indicate percent

Table3. Basic anthropometric measurements of normal and obese girls

	Urban		Rural		P value	
	Normal (n=30)	Obese (n= 30)	Normal (n=30)	Obese (n= 30)	Urban/ Rural	Normal/ Obese
Height (m)						
Mean	1.59	1.58	1.58	1.59	0.9377 ^{NS}	0.8659NS
Weight (kg)						
Mean	54.88	69.68	52.63	69.74	0.4931 ^{NS}	<0.0001***
Waist circumference (cm)						
Mean	72.68	84.26	70.32	84.15	0.3725 ^{NS}	<0.0001***
Hip circumference (cm)						
Mean	98.01	107.69	97.22	111.12	0.5190 ^{NS}	<0.0001***
Wrist circumference (cm)						
Mean	16.19	17.36	16.40	17.80	0.1885 ^{NS}	<0.0001***
Biceps (mm)						
Mean	13.04	21.40	13.15	21.50	0.9140 ^{NS}	<0.0001***
Triceps (mm)						
Mean	11.22	29.83	10.46	19.81	0.2399 ^{NS}	0.0027***
Sub scapular (mm)						
Mean	11.95	19.29	10.13	19.48	0.2866 ^{NS}	<0.0001***
Supra iliac (mm)						
Mean	9.85	18.08	10.04	20.64	0.4921 ^{NS}	<0.0001***

NS Non significant;*** Significant at 1%

themselves underweight. On the other hand, 27% of obese urban girls considered themselves normal. In rural group, only 7% of normal girls considered themselves as overweight or obese while 27% of obese girls perceived themselves normal. The results indicated that the perception of normal girls towards their body weights was better in rural group in comparison to urban group, however, no difference in body perception was found among rural normal and obese girls. Combs (2009) showed that gender differences existed in the accuracy of weight perception with females overestimating their weight.

In normal group, 17 and 3% of urban and rural parents perceived their child as obese while only 3% considered their child as underweight in both urban and rural group. Contrary to this, 50 and 12% of the parents of urban

and rural obese girls considered their child as normal. The findings clearly reflected that half of the parents in urban group under reported the weight of their ward. The subjects in urban and rural group reported that 80 and 9 % of their siblings and friends perceived their weights correctly. In case of obese group, 67 and 77% siblings and friends of urban and rural subjects accurately perceived their body weights, respectively. The results showed that there is lesser accuracy in body perception by self, parents, siblings and friends among urban subjects which may be the causative factor of higher incidence of overweight and obesity among urban girls in comparison to rural girls.

The study concluded that prevalence of overweight and obesity was higher in urban college girls in comparison to rural girls. The body weight of normal girls in both urban and

Table 4. Derived anthropometric measurements of normal and obese girls

	Urban		Rural		P value	
	Normal	Obese	Normal	Obese	Urban/ Rural	Normal/ Obese
Body Mass Index, Kg m ⁻²						
Mean	21.62	27.97	21.20	27.44	0.3706 ^{NS}	<0.0001***
Waist/hip ratio						
Mean	0.74	0.78	0.73	0.76	0.1675 ^{NS}	0.0167**
Wrist/height ratio						
Mean	9.84	9.22	9.63	8.96	0.0996*	<0.0001***
Percent body fat						
Mean	25.39	33.85	24.70	33.55	0.4921 ^{NS}	<0.0001***

^{NS} Non significant; *Significant at 10%; **Significant at 5%; *** Significant at 1%

Table 5. Body Perception of normal and obese girls studying in an urban and rural colleg

Body perception	Urban College (%)		Rural College (%)	
	Normal (n=30)	Obese (n= 30)	Normal (n=30)	Obese (n= 30)
Self perception of body weight				
Underweight	3	0	0	0
Normal	80	27	93	27
Overweight	17	60	7	56
Obese	0	13	0	17
Parental perception of body weight				
Underweight	10	0	3	0
Normal	73	50	94	40
Overweight	17	47	3	50
Obese	0	3	0	10
Friends and siblings perception regarding body				
Underweight	3	0	0	0
Normal	80	33	97	23
Overweight	17	57	3	60
Obese	0	10	0	17

Table 6. Body satisfaction among selected normal and obese girls

	Urban (%)		Rural (%)	
	Normal (n=30)	Obese (n=30)	Normal (n=30)	Obese (n=30)
Body Satisfaction				
Satisfied	77	50	93	33
Unsatisfied	23	50	7	67
Personality Satisfaction				
Yes	87	63	80	30
No	13	37	20	70
Parental Trust				
Father				
Yes	93	100	100	97
No	7	0	0	3
Mother				
Yes	100	100	100	100
No	0	0	0	0
Siblings and Friends Trust				
Siblings				
Yes	100	93	100	100
No	0	7	0	0
Friends				
Yes	100	90	100	100
No	0	10	0	0
Communication with Parents				
Father				
Good	100	90	100	100
Poor	0	10	0	0
Mother				
Good	100	100	100	100
Poor	0	0	0	0
Communication with Siblings and Friends				
Communication with siblings				
Good	100	93	100	100
Poor	0	7	0	0
Communication with Friends				
Good	100	90	100	100
Poor	0	10	0	0

rural groups was close to the weight of reference Indian woman. The perception of normal girls towards their body weights was better in rural group in comparison to urban group. About one-fourth of obese girls both urban and rural groups under reported their weights. Based on the results of the study, it is recommended that nutrition awareness, importance of physical activity and self-discipline need to be promoted among young girls through various educational programs so that, they can achieve optimum body composition and desirable body weights despite their heredity and family history of obesity.

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Received 30 September, 2015; Accepted 1 February, 2016

Oh y for Members



Antifungal Potential of Coriander Seed Essential Oil and its Constituents

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Abstract: Forty one compounds were identified, which constituted approximately 98% of the coriander seed essential oil. The major components were linalool (75.2%), geraniol (5.24%), geranyl acetate (4.98%), camphor (1.76%), α -pinene (1.67%) and limonene (1.49%). The antifungal activity of the coriander seed essential oil, its fractions and isolated compounds was evaluated against plant pathogens *Alternaria triticina* and *Bipolaris sorokiniana* using spore germination technique. ED₅₀ and ED₉₀ values for all components against the tested fungi were observed in the range 0.565-1.0 and 1.5-2.6 mg mL⁻¹, respectively.

Key Words: Antifungal activity, *Coriander sativum*, Essential oil, Hydrodistillation, Linalool

Bioactive phyto-chemicals are often recovered as essential oils by hydro-distillation of whole tissues and seeds of plants (Delaquis *et al.*, 2002). Essential oils and several plant extracts, rich in monoterpenes are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents. Monoterpenes are abundant natural compounds that meet the non-toxicity and low cost criteria required for new pesticide candidates. Essential oils are aromatic oily liquids obtained from different plant parts and have broad spectrum of activities. Due to antifungal properties of the essential oils, research on structurally unique biologically active compounds progressed (Matasyoh *et al.*, 2009).

Coriander sativum is a glabrous, aromatic, herbaceous annual herb belonging to the family Apiaceae (Duke *et al.*, 2002). Coriander seed oil was included among the 20 major essential oils in the world market and its commercial value depends on its physical properties, chemical composition and aroma (Smallfield *et al.*, 2001). Linalool was found to be the main volatile compound in seeds, typically constituting more than 50% of the total essential oil (Ramadan and Morsel, 2003). Many phyto-chemical studies carried out on this plant resulted in isolation of bioactive ingredients. Coriander oil has also been reported to possess antifungal (Garg and Siddiqui, 1992), antimicrobial (Baratta *et al.*, 1998) and antibacterial (Cantore *et al.*, 2004; Kubo *et al.*, 2004). The present investigation was carried out to study the chemical composition and the antifungal potential of coriander seed oil, its non-polar and polar fractions and isolated compounds against two wheat fungi i.e. *Alternaria triticina* and *Bipolaris sorokiniana*.

MATERIAL AND METHODS

The powdered coriander seeds (var. Punjab Sugandh) were dipped in water overnight and the essential oil was extracted using Dean and Stark apparatus. The yield of oil was determined and the essential oil was stored at 4°C for further use. The coriander seed essential oil was analyzed for chemical composition using GC-MS. The GC-MS (QP2010 Plus, Shimadzu, Japan) was equipped with an Rtx-5 MS capillary column (30.0 m × 0.20 mm i.d., 0.25 μ m film thickness). The GC injector was maintained at 250°C and operated in split injection mode with the split valve closed for 1 min. Helium gas was used as the carrier gas at a constant pressure of 69 kPa. The column oven was initially maintained at 50°C for 2 min, raised to 180°C at 3°C min⁻¹, then to 280°C at 10°C min⁻¹. The interface temperature was 260°C and the ionization mode was electron impact (70 eV). The mass selective detector was operated in the scan mode between 40 and 600 m/z. Data acquisition was started 3.0 min after injection. MS parameters used were; Ionization Voltage (EI) 70 eV, peak width 2s, mass range 40–600 amu and detector voltage 1.5 V. Peak identification was carried out by comparison of the mass spectra with mass spectra data available on database of NIST08, WILEY8, Perfumery and Flavor and Fragrance libraries.

The coriander seed oil was dissolved in ether and applied on the chromatoplate coated with silica gel G. The chromatographic plates were developed using benzene: ethyl acetate (19:1) as the solvent system, air dried, sprayed with sulfuric acid: methanol (1:19) and heated in an electric oven at 100°C for 5 min in order to reveal the spots. The R_f values and colours of the spots were noted. The coriander seed essential oil (10g) was fractionated into non-polar and

polar fractions using column chromatography. Column was packed with silica gel 60-120 mesh size activated at 110°C for 1 hour. The essential oil was adsorbed on silica gel for 5 min and column was eluted with petroleum ether and dichloromethane as the solvents to obtain non-polar and polar fractions. The coriander seed essential oil was subjected to extensive column chromatography to isolate pure compounds using petroleum ether as solvent and polarity was increased using dichloromethane.

Antifungal activity: Pure cultures of *A. triticina* and *B. sorokiniana* were obtained from the Department of Plant Pathology, PAU, Ludhiana, Punjab, India and were maintained on Potato Dextrose Agar (PDA) slants and plates at 4°C. The antifungal activity was evaluated at different concentrations i.e. 0.5, 1.0, 2.0 and 3.0 mg mL⁻¹ by spore germination inhibition method.

Spore germination inhibition method: Antifungal activity was tested using spore germination inhibition technique. Ten-day old cultures of the test fungi (*A. triticina* and *B. sorokiniana*) were taken from PDA slants and spore suspension was made by adding sterilized distilled water. In order to remove mycelial and agar bits, the suspension was filtered through three layers of sterilized cheese cloth after shaking the tube thoroughly under aseptic conditions. Haemocytometer was used to form standardized spore suspension. Small droplets (0.02 mL) of test solution and spore suspension in equal amount were seeded in the cavities of the cavity slides. These slides were placed in petriplates lined with moist filter paper and incubated for 20 hrs at 24±1°C. The number of spores germinated were counted and per cent spore germination inhibition was calculated. The fungicide Bavistin 50WP was used as the standard to compare the activity of the compounds. The antifungal activity was expressed in terms of ED₅₀ and ED₉₀

values i.e. effective dose to inhibit 50 and 90 percent spore germination, respectively.

RESULTS AND DISCUSSION

The coriander seed essential oil was pale yellow coloured liquid, having density and refractive index of 0.886 gm cm⁻³ and 1.504, respectively at 20°C. The essential oil was soluble in organic solvents like acetone, benzene and dichloromethane and insoluble in water. The yield of coriander seed oil was 0.18%. Thin layer chromatography of coriander seed essential oil revealed four spots having R_f values of 0.44 (yellow), 0.62 (purple), 0.68 (dark green) and 0.90 (pinkish-red). The non-polar fraction showed one spot having R_f value of 0.90 (pinkish-red). The polar fraction gave three spots having R_f values of 0.44 (yellow), 0.62 (purple) and 0.68 (dark green).

Chemical composition of coriander seed oil: Gas chromatography-Mass spectrometry (GC-MS) data of coriander seed essential oil revealed the presence of forty one compounds of which linalool (75.2%) was the major compound. Other compounds were geraniol (5.24%), geranyl acetate (4.98%), camphor (1.76%), alpha-pinene (1.67%), limonene (1.49%) etc. Linalool mainly contributes to the aromatic odour of the essential oil. The retention time and percentage area of each compound are given in Table 1.

Three pure compounds limonene, geraniol and linalool were isolated using extensive column chromatography and were characterized with the spectral analysis (Table 2).

Antifungal Activity

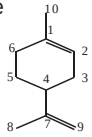
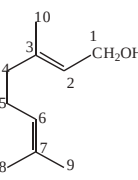
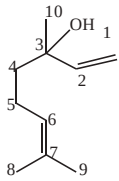
Coriander seed essential oil, its fractions and compounds isolated were evaluated *in vitro* for their antifungal potential against *A. triticina* and *B. sorokiniana* using spore germination inhibition method.

A. triticina: The antifungal activity of oil and its components

Table 1. GC-MS data of Coriander seed essential oil

Name	Retention time (min)	Area (%)	Name	Retention time (min)	Area (%)
α-Pinene	7.730	1.67	n-Undecanal	24.095	0.08
Camphene	8.267	0.10	4-vinyl-Guaiacol	24.385	0.20
β-Pinene	9.348	0.24	Myrtenyl acetate	24.899	0.05
Myrcene	9.966	0.22	Citronellyl acetate	26.111	0.09
p-Cymene	11.342	0.66	Neryl acetate	26.599	0.06
Limonene	11.516	1.45	Geranyl acetate	27.472	4.98
γ-Terpinene	12.854	0.49	Dodecanal	28.441	0.12
Linalool oxide	13.470	0.65	(E)-Caryophyllene	28.862	0.18
1-Octanol	13.776	0.21	Dodec-(2e)-enal	30.790	0.29
Linalool	15.300	75.02	trans-2-Dodecen-1-ol	31.002	0.13
Camphor	16.797	1.76	Butylated hydroxytoluene	32.696	0.09
Borneol	17.747	0.86	Trans-tetradec-2-enal	38.685	0.10
1-Nonanol	18.047	0.09	4-Nonyl-phenol	40.290	0.04
Terpin-4-ol	18.259	0.24	(z)-9-Octadecenoic acid	41.954	0.07

Table 2. Spectral data of isolated compounds

Structure	IR data(cm^{-1})	^1H NMR (400 MHz) data (δ in ppm)	^{13}C NMR data (δ in ppm)
Limonene 	3070 (=C-H stretching), 2925 and 1446 (C-H stretching of $-\text{CH}_2-$), 1643(C=C stretching), 1375 (C-H bending of CH_3)	1.65(s, 3H), 1.73(s, 3H), 1.42-1.52(m), 1.72-1.82(m, H), 4.70(s, 2H), 5.40(m, H).	133.75(C_1), 120.65(C_2), 30.60(C_3), 41.09(C_4), 27.92(C_5), 30.81(C_6), 150.28(C_7), 108.3(C_8), 20.82(C_9), 23.47 (C_{10}).
Geraniol 	3368(-OH), 2966 (C-H stretching), 2926 and 1450(C-H stretching of $-\text{CH}_2-$), 1641(C=C stretching), 1377 (C-H bending of CH_3), 1057(C-O stretching).	1.60(s, 3H), 1.66(s, 3H), 1.68(s, 3H), 4.14 (d, 2H), 5.09(t, 1H), 5.39(t, 1H)	59.15(C_1), 124.7(C_2), 139.33 (C_3), 39.52 (C_4), 26.37 (C_5), 123.9 (C_6), 131.64 (C_7), 19.4 (C_8), 25.63 (C_9), 16.20 (C_{10}).
Linalool 	3400(-OH), 3086 (=C-H stretching), 2970 (C-H stretching), 2926 and 1452 (C-H stretching of $-\text{CH}_2-$), 1641(C=C stretching), 1375 (C-H bending of CH_3) and 1113(C-O stretching)	1.25(s, 3H), 1.58(s, 3H), 1.66(s, 3H), 1.46-1.55(m, 2H), 1.96-2.05(m, 2H), 4.99-5.21(dd, 2H, $J_{\text{trans}}=17.4$ Hz, $J_{\text{cis}}=10.7$ Hz, $J_{\text{gem}}=1.3$ Hz), 5.84-5.91(dd, 1H, $J_{\text{trans}}=17.4$ Hz, $J_{\text{cis}}=10.7$ Hz), 5.07-5.11(t, 1H).	111.44(C_1), 145.01 (C_2), 73.08 (C_3), 42.08 (C_4), 22.66 (C_5), 124.45 (C_6), 131.10 (C_7), 26.01(C_8), 17.43 (C_9) and 27.40 (C_{10}).

increased with increase in concentration (Table 3). The ED_{50} and ED_{90} values of coriander seed oil, its non-polar and polar fractions were 1.00, 0.995, 0.985 and 1.98, 1.91, 1.97 respectively. Linalool, geraniol and limonene showed 50 % inhibition and 90 % inhibition at 0.87, 0.85, 0.94 mg mL^{-1} and 1.80, 1.82, 1.81 mg mL^{-1} , respectively (Fig. 1). The isolated compounds i.e. linalool, geraniol and limonene were found to be more effective than oil and its fractions.

Linalool and geraniol were significantly more effective than coriander seed oil and other components. This may be attributed to alcoholic moiety present in these compounds. Statistical analysis of data showed that there was a positive correlation between concentrations and antifungal activity (Table 3). The standard fungicide used (Bavistin 50WP) showed ED_{50} and ED_{90} values 0.08 and 0.15 mg mL^{-1} . It was more effective than coriander seed oil and its components.

B. sorokiniana: Fungicidal activity of oil and its components increased with increase in concentration (Table 4). The ED_{50} and ED_{90} values of coriander seed oil, its non-polar and polar fractions were 0.59, 0.657, 0.61 and 2.62, 2.32, 2.22 respectively. The ED_{50} and ED_{90} values of isolated compounds linalool, geraniol and limonene were 0.612, 0.675, 0.655 mg mL^{-1} and 2.00, 2.06, 2.62 mg mL^{-1} , respectively (Fig. 2). The order of antifungal activity was:

Linalool > Geraniol > Polar-fraction > Coriander seed oil > Limonene > Non-Polar fraction

Statistical analysis of data (Table 4) showed that there was a positive correlation between concentration and antifungal activity. The standard fungicide (Bavistin 50WP)

Table 3. Antifungal activity against *A. triticina*

Components	Concentrations (mg mL^{-1})			
	0.5	1.0	2.0	3.0
Coriander seed essential oil	22.83 (28.52)	49.99 (44.97)	90.90 (72.42)	100.00 (89.96)
Polar fraction	21.07 (27.32)	51.38 (45.77)	95.63 (78.20)	100.00 (89.96)
Non-polar fraction	21.09 (27.30)	50.85 (45.47)	93.63 (75.38)	98.35 (82.72)
Linalool	26.60 (31.03)	58.04 (49.61)	97.56 (81.43)	100.00 (89.96)
Geraniol	25.65 (30.41)	60.63 (51.12)	95.90 (78.34)	100.00 (89.96)
Limonene	18.01 (25.08)	54.58 (47.61)	97.96 (81.76)	98.65 (84.51)
Bavistin 50WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)

Figures in parentheses are arc sine transformed values
CD ($p=0.05$) Component: 1.46, Concentrations: 1.10, Interaction: 2.93

showed ED_{50} and ED_{90} values 0.04 and 0.08 mg mL^{-1} , respectively.

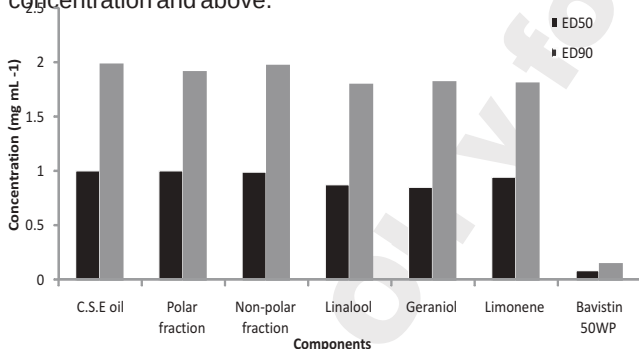
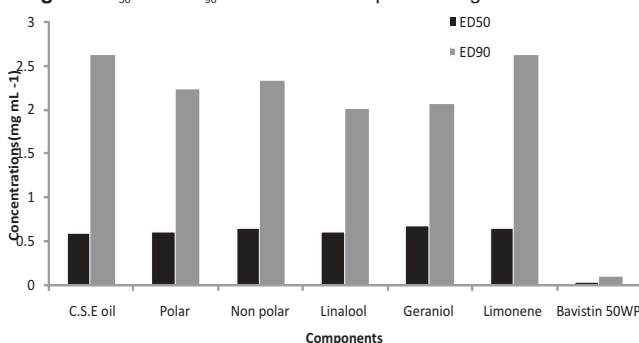
In our studies, it was found that ED_{50} of coriander seed oil and its tested components was 0.85-1.00 mg mL^{-1} and 0.59-0.655 mg mL^{-1} , respectively against *A. triticina* against *B. sorokiniana*. In earlier studies, Lalitha *et al.* (2011) reported that essential oil of *C. sativum* showed complete inhibition at 2.0 mg mL^{-1} concentration against *Bipolaris oryzae*, *Alternaria alternata*, *Drechslera halodes* and *Fusarium oxysporum* while *Pyricularia oryzae*, *Tricoconis padwickii* and *Fusarium moniliforme* were completely inhibited at 1 mg mL^{-1} concentration. Coriander seed

Table 4. Antifungal activity against *B. sorokiniana*

Components	Concentration (mg mL ⁻¹)			
	0.5	1.0	2.0	3.0
Coriander seed essential oil	44.75 (41.97)	74.25 (59.48)	86.90 (68.07)	95.66 (77.95)
Polar fraction	42.81 (40.84)	75.08 (60.03)	87.09 (69.05)	100.00 (89.96)
Non-polar fraction	43.09 (41.01)	65.19 (53.82)	86.53 (68.47)	97.09 (80.28)
Linalool	43.19 (41.07)	73.32 (58.87)	89.62 (71.41)	100.00 (89.96)
Geraniol	35.99 (36.85)	76.00 (60.65)	88.61 (70.28)	100.00 (89.96)
Limonene	40.163 (39.31)	71.65 (57.81)	84.72 (66.98)	97.82 (81.47)
Bavistin 50WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)

Figures in parentheses are arc sine transformed values
CD (p=0.05) Components: 1.03, Concentrations: 0.78,
Interaction: 2.07

essential oil showed maximum activity of 84.75 and 84.31% against *Drechslera tetramera* and *Fusarium solani* at 2 mg mL⁻¹ concentration. Darughe *et al.* (2012) reported that coriander seed essential oil completely inhibited the growth of moulds in cakes at 1.5 mg mL⁻¹ concentration upto 10 days and 63% upto 60 days. Sumalan *et al.* (2013) reported that the coriander seed oil inhibited the growth of various fungi i.e. *Alternaria*, *Fusarium*, *Saccharomyces*, *Aspergillus* and *Hyphopichia* species in stored wheat grains at 2 mg mL⁻¹ concentration and above.

**Fig. 1.** ED₅₀ and ED₉₀ values of all components against *A. triticina***Fig. 2.** ED₅₀ and ED₉₀ values of all components against *B. sorokiniana*

CONCLUSION

Forty one compounds were identified in GC-MS analysis and linalool was the major constituent. The components like linalool and geraniol having alcoholic moiety were more effective than coriander seed oil and its other components, so antifungal activity of these compounds may be related to their alcoholic moiety. Although the tested components showed activity at higher concentration as compared to standard fungicide, yet essential oil being non-toxic and environment friendly can be used as alternative to synthetic fungicides. Coriander seed oil and its tested components may be used to control fungal infestation.

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Received 22 February, 2016; Accepted 7 May, 2016



Appraisal of Status of Metacognitive Skills, Emotional Intelligence and Achievement Motivation among Adolescents

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Abstract: The present research is an attempt to assess the levels of metacognitive skills, emotional intelligence and achievement motivation among college students of Ludhiana District. The sample included 200 college students, in age range of 17-19 years. The major proportion of adolescents had a high level of metacognition. Boys had higher mean scores as compared to their female counterparts, across various components and sub-components of metacognition. A majority of respondents was in average and low level of emotional intelligence whereas irrespective of gender, the respondents were found to possess low level achievement motivation.

Key Words: Achievement motivation, Emotional intelligence, Metacognitive skills

Metacognition is important in learning and is a stronger predictor of academic success (Dunning *et al.*, 2003). Students with good metacognition demonstrate good academic performance compared to students with poor metacognition who may be benefitted from metacognitive training to improve their metacognition and academic performance. Individual differences exist in metacognition and people with poor metacognition are deemed "incompetent" as they perform inadequately relative to their peers (Kruger and Dunning, 1999). Emotional Intelligence is defined as the capacity to reason about emotions, and of emotions to enhance thinking and it determines the ability to handle one's relationships and exert self-control to display her/his emotions in a mature manner. Further, achievement motivation is an important determinant of aspiration, effort, and persistence when an individual expects his performance will be evaluated in relation to some standard of excellence. People who are oriented towards achievement, in general, enjoy life and feel in control. It is also seen that achievement motivated people seem to be more concerned with their personal achievement rather than the rewards of success.

Thus, the foregoing discussion emphasizes three important traits viz. metacognition, emotional intelligence and achievement motivation as precursors of academic excellence and personal adjustment. Therefore, it seems imperative to explore the status of these three variables for stability and success in professional and personal life. So, the present study was planned to explore the socio-personal characteristics of the college adolescents and assess their emotional intelligence, achievement motivation and metacognitive skills.

MATERIAL AND METHOD

The study was conducted in the four constituent colleges of Punjab Agricultural University, Ludhiana. The final sample comprised 200 college going adolescents aged 17 to 19 years (N=200) studying in the second and third year classes of various undergraduate programmes. The subjects were proportionately drawn from each class and were equally distributed across both the genders (males=100 & females=100). To assess the achievement motivation of the respondents Academic Achievement Motivation Test, designed by Sharma (1984), was used. Before its final use, the Academic Achievement Motivation Test was translated to English language for the convenience of the adolescents, pretested and modified. Mayer-Salovey-Caruso Emotional Intelligence Test (2000) was used to assess the emotional intelligence of the selected subjects, whereas metacognitive skills were assessed from Metacognitive Awareness Inventory (MAI) designed by Schraw and Dennison (1994).

RESULTS AND DISCUSSION

The adolescents fell in almost an equal number belonged to 17 years and 18 years of age. 5.50 per cent subjects had no siblings, whereas 27.50 per cent had one sibling, and rest 30.50 per cent had three or more siblings. The majority of the mothers were housewives and the percentage was slightly higher in case of mothers of male respondents as compared to female respondents. The occupations of the fathers of the respondents, major were engaged in business while very small proportion was non-working. Sixty three per cent of adolescents belonged to the nuclear families and rest belonged to joint families.

Table 1. Socio-personal profile of the respondents (%) (N=200)

Socio-personal characteristics	Male (n=100)	Female (n=100)	Overall (N=200)
Age (years)			
17	29	28	28.5
18	30	33	31.5
19	41	39	40
No. of siblings			
Only child	6	5	5.5
One	34	21	27.5
Two	35	38	36.5
> two	25	36	30.5
Maternal education			
Illiterate	13	15	14
Up to 5 th	45	42	43.5
Matric	27	25	26
Up to +2	10	12	11
Graduate	3	4	3.5
Postgraduate	2	2	2
Paternal Education			
Illiterate	8	11	9.5
Up to 5 th	15	18	16.5
Matric	45	39	42
Up to +2	25	23	24
Graduate	4	5	4.5
Postgraduate	3	4	3.5
Maternal occupation			
House wife	82	73	77.5
Business	5	10	7.5
Service	11	14	12.5
Farming	1	1	1
Labourer	1	2	1.5
Paternal occupation			
Non-working	2	0	1
Business	53	63	58
Service	20	18	19
Farming	16	13	14.5
Labourer	9	6	7.5
Family Type			
Nuclear	60	66	63
Joint	40	34	37

Level of metacognitive skills, emotional intelligence and achievement motivation: Major proportion of respondents represented the category of 'high level' of metacognition, followed by average level of metacognition. Gender-wise distribution revealed that the major proportion of boys and girls had a 'high level' of metacognition. The number of boys falling in the average level of metacognition was slightly more

than girls. The picture was quite similar in the 'low level' of metacognition with only one per cent boys and girls falling in this level of metacognition. However, the gender differences at all levels of metacognition were found to be statistically non-significant.

Table 2. Per cent distribution of adolescents as per levels of metacognition

Levels of Metacognition	Male (n=100) f (%)	Female (n=100) f (%)	z-value	Overall (N=200) f (%)
High (>34.67)	64	66	0.21	65
Average (17.33-34.66)	35	33	0.44	34
Low (up to 17.33)	1	1	NA	1

NA: Not applicable

Across the three sub-components of the knowledge of cognition, more than half of the respondents were falling in high level of metacognition (58, 71.5 and 57.5, respectively). In case of conditional knowledge, 39.50 per cent of respondents were falling in the category of average level of metacognitive skills followed by declarative knowledge and procedural knowledge. Almost, 9 per cent respondents showed up in the low level of metacognitive skills in procedural knowledge followed by 4 per cent in 'declarative knowledge' and 3 per cent in conditional sub-component. It is indicated that among these three sub-components, the respondents exhibited better knowledge about the execution of procedural skills and were therefore, more likely to sequence their strategies effectively during studying. The declarative knowledge sub-component was found in between moderate and low level which indicates that respondents' knowledge and beliefs about oneself as a thinker or learner, and what he/she believes about other people's thinking processes was not so good. However, it was found that conditional knowledge indicating their judgment about assessment of their comprehension errors was moderately developed. This suggests that parents and teachers need to encourage adolescents in low and average category by making the home and classroom environment more conducive for developing high level of metacognitive skills.

With respect to regulation of cognition, the respondents performed better in information management strategies and 'debugging' sub-components with majority of subjects falling under 'high level' of metacognition. It indicates that respondents were good at processing information and use strategies to correct their learning. On the other hand, 14 per cent respondents were found in evaluation sub-component of metacognition followed by 11 per cent in 'planning', very few in 'information management strategies' and 'debugging' sub-components. The per cent of

Table 3. Gender-wise per cent distribution of adolescents across varying levels of the sub-components of metacognition (%)

Levels of metacognitive skills	Male (n=100)	Female (n=100)	z-value	Overall (N=200)
I- Knowledge of cognition				
Declarative knowledge				
High (5.34-8.00)	60	56	0.57	58
Average (2.67-5.34)	35	41	0.89	38
Low (up to 2.67)	5	3	0.72	4
Procedural knowledge				
High (2.67-4.00)	73	70	0.48	71.5
Average (1.34-2.66)	18	21	0.53	19.5
Low (up to 1.33)	9	9	NA	9
Conditional knowledge				
High (3.34-5.00)	61	54	1.00	57.5
Average (1.67-3.33)	36	43	1.01	39.5
Low (up to 1.66)	3	3	NA	3
II-Regulation of cognition				
planning				
High (4.67-7.00)	61	58	0.43	59.5
Average (2.34-4.66)	28	31	0.46	29.5
Low (up to 2.33)	11	11	NA	11
Information management strategies				
High (6.67-10.00)	73	70	0.48	71.5
Average (3.34-6.66)	22	27	0.81	24.5
Low (up to 3.33)	5	3	0.72	4
Comprehension monitoring				
High (4.67-7.00)	66	62	0.59	64
Average (2.34-4.66)	28	29	0.16	28.5
Low (up to 2.33)	6	9	0.77	7.5
Debugging				
High (3.34-5.00)	73	69	0.63	71
Average (1.67-3.33)	23	26	0.48	24.5
Low (up to 1.66)	4	5	0.32	4.5
Evaluation				
High (5.00-6.00)	41	44	0.43	42.5
Average (3.00-4.00)	43	44	0.14	43.5
Low (up to 2)	16	12	0.82	14
Total				
High (>34.67)	64	66	0.21	65
Average (17.33-34.66)	35	33	0.44	34
Low (up to 17.33)	1	1	NA	1

NA: Not applicable

'evaluation' subcomponent of metacognition indicates that adolescents have to work on their analyzing skills of their performance after the completion of learning episode. Therefore, among the various sub-components of regulation of cognition component of metacognition adolescents were weak in the self evaluation and planning aspect. However, the gender differences were found to be statistically non-significant across all the components and sub-components of metacognition.

The boys had slightly better mean scores as

compared to their female counterparts, across all the sub-components except 'evaluation' with respect to the two components of metacognition (Table 4). But statistically the gender differences in the mean scores of respondents were found to be non-significant. It could be inferred from these observations that boys and girls were comparable in their 'knowledge as well as regulation of their own thinking and learning activities. Although the boys were slightly better aware and had good management of cognition, including knowledge about appropriate strategies as compared to their female counterparts as they had better mean scores in declarative, procedural as well as conditional knowledge as compared to girls. Similarly, gender differentials were non-significant in case of regulation of cognition component. However, girls were found to obtain better mean scores in evaluation strategies suggestive of that they are better at appraising the products and efficient of one's own learning. The above findings reflect that the respondents regulate their planning, information management strategies, comprehensive monitoring, debugging strategies with proper attention but they were inattentive at evaluation strategies. Further, it is interesting to note that both boys and girls had overall better mean scores of 5.07 and 4.95, respectively on regulation of cognition component of metacognition as compared to the knowledge of cognition component. Hence, it can be concluded that parents and teachers need to work harder in building up the self-awareness aspect more than the self-regulation aspect of adolescents.

It is evident that the gender differentials in mean scores across various sub-components of metacognition were better in males as compared to their female counterparts at almost all levels and sub-components of metacognition except few. However, the gender differentials were found to be statistically significant only at the higher level of the debugging (t-value = 3.45; $p < 0.01$) sub-component of metacognition. Thus, the foregoing discussion suggests that although the gender did not impact much across various components and sub-components of metacognition yet boys surpassed girls by being significantly outstanding in Debugging, which reflects boys assess their comprehension errors better than girls.

The findings of the study conducted by Liliana and Lavinia (2011) also indicated that generally both boys and girls use their metacognitive skills in learning. In addition, the results indicated that there were significant differences between boys and girls solely on the following dimensions: the perception of performance as a result of one's will and effort, the perception regarding teachers' expectations about learning, the use of prior knowledge in problem-solving, planning, knowledge about one's own intellectual strengths

Table 4. Gender-wise distribution of scores (Mean $\pm$ SD) across two components of metacognition among adolescents

Components of metacognition	Male	Female	t-value	Overall
I-Knowledge of cognition				
Declarative knowledge	5.64 $\pm$ 1.59	5.53 $\pm$ 1.36	0.523	5.59 $\pm$ 1.48
Procedural knowledge	2.94 $\pm$ 1.01	2.88 $\pm$ 1.00	0.420	2.91 $\pm$ 1.01
Conditional knowledge	3.67 $\pm$ 1.04	3.57 $\pm$ 1.00	0.689	3.62 $\pm$ 1.02
Sub-total: I	4.08 $\pm$ 1.21	4.0 $\pm$ 1.12	0.751	4.04 $\pm$ 1.17
II-Regulation of cognition				
Planning	4.70 $\pm$ 1.55	4.62 $\pm$ 1.52	0.367	4.66 $\pm$ 1.54
Information management strategies	7.44 $\pm$ 1.86	7.37 $\pm$ 1.74	0.274	7.41 $\pm$ 1.80
Comprehension monitoring	5.00 $\pm$ 1.42	4.86 $\pm$ 1.47	0.683	4.93 $\pm$ 1.45
Debugging	3.98 $\pm$ 1.08	3.81 $\pm$ 1.07	1.120	3.90 $\pm$ 1.08
Evaluation	3.93 $\pm$ 1.47	4.08 $\pm$ 1.41	0.735	4.01 $\pm$ 1.44
Sub-total: II	5.01 $\pm$ 1.48	4.95 $\pm$ 1.44	0.459	4.98 $\pm$ 1.46
Total	4.55 $\pm$ 1.35	4.48 $\pm$ 1.28	0.078	4.51 $\pm$ 1.32

and weaknesses, the use of various learning strategies and monitoring the learning process.

However, in contrast to these findings, investigations conducted by Fazal *et al.* (2012) indicated significant relationship of time management skills, reading and note taking skills with academic achievement. Students with higher academic achievement use a wide range of study skills as compared to students with lower academic achievement. Analysis further revealed that the girls were better in using study skills as compared to boys.

Table 6. Per cent distribution of adolescents as per levels of emotional intelligence and achievement motivation

	Male	Female	z-value	Overall
Levels of emotional intelligence				
High (80.00 - 119.85)	0	0	NA	0
Average (39.96 - 79.90)	49	51	0.28	50
Low (up to 39.95)	51	49	0.28	50
Levels of achievement motivation				
High (25.34 - 38.00)	4	0	2.08	2
Average (12.67- 25.33)	30	25	0.80	27.5
Low (up to 12.66)	66	75	1.41	70.5

NA: Not applicable

The per cent distribution of respondents across varying levels of emotional intelligence indicates that average and low categories of emotional intelligence comprised of same proportion of respondents (Table 6). In the case of high level category, the respondents could reach to that level. It shows that there is lack in the proper facilitation of thought and management of one's emotions. Furthermore, the gender-

wise analysis revealed non-significant differences in the emotional intelligence of boys and girls at all the levels of Emotional Intelligence. It, therefore, could be concluded that almost all the respondents were divided into average and low category of emotional intelligence while there were no respondents who could reach high level of emotional intelligence. The findings point towards a major gap which needs to be fulfilled to train adolescents to be emotionally more mature and stable individuals. Besides this it was further observed that irrespective of gender none of the respondent could reach the high level of emotional intelligence.

The percentage distribution of respondents across varying levels of achievement motivation revealed that a major proportion of respondents were in 'lower level' (70.5%) of achievement motivation and 27.5 per cent of respondents showed moderate achievement motivation. However, a very few respondents were in higher level of achievement motivation. The findings corroborate with the investigations conducted by Zulkipli *et al.* (2006) observed a significant positive relationship between students' academic performance and metacognitive awareness, and no significant difference in metacognitive awareness between male and female across all academic years.

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Table 5. Gender differentials in scores (Mean $\pm$ SD) across various levels and sub-components of metacognition among adolescents

Levels of metacognitive skills	Male	Female	t-value	Overall
1. Declarative knowledge				
High (5.34-8.00)	6.75 $\pm$ 0.70	6.54 $\pm$ 0.60	1.77	6.65 $\pm$ 0.65
Average (2.67-5.34)	4.26 $\pm$ 0.70	4.41 $\pm$ 0.71	0.97	4.34 $\pm$ 0.71
Low (up to 2.67)	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	0	2.00 $\pm$ 0.00
2. Procedural knowledge				
High (2.67-4.00)	3.46 $\pm$ 0.50	3.43 $\pm$ 0.50	0.36	3.45 $\pm$ 0.50
Average (1.34-2.66)	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	0	2.00 $\pm$ 0.00
Low (up to 1.33)	0.67 $\pm$ 0.50	0.67 $\pm$ 0.50	0	0.67 $\pm$ 0.50
3. Conditional knowledge				
High (3.34-5.00)	4.37 $\pm$ 0.49	4.35 $\pm$ 0.48	0.28	4.36 $\pm$ 0.49
Average (1.67-3.33)	2.69 $\pm$ 0.47	2.77 $\pm$ 0.43	0.72	2.73 $\pm$ 0.45
Low (up to 1.66)	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	0	1.00 $\pm$ 0.00
4. Planning				
High (4.67-7.00)	5.75 $\pm$ 0.75	5.71 $\pm$ 0.68	0.36	5.73 $\pm$ 0.72
Average (2.34-4.66)	3.54 $\pm$ 0.51	3.65 $\pm$ 0.49	0.85	3.60 $\pm$ 0.50
Low (up to 2.33)	1.82 $\pm$ 0.40	1.64 $\pm$ 0.50	0.93	1.73 $\pm$ 0.45
5. Information management strategies				
High (6.67-10.00)	8.33 $\pm$ 1.05	8.24 $\pm$ 1.07	0.48	8.29 $\pm$ 1.06
Average (3.34-6.66)	5.59 $\pm$ 0.67	5.70 $\pm$ 0.47	0.67	5.65 $\pm$ 0.57
Low (up to 3.33)	2.60 $\pm$ 0.89	2.00 $\pm$ 1.00	0.56	2.30 $\pm$ 0.95
6. Comprehension monitoring				
High (4.67-7.00)	5.83 $\pm$ 0.85	5.82 $\pm$ 0.78	0.08	5.83 $\pm$ 0.82
Average (2.34-4.66)	3.68 $\pm$ 0.48	3.69 $\pm$ 0.47	0.09	3.69 $\pm$ 0.48
Low (up to 2.33)	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	0	2.00 $\pm$ 0.00
7. Debugging				
High (3.34-5.00)	4.53 $\pm$ 0.50	4.41 $\pm$ 0.49	3.45**	4.47 $\pm$ 0.50
Average (1.67-3.33)	2.74 $\pm$ 0.45	2.77 $\pm$ 0.43	0.24	2.76 $\pm$ 0.44
Low (up to 1.66)	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	0	1.00 $\pm$ 0.00
8. Evaluation				
High (5.00-6.00)	5.39 $\pm$ 0.49	5.39 $\pm$ 0.49	0.04	5.39 $\pm$ 0.49
Average (3.00-4.00)	3.42 $\pm$ 0.50	3.48 $\pm$ 0.51	0.55	3.45 $\pm$ 0.51
Low (up to 2)	1.56 $\pm$ 0.63	1.50 $\pm$ 0.67	0.15	1.53 $\pm$ 0.65
Total				
High (>34.67)	40.94 $\pm$ 6.36	40.55 $\pm$ 3.65	0.40	40.75 $\pm$ 5.00
Average (17.33-34.66)	30.17 $\pm$ 3.38	30.00 $\pm$ 4.02	0.19	30.09 $\pm$ 3.70
Low (up to 17.33)	14.00 $\pm$ 0.00	14.00 $\pm$ 0.00	NA	14.00 $\pm$ 0.00

NA: Not applicable ; ** Significant differences at 0.01 level

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Shift in Land Use Pattern and Constraints Faced by Farmers in Crop Diversification in Kashmir Valley

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Abstract: An empirical relation between crop diversification and selected independent variables was established. The regression analysis suggested that most of the independent variables had significant contribution towards components of diversification and divers of diversification. Effective use of land and water resources, employment generation, food security, changing consumer demand and cropping pattern were found most important determinants of crop diversification.

Key Words: Constraints, Crop diversification, Food security, Land use pattern

Crop diversification has a lot of benefits for food and nutrition security, income growth, poverty alleviation, employment generation, judicious use of land and water resources, sustainable agricultural development and environmental improvement (Reddy and Suresh, 2009). Improvement in income, gainful employment and stabilization of income flow, diversification in crops are major strategy for diversification in traditional crop rotations (Ojo *et al.*, 2014; Gill *et al.*, 2014). The concept in India is seen as referring to the shift from regional dominance of one crop to regional production of number of crops, which takes into account the economic returns from different value added crops with complementary marketing opportunities (Singh, 2009). The process of diversification is triggered by the availability of improved rural infrastructure, rapid technological advancements in agricultural production, and changing food demand patterns. Hence, this process of diversification towards high-value crops is likely to accelerate agricultural growth and usher in a new era of rural entrepreneurship and generate employment opportunities (Parthasarathy *et al.*, 2008).

In farm planning, farmer as a decision maker takes three decisions - what to produce, how to produce and how much to produce (Van and Keller, 2006). At present, diversification is considered as policy initiative to tackle or improve the situation in the agricultural sector. It is now emerging as an important option because of several key developments in the economy such as booming economy, changing consumption pattern towards non –staple crops both in rural and urban areas, trade liberalization. In this context, diversification is seen mainly as a shift in cropping pattern from low value crops towards high value crops. The state of Jammu and Kashmir has varied climatic features, topography and many crops such as cereals, fruits,

vegetables, medicinal and aromatic plants are being cultivated throughout the length and breadth of the state. Agricultural diversification in Kashmir is slowly picking up momentum in favour of high-value food commodities. The findings of the study and the inference drawn can be generalised for the whole valley. The present study was of great importance for researchers, extension workers, farmers, planners and the organisations engaged in promoting diversification as the high value crops can improve the economic condition of the farmers, help in overcoming menace of unemployment.

MATERIAL AND METHODS

The present study was conducted in ten districts of the Kashmir Valley (Jammu and Kashmir, India) comprising extreme sector of the Himalayas and occupies a central geographical location in the Asian continent. It is the northern most state of India extending from 32°17' to 37° 05'N latitude and 72°40' to 80° 30'E longitude. The altitude ranges from 215-7012 meters above mean sea level.

A multistage sampling procedure was adopted for the selection of districts, tehsils, villages and sample respondents. Pulwama and Budgam districts were randomly selected for the study. Two tehsils each (Tral and Pulwama from district Pulwama, and Chadoora and Budgam from district Budgam) were selected from two districts. Eight villages in total from the four tehsils were shortlisted for the purpose of study (Table 1).

Selection of the respondents: Comprehensive lists of farmers engaged in crop diversification from each village were framed in consultation with village Lumberdar and out of this only 20 farmers or respondents who showed higher level of diversification. Broadly the indicators of crop diversification for selecting the respondents were cropping pattern,

Constraint: The constraints in crop diversification were grouped under six categories, i.e., technical, economic, production, inputs, social, and marketing constraints. Percentage of each constraint from frequency thus calculated was obtained and accordingly ranking was done on the basis of maximum percentage.

Change in cropping pattern of major crops among the respondents : The percentage area under cereals between 2006 and 2008 was 46.48 % while as during period 2009 to 2011, it was 39.82% indicating that the area under paddy had declined over the years. It was observed that percentage of area under vegetables during period 2006-2008 was 19.51%, while it was 25.92% during 2009-2011 implying that area under vegetables had increased during these years. In

Constraints perceived by the farmers in crop diversification: In technical constraints, 98.13% of respondents were unaware of high yielding varieties, 97.5% were constrained by lack of technical know how about soil testing. 95% faced constraint of lack of guidance for optimum pesticide use while 92.5 % faced lack of guidance for optimum fertilizer dose and only 25% faced lack of technical guidance in selection of appropriate fruit varieties a constraint. Minor constraints included unawareness about proper spray schedule, difficulties in establishment of young trees, lack of proper technical guidance about training and pruning of fruit trees and improper layout of orchard. In case of economic constraints, hundred per cent respondents faced the constraint of lack of crop insurance followed by 87 (54.375%) respondents who faced lack of own capital as a constraint. The constraint of inadequate loan from financial agencies was faced by 23.12% respondents. In production constraints, majority of respondents were constrained by uncertainty about weather conditions. This was followed by 89 (55.625%) respondents who faced lack of water facilities particularly when spraying was done on table lands. 53.12% farmers faced constraint of lack of irrigation facility.

In case of constraints related to inputs, all the respondents faced the constraint of spurious chemical available in the market and high cost of fertilizers followed by 93.12% farmers who faced the constraint of unavailability of

Kashmir valley (comprises of 10 districts)

Kashmir valley (comprises of 10 districts)				
Selected districts (random sampling)	Pulwama		Budgam	
Selected tehsils (purposive sampling)	Tral	Pulwama	Chadoora	Budgam
Selected villages (random sampling)	Pinglish Nageenpore	Malikpora Barpora	Daulatpora Boogam	Narkoora Duru
Selection of respondents (purposive sampling)	20 from each village			
Total number of respondents (N)	180			

[illegible]

Table 3. Constraints faced by farmers of Kashmir valley in crop diversification

Constraints	Frequency	Percentage	Rank
Technical constraints			
Unawareness about proper spray schedule	20	12.5	VI
Difficulty in establishment of young trees	11	6.88	VII
Lack of technical guidance in selection of appropriate fruit varieties	40	25.0	V
Lack of technical know how about soil testing	156	97.5	II
Lack of guidance for optimum fertilizer dose	148	92.5	IV
Lack of guidance for optimum pesticide dose	152	95.0	III
Unawareness about new high yielding varieties	157	98.13	I
No proper layout of orchard	07	4.38	IX
Lack of proper technical guidance about training and pruning of fruit trees	09	5.63	VIII
Economic constraints			
Lack of own capital	87	54.38	II
Inadequate loan from financial agencies	37	23.13	III
Lack of crop insurance	160	100.00	I
Production constraints			
Uncertainty of weather conditions	160	100.00	I
Lack of irrigation facilities	85	53.13	II
Lack of water facilities particularly when spraying is done on table lands	89	55.63	III
Constraints related to inputs			
Unavailability of suitable equipments/machinery	79	49.37	III
Spurious chemicals available in the market	160	100.00	I
High cost of fertilizers	160	100.00	I
Unavailability of chemicals in time	149	93.13	II
Social constraints			
Pesticides are harmful to human beings and animals	48	30	II
Family members prefer jobs than self-employment	73	45.63	I
Marketing constraints			
Lack of market or local mandi near your village.	42	26.25	IV
Improper road linkage	83	51.88	III
Improper transport facility to the market	97	60.63	II
Lack of storage facility in the market place or local mandi	102	63.75	I

chemicals on time. The constraint of unavailability of equipment's/machinery was faced by 49.37% respondents. In case of social constraint, 45.62% respondents felt the constraint that family members prefer jobs than self-employment, followed by 30% farmers who faced the constraint that pesticides are harmful to human beings and animals. In marketing constraints majority of 102 (63.75%) respondents faced the constraint of lack of storage facility in the market place or local mandi followed by 60.62% farmers who faced the constraint of improper transport facility to the market followed by 51.87% respondents who faced the

constraint of improper road linkage. The development of farm diversification faces a range of financial and political barriers such as lack of capital for investment or financial support, difficulty in obtaining planning permission, lack of experienced labour and difficulties in obtaining the right information or advice (Chaplin *et al.*, 2004; Desai *et al.*, 2014). Area under cereals has markedly decreased, whereas, area under spices has also shown decreasing trend to some extent. The area under vegetables and horticulture has considerably increased. The study has shown that diversification into a number of income sources

and crops grown are very high in the study area. Diversification can also help to relax credit or capital constraints among farmers.

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Received 6 October, 2015; Accepted 2 March, 2016



Current Distribution of Invasive Alien Flora of Arookkutty Panchayath, Kerala, India

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Abstract: In this ecological investigation using quadrat methods seventeen species were observed, out of which 11 were terrestrial invasive plants. Four terrestrial species were minor and distributed along the roadsides; 2 of them were aquatic plant species reported from lake water and fresh water ponds respectively. Among these 17 problematic plants, 11 species (61.11%) were native to South America, followed by North America (2 species), Africa (4 species), and China (1 species). *Mikania micrantha* Kunth was the frequent species and Asteraceae was dominant family among the quadrats with wide range of habitats and hosts. Less competitive environments, high resource availability, salinity of the soil, easy dissemination of propagules, and presence of bare lands may cause the easy colonization of invasive alien species in the study area.

Key Words: Alien plants, Aquatic weeds, Invasive *Mikania micrantha* Kunth, Plant invasions

The term "Alien Invasive Species" refers to introduced (alien) species that are able to establish and naturalize in a new area then cause problems for biodiversity, human development by uncontrolled spreading (Howard, 2012). Biological invasions are distributed among all categories of living organisms as well as all kinds of ecosystem throughout the world (Sujay *et al.*, 2010). IUCN defines invasive alien plant species as an alien species which become established in natural or semi natural ecosystems or habitat, an agent of change, threatens to native biological diversity. Introduced plants with high reproductive rates and the potential to spread rapidly over large areas are regarded as invasive alien plants Pysek *et al.*, (2004). Invasive species cause loss of biodiversity including species extinction, change in hydrology and ecosystem function (McGeoch *et al.*, 2010). The exotic plants vary from native one by their requirements, and modes of resource acquisition. More consumption may cause changes in soil structure, its profile, decomposition nutrient content and moisture. Invasive species are a serious hindrance for conservation and sustainable use of biodiversity. The present study enumerated the invasive alien species of Arookkutty Grama Panchayath local self government those are creating major threats for agricultural fields, ponds, canals, road sides, etc. for future implications of prevention methods.

Arookkutty Grama Panchayath is situated at 9.87° North latitude and 76.35° East longitude and 1 meter elevation above the sea level. It is a small city, located in the banks of Vembanadu Lake, Northern end of Alappuzha district, Kerala having predominant mangrove vegetation.

Field studies were adapted to record maximum number of invasive alien plant species of study area. In this

investigation, enumeration of invasive alien species were done in the study area by randomly placing 20 quadrats measuring 4m x 4m in invaded and non-invaded localities of vegetation after preliminary studies.

The observed plants were confirmed through the available literature (Gamble, 1921) and available online checklists of global invasive species database. Abundance and frequency of these plants were calculated and enumerated alphabetically in tabular form.

A total of 17 invasive alien species were recorded from study area, which is creating major problems in different aspects (Table 1), 15 of them were terrestrial invasive weeds and two of them were aquatic species, distributed in both fresh water and brackish water. Eleven terrestrial weeds were recorded from Quadrats, placed in both invaded and non invaded localities (Frequency > 5% or Frequency = 5). *Cleome burmanni* Wt & Arn, *Hyptis suaveolens* (L) Poit, *Ipomea palmata* Forssk, and *Stachytarpheta dichotoma* Vahl were four species, with is having minor distribution. It was observed along the road sides and not in any quadrats.

As per the reports, aquatic species is a major problem than the terrestrial one in study area. It adversely affects the ecosystem by polluting of canals, preventing transportation through lakes. *Colocasia esculenta*. L was a common invasive species along the quadrats, can be considered as invasive but not alien because it is indigenous in origin. The South American continents contributed majority of species (10 species, 61.11%) followed by Africa (4 species), North America (2 species) and China (1 species). Among these 17 plant species, highest number of plants (6 plants species) belongs to the family Asteraceae, four from Fabaceae and two from Verbenaceae. According to Rejmnek

Table 1. Common invasive alien plants reported from Arookkutty Grama Panchayath

Scientific name	Family	Place of origin	Growth form	Abundance	Frequency
<i>Ageratum conyzoides</i> Linn	Asteraceae	South America	Annual herb	20.5	10
<i>Alternanthera pungens</i> Kunth	Amaranthaceae	Tropical America (Brazil and Peru)	Prostrate perennial herb	18	15
<i>Cleome burmanni</i> Wt&Arn	Capparidaceae	West Africa	Annual herb		
<i>Crotalaria pallida</i> Aiton	Fabaceae	Africa	Erect perennial shrub	1	10
<i>Eichhornia crassipes</i> (Mart.) Solms	Pontederiaceae	South America (Brazil)	Aquatic floating perennial		
<i>Eupatorium odoratum</i> . L	Asteraceae	North America	Aromatic Perennial shrub	3.37	40
<i>Hyptis suaveolens</i> (L) Poit (Bush mint)	Lamiaceae	South America	Annual or perennial herb or sub shrub		
<i>Lantana camara</i> Linn	Verbenaceae	South America and Central America	Woody shrub	4.5	20
<i>Mikania micrantha</i> Kunth	Asteraceae	South America	Perennial climber	5.53	75
<i>Mimosa diplotrica</i> C. Wright ex Sauvalle	Fabaceae	South America	Annual shrub	7	10
<i>Mimosa pudica</i> Linn	Fabaceae	South America	Creeping herb	5.53	40
<i>Pistia stratiotes</i> Linn	Araceae	Uncertain origin. First discovered from	Aquatic, floating perennial		
<i>Phaseolus</i> sp Linn	Fabaceae	South America	Climber	7.5	40
<i>Spilanthes americana</i> (Mutia ex L.F) Hieronvar <i>repens</i> (Walter) A.H Moore	Asteraceae	South America	Marginal aquatic perennial	21	15
<i>Stachytarpheta dichotoma</i>	Verbenaceae	South America	Perennial herb		
<i>Trifolium repens</i> Linn	Fabaceae	Europe	Annual creeper		
<i>Wadelia chinensis</i>	Asteraceae	China	Perennial creeper	17	15

and Randall (1994), 55 per cent of non indigenous plants were native to American continents. Dominant family Asteraceae was contributed most exotic weeds to the country (Yasuyuki *et al.*, 2010). Asteraceae is having large number of seeds, high reproductive rates and vegetative growth. Fabaceae is one of the largest families of exotic weeds having the capacity of nitrogen fixation. *Mikania micrantha* Kunth had highest frequency among the quadrats. It was observed in different habitat like roadsides, ponds, marshy places, waste lands and in a wide range of hosts such as herbs, shrubs, climbers, trees, mangroves, even in other weeds. Majority of the invasive plants were herbs characterized by large number of seeds, high reproductive rates and vegetative growth. Quadrat studies showed that the common species *Mikania micrantha* Kunth was associated with naïve flora such as trees, shrubs, herbs, climbers and mangroves. Flowering of these invasive species may attract butterflies, which ensures the pollination

as well as dispersal of seeds made it a common weed in the study area. According to Avevalo *et al.* (2005) road construction facilitated plant invasion. Most of the invasive alien flora incorporated to native plants to overcome the dispersion barriers as a step of invasion process. Native flora of the area was badly affected by invasive species. It competes with the host plant of agricultural fields. Its greater adaptability and vigorous growth may helps for easier invasion in study area. Empty niches may also cause invasion leads to the biodiversity loss and ecosystem functioning. A better detection process and the knowledge about the mode of introduction, morphology, and phenology is required for effective management of invasive alien species.

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Received 16 November, 2015; Accepted 4 April, 2016



Mangroves Species Composition in Rajpuri creek of Maharashtra, India

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Abstract: Mangrove habitat of Rajpuri creek was screened for ecological composition and distribution using quadrat method at fifteen sites. A total number of eight species were reported of which *Avicennia marina*; *Sonneratia alba*, *Rhizophora mucronata* and *Aegiceras corniculatum* are the dominant species. Maximum frequency is recorded for *A. marina*. Simpson diversity index, reciprocal index, Shannon Weiner index reveals medium complexity in the community.

Key Words: Diversity indices, Ecology, Maharashtra, Mangroves, Rajpuri creek

Mangroves are important salt tolerant forest ecosystem found mainly in tropical and subtropical intertidal regions of the world. These are the world's most productive ecosystem with complex association of flora and fauna. The mangroves habitat is characterized by dynamism caused by tidal activity and seasonal influence on originally salt dominated substratum. These species possesses common characteristics of tolerating high salinity and conversion adaptation of these species (Gokhale *et al.*, 2012). The great importance of mangrove is related in the maintenance of fish stock and sea biodiversity as it acts as shelter for species as well as important source of organic material for adjoining waters (Spalding *et al.*, 2012). The mangrove forests also get frequently affected by rapid change of different environmental factors (Kjerfve *et al.*, 1999). Now a day, encroachment in the mangrove habitat causes damage to this most productive ecosystem and the urbanization is the most important factor disturbing this system. In the present work, attempt is made to study the ecological characteristics of Rajpuri creek.

Rajpuri creek lies between 18°14' to 18°34' latitude and 72°97' to 73°11' longitudes (Fig. 1), on the Raigad coast of Maharashtra. This is comparatively largest creek covers the area from Murud, Mhasala, Roha Srivardhan. Fifteen quadrates of 20X20 meter were placed throughout the creek and distribution of the species was recorded. The data obtained was processed using the formulae given by Ram and Shaji (2013). The diversity indices were calculated using online biodiversity calculator (http://www.alyoung.com/labs/biodiversity_calculator.html) and further confirmed by the formulae.

The *Avicennia marina* was dominant species in the Rajpuri creek followed by *Acanthus illicifolius* and *Sonneratia alba* (Table 1). The frequency of *A. marina* was 86.6 per cent

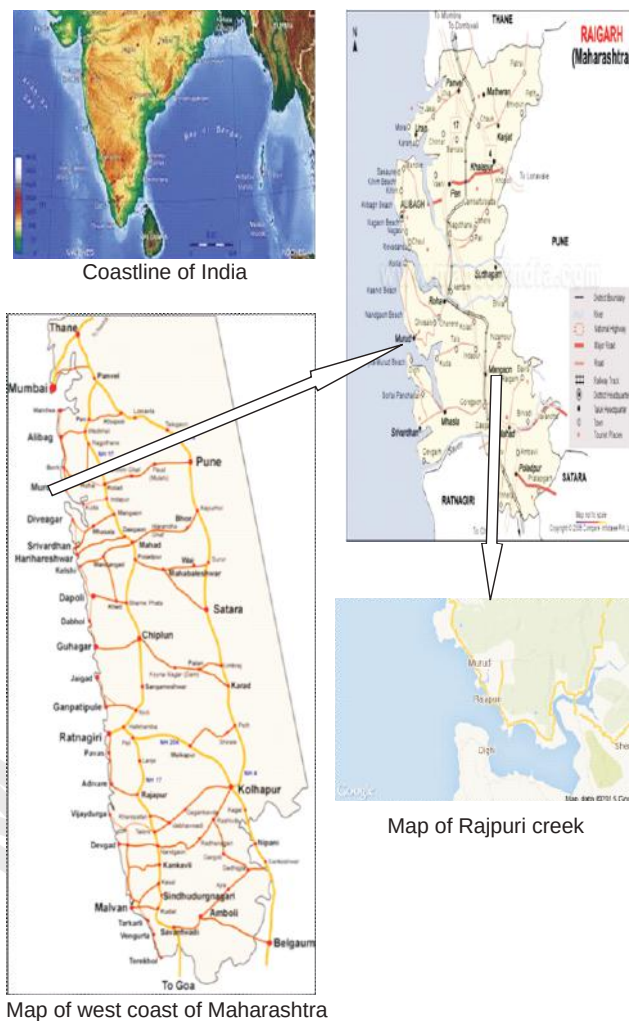
and minimum was in *Avicennia officinalis*. *A. marina* is more dominant species in this area indicating pollution in study site and it is tolerant to relatively high salinity, together with low rainfall and high temperatures (Loughland and Al-abdulkader, 2011). The frequency of *S. alba*, and *R. mucronata* and *A. illicifolius* is 66.6 per cent. Similar observations were recorded by Ram and Shaji (2013) and reported the density in *E. agallocha* as 0.3, whereas, in the study site density of *E. agallocha* is 1.0 as the species grows as backward mangrove and is less salt tolerant. The Important Value Index (IVI) for *A. marina* was 95.79 and 23.90 for *A. officinalis*. Nabi and Rao (2012) show IVI value for *A. marina* as 30.61 and for 22.91 *A. Officinalis* from Machilipatnam coastal region of Andhra Pradesh. Kumbalam Island of Kerala shows higher percentage frequency for *A. illicifolius*, *A. officinalis*, *R. mucronata* and *Kandelia candal*. Maturity index was 49.12 (Table 1), which was much lower than i.e., Nabi and Rao (2012) 78.43, 68.63, 84.31 from Pedapatnam, Gilakaladindi and P.T. Palem of Krushna district, respectively. The maximum index values shows highly matured mangroves community.

Simpson's diversity index was 0.371 indicating diversity in the studied vegetation. The diversity value (H')

Parameter	Index value
Maturity index value	49.12
Shimpson's index	0.629
Shimpson's diversity index	0.371
Reciprocal index	1.589
Shannon Weiner index	1.51
Species evenness	0.729

Table 1. Ecological status of different species on the Rajpuri creek

Name of plant	Total number of individuals	Frequency	Relative frequency	Density	Relative density	Population density	Relative Population density	Abundance	Relative abundance	Species richness	IVI Index
<i>Avicennia marina</i>	986	86.6	22.03	65.73	44.86	4.382	44.9	75.84	28.9	0.254	95.79
<i>Avicennia officinalis</i>	15	6.66	1.694	3.46	2.36	0.23	2.35	52.00	19.85	1.109	23.90
<i>Sonneratia apetala</i>	76	26.6	6.76	5.06	3.45	0.33	3.3	19.00	7.25	0.918	17.46
<i>Sonneratia alba</i>	232	66.6	16.94	15.46	10.55	1.031	10.5	23.2	8.86	0.525	36.35
<i>Rhizophora mucronata</i>	147	66.6	16.94	9.8	6.68	0.653	6.69	14.37	5.48	0.660	29.1
<i>Aegiceras coriiculatum</i>	115	53.3	13.56	7.66	5.22	0.511	5.2	14.37	5.48	0.746	24.26
<i>Acanthus illicifolius</i>	575	66.6	16.94	38.33	26.16	2.55	26.1	57.5	21.95	0.333	65.05
<i>Excoecaria agallocha</i>	52	20.0	5.08	1.00	0.68	0.06	0.61	5.00	1.90	2.067	7.66

**Fig. 1.** Location map of study site

ranging between 0 (indicating low community complexity) and 4 (indicating high community complexity). The value for Shannon Weiner index was 1.51 indicates medium complexity of the studied community. In the studied ecosystem, the species evenness was 0.729. Increasing human encroachment and urbanization creating more pressure on this ecosystem and these studies should be useful to undertake certain measures and conservation strategies in order to minimize human impact on this important coastal ecosystem.

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Received 23 September, 2015; Accepted 8 December, 2015



Effect of Coloured Shade Nets on Softwood Graft Success in Jackfruit (*Artocarpus heterophyllus* Lam.)

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Abstract: The influence of different coloured shade nets viz., black, red, blue and green on graft take, survival and growth parameters in jackfruit was observed during 2011-2013. The effect of coloured shade nets on graft after 3 months grafting was significant. The graft kept under red shade net recorded the highest graft success (34.68 %) and graft survival (79.91%). Significantly lowest per cent graft success and graft survival was in graft kept under green shade net. With respect to growth parameters viz., number of sprouts, leaves, height and, graft girth was not significant under different shade nets.

Key Words: Coloured shade net, Grafting, Graft take, Jackfruit

Jackfruit tree (*Artocarpus heterophyllus* Lam), indigenous to Western Ghats of India, is one of the most popular fruits of South India (Mamatha *et al.*, 2014). In spite of its uniqueness in size, high productivity, adaptability to varying agro-climatic soil conditions, high nutritive value and suitability for making an array of products, jackfruit is not cultivated on plantation scale. South India is blessed with a large number of varieties. Though cultivation of the crop is high in Kerala, its utilization as a popular table fruit is confined more to other states. The crop is propagated mainly through seed and there is very large variability in fruit quality attributes. The crop is also not given adequate care with no manuring, irrigation and plant protection more so when it is part of the homesteads. The seed derived plants start yielding from 5-8 years while graft plants from 4-5 years and continue to produce very high yields over a long period and the yield varies from tree to tree.

The light intensity and its quality are effectively manipulated by different colours of polyhouse or shade nets, which directly or indirectly affect the physiological processes of the plant. With the installation of protective netting over the orchard, we would expect changes in the physiology of trees as they react to the microclimate changes in the humidity, temperature and available light, which are the major factors influenced by netting (Guerrero *et al.*, 2002 and Shahak *et al.*, 2004). Light quality (red: far-red ratio) can affect plant growth and can be used to manipulate stock plant management, seed germination and shoot development. Coloured netting is not used on a routine in agroforestry nurseries but an easy way to change the light quality favourably for vegetative growth, for example in stock plant management. Use of coloured shade nets or polyhouses for production of fruit crops is being practiced as a new technology to know their effects on growth of plants. Hence an attempt was made to

know the effect of coloured shade nets on jackfruit grafts.

An investigation was carried out at the Department of Fruit Science, Kittur Rani Channamma College of Horticulture, Arabhavi, Karnataka state. The experiment was designed in CRD with four treatments and five replications, per replication 10 grafts were selected. Totally 200 grafts of uniform size healthy, disease and pest free vigorously grown rootstocks were selected per treatment. The treatment included different coloured shade nets viz. black, red, blue and green. The scions shoots were separated from the selected tree and were kept in water to get rid of the white latex and used for grafting next day. The observation on per cent success (90 days after grafting), per cent survival (120 days after grafting) and growth parameters like height of graft, number of sprout, number of leaves and graft girth (at monthly intervals) were recorded.

The coloured shade nets have varying effects on success and growth of jack fruit grafts. The influence of different coloured shade nets on graft success and survivability was significant. The grafts kept under red coloured shade nets recorded highest graft success (34.68 %), which was at par with grafts under black shade net (33.94 %) and blue (33.08 %). Significantly lowest graft

Table 1. Effect of coloured shade nets on soft wood grafting success and graft survivability

Colour shade net	Per cent success	Per cent survival
Black	33.94 (31.20) *	76.24 (94.20)
Red	34.68 (33.00)	79.91 (96.20)
Blue	33.08 (29.80)	69.44 (87.60)
Green	30.09 (25.20)	61.91 (77.80)
CD (p=0.05)	2.00	4.40

DAG – Days after grafting.

*Values in parenthesis are angular transformation data

Table 2. Effect of coloured shade nets on number of sprouts, number of leaves per graft, sprout height on graft and girth of graft

Treatments	Number of sprouts		Number of leaves		Sprout height (cm)		Graft girth (mm)	
	30 DAG	90 DAG	30 DAG	90 DAG	30 DAG	90 DAG	30 DAG	90 DAG
Black	1.46	2.11	3.43	6.46	17.68	23.07	4.68	5.59
Red	1.53	2.12	3.73	6.71	18.20	23.23	5.16	5.87
Blue	1.42	2.01	3.28	5.77	16.98	21.56	4.47	5.54
Green	1.40	1.97	2.66	5.53	15.61	19.90	4.34	4.82
CD (p=0.05)	NS	NS	NS	NS	NS	2.24	0.73	0.89

DAG – Days after grafting NS- Non significant

success was recorded in grafts under the green shade net. Graft survival was highest in red colour shade nets (79.91 %), which was at par with grafts under black shade net (76.24 %). Significantly lowest graft success of 60.50 per cent was in grafts under the green shade net.

The results on vegetative growth parameters viz., number of sprouts and leaves were non significant while there was significant difference for sprout height and graft girth of graft (Table 2).

At 90 DAG, significantly highest sprout height was recorded in grafts kept under red shade net (23.23 cm), which was statistically on par with black colour shade net (23.07 cm) and blue shade net (21.56 cm). The minimum number of leaves was recorded in grafts under green shade net. At 30 days after grafting the sprout height was found non significant.

The graft girth was significant in 30th and 90th day after grafting. On the 30th and 90th day after grafting, the maximum graft girth was in red colour shade net (5.16 and 5.87 mm), which was at par with black and blue shade net. The higher success rate of grafts kept under red and black shade nets may be due to higher number of sprouts and leaves. This may be influenced by various factors like variation in the light quality, increased photosynthesis, moderate temperature and relative humidity. Vegetative growth and yield increased with black shading than without shading in strawberry (Hasanein *et al.*, 2011). Shamir *et al.* (2001) reported that blue nets do not transmit light between

580 and 750 nm, thus keeping the R/FR ratio similar to that of natural sunlight. Thus, the lack of FR (700-750 nm) may be a major inducer of dwarfing and other inhibition effects in blue nets. The results of the study reveal that growth parameters of the grafts can be manipulated by use of different coloured shade nets. Unravelling the physiological response to the red net awaits further studies.

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Effect of Tree Spacing and Organic Manures on the Seed Production of *Calendula officinalis* L. under *Grewia optiva* Drummond. based Agroforestry System

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Abstract: Seed production parameters of *Calendula officinalis* were suppressed under the close spacing and these parameters were highly influenced by the *Grewia optiva* trees in addition to application of organic manure. Maximum number of seeds and higher seed yield plant⁻¹ was recorded in open condition as compared to agroforestry system. Organic manure enhanced the seed yield both in open condition and within agroforestry system, however 1,000 seed weight was recorded more in agroforestry system than that of open condition (control). Integrating winter annual flower crop with *Grewia optiva* offers an excellent opportunity for diversification and more income generation than the monoculture.

Key Words: Seed production parameters, *Calendula*, *Grewia optiva*, Spacing, Manures

In agroforestry systems, the use of organic manures is found to increase the efficiency because the leaves or small twigs fall directly on to the floor/ surface where crop is growing. This adds organic matter into the soil, and also acts as mulch that helps to suppress the weed growth and conserve moisture by reducing summer temperature. Organic manure is well suited to achieve both production and conservation goals. Sometimes yield reductions in various intercrops have been reported due to such interactions but the crop yield loss may well be compensated by economically through yield of timber, fodder and fuel wood tree species. Due to the acute shortage of the green fodder in winter season, *Grewia optiva* is one of the most suitable tree species for agroforestry system. It provides sustainable production of biomass on a short rotation basis and enriches the soil by adding leaf litter. The cultivation of flower crop under *Grewia optiva* tree could be an economically viable option with least expenses because of the use of organic manures. However, successful cultivation of flower crop under agroforestry requires information on species adaptability under shade, their cultivation, management and economic assessment of the system (Rani *et al.*, 2011). *Calendula officinalis* is widely cultivated in sunny locations and different kinds of soils. Although perennial in nature, but commonly treated as an annual plant, particularly in colder regions where its winter survival is poor. It is grown for a drug, calendulae floss, obtained from the flowers, particularly the petals. The flower is used in poultry feed and manufacturing of food colours while seed oil of *Calendula officinalis* has similar properties to tung oil and, therefore could be used as

binder in paints, coatings and cosmetics (Muuse *et al.*, 1992). Organic farming comprises of diversified agriculture techniques like intercropping, crop rotation, green manuring, organic manures (FYM and compost), organic residues, biofertilizers, etc. Most commonly used organic manure is FYM in which cattle dung constitutes the major source of nutrients and contains 0.72, 0.35 and 0.80 per cent N, P and K, respectively.

The study was conducted at the experimental field of Department of Silviculture and Agroforestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) during the years 2010-2011. An agroforestry system comprising of *Grewia optiva* + *Calendula officinalis* was developed to study the effect of different tree spacing and organic manures on seed production parameters of winter flower annual (*Calendula officinalis* L.). The *Grewia optiva* was planted at different spacing viz. 1m, 2m and 3m apart. *Calendula officinalis* an important winter annual flower was sown as intercrop and applied with four manure doses i.e. 2.50 kg, 5.00 kg FYM and 1.50 kg, 3.00 kg vermicompost per plot. Nursery raised seedlings of *Calendula officinalis* were transplanted in the system during the month of November 2010 and data recorded on various growth parameters from December 2010 - May 2011. Raised nursery beds of dimension 2m×1m were prepared. Seeds of flower crop (100 g) were sown in the month of September in raised nursery beds. Nursery beds were kept moist by sprinkling water and free from weeds. Seedlings were ready for transplanting in 40-45 days after sowing. Experimental fields were prepared by ploughing the field twice and made smooth

by harrowing, followed by planking. The plots were prepared to accommodate all the treatments for the flower crop. Different doses of organic manures viz; FYM and vermicompost were applied to all the plots consisting flower crop, except control. Manures were evenly spread and thoroughly mixed with the soil before transplanting.

Uniform and healthy seedlings of *Calendula officinalis* were lifted from the nursery on 4th November 2010 and transplanted in the experimental field at 30×30 cm spacing between the plants and rows. Light irrigation after transplanting was given to facilitate the establishment of seedlings. Hand weeding and irrigation was done, as and when required.. No major diseases or attack of insect pest was observed during the course of investigation, but during the month of February the attack of leaf minor was noticed on some seedling and it was controlled by applying the Chlorpyrifos @20 ml/20lt of water. Field was also covered from all the sides with the help of net to prevent the crop from the attack of rabbits and other animals. The number of seeds per flower were counted, final numbers of seeds were based on the ten flowers. Seed yield plant⁻¹ was recorded separately for all treatments. Thousand seeds were counted from each experimental plot and finally the weight of thousand seeds was recorded. The data generated was analyzed in spit plot design.

The tree spacing, organic manure and interaction effect registered a significant variation for number of seeds per flower (Table 1). Among three spacing, maximum (29.54) number of seeds flower⁻¹ was recorded in Sp₀, and minimum (27.23) number of seeds flower⁻¹ was recorded in Sp₁. Maximum number of seeds flower⁻¹ (31.78) was recorded with 2.5kg vermicompost, which was also found statistically at par with 3 and 5 kg FYM and minimum number of seeds flower⁻¹ (25.79) was recorded in untreated plots. Cumulative effect of treatment and spacing reveals that, maximum number of seeds flower⁻¹ (38.53) was recorded in T₃ Sp₃, whereas, minimum (20.03) in treatment combination of T₅Sp₁. The increase in numbers of seeds flower⁻¹ with the increase in the tree spacing might be due to the combined effect of shade from tree canopy and organic fertilizer. These effects can be seen in the total seed yield plant⁻¹ and seed yield per plot⁻¹. The results of present investigation may be endorsed with the findings of Kaushal and Verma (2006) where they reported that number of wheat tillers per plant in wheat crop decreased significantly at 1m spacing as compared to wider spacing. Applications of organic manures have a significant influence on the number of seeds per flower. Vermicompost dose @ (2.5kg) were found to be the best dose followed by the FYM @ (5kg) per plot. Kusuma

Table 1. Effect of tree spacing and organic manures on seed production parameters of *Calendula officinalis* L. under *Grewia optiva* Drummond. based agroforestry system

Manure doses	Number of seeds per flower				Mean	Seed yield plant ⁻¹ (g)				Mean	Seeds yield plot ⁻¹ (g)				Mean	1000 Seed weight (g)				Mean
	Tree spacing					Tree spacing					Tree spacing					Tree spacing				
	Sp ₁	Sp ₂	Sp ₃	Sp ₀		Sp ₁	Sp ₂	Sp ₃	Sp ₀		Sp ₁	Sp ₂	Sp ₃	Sp ₀		Sp ₁	Sp ₂	Sp ₃	Sp ₀	
T ₁ (5 kg FYM)	31.63	27.60	31.50	33.07	30.95	4.44	4.27	4.21	5.79	4.68	59.61	52.73	59.63	64.33	59.08	6.63	7.00	7.07	6.60	6.83
T ₂ (3 kg FYM)	31.77	28.40	30.73	29.13	30.01	4.24	4.38	4.30	5.02	4.48	53.60	52.17	58.80	66.57	57.78	5.73	7.17	6.73	6.57	6.55
T ₃ (2.5 kg VC)	27.37	31.50	38.53	29.70	31.78	4.03	4.72	4.18	5.59	4.63	48.30	57.37	52.03	66.93	56.16	6.53	7.17	7.03	6.40	6.78
T ₄ (1.5 kg VC)	25.33	27.80	26.17	24.07	25.84	3.58	4.48	4.55	4.63	4.31	56.17	49.30	45.67	66.53	54.42	6.37	6.83	6.43	6.77	6.60
T ₅ (Control)	20.03	31.30	20.10	31.73	25.79	3.69	3.68	4.71	5.07	4.28	44.03	56.67	55.17	60.83	54.18	6.60	6.17	6.07	6.50	6.33
Mean	27.23	29.32	29.41	29.54		4.00	4.30	4.39	5.22		52.34	53.65	54.26	65.04		6.37	6.87	6.67	6.57	
CD (p=0.05)	S	2.43				S	0.86				S	3.69				S	0.39			
	T	1.20				T	0.25				T	3.11				T	0.32			
	S×T	4.87				S×T	0.50				S×T	6.22				S×T	0.65			

(2001) reported that golden rod (flower crop) produce the maximum number of suckers plant⁻¹ when fertilized with different levels of NPK and vermicompost @ 10 t ha⁻¹. Tree spacing, organic manure and interaction effect had a significant effect on the seed yield per plant. Cumulative effects of treatment and spacing (S×T) shows that treatment combination T₃Sp₀ recorded higher (66.93 g) seed yield plot⁻¹ where it was recorded minimum (44.03 g) in treatment combination of T₅Sp₃. The remaining treatment combinations (T₂Sp₀, T₁Sp₀ and T₅Sp₀) were statistically at par with the T₃Sp₀. The highest seed yield per plant and highest seed yield per plot in open condition (Sp₀) might be due to the light availability, which is important factor for photosynthesis, growth/development and yield. Shade imposes a limitation to biological productivity in plant, although the extent of the limitation varies with shade tolerance of the species (Chauhan *et al.*, 2010; 2011; Rani *et al.*, 2015). The decrease in seed yield per plant was mainly due to the shadowing effect caused by *Grewia optiva* canopy. The present findings are in line with the findings of Sheik and Haq (1986) where they observed depressed wheat yield upto a distance of 5m from tree rows. Similarly khybri *et al.* (1992) in a Wheat–*Grewia*, Wheat–*Morus* and Wheat–*Eucalyptus* intercropping system observed 39% yield reduction upto 1m from tree rows, 33% upto 2m, 25% upto 2-3m and 12% upto 3-5m from tree row. Application of organic manures showed that application of FYM @ 5 kg plot⁻¹ recorded the highest seed yield per plant, which is closely followed by the Vermicompost (2.5kg) plot⁻¹. These results are in the conformity with the findings of Hari Priya and Sriramachandrasekharan (2002) where they noticed that, better growth and yield of marigold with different doses of FYM. Similar results were also reported by Prakash *et al.* (2002) and Yadav *et al.* (2000) in marigold.

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Chauhan SK, Gupta N, Walia R, Yadav S, Chauhan R and Mangat

Received 17 November, 2015; Accepted 22 April, 2016



Karanj Extract as an Antimicrobial Textile Finish for Cotton Fabric

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Abstract: Karanj plant parts used for inhibiting the growth of micro-organisms. It was observed that leaves and seeds extract in viscous state was found more effective as compared to treatments with solution of dry leaves of karanj and seeds powder upto 7th day. Among leaves and seed, leaves extract was more effective and it was found hundred per cent reduction effective after 30 minutes and upto 7th day of inoculation of *Aspergillus* fungus.

Key Words: Antiseptic, *Aspergillus*, Chloroxylenol, Soxhlet method, Viscous

Anti microbial activity is being considered to be important and inevitable property especially for cellulosic fabric products used in direct contact with human body and will improve functionality of textile products, especially when garments worn close to the skin and also in several medical applications, such as infection control and barrier material (Malpani, 2013). Antimicrobial property of the textile reduces microbial colonization as well as adds therapeutic value to the material (Senti *et al.*, 2011). Cotton fabrics are generally worn next to skin hence direct contact of textiles with human body provides warmth, humidity and nutrients; an excellent environment for micro-organism growth. These micro-organisms create unpleasant odour, discoloration, staining, degradation of textile materials and spread of diseases. Therefore, the present study was planned to develop renewable treatment from Karanj plant parts for inhibiting the growth of micro-organisms in cotton fabric against washing.

Hundred per cent cotton (scoured) fabric and an antiseptic containing chloroxylenol were procured from local market of Hisar city and leaves/seeds of Karanj (*Pongamia pinnata*) plant were collected from different places in campus CCS HAU Hisar. *Aspergillus* fungus was also readily available in the department of Microbiology, College of Basic Sciences and Humanities, CCS HAU, Hisar.

Cotton fabric was desized in a solution containing 1% H₂SO₄ at 50°C for 60 minutes with material to liquor ratio 1:40. The fabric was rinsed thoroughly to remove any starch and acid residues. The fabric was weighed, soaked, squeezed, and treated in a solution containing 1% soap, 3% soda ash and 0.5% sodium sulphite at boiling temperature (100°C) with 1:40 material to liquor ratio for 60 minutes. After that the fabric was rinsed thoroughly and dried on a flat surface (Gaba, 1999) Sample taken from scoured cotton was kept as control sample.

Preparation and application of karanj a extract:

Extraction was carried out by refluxing (soxhlet) and distillation methods. Half quantity of leaf/ seed extracts separately converted from viscous to dry form by keeping in open place for four to five days. Through silica gel viscous extracts were converted in dry state. Solution of Karanj extracts in viscous and dry state in two concentrations i.e. 2.5g l⁻¹ and 5g l⁻¹ were prepared. On the basis of weight of the fabric quantity 1:20 material to liquor ratio of Karanj extracts were calculated. Four pieces of scoured cotton fabric were sterilized and impregnated with these four prepared solutions separately. The samples were immersed in the treatment bath for 30 minutes. Without squeezing, the fabric was placed in the dry place.

Preparation and application of an antiseptic containing chloroxylenol on cotton fabric: Solution of an antiseptic containing chloroxylenol was prepared in distilled water in 2.5g l⁻¹ concentration. Material to liquor ratio was taken as 1:20. Another piece of cotton fabric was sterilised and immersed in the solution for 30 minutes. Without squeezing the fabric was kept overnight for drying. The antiseptic treatment was taken as standard.

Determination of microbial population: The microbial population (total colony forming units) of treated samples without and with inoculation of *Aspergillus* was determined quantitatively using AATCC-100 test method. The size of sample for determination of *Aspergillus* population was 2"×2" inches were taken from *Karanj extract* treated cotton fabric samples. To assess the fungal count and per cent reduction of treated/untreated samples, the sample was dipped in PDA (Potato Dextrose Agar) medium sterilized water overnight in a petri dish. Serial dilution (10⁻¹, 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵) was carried out to fungal count. From each of prepared dilutions, 0.1 ml was transferred onto the prepared petri-dish and spread with the help of spreader under laminar flow. The petri-dishes were placed in the incubator set as 30°C for 24

hours for the growth of fungus. The colonies of fungus were counted manually and per cent reduction was also calculated.

Samples treated with leaves extract in viscous state (LEVS), seeds extract in viscous state (SEVS), solution of dry leaves powder (SDPS), solution of dry seed powder (SDLP) and solution of antiseptic containing chloroxylenol (standard), without inoculation of *Aspergillus* were kept as control samples for determining the fungus growth and population against inoculated samples. These were coded as C₆, C₇, C₈, C₉ and C₁₀, respectively.

The effect of leaves and seeds extracts in viscous and dry state after inoculation on samples (treated samples) was compared with samples treated with antiseptic containing chloroxylenol (standard) samples. These samples were inoculated with *Aspergillus* separately. The observations were recorded after 30 min. of inoculation of *Aspergillus* on the same day. The observations were also

recorded at time intervals of 7th, 14th and 21st days with dilution factors 10⁻⁴ and 10⁻⁵ mean. Mean values of 10⁻⁴ and 10⁻⁵ dilution factors were calculated.

Assessment of efficacy of treatments with 2.5 g l⁻¹ concentration of leaf and seed extracts in viscous and dry states at different intervals on *Aspergillus* inoculated samples. The per cent reduction was compared with the both control samples i.e. scoured untreated samples and samples treated with leaf and seed extracts both in viscous and dry state and without any fungus inoculation (C₆-C₁₀). The colony forming units on control samples (C₆-C₁₀) were observed nil after 30 minutes, 7th, 14th and 21st days of inoculation in both dilution factors 10⁻⁴ and 10⁻⁵. There was confluent growth on control, untreated samples after 30 minutes, 7th, 14th and 21st days of inoculation with both dilution factors 10⁻⁴ and 10⁻⁵.

Efficacy of karanj extracts: The data in Table 1 indicated that there was 100 per cent reduction in *Aspergillus* growth after 30 min. of inoculation in leaf and seed extracts in

Table 1. Assessment of efficacy of Karanj a extracts* at different intervals on *Aspergillus* inoculated samples

Incubation Period	Dilution Treatments	After 30 min.	7 Days	14 Days	21 Days
		<i>Aspergillus</i>	<i>Aspergillus</i>	<i>Aspergillus</i>	<i>Aspergillus</i>
		10 ⁴ & 10 ⁵ Mean	10 ⁴ & 10 ⁵ Mean	10 ⁴ & 10 ⁵ Mean	10 ⁴ & 10 ⁵ Mean
Leaves extract in viscous state (LEVS)		100	99.7	98.7	98.00
Seed extract in viscous state (SEVS)		100	99.65	97.55	97.4
Solution of dry leaves powder (SDLP)		100	98.6	97.00	96.35
Solution of dry seed powder (SDSP)		100	98.6	97.00	95.8
Chloroxylenol (Standard)		100	99.65	97.55	97.45
Control (C)		Confluent growth			
Without fungus inoculation (C ₆ -C ₁₀)		Nil			

*Concentration 2.5g/l

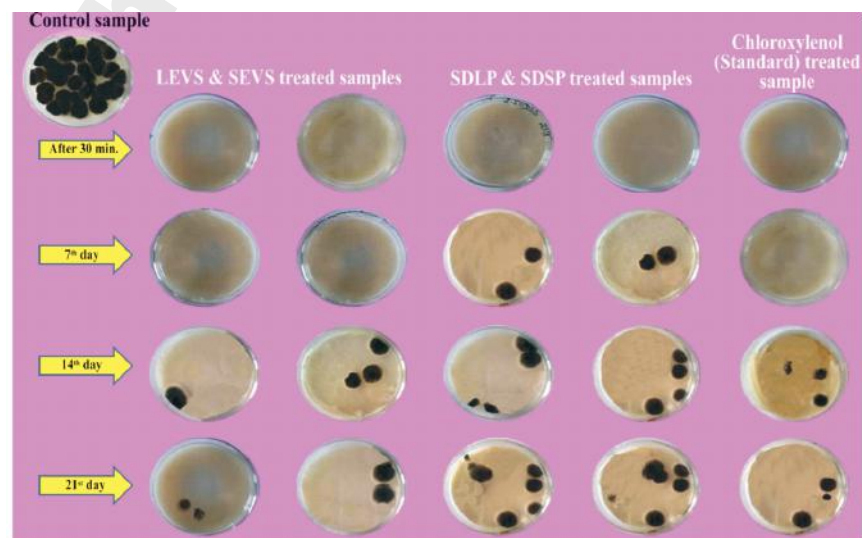


Plate 1. *Aspergillus* growth on control and treated samples upto 21st day (2.5g/l)

viscous and dry state and antiseptic containing chloroxylenol (standard) treated samples in both dilution factors. On 7th day, 99.7, 99.65, 98.6, 98.6 and 99.65 per cent reduction in *Aspergillus* growth was observed samples treated with leaves extract in viscous state, seeds extract in viscous state, solution of dry leaves powder, solution of dry seeds powder and antiseptic containing chloroxylenol (standard) with mean CFUs in 10^{-4} and 10^{-5} dilution factors respectively.

There were 98.7, 97.55, 97, 97 & 97.55 per cent reduction in *Aspergillus* growth on 14th day on samples treated with leaves extract in viscous state, seeds extract in viscous state, solution of dry leaves powder, solution of dry seeds powder and antiseptic containing chloroxylenol (standard) treated samples with mean CFUs in 10^{-4} and 10^{-5} dilution factors, respectively. On 21st day also same trend was observed. The leaf and seed extracts in viscous state was

found more effective as compared to treatments with solution of dry leaf and seed powder upto 7th day. Among leaf and seed, leaf extract was more effective.

The leaf extract in viscous state was the most effective among all treatments even as compared to standard. There was 100 per cent reduction in *Aspergillus* growth in LEVS treated sample upto 7th day.

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Received 26 October, 2015; Accepted 2 February, 2016



Biochemical Constituents of Different Mulberry Varieties Grown under “Seri Suvarna” Technology with Protective Irrigation

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Abstract: Seven popular mulberry varieties of *Morus alba* viz., V1, S36, S34, S30, S13, MR2 and RFS175 were analysed for bio-chemical constituents like total soluble proteins, carbohydrates, starch, chlorophyll and total soluble sugars. Among the different leaf constituents, total soluble proteins, carbohydrates, starch and total soluble sugars were found significantly higher in V1 mulberry leaves followed by S36, RFS175, S13, S34 and MR2 mulberry leaves. The chlorophyll – a, b and total chlorophyll contents were significantly more in S36 leaves followed by V1, S34, RFS175, S13 and S30 mulberry leaves.

Key Words: Biochemical constituents, Mulberry, Protective irrigation, Seri Suvarna

One of the major factors responsible for yield gap in sericulture is the nutritional status of mulberry leaf. It is well-known that the silkworm, *Bombyx mori* L. is a typical monophagous insect feeding only on mulberry (*Morus* spp.). It derives all the essential nutrients from the mulberry leaf for its growth and development and is very efficient bio-reactor for protein synthesis just like yeast in fermentation industry. Therefore, the nutrition of the silkworm is of due importance not only for the maintenance of its life but also for both seed and silk production and fed with good quality mulberry leaves in optimum quantity for the successful cocoon production. The various factors responsible for successful cocoon harvest are mulberry leaf (38.20%), climate (37.00%), rearing techniques (9.30%), silkworm race (4.20%), silkworm seed (3.10%) and other factors (8.20%). Therefore, a study has been undertaken to select mulberry variety/varieties containing higher biochemical constituents for qualitative and quantitative improvement of cocoon production in the silkworm, *Bombyx mori*.

The field experiments were conducted at Department of Sericulture, University of Agricultural Sciences, Bengaluru. The experiments were initiated in an established gardens of seven mulberry varieties viz., V1, S36, S34, S30, S13, MR2 and RFS175, which were grown under UAS - Seri Suvarna Technology (trenching and mulching) with protective irrigation, which includes opening of trenches (2' × 1.5') between the row, filling of biomass at the rate of four tonnes per acre (Mulberry, Neem, *Glyricidia* sp., weeds, etc.). The first layer was filled with biomass and second layer filled with FYM @ five tonnes per acre (Chinnaswamy *et al.*, 2006). Weeding and intercultural practices were carried out after harvest of each crop. The experimental plots were given protective irrigated by furrow method once in 25 days

depending on the soil moisture condition. To record all the qualitative parameters of mulberry, five plants were selected at random and labelled in net area excluding the border rows. The collected leaf samples were dried in an oven at 70°C until constant weight was obtained. The total soluble protein (Lowry *et al.*, 1951), carbohydrate (Dubios *et al.*, 1956), starch (Mc Cready *et al.*, 1960), chlorophyll (Hiscox and Israelstam, 1979) and total soluble sugar (calorimetric method *i.e.*, phenolsulphuric acid method of Dubios *et al.* (1956) were estimated by standard methods.

Total soluble protein (%): The total soluble protein content among the mulberry varieties differed significantly and was highest in V1 mulberry leaves (23.74%) followed by S36, RFS175, S13, S34 and MR2 mulberry leaves. However minimum soluble protein was in S30 mulberry leaves.

Carbohydrates starch and soluble sugar (%): Among the mulberry varieties, V1 mulberry leaves recorded higher carbohydrate starch and sugar followed by S36, S13, RFS175, S34 and MR2, whereas minimum was registered in S30 mulberry leaves.

Chlorophyll (mg g⁻¹): The chlorophyll – a, chlorophyll – b and total chlorophyll contents were recorded significantly higher in S36 mulberry leaves (1.450 mg g⁻¹, 0.630 mg g⁻¹ and 2.080 mg g⁻¹) followed by V1, S34, RFS175, S13 and S30 mulberry leaves. The least chlorophyll-a, chlorophyll-b and total chlorophyll contents was recorded in MR2 mulberry leaves.

The above findings are also in harmony with Bose and Bindroo (2001), who reported that Chinese variety, which has higher sugars, starch and total carbohydrate contents compared to other selected mulberry varieties.

Further, the present results are also closely agreeable to those of Chakravorty *et al.* (2006) who reported that TR 10 variety showed best results for carbohydrate content along

Table 1. Biochemical parameters of different mulberry varieties

Varieties	Total soluble protein (%)	Carbohydrates (%)	Starch (%)	Total soluble sugar (%)	Chlorophyll-a (mg g ⁻¹)	Chlorophyll-b (mg g ⁻¹)	Total chlorophyll (mg g ⁻¹)
V1	23.74	23.87	12.23	13.41	1.446	0.625	2.071
S36	22.65 (28.42)	21.43 (27.57)	11.02 (19.39)	12.71 (20.89)	1.450	0.630	2.080
S34	21.76 (27.81)	20.43 (26.87)	9.42 (17.87)	11.52 (19.84)	1.414	0.556	1.970
S30	20.01 (26.57)	17.76 (24.92)	8.25 (16.69)	10.58 (18.98)	1.380	0.489	1.869
S13	22.18 (28.09)	21.21 (27.42)	10.48 (18.89)	12.23 (20.47)	1.402	0.499	1.901
MR2	20.42 (26.86)	19.21 (25.99)	8.95 (17.41)	11.03 (19.40)	1.370	0.300	1.670
RFS175	22.64 (28.41)	20.71 (27.06)	9.82 (18.26)	11.79 (20.08)	1.418	0.509	1.927
CD (p=0.05)	1.700	1.771	0.409	0.500	0.062	0.037	0.084

Note: Figures in the parentheses are angular transformed values.

with other biochemical contents like crude proteins, total free amino acids, crude fibre, total chlorophyll, lipid and β -sterols compared to other varieties. Further, Ramachandra *et al.* (2008) also reported maximum soluble protein content in V1 variety compared to all other varieties.

It is concluded that V1 mulberry variety is the best among the seven mulberry varieties followed by S36 and S13 in respect of considered biochemical constituents. Apart from the chemical constituents, various morphological, physiological and biochemical characters of the plant may have influence on the quality of the leaves.

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Received 2 August, 2015; Accepted 8 April, 2016



Effect of Phosphorus Levels on Nutrient Uptake and Yield in Rice bean

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Abstract: The cultivar JRGBJ-05-4 with 90 kg P₂O₅ ha⁻¹ recorded maximum green fodder yield (438.14 q ha⁻¹) at 50% flowering. The maximum nutrient uptake was in JRGBJ05-4 with 90 kg of phosphorus (438.14 q ha⁻¹) followed by Bidhan ricebean -1 with 90 kg of phosphorus (415.01 q ha⁻¹), JRGBJ05-4 with 30 kg phosphorus (402.05 q ha⁻¹) and Bidhan ricebean -1 with 30 kg phosphorus (360.41 q ha⁻¹).

Key Words: Nutrient uptake, Ricebean, Soil properties

Rice bean (*Vigna umbellata*) is an important legume *Kharif* crop of North Eastern hill region of India and is identified as highly nutritious fodder than other leguminous fodders like cowpea and moth bean (*Vigna aconitifolius*) with respect to protein, calcium and phosphorus contents and more fodder yield. The availability of adequate fodder with better nutritive profile is pre-requisite for exploiting production potential of reared animals. Due to increasing population pressure of human beings, land is mainly used for food and cash crops, thus there is little chance of having good-quality land available for fodder production, unless milk production becomes remunerative to the farmer as compared to other crops. To meet the current level of livestock production and its annual growth in population, the deficit in all components of fodder, dry crop residues and feed has to be met from either increasing productivity, utilizing untapped feed resource, increasing land area (not possible due to human pressure for food crops) or through imports. The prevailing feed stuff is not enough in energy and protein (Riaz *et al.*, 2008) and feed alone constitute approximately 60-70 per cent of the total cost of milk production. The phosphorus is responsible for better root development of rice bean which enhances the uptake of nutrients higher as compare to without phosphorus condition and biomass production occurs greater with increasing phosphorus level was also reported by Bhattacharya and Mukherjee (1998) and Raj and Patel (1991). More leaf area index, crop growth and higher biomass induced by higher dose of phosphorus level which is essentially necessary for fodder ricebean. Similar study was done by Zaman and Malik (1999). The yield and soil fertility in rice bean was improved by different combinations of phosphorus with the cultivars of ricebean and uptake of N, P and K enhanced at the optimum level was also seen by Choubey *et al.* (2001). It was reported that

Ricebean supplied the highest amount of phosphorus (90 kg/ha) through incorporation of stover by improving organic matter in soil same result was found by Ghosh *et al.* (2007).

Field experiment was conducted at Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) during *Kharif* 2012. The soil of the experimental field was predominantly sandy clay loam in texture. The organic carbon content (0.40%) and available nitrogen (232 kg ha⁻¹) were low. The available phosphorus (17.18 kg ha⁻¹) and potash (310 kg ha⁻¹) were medium and high, respectively. The soil pH was neutral (7.2) with normal electrical conductivity (0.32 dS m⁻¹). The experiment was carried out in Factorial Randomized Block Design with three replications. Each replication consisted of two varieties V₁ JRGBJ-05-4 and V₂ Bidhan ricebean-1 with four levels of phosphorus (0, 30, 60 and 90 kg ha⁻¹) treatments. The crop was raised as per recommended practices. Phosphorus was given as per treatments as basal application through single super phosphate. The soil samples were collected after the land preparation and before sowing of the crop to a depth of 0-30 cm and were analyzed as per standard method. The nutrient uptake was calculated by following formula.

Uptake of nutrients: The maximum (201.36 kg ha⁻¹) nitrogen uptake by ricebean plant was recorded in the JRGBJ05-4 with 90 kg of phosphorus followed by Bidhan ricebean -1 with 90 kg of phosphorus (190.96 kg ha⁻¹), JRGBJ05-4 with 30 kg phosphorus (181.42 kg ha⁻¹) and Bidhan ricebean -1 with 30 kg phosphorus (167.72 kg ha⁻¹). Therefore the lowest nitrogen uptake (94.11 kg ha⁻¹) was recorded in Bidhan ricebean -1 with 0 kg phosphorus. Phosphorus uptake was markedly influenced due to different treatments. JRGBJ05-4 with 90 kg of phosphorus had higher phosphorus uptake (3.63 kg ha⁻¹) followed by JRGBJ05-4 with 30 kg phosphorus (3.10 kg ha⁻¹) and Bidhan ricebean -1 with 90 kg of

Table 1. Effects of different treatments on green fodder yield (q ha^{-1}), nutrient uptake (kg ha^{-1}) and soil properties in ricebean

Treatment	Green fodder yield (q ha^{-1})	Nutrient uptake by crop plants (kg ha^{-1})			Total uptake (kg ha^{-1})	Soil PH	EC (ds/m)	OC (%)	Available nutrient (kg ha^{-1})		
		N	P	K					N	P	K
$V_1 P_0$	286.39	117.42	1.42	26.89	145.73	7.2	0.37	0.41	225	16.0	275.0
$V_1 P_1$	326.64	139.06	1.91	31.63	172.6	7.2	0.37	0.42	225	16.1	275.5
$V_1 P_2$	402.05	181.42	3.10	47.58	232.1	7.3	0.38	0.43	227	16.5	276.4
$V_1 P_3$	438.14	201.36	3.63	52.20	257.19	7.4	0.39	0.44	229	16.9	277.0
$V_2 P_0$	267.88	94.11	1.08	19.36	114.55	7.2	0.38	0.41	227	16.0	276
$V_2 P_1$	298.88	127.71	1.58	26.27	155.56	7.2	0.38	0.42	227	16.2	276.3
$V_2 P_2$	360.41	167.72	2.27	35.89	205.88	7.3	0.39	0.42	228	16.8	279.1
$V_2 P_3$	415.01	190.96	2.63	49.47	243.06	7.4	0.40	0.43	228	17.1	280.0
Initial Status CD ($p=0.05$)	9.05					7.2	0.32	0.40	232	17.18	310.0

V_1 = JRGBJ-05-4, V_2 = Bidhan ricebean-1; P_0 = 0 kg $P_2O_5 \text{ ha}^{-1}$, P_1 =30 kg $P_2O_5 \text{ ha}^{-1}$, P_2 =60 kg $P_2O_5 \text{ ha}^{-1}$ and P_3 =90 kg $P_2O_5 \text{ ha}^{-1}$

phosphorus (2.63 kg ha^{-1}). While the lowest (1.08 kg ha^{-1}) phosphorus uptake was recorded by Bidhan ricebean -1 with 0 kg phosphorus. Different treatments varied for potassium uptake. It was maximum (52.20 kg ha^{-1}) under treatment combinations of JRGBJ05-4 with 90 kg of phosphorus which was followed by Bidhan ricebean -1 with 90 kg of phosphorus (49.47 kg ha^{-1}), JRGBJ05-4 with 30 kg phosphorus (47.58 kg ha^{-1}) and Bidhan ricebean -1 with 30 kg phosphorus (35.89 kg ha^{-1}). However, the lowest 19.36 kg ha^{-1} potassium uptake was recorded by Bidhan ricebean -1 with 0 kg phosphorus. Uptake of nutrients were similarly studied by Kumawat *et al.* (2009).

Green fodder yield: The green fodder yield increased significantly with the ricebean varieties. The variety JRGBJ-05-4 was found significantly superior and recorded maximum green fodder yield 363.30 q ha^{-1} at 50% flowering stage. Whereas, Bidhan ricebean-1 variety was noted minimum green fodder yield 335.54 q ha^{-1} for the same character. The application of treatment P_3 was found significantly superior (426.57 q ha^{-1}) followed by P_2 (381.23 q ha^{-1}). While, the lowest (277.13 q ha^{-1}) green fodder yield was obtained in P_0 . JRGBJ05-4 with 90 kg of phosphorus was noted superior and recorded maximum green fodder yield (438.14 q ha^{-1}) at 50% flowering as compared to other treatment combination. While, the minimum 267.88 q ha^{-1} green fodder yield was recorded in the Bidhan ricebean -1 with 0 kg phosphorus at 50% flowering. Similar result was observed by Jamadagni *et al.* (2002).

Changes in chemical properties of soil: The chemical properties of soil viz., soil pH, electrical conductivity (EC) and organic carbon % (OC) available nitrogen and phosphorus contents did not deviate much from their initial status after the completion of the crop whereas, available potassium content of soil was deviate from their initial status after the completion of crop cycle in almost all the treatments due to effect of crops.

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Effect of Spacing and NPK on Yield and Alkaloid Content of *Solanum nigrum* L. Under Hill Zone of Karnataka

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Abstract: The spacing of 60 cm x 45 cm and application of NPK fertilizer @ 125:75:75 kg ha was found beneficial for getting higher fruit yield and alkaloid content by makoi. Among different spacing levels, higher fresh and dry yield (14.33 and 2.58 t ha⁻¹, respectively) and alkaloid content (0.33 %) by black night shade. Among the different fertilizer levels, application of 125:75:75 kg NPK ha⁻¹ recorded maximum fresh and dry yield and higher alkaloid content (0.37 %). The interaction of spacing and fertilizer levels showed non-significant effect in increasing fresh and dry yield and alkaloid content of black night shade.

Key Words: Alkaloid content, Black night shade, Medicinal, NPK, Spacing, Solanaceae

Makoi (*Solanum nigrum* L.) is a newly emerging herbaceous medicinal crop, belongs to the family Solanaceae. It is an medicinal plant and occurs throughout India, particularly in drier parts up to an elevation of 2,100 m and is distributed in all tropical and temperate regions of the world (Anonymous, 1969). The leaves, berries and the whole herb plants are economically important. The alkaloids, alphasolanomarginine and alpha-solanosine have been isolated and identified from the green, unripe fruits (Ridout *et al.*, 1989). The berries also contain four steroidal glycoalkaloids namely alpha-solanigrine, beta-solanigrine, solamarginine and solanosine. Solamarginine and solanosine are present in leaves. Although, work has been done on the therapeutic uses of this crop, the information on the cultural and nutrient management practices suited to the hill zone condition of Karnataka is very meagre. Hence, the present investigation was planned to standardize the optimum spacing and nutrient requirement of this crop for enhancing the yield and alkaloid content of makoi. The present investigation on the effect of spacing and NPK on yield and alkaloid content of black night shade under hill zone of Karnataka at Horticulture Research Station, Thirthahalli were conducted during 2013. In all, there were sixteen treatments comprised of two levels of spacing and eight levels of fertilizer (Table 1). The experiment was laid out with factorial concept in randomized block design with three replications. The gross plot size was 3.0 x 3.0 m (9 m²). The treatments were allocated to individual plots at random. Ridges and furrows were formed as per the row spacing. Farm yard manure of 9 kg plot⁻¹ (@10 tons ha⁻¹) was evenly spread in all plots and thoroughly mixed in to the soil. Before sowing, the seeds were pre-soaked in 500 ppm GA3 solution for 12 hours to overcome the dormancy and to ensure good germination. Thirty days old

healthy, uniform sized seedlings were selected and transplanted in the experimental plots as per the treatments. The experimental plots were applied with the calculated quantity of fertilizers as per the treatments. Out of total quantity, 50% of nitrogen and full dose of phosphorous and potash were supplied as basal dose a day before transplanting. The remaining 50% of nitrogen was given as top dressing at 30 days after transplanting. The crop was harvested three months after planting at mature green berry stage. Observations on yield parameters were recorded at 90 DAP (At harvest) and total alkaloid content of whole plant was estimated from the powdered sample using gravimetric method. The data collected on different parameters were subjected to statistical analysis. The effect of different spacing levels on the total alkaloid content was significant (Table 1). The maximum total alkaloid content (0.33 % w/w) was recorded in plant spaced at 60 x 45 cm. The minimum alkaloid content (0.31 % w/w)

Among the different spacing levels, plants spaced at 60 x 45 cm recorded significantly higher fresh and dry yield per hectare (14.33 and 2.58 t, respectively). This was due to the fact that the more number of plants per unit area at this spacing to accumulate higher fresh and dry yield per hectare. Among the different fertilizer levels, application of 125:75:75 kg NPK ha⁻¹ recorded the maximum fresh and dry yield per hectare. This was due to positive role played by nutrient on growth and metabolism of plants, which increased the accumulation of matter in the plant. These findings are in line with the results of Lokesh and Gangadharappa (2007) in *S. nigrum*. Among the interaction no significant difference was noticed in fresh and dry yield per hectare was due to interaction of spacing and fertilizer.

The effect of different spacing levels on the total

Table 1. Effect spacing and NPK on yield and alkaloid content of black night shade

Treatments	Fresh yield (t ha ⁻¹)	Dry yield (t ha ⁻¹)	Alkaloid (%)
Spacing level (S)			
S ₁ : 60x45 cm	14.33	2.58	0.33
S ₂ : 60x60 cm	13.43	1.60	0.31
CD (p=0.05)	0.82	0.22	0.003
Fertilizer level (F)			
F ₁ : 100:50:50 kg NPK ha ⁻¹ (control)	12.18	1.31	0.27
F ₂ : 100:50:75 kg NPK ha ⁻¹	12.74	1.59	0.29
F ₃ : 100:75:50 kg NPK ha ⁻¹	13.16	1.74	0.29
F ₄ : 100:75:75 kg NPK ha ⁻¹	13.56	1.74	0.31
F ₅ : 125:50:50 kg NPK ha ⁻¹	14.02	2.17	0.32
F ₆ : 125:50:75 kg NPK ha ⁻¹	14.45	2.17	0.33
F ₇ : 125:75:50 kg NPK ha ⁻¹	14.90	2.29	0.34
F ₈ : 125:75:75 kg NPK ha ⁻¹	16.03	3.71	0.37
CD (p=0.05)	1.65	0.45	0.02
Interaction effects (S x F)			
S ₁ F ₁	12.30	1.44	0.30
S ₁ F ₂	13.03	1.95	0.28
S ₁ F ₃	13.59	2.14	0.31
S ₁ F ₄	13.87	2.11	0.32
S ₁ F ₅	14.59	2.79	0.32
S ₁ F ₆	15.07	2.79	0.33
S ₁ F ₇	15.47	2.89	0.35
S ₁ F ₈	16.74	4.52	0.38
S ₂ F ₁	12.05	1.19	0.24
S ₂ F ₂	12.45	1.22	0.29
S ₂ F ₃	12.74	1.33	0.27
S ₂ F ₄	13.26	1.37	0.30
S ₂ F ₅	13.44	1.55	0.32
S ₂ F ₆	13.84	1.55	0.32
S ₂ F ₇	14.33	1.70	0.33
S ₂ F ₈	15.33	2.91	0.37
CD (p=0.05)	NS	NS	0.03

alkaloid content was significant (Table 1). The maximum total alkaloid content (0.33 % w/w) was recorded in plant spaced at 60 x 45 cm. The minimum alkaloid content (0.31 % w/w) was noticed in 60 x 60 cm spacing. This may be due to the ideal growing conditions for plant growth prevailed at this spacing resulting in higher total alkaloid content. The results

are in line with the findings of Pushpalatha *et al.* (2003) and Lokesh and Gangadharappa (2007) in makoi. Significant variation for alkaloid content was observed due to application of different fertilizer which ranged from 0.27 to 0.37 % w/w. A linear increase in alkaloid content was observed with increase in fertilizer level. Application of 125:75:75 kg NPK ha⁻¹ (F₈) recorded the maximum alkaloid content (0.37 % w/w) 8 followed by F₇ (0.34 % w/w). The total alkaloid content was 7 lowest (0.27 % w/w) in control. The higher alkaloid content at higher level of nutrients was due to the availability of balanced nutrients.

Total alkaloid content was also differed significantly due to interaction of spacing and fertilizers and ranged between 0.24 to 0.38 % w/w. Among the interactions of spacing and fertilizers, S F treatment recorded maximum 1 8 total alkaloid content (0.38 % w/w), which was statistically at par with S₁F₈ (0.37 % w/w) and S F (0.35 % w/w) and significantly superior over other treatment combination. Lowest alkaloid content of 0.24 % w/w was recorded in control (S₁F₁). This could be attributed to total alkaloid content, which is mainly dependent on timely nutrient application and utilization of light, moisture and space. The results are comparable with the findings of (0.40 % w/w with a spacing of 60X30 cm) Pushpalatha *et al.* (2003) and Lokesh and Gangadharappa (2007) in makoi.

Planting of makoi at 60 x 45 cm spacing and supplemented the crop with 125:75:75kg NPK ha⁻¹ is beneficial for obtaining the higher fresh and dry herbage yield per hectare and maximum alkaloid content under hill zone of Karnataka.

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Yield Attributes Yield and Quality of Soybean Influenced by Crop Varieties and Phosphorus

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Abstract: Application of phosphorus 90kg P_2O_5 ha⁻¹ significantly influenced the number of pod/plant, number of seed/pod, test weight seed yield stover yield and harvest index. The highest seed yield and oil content was also recorded with the 90 kg P_2O_5 ha⁻¹ and it was at par with 60 kg P_2O_5 ha⁻¹. Better yield attributes and yield were obtained in Local variety, which was significantly higher than other varieties, but oil content and protein was significantly higher in Bragg.

Key Words: Phosphorus, Protein, Quality, Soybean

Among the macronutrients, phosphorous is one of the major limiting nutrients in acids soils of Manipur for proper growth and yield of soybean. There are many reports from different parts of the country about the use of phosphorous for better crop performance and seed yield of soybean oil content (Hussan *et al.*, 2011). Growing of suitable variety well adapted to climatic condition of the area is one of the means to increase the plant height, fresh weight, dry weight of plant and nodules as well as production of this crop (Kazi *et al.*, 2002). According to Ankomah *et al.* (1995), soil deficient in phosphorus limit the extent of nodulation, nitrogen fixation and seed yield of legume crops. Phosphorus, apart from its effect on nodulation process and plant growth, has been found to exert some direct effects on soil rhizobia. Application of phosphorus significantly improved the performances of soybean.

An experiment was conducted during the *kharif* season of 2010 at Central Agricultural University, Imphal, Manipur with twelve treatment combinations consisting of four levels of phosphorus viz. 0, 30, 60 and 90 kg P_2O_5 ha⁻¹ and three varieties of soybean (Bragg, JS-335 and Local) in a randomized block design with three replication. Representative soil sample were collected from 0 to 15 cm depth randomly from several spots of the experimental field before starting the experiment and physio-chemical properties of soil were analysed. The soil of experimental field was clay in texture with pH 5.1 and contained organic carbon 1.8%, available nitrogen 374 kg ha⁻¹, available phosphorus 30.32 kg ha⁻¹ and available K 120 kg ha⁻¹. NPK were applied as basal of recommended dose except the phosphorus, which was applied as per treatments. Five representative sample plants were randomly selected from each of the plots for biometric observations. The selected plants were tagged for accurate observation. The sun dried

harvested crop was recorded from net plot area after subtracting the seed yield. The estimation of per cent nitrogen was done using automatic Nitrogen Analyzer and percentage of crude protein was calculated by multiplying the per cent nitrogen value by 6.25. Oil content in seed was determined by soxhlet extraction method as described by AOAC (1980).

The application of phosphorus significantly increased the yield attributes. The maximum no. of pods plant⁻¹, seeds pod⁻¹, test weight was recorded under application of phosphorus 90 kg P_2O_5 ha⁻¹ and it was significantly superior to rest to phosphorus treatments. The similar results were also reported by Atere and Olayinka (2012).

Among the varieties, the maximum no. of pods plant⁻¹ seeds pod⁻¹ under Braggs cultivar, which was significantly superior to Local control and JS-335. However, test weight (160.55) was recorded under Local variety, which was significantly superior to Braggs and JS-335. The seed and stover yield was significantly affected by phosphorus application. Application of 90 kg P_2O_5 ha⁻¹ showed a significant superiority over that of control and 30 kg P_2O_5 ha⁻¹ and was at par to 60 kg P_2O_5 ha⁻¹ in respect of seed yield (22.74 q ha⁻¹). Application of phosphatic fertilizer therefore provides better nutrition to soybean which resulted in higher seed yield. Increased in seed yield with the application of phosphorus may be due to better growth of the plant as expressed in term of plant height, fresh and dry weight of plant and nodulation, which were favourably affected by phosphate fertilization. This finding is also supported by Gosavi *et al.* (2008) and Luo *et al.* (2005). The highest seed and stover yield was recorded with the Local variety, which remained at par to Bragg but significant superior to JS-335.

Table 1. Effect of phosphorus and varieties on yield attributes of soybean

	Pods plant ⁻¹				Seeds pod ⁻¹				Test weight (g) of soybean grain			
	V ₁	V ₂	V ₃	Mean	V ₁	V ₂	V ₃	Mean	V ₁	V ₂	V ₃	Mean
P ₀	45.40	42.00	37.93	41.78	3.07	2.78	2.75	2.87	80.81	78.47	155.98	105.09
P ₁	49.93	45.27	38.00	44.40	3.22	2.90	2.87	2.99	82.19	79.10	157.83	106.37
P ₂	50.07	45.67	38.27	44.67	3.30	2.92	2.90	3.04	82.27	80.89	160.56	107.90
P ₃	50.10	46.53	41.20	45.94	3.35	3.07	3.03	3.15	82.40	80.92	167.86	110.39
Mean	48.88	44.87	38.85		3.23	2.92	2.89		81.92	79.85	160.55	
CD (p=0.05)												
Variety (V)		0.21				0.073				1.34		
Phosphorus (P)		0.24				0.086				1.55		
V*P		0.41				NS				2.68		

Table 2. Effect of phosphorus and varieties on yield and harvest index

	Grain yield (q ha ⁻¹)				Stover yield (q ha ⁻¹)				Harvest index (%)			
	V ₁	V ₂	V ₃	Mean	V ₁	V ₂	V ₃	Mean	V ₁	V ₂	V ₃	Mean
P ₀	14.28	13.46	13.27	13.67	45.38	48.60	49.33	47.77	37.31	41.19	44.10	40.87
P ₁	17.20	17.87	18.41	17.83	53.81	54.98	55.89	54.89	38.00	43.22	46.58	42.60
P ₂	22.55	21.21	23.15	22.30	60.38	60.44	62.29	61.04	38.28	43.79	48.54	43.53
P ₃	22.99	21.54	23.70	22.74	65.25	66.29	67.89	66.47	38.54	43.93	49.29	43.92
Mean	19.25	18.52	19.63		56.20	57.58	58.85		38.03	43.03	47.13	
CD (p=0.05)												
Variety (V)		0.52				0.34				0.39		
Phosphorus (P)		0.60				0.40				0.45		
V*P		1.04				0.69				0.79		

Table 3. Effect of phosphorus and varieties on nitrogen, protein and oil content in seed

	Protein content (%)				Oil content (%)			
	V ₁	V ₂	V ₃	Mean	V ₁	V ₂	V ₃	Mean
	40.35	39.59	38.50	39.48	20.22	19.37	20.33	19.97
	40.29	39.85	39.32	39.82	21.68	19.71	20.14	20.51
	40.33	39.63	39.69	39.88	21.62	20.45	20.16	20.75
	40.44	39.73	39.58	39.92	22.02	20.63	20.09	20.91
	40.35	39.70	39.27		21.39	20.04	20.18	
CD (p=0.05)								
Variety (V)			0.34				0.39	
Phosphorus (P)			0.40				0.45	
V*P			0.69				0.79	

On the other hand, the Local variety recorded more number of pods plant⁻¹ and number of seeds pod⁻¹, which ultimately contributed to obtain higher seed yield. These finding are also supported Sharma *et al.* (1999). The phosphorus and varieties interaction also give significantly superior grain and stover yield. The maximum yield was recorded with the application of phosphorus 90 kg ha⁻¹ in Bragg.

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Effect of Seed Rhizome Size on Yield and Quality of Turmeric (*Curcuma longa* L.) cv. Salem

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Abstract: Effect of seed rhizome size on yield and quality of turmeric cv. Salem was carried out during 2012-13 at College of Horticulture, Arabhavi, Karnataka. The highest cured yield (10.41 t/ha), processing percentage (30.93), curcumin content (4.83 %) and volatile oil (1.34 %) was obtained by planting of whole mother rhizome of size 25 g followed by cut mother rhizome of size 25 g.

Key Words: Curcumin, Seed rhizome, Turmeric

Turmeric is propagated vegetatively using both mother as well as finger rhizomes. The type and weight of planting material affects the vigour of the plant and crop yield per unit area as well as the cost of production. The cost incurred on planting material accounts to nearly 50 per cent of total crop production in turmeric. Studies on the use of different type and sizes of rhizome are scanty. Therefore, it is necessary to determine the optimum size and type of seed rhizome for turmeric cultivation. Hence, the present investigations were undertaken to assess the performance of turmeric in response to different type and size of seed rhizomes. The study was carried out at the Kittur Rani Channamma College of Horticulture, Arabhavi, Karnataka during 2012-2013.

Well-matured, healthy, different type and different sized rhizomes of turmeric cv. Salem obtained by the harvest of crop grown during 2011-12, were utilized for the investigation during 2012-13. The seed rhizome treatments were T₁-Single eye bud (2g) sprouted on moist sand (45 days before planting), T₂-Single eye bud (2g) sprouted on moist sand (30 days before planting), T₃-Single eye bud sprouted (2g) in prostrays (30 days before planting), T₄-Whole mother rhizome (25 g), T₅-Cut mother rhizome (25 g), T₆-Primary rhizome (25 g), T₇-Secondary rhizome (25 g). The seed rhizomes were planted at 45 cm between rows and 22.50 cm between the plants (ridge and furrow method). The recommended practices were adopted for raising of crop.

Observations recorded by using five randomly selected clumps from each treatment on the yield and yield attributes and quality parameters viz., volatile oil percentage (ASTA, 1968), curcumin content (Manjunath *et al.*, 1991), processing percentage and cured yield were recorded after harvest.

Yield and yield attributes: The fresh rhizome yield (yield per clump, yield per plot and yield per hectare) and yield attributes such as number of rhizomes per clump and rhizome clump size were significantly higher in T₄ followed by T₅, whereas, fresh rhizome yield and yield attributes were lower in the treatments T₁, T₂ and T₃. Increased rhizome yield in mother rhizome as planting material might be attributed to better crop growth in terms of quick emergence, higher plant height, higher leaf area index and tillers per clump, which intercepted more photosynthetically active radiation and resulted in higher values of yield attributing characters which ultimately contributed towards higher yield of the crop. On the other hand, the plants emerged from single eye bud did not grow vigorously and satisfactorily. Yield attributing parameters like number of rhizomes per clump, girth of rhizome and rhizome clump size were significantly lower in plants raised from single bud compared to the plants emerged out of mother rhizomes. Thus, the plants resulting from mother rhizomes are vigorous and yield better as compared to finger plant material. The results obtained in the present study are in conformity with the reports of Sengupta and Dasgupta (2011) in ginger, Olojede *et al.* (2009), Datt *et al.* (2007) and Hossain *et al.* (2005) in turmeric.

Quality parameters: Among the quality parameters curcumin, volatile oil content and processing percentage were influenced significantly by the size of rhizomes and significantly highest curcumin, volatile oil content and processing percentage was observed in T₄ followed by T₅ compared to the T₁. There was decrease in crop duration and poor development of fingers in T₁, which lead to decreased curing percentage, curcumin and volatile oil content. On the other hand, increase in seed rhizome weight/ size (T₄, T₅, T₆) increase the yield and quality of the finger rhizomes and

Table 1. Yield attributes and quality parameters in turmeric cv. Salem as influenced by different size of seed rhizome

Treatment	Number of rhizomes per clump	Rhizome clump size (cm ³)	Fresh yield (g clump ⁻¹)	Yield to seed ratio (%)	Fresh plot yield (kg/4.86 m ²)	Fresh yield (t ha ⁻¹)	Processing percentage	Estimated cured yield (t ha ⁻¹)	Curcumin content (%)	Volatile oil (%)
T ₁	12.37	8.11	53.87	27.10	9.13	18.79	22.17	4.16	3.70	0.69
T ₂	13.20	8.33	56.13	28.07	9.29	19.11	22.43	4.28	4.18	0.78
T ₃	13.50	8.64	58.33	31.33	9.61	19.77	22.77	4.49	4.23	0.81
T ₄	29.97	18.74	417.33	16.71	16.37	33.69	30.93	10.41	4.83	1.34
T ₅	23.23	14.54	360.07	13.78	15.32	31.52	27.97	8.79	4.53	1.00
T ₆	20.90	12.17	291.47	11.26	14.04	28.90	25.90	7.48	4.34	0.87
T ₇	18.80	10.99	207.40	8.30	12.95	26.65	23.80	6.34	4.30	0.79
CD (p=0.05)	2.35	1.43	20.64	1.84	1.20	2.46	2.50	0.83	0.45	0.22

T₁: Single eye bud (2g) sprouted on moist sand (45 days before planting), T₂: Single eye bud (2g) sprouted on moist sand (30 days before planting), T₃: Single eye bud sprouted (2g) in portrays (30 days before planting), T₄: Whole mother rhizome (25g), T₅: Cut mother rhizome (25g), T₆: Primary rhizome (25g), T₇: Secondary rhizome (25g)

fetches higher prices in the market. Higher yield is attributed to the bigger rhizome size, which has enough reserved food to influence better vegetative growth through accumulation of more photosynthates in rhizomes and secondary metabolites like curcumin and essential oil. The results obtained in the present investigations are in conformity with the findings of Balwinder and Gill (2010); Manhas and Gill (2010) in turmeric.

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Received 26 November, 2015; Accepted 1 February, 2016



Quality Characterization in Carrot (*Daucus carota* L.) Germplasm

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Abstract: Thirty eight genotypes were tested in a randomized complete block design with three replications to determine the variation in quality characters like total soluble solids, sugar content, dry matter, β carotene content, anthocyanin content and juice yield. The highly significant differences for all the traits indicated the presence of wide variability in the genotypes. The juice yield ranged from 543.33-425 ml/kg, TSS from 8.28-6.55% and dry matter from 8.70-6.78%. Genotypic and phenotypic coefficients of variation were found to be higher for β carotene (GCV=4.62) and (PCV=33.26). High heritability (98.11%) was observed for β carotene followed by sugar content (89.62%) and juice content (89.35%) which was associated with high genetic advance as percentage of mean for β carotene and sugar content.

Key Words: Carrot (*Daucus carota* L.), β -carotene content, Juice yield and TSS

Carrot juice is a rich source of carotene, which is an important source of pro-vitamin A, fiber and other dietary nutrients (Simon 1990). Carrot is also an important source of calcium, magnesium and potassium. Around 35 million tonnes of carrots are produced globally each year, giving the crop a financial and horticultural significance. In India, total area under carrot is 68.32 thousand hectares with annual production of 1172.71 thousand metric tonnes with average productivity of 17.17 mt hectare⁻¹. Diversity exists in pigmentation of wild and cultivated carrot roots. White, yellow, orange, red, purple and pink types are also known to exist. Yellow carrots contain xanthophylls, which help develop healthy eyes and may prevent lung and other cancers. Red carrots also contain lycopene, which help to prevent heart diseases and some cancers including prostate cancer. Purple carrots contain pigments called anthocyanins (Montilla *et al.*, 2011) that act as powerful antioxidants, grabbing and holding on to harmful free radicals in the body. Anthocyanins also help prevent heart diseases by slowing blood clotting. On the basis of these studies, the importance of component characters are marked to facilitate the selection programme for better gains. Hence, an investigation was carried out at Punjab Agricultural University, Ludhiana, during the year 2012-13 for estimating genetic variability, heritability and genetic advance among various qualitative traits in 38 genotypes of carrot.

The thirty eight genotypes were collected from diverse agroclimatic conditions under the presumption that wider genetic divergence could be assessed (Table 1). The experiment was laid out in a Randomized Complete Block Design with three replications. Each entry was planted on a 5 meter long ridge at a spacing of 60 cm between rows and 7.5 cm between plants with a plot size of 3.0m² and standard

agronomic practices were followed to raise a successful crop. The plants were thinned to maintain plant to plant distance of 7.5 cm about a fortnight after seedling emergence. Ten randomly selected plants from each genotype in each replication were used for recording the observations. Observations were recorded for the characters were total soluble solids, sugar content, dry matter, β carotene content and juice content. The extent of variability was measured in terms of range, mean, genotypic coefficient of variation (GVC), phenotypic coefficient of variation (PVC) along with per cent of heritability (h^2).

The significant differences indicated the presence of variability and considerable opportunity for improvement of different qualitative characters. Total soluble solids ranged from 6.54% (PC-120) 8.27%(PC 112). Other genotypes which had more TSS but less than PC-112 were PC-115 (8.05%), PC-80 (7.91%), PC-61(8.32%). Sugar content ranged from 3.84% - 4.91% with a mean of 4.44%. Genotype PC-79-A had the maximum percentage sugar content 4.91%. Genotype PC-106 has the lowest sugar content of 3.88% (Table 1). Yadav *et al.* (2009) observed that percentage sugar content ranges between 7.00-7.80. Dry matter content ranged from 6.68% 8.70% with a mean of 7.66%. Genotype PC-106-1 had the maximum dry matter content of 8.70%. Fikselova *et al.* (2010) observed mean higher contents of dry matter in the year 2003 compared to the year 2002. The β carotene content ranged from 4.109.70 mg/100 g of roots (Table 1). PCO-2 had a highest β carotene content of 9.70 (mg/100g) followed by PCO-1(8.86 mg/100g). The minimum β carotene content of 4.10 (mg/100g) was found in PC-105. Anthocyanin content of PCB-5 was 185.16 mg/100g. Juice yield varied from 425.00 - 543.33 ml/kg of roots. The maximum juice yield was recorded in PCB-5 (573.33 ml/kg) followed by PC-73 (530.00). The

Table 1. Mean value of different genotypes for various traits

Source	Accession	TSS (%)	Sugar content (%)	Dry matter (%)	β carotene (mg/100g)	Juice content (ml/kg)
PAU, Ludhiana	PCB-5	7.69	4.44	7.61	4.52	573.33
PAU, Ludhiana	SEL-80-1	7.46	4.72	7.21	4.53	478.33
PAU, Ludhiana	PC-43	7.87	4.67	7.56	4.82	500.33
PAU, Ludhiana	PC-73	6.64	4.69	7.94	5.34	530.00
PAU, Ludhiana	PC-79	7.52	4.83	7.93	6.91	518.33
PAU, Ludhiana	PC-79 A	7.43	4.91	7.65	6.75	473.33
PAU, Ludhiana	PC-79 B	7.64	4.61	6.68	6.30	480.00
PAU, Ludhiana	PC-80	7.91	4.58	7.25	6.38	436.66
PAU, Ludhiana	PC-80-1	7.11	4.20	7.19	6.64	501.66
PAU, Ludhiana	PC-100	7.50	4.14	7.46	5.56	491.66
Punjab	PC-101	7.75	4.90	8.62	5.52	484.00
Punjab	PC-103	7.61	4.02	7.60	5.13	436.66
Punjab	PC-104	7.55	4.60	7.28	4.89	485.00
Punjab	PC-105	7.03	4.48	8.69	4.10	501.66
Punjab	PC-106	7.66	3.88	7.09	4.76	440.00
Punjab	PC-106-1	7.46	4.11	8.70	4.56	455.00
Haryana	PC-107	6.60	4.60	7.39	4.87	455.00
Haryana	PC-108	7.42	4.16	7.66	4.88	438.33
Haryana	PC-109	7.42	4.52	7.49	4.13	493.33
Punjab	PC-110	7.65	4.17	7.23	4.65	463.33
Punjab	PC-111	7.48	4.18	8.19	4.99	481.33
Punjab	PC-112	8.27	4.18	7.90	5.77	478.33
Rajasthan	PC-113	7.78	4.59	7.71	5.13	465.66
Rajasthan	PC-114	7.26	4.75	8.17	4.69	486.66
Rajasthan	PC-115	8.05	4.14	7.24	4.46	500.00
Punjab	PC-116	7.42	4.20	8.25	4.83	456.66
Punjab	PC-117	7.63	4.69	7.86	5.60	436.66
Punjab	PC-118	7.69	4.75	7.19	4.70	425.00
Punjab	PC-119	7.81	4.52	7.49	5.44	480.00
Punjab	PC-120	6.54	4.34	7.86	4.76	465.00
Punjab	PC-121	7.50	4.64	8.31	6.88	498.33
Punjab	PC-122	7.36	4.36	7.40	4.31	482.00
Punjab	PC-123	7.31	4.55	7.27	4.18	488.33
Punjab	PC-124	7.52	4.71	7.70	5.35	458.33
Jammu & Kashmir	PCO-1	7.26	4.14	7.12	8.86	471.66
Jammu & Kashmir	PCO-2	7.75	4.35	7.30	9.70	491.66
Jammu & Kashmir	PCO-3	7.40	4.56	7.49	8.77	486.00
PAU, Ludhiana	PC-34 (SC)	7.35	4.48	8.11	5.75	461.66
	CD (P=0.05)	0.35	0.15	0.52	0.23	13.31

minimum juice yield was recorded in PC-118 (425.00 ml/kg). Amin *et al.* (2009) observed juice yield varied between 415.83-581.67 ml/kg of roots with maximum being in genotype PC-5 and minimum in Early Nantes.

The estimates of genetic parameters of variability, viz., phenotypic and genotypic coefficient of variation along

with heritability in broad sense (h^2) for different characters are given in (Table 2). Phenotypic coefficient of variation ranged from 04.49-33.26. The highest phenotypic coefficient of variation existed for β carotene content followed by sugar content and dry matter. The lowest phenotypic coefficient of variation was shown by TSS content (4.49). Amin *et al.* (2013)

Table 2. Range mean, GCV, PCV, heritability in broad sense and genetic advance as percentage of mean for different characters in carrot

Character	Range	Mean	GCV	PCV	Heritability (%)	G.A (%)
TSS (%)	8.28-6.55	7.49	2.87	4.49	71.01	7.79
Sugar content (%)	4.91-3.84	4.44	2.13	6.26	89.62	12.20
Dry matter (%)	8.70-6.78	7.66	4.19	5.60	64.07	9.23
carotene content (mg 100g ⁻¹)	9.70-4.10	3.33	4.62	33.26	98.11	67.87
Juice content(ml kg ⁻¹)	543.33-425	475.24	1.72	4.98	89.35	9.71

also studied the coefficient of variation in carrot, which was highest for carotene content and TSS content. Genotypic coefficient of variation ranged from 1.72-04.62. Maximum genotypic coefficient of variation was observed in β carotene content followed by dry matter. However, minimum genotypic coefficient of variation was found in case of juice content (1.72).

In the present study heritability ranged from 64.04-98.11. Very high heritability was in β carotene followed by sugar and juice yield. The lowest heritability was found in case of dry matter (Table 3). Bhatia *et al.* (2002) found high heritability estimates for juice yield. The highest genetic advances as percentage of mean was observed for carotene content (67.87) followed by sugar content (12.20) and juice yield (09.71). Kumar *et al.* (2010) found varied genetic advances as percentage of mean in different cultivars. The results are of significance as they support more efficient utilization of available genetic variability in carrot germplasms breeding programmes which delivers novel cultivars with enhanced nutritional value.

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Received 10 February, 2016; Accepted 14 May, 2016



Response of Drip Irrigated Spring Maize to Different Levels of Salinity

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Abstract: The four treatments of drip irrigation with saline water of 2000 mmho/cm (EC-2), 4000 mmho/cm (EC-4), 6000 mmho/cm (EC-6) and 8000 mmho/cm (EC-8) electrical conductivity were compared with drip irrigation by normal water, having electrical conductivity in the range of 600-700mmho/cm. Maximum plant height was recorded under normal irrigation water, which was statistically at par with EC-2 and EC-4 irrigation treatments. Significant differences in the number of grain per cob were observed and maximum number of grains per cob were recorded under EC-2 irrigation water. Significantly higher yields were recorded under EC-2 and EC-4 irrigation treatment as compared to higher saline water treatments of EC-6 and EC-8. No significant difference was observed in protein content, carbohydrates and oil content of the grains under different treatments.

Key Words: Drip irrigation, Saline water, Spring maize

Maize (*Zea mays* L.) is the third most important food crop in India after wheat and rice. In Punjab State, spring maize is grown during the end of January and before 15th February and harvested in the end of May. The irrigation demand in the month from March to May is quite high and water quality can have a profound impact on crop production. Maize is moderately salt tolerant crop. Assured irrigation is very important during spring season as total water requirement is to be met only through irrigation in the absence of rains. Due to shortage of good quality water, use of brackish water for irrigation can increase availability of water to fulfil the basic need of water for crop production. This water can be applied through drip irrigation and frequent application of small quantities of water through drip irrigation prevents the built up of salts in the soil near the root zone, as salts are pushed to the periphery of the wetted area around the roots. By delivering water directly near the roots, the plant experiences less stress and thrives (Gupta and Yasmin, 2013; Gupta and Chattoo, 2014). It may imply that a higher yield can be produced per unit brackish water input, thus enabling an expansion of irrigated agriculture, by using brackish water through drip irrigation system. The proposed study was intended to identify threshold levels of salinity for drip irrigated spring maize.

A field experiment was conducted at Department of Soil and Water Engineering, Punjab Agricultural University, Ludhiana in the spring season of the year 2013. The maize variety PMH-1 was sown with randomized block design with three replications for four treatments of saline water drip irrigation of 2000 mmho/cm (EC-2), 4000 mmho/cm (EC-4), 6000 mmho/cm (EC-6) and 8000 mmho/cm (EC-8) electrical conductivity compared with normal water (electrical

conductivity in the range of 600-700 mmho/cm). The recommended agronomic practices were adopted for raising the crop. The observations for growth and yield attributing parameters were recorded from five tagged plants. The soil of the experimental site was loamy sand in texture, low in organic carbon (0.15%) and available nitrogen and medium in available phosphorous. The results of growth parameters, grain yield and quality parameters of spring maize due to the application of different levels of saline irrigation water are shown in Table 1.

The maximum plant height at harvest, was recorded with normal water and statistically at par with EC-2 irrigation but significantly better to EC-4, EC-6 and EC-8. The minimum plant height was recorded in EC-8 irrigation treatment where salinity of irrigation water was maximum. This might be due to the presence of salt in soil solution, which decreases the osmotic potential of soil, creating water stress and making it difficult for the plant to absorb water necessary for growth and thus there was decrease in the plant height along with increase in salinity. Blanco *et al.* (2008) also reported at Piracicaba (Brazil) decrease in plant height of maize along with increase in salinity.

There was no significant differences in cob length and circumference due to different irrigation water treatments. The maximum length of cob 17.7cm was recorded in normal treatment. Maximum number of grains per cob were in EC-2 irrigation water treatment and was statistically at par with normal irrigation water treatment but was significantly higher as compared to EC-4, EC-6 and EC-8 irrigation water treatments. Increasing salinity of irrigation water resulted in decrease in number of grains per cob. The irrigation with saline water might have resulted in non-filling of

Table 1. Effect of saline water irrigation on growth parameters, grain yield and quality of spring maize

Treatments	Plant height (cm)	Length of cob	Circumference of cob (cm)	Number of grains per	1000-grain	Grain yield	Protein content %	Oil content%	Carbohydrates %
EC-2	205.0	16.4	13.8	338.8	270.5	55.0	11.7	4.0	2.8
EC-4	192.0	16.0	13.6	320.6	267.3	52.8	11.8	4.2	2.7
EC-6	179.0	15.7	13.7	316.1	258.6	47.9	11.5	4.2	2.5
EC-8	166.0	16.1	13.4	303.9	251.5	45.4	11.7	4.4	2.5
Normal	216.3	17.7	14.0	337.9	271.9	54.0	12.0	3.9	2.5
CD (p=0.05)	23.9	NS	NS	9.3	14.3	3.1	NS	NS	NS

cobs. Kang *et al.* (2010) also found decrease in number of grains per cob along with increase in salinity of irrigation water. The highest value of 1000-grain weight was observed under normal irrigation, which was statistically at par with EC-2, EC-4 and EC-6 but was significantly higher over EC-8. There was a decreasing trend in grains with increase in salinity, but significant difference of 20.4 g decrease in 1000-seed weight was observed in EC-8 irrigation water treatment as compared to irrigation with normal water. Kang *et al.* (2010) also recorded decrease in 1000-grain weight along with increase in salinity of irrigation water.

There were significant differences in grain yield due to various irrigation treatments. The highest grain yield was in EC-2 irrigation and this was found statistically at par with normal irrigation and EC-4 but was significantly higher than EC-6 and EC-8. The data clearly shows a decreasing trend in grain yield with increase in level of salinity of irrigation water. This might be due to the fact that increasing salinity treatments also resulted in decrease in number of grains per cob and 1000-grain weight, which ultimately resulted in decrease in grain yield among these saline water treatments as compared to normal water treatment. The threshold level of saline water in the study was 4000 mmho/cm, as maize yield decreased significantly for saline water application above this level. The result of the study was in conformity with Yazar *et al.* (2003) who reported that the maize yield decreased in relation to the increase in salt concentration level of irrigation water applied with trickle system and

Barbosa *et al.* (2012) also recorded low yield in plants subjected to continuous irrigation with high salinity water of EC 4.5 dS/m. Kaur *et al.* (2015) recorded 28.07% higher maize yield through drip irrigation than conventional irrigation. There were non-significant differences in protein, oil and carbohydrate content due to different levels of salinity of irrigation water applied (Table 1).

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Effect of Seaweed Extract on Morpho-Physiological Parameters of Rice

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Abstract: Total leaf area, number of leaves, relative growth rate and vigour index enhanced with application of seaweed extract (SWE). A significant increase in photosynthetic pigments i.e. chl a, chl b, total chlorophyll and carotenoid content was observed with increase in SWE concentrations. The maximum Hill reaction activity was recorded under anaerobic conditions at 15 percent concentration SWE.

Key Words: Aerobic and anaerobic, Photosynthetic pigments, Seaweed extract, Vigour index

Among cereal crops, rice (*Oryza sativa* L.) is the most important crop of India and second most important crop of the world, grown under more diverse environmental conditions and in wide range of climatic zones, to nourish the mankind (Meena *et al.*, 2014). Among the rice growing countries in the world, India has the largest area under rice crop (about 45 million ha) accounting for 29.4 per cent of the global rice area. Of the total area, about 46 per cent is irrigated, 28 per cent is rainfed lowland, 12 per cent is rainfed upland and 14 per cent is flood prone (Budhar *et al.*, 2006). Traditionally rice was grown as irrigated, puddled and transplanted crop which involved raising nursery and manual transplanting of seedlings. Increased competition for water and climate change reduced the amount of water available for agriculture and it was estimated that by 2025, 15-20 million hectares of irrigated rice would experience water scarcity. Aerobic rice production system saved total water usage (27-51%) as compared to flooded system by eliminating continuous seepage, percolation, reducing evaporation and increasing water productivity (32-88%) (Bouman *et al.*, 2006). In this system, fields remain unsaturated throughout the season like an upland crop, such as wheat on non-flooded soils. Use of biofertilizers reduced the use of chemical inputs without negatively affecting crop yield or the farmers' income. These contain complex range of plant growth regulators, antibiotics, macro and micronutrients, which enhanced photosynthesis by increasing plant chlorophyll level to harness the sun energy efficiently and accelerated the health and yield of plants (Khan *et al.*, 2009; Prakesh *et al.*, 2013).

The present investigation was conducted at Punjab Agricultural University, Ludhiana in completely randomized design (CRD) with three replications. The seeds of rice cultivar PR 120 were surface sterilized with 0.1 % mercuric

chloride for 3 minutes to avoid any fungal infection and sown in pots under aerobic and anaerobic growth conditions. The pots were placed in incubator with a temperature of $30 \pm 2^\circ\text{C}$. After 3 days, foliar application of seaweed extract @ 10.0, 12.5 and 15.0 per cent (w/v) was given. The morpho-physiological and biochemical parameters were recorded at 14 days interval.

Leaf area was calculated by using formula given by Bhan and Pande (1966). The photosynthetic pigments were extracted from the leaves in 5 ml of the dimethylsulfoxide (DMSO) and concentration was calculated from the absorbance values at 665, 645 and 480 nm (Hiscox and Israelstam, 1979). To determine Hill reaction activity, intact chloroplasts were obtained from pre-weighed leaves by gently grinding in extraction medium (0.067 M phosphate buffer with pH 5.5 containing 0.35 M sucrose). The Hill activity was expressed as a decrease in absorbance $\text{mg chlorophyll}^{-1} \text{hr}^{-1}$.

Photosynthetic pigments: The chlorophyll content (chl a, chl b) and carotenoid varied significantly in selected rice cultivar under aerobic and anaerobic conditions (Table 1). Chlorophyll content (chl a, chl b and total chlorophyll) was higher under anaerobic conditions than aerobic conditions. The minimum carotenoid content was observed under aerobic conditions. The results showed that the application of SWE in studied range of concentration increased chlorophyll and carotenoid content in the leaves under both conditions but the increase was more pronounced under anaerobic condition. Maximum amount of photosynthetic pigments (chl a, chl b, total chlorophyll and carotenoid content) were recorded in the leaves treated with SWE @ 15 %. It was attributed to presence of magnesium and cytokinins in SWE (Kalaivanan and Venkatesalu, 2012). Selvam and Sivakumar (2014) reported that application of SWE induced absorption of essential nutrients, which led to enhancement in

photosynthetic pigments and photosynthetic activity.

Hill reaction activity: Application of SWE had significantly increased the hill reaction activity under both aerobic and anaerobic conditions but the effect was more significant under anaerobic condition (Table 1). SWE @ 15% was more effective to increase the hill activity under both conditions. Anjum *et al.* (2011) and Nagaraj *et al.* (2002) proposed that there was a positive correlation between chlorophyll content and photosynthetic rate, and small drop in chlorophyll content caused drastic reduction in photosynthetic rate (Sutharsan *et al.*, 2014). The increase in chlorophyll content attributed to reduction in chlorophyll degradation, which might be caused partly by betaines and betaine-like compounds present in seaweed extract (Khan *et al.*, 2009).

Morpho-physiological parameters: The leaf number varied non-significantly under both aerobic and anaerobic conditions. SWE application at lower concentrations viz 10.0 and 12.5 % showed no stimulatory effect on leaf number in both conditions while higher concentration viz 15% showed positive effect on leaf number. The leaf number was maximum (2.57) under anaerobic conditions as compared to aerobic conditions (2.33) when higher dose of SWE was applied. Rayorath *et al.* (2008) reported that extracts of *Ascophyllum nodosum* affected number of leaves of *Arabidopsis* at very low concentration. Sutharsan *et al.* (2014) recorded maximum average leaves number in tomato with application of seaweed liquid extract (*Sargassum crassifolium*) at lower concentration while higher concentration (100% SLE) exhibited inhibitory effect on leaves number. The non significant difference in total leaf

area was observed under both aerobic and anaerobic conditions (Table 2). The results revealed that the application of SWE at all concentrations showed positive effects on total leaf area but effect was more pronounced under anaerobic conditions. Total leaf area between 3.41 m² and 5.61 m². The beneficial effect of seaweed extract application on leaf area could be attributed to its many components working synergistically at different concentrations (Fornes *et al.*, 2002). Saa *et al.* (2015) reported that the increment in shoot leaf area with SWE application was due to a small increment in total leaf number per shoot and a significant increment in average leaf size.

There was a direct relationship between relative growth rate and plant biomass. The RGR was recorded low under aerobic conditions as compared to anaerobic conditions. The results revealed that application of SWE had significantly increased the relative growth rate under aerobic and anaerobic conditions but the effect was more pronounced under anaerobic conditions (Table 2). The maximum RGR (0.265) was under anaerobic conditions when higher concentration of SWE viz 15 % was applied. In other concentrations of SWE also, better RGR was noted as compared to control. This phenomenon may be due to presence of macro or micro nutrients in the SWE which stimulated plant growth as a result of enhancement of protein synthesis, cell division and mobilization of nutrients needed for growth (Pascale, 1993). Mansori *et al.* (2014) reported that application of seaweed extract of *Fucus* and *Ulva* increased dry biomass in bean plants. The positive effect of SWE in all concentrations on vigour index over control

Table 1. Photosynthetic attributes of rice as influenced by levels of SWE application under aerobic and anaerobic conditions

Treatments	Chl a content		Chl b content		Total chlorophyll content		Carotenoid content		Hill activity	
	AC	ANC	AC	ANC	AC	ANC	AC	ANC	AC	ANC
Control	0.657	0.718	0.406	0.600	1.158	1.469	0.0694	0.0729	0.126	0.243
SWE @ 10 %	0.680	0.764	0.439	0.651	1.225	1.514	0.0787	0.0804	0.156	0.272
SWE @ 12.5 %	0.696	0.794	0.463	0.680	1.285	1.580	0.0856	0.0875	0.196	0.304
SWE @ 15%	0.711	0.809	0.488	0.716	1.344	1.631	0.0890	0.0970	0.244	0.356
CD (p=0.05)	0.177		0.169		NS		0.169		0.172	

Table 2. Morpho-physiological attributes of rice as influenced by levels of SWE application under aerobic and anaerobic conditions

Treatments	Number of leaves		Total leaf area (m ²)		Relative growth		Vigour index	
	AC	ANC	AC	ANC	AC	ANC	AC	ANC
Control	2.30	2.33	3.41	4.56	0.211	0.216	356.75	670.00
SWE @ 10%	2.30	2.33	3.60	4.90	0.219	0.223	371.00	676.00
SWE @ 12.5%	2.30	2.33	3.84	5.24	0.225	0.242	384.00	687.00
SWE @ 15%	2.33	2.57	4.04	5.61	0.231	0.265	390.71	699.77
CD (p=0.05)	NS		NS		0.172		1.746	

was observed. The maximum vigour index (699.77) was with 15 % concentration of SWE application under anaerobic conditions as compared to aerobic conditions (390.71). In concentrations 10 % and 12.5 %, vigour index was 676.00 and 687.00 under anaerobic conditions and 371.00 and 384.00 under aerobic conditions, respectively. Seaweed extracts improved nutrient and water uptake by roots efficiency thereby causing enhanced general plant growth and vigour (Crouch *et al.*, 1990). The presence of precursors of elicitor compounds in SWE also promoted germination and seedling vigour.

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Evaluation of Different Substrates for Cultivation of *Ganoderma lucidum* (M.A. Curtis : Fr.) P. Karst

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Abstract: Two strains of *Ganoderma lucidum* were collected and cultured on malt extract medium. Malt extract medium, temperature range of 25-35°C and acidic pH (5.0) were conducive for the mycelial growth of *G. lucidum*. Mango sawdust supplemented with 10 per cent wheat bran at light intensity 500-800 lux was best in terms of spawn run, primordial initiation, number of primordia, fruit body expansion and development, yield and biological efficiency among all the substrate combinations. Supplementation of wheat bran (10%) in mango sawdust as a base substrate can be used commercially for the cultivation of *G. lucidum* by the mushroom growers and entrepreneurs.

Key Words: Fruiting, *Ganoderma lucidum*, Primordia, Saw dust, Wheat bran

Ganoderma lucidum (M.A. Curtis : Fr.) P. Karst is a basidiomycetous fungus of Polyporaceae family, is a high value medicinal mushroom which grows wild on a variety of non-resinous woody substrates in a wide range from temperate to tropical zones. In India, fruiting bodies of *G. lucidum* have successfully been cultivated on different substrates like woodlogs, wood chips and sawdust (Tripathy *et al.*, 2011). Sawdust of various tree species *i.e.* *Dalbergia sisoo*, *Albizia procera*, *Alnus nepalensis*, *Mangifera indica* and their mixed sawdust were evaluated for the cultivation of *Ganoderma lucidum* (Gurung *et al.*, 2012). For the purpose of increasing biological yield while reducing the time required for mushroom production, supplementation of substrate becomes one of the major aspects in mushroom cultivation. The aim of the present study was to evaluate different substrate combinations for the cultivation of *G. lucidum* due to its commercial medicinal importance its trade is growing fast due to import from other countries with global trade increasing more than two billion dollars.

Two strains (OE-53 and OE-52) of *Ganoderma lucidum* were collected from Directorate of Mushroom Research, Solan for evaluation for their mycelial growth, number of pinheads and total yield.

Mycelial growth optimization: Studies on *Ganoderma lucidum* were carried out in petriplates (radial growth), glass tubes (linear growth) and conical flasks (visual) to ascertain optimum temperature, pH and incubation period for mycelial growth of *G. lucidum*. Effect of different temperatures and pH on the mycelial growth was observed. The pH of the medium before and after sterilization was adjusted with the help of systronic pH meter by adding either N/10 NaOH or N/10 HCl.

Substrate preparation: Sawdust from different broad leaf tree species (*Mangifera indica*, *Populus sp.*, *Dalbergia sisoo*

and *Eucalyptus sp.*) were collected from sawmills and evaluated in various combinations with wheat bran, 10% wheat bran + 90% sawdust, 20% wheat bran + 80% sawdust and 30% wheat bran + 70% sawdust and wetted to 65% moisture level. Calcium sulphate (gypsum) and calcium carbonate (chalk powder) were added to get a pH of 5.0. Then the mixed substrates (500 gm dry wt.; 660 gm wet wt.) were filled in polypropylene bags and plugged with non-absorbent cotton after putting a plastic (PVC) ring at the neck. For each treatment, three replications were maintained. The substrates were sterilized at 15 p.s.i for 3 hours followed by cooling under room temperature. Each bag was inoculated with spawn @ 3% on dry weight basis and mixed with substrate and incubated at a temperature ranging between 28-35°C, humidity 80-85 per cent for about 30 days. Light intensity was adjusted at 150-200 lux. Observations on the pattern of mycelial colonization were recorded.

Fruiting: After complete mycelial colonization of the substrate in about 25-30 days, top of polypropylene bags was cut at the level of the substrate exposing the top surface. The precise conditions for pinning and fruiting (temperature 28°C, 1500 ppm CO₂, 800 lux light, 85-90% RH) were maintained in cropping room. After the pin head change into flat cap, humidity was reduced to 80-85%, fresh air was introduced to lower concentration to 1000 ppm CO₂ and 500 lux brightness. Once the cap was fully formed, with yellowing of the cap margin, temperature and relative humidity were lowered to 25°C and 70%, respectively, for thickening, reddening and maturation of the fruit bodies. On maturity, caps were reddish brown and spores were shed on the top surface. At this stage, fruit bodies were harvested and weighed. Second and third flushes appeared after about 15-20 days interval. Cropping was continued upto 2 months after opening of the bags. One

cropping cycle took 15-20 days. Entire crop duration took about 110-120 days. Data pertaining to primordial initiation, fruiting development, maturity of basidiocarp and yield were recorded during cropping period. Experiment was conducted in 36 bags of 500 g each with three replications.

In the preliminary yield trials, cultures of strain OE-53 fruited, hence it was selected over OE-52 due to its higher fruiting potential. OE-53 procured from DMR, Solan was selected on the basis of its better mycelial growth, number of pinheads and total yield.

Mycelial growth optimization: Malt Extract Agar supported maximum radial growth of both the strains at the temperature ranging from 25-35°C. Cultural growth of both the strains was favoured by temperature of 30°C and pH 5.0. Earlier workers i.e. Rai (2003) have reported mycelial growth of the *G. lucidum* at acidic pH. Lee *et al.* (2003) found pH 4.2 to be optimum for mycelial production of *G. lucidum*. Veena and Pandey (2011) reported pH range of 4.0- 6.5 to be the best for growth of *G. lucidum*. The present findings on the OE-52 and OE-53 strains of *G. lucidum* are also comparable to earlier reports.

Substrate evaluation: On the basis of results obtained from preliminary fruiting trial, strain OE-53 was selected for substrate evaluation. Fastest mycelial colonization (>3mm/day) was observed in mango sawdust + wheat bran but fast (2 mm/day) mycelial colonization was in eucalyptus sawdust + wheat bran. Shisham and poplar gave the slowest mycelial growth (<2 mm). Mango sawdust based substrate took significantly less time for spawn run as compared to other combinations. All the substrates were well colonized by the mushroom mycelium except Shisham where the colonization was in patches. Mango sawdust supplemented with 10% wheat bran showed quick and complete spawn run among all the substrate combinations. The addition of wheat bran or rice bran is an essential ingredient for the cultivation of *G. lucidum* as it might be taking care of thiamine requirement due to which mycelial growth and reproduction of *G. lucidum* is enhanced. Sawdust of *Alnus nepalensis* (Utilis) mixed with rice bran (10 per cent) was found a good substrate for the cultivation of *G. lucidum* (Negi *et al.*, 2008).

The earliest fruiting primordial initiation was observed on mango sawdust supplemented with 10% wheat bran, whereas, similar results were recorded on poplar and *Eucalyptus* sawdust supplemented with 20% and 30% wheat bran, respectively. In case of Shisham sawdust based combinations no primordia formation took place. Sawdust of *Alnus nepalensis* (Utilis) mixed with rice bran (10 per cent) was good substrate for the cultivation of *G. lucidum* (Negi *et al.*, 2008). Earliest fruit body expansion and development was observed on mango sawdust supplemented with 10%

Table 1. Fruiting primordial initiation behaviour of *Ganoderma lucidum* on different substrate combinations

Substrates**	Primordial initiation (Days)	No. of primordia*	Fruit expansion (Days)*	First harvest (Days)*	Length of stipe*	Diameter of pileus*	Thickness of stipe*
10% wheat bran +90% mango sawdust	28.00	4.30	33.00	38.00	4.50	4.80	1.50
20% wheat bran +80% mango sawdust	31.00	2.60	36.00	41.00	4.60	3.80	1.40
30% wheat bran +70% mango sawdust	34.00	2.30	39.00	45.00	4.50	4.70	0.90
10% wheat bran +90% eucalyptus sawdust	40.00	2.70	48.00	52.00	2.70	2.10	0.90
20% wheat bran +80% eucalyptus sawdust	40.00	3.30	43.00	48.00	1.40	2.40	0.80
30% wheat bran +70% eucalyptus sawdust	35.00	3.30	39.00	44.00	1.90	3.10	1.00
10% wheat bran +90% poplar sawdust	45.00	2.30	49.00	55.00	1.60	2.00	0.80
20% wheat bran +80% poplar sawdust	42.00	3.70	44.00	49.00	2.10	2.60	0.90
30% wheat bran +70% poplar sawdust	47.00	1.70	50.00	56.00	1.80	2.10	0.90
CD (p=0.05)	1.09	0.98	1.14	0.99	0.99	0.33	0.18

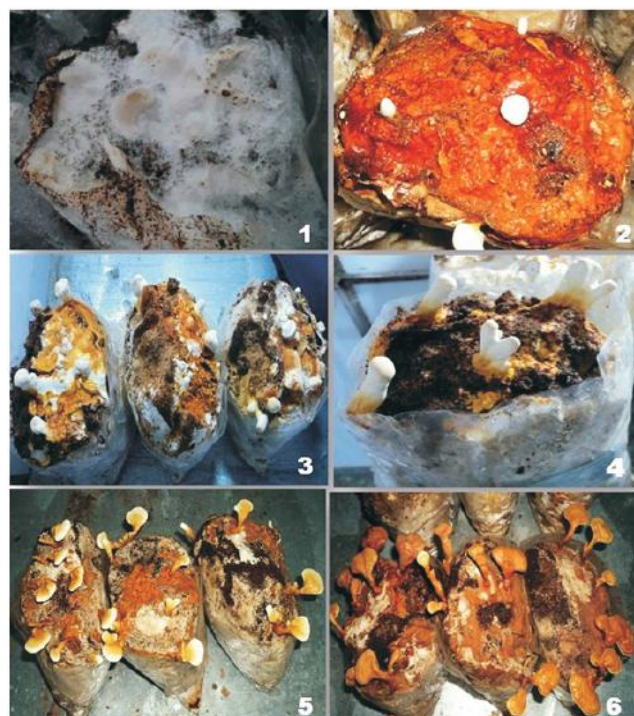
*average of three replications

**days for fruit expansion and harvesting after spawning at light intensity of 500-800 lux

Table 2. Yield potential of *Ganoderma lucidum* on different substrate combinations**

Substrate combinations	First flush	Second flush	Third flush	Total yield	Biological efficiency
10%Wheat bran + 90% Mango sawdust	33.70	22.80	13.30	69.60	17.40
20% Wheat bran + 80% Mango sawdust	27.30	15.70	8.70	51.60	12.90
30% Wheat bran + 70% Mango sawdust	22.30	14.30	9.30	46.00	11.50
10% Wheat bran + 90% Eucalyptus sawdust	8.70	3.30	3.00	15.30	3.80
20% Wheat bran + 80% Eucalyptus sawdust	12.70	8.70	7.30	28.70	7.20
30% Wheat bran + 70% Eucalyptus sawdust	16.70	13.30	10.70	40.70	10.20
10% Wheat bran + 90% Poplar sawdust	13.30	10.00	6.70	30.00	7.50
20% Wheat bran + 80% poplar sawdust	14.30	12.30	8.30	35.00	8.70
30% Wheat bran + 70% poplar sawdust	11.30	7.00	5.00	23.30	5.80
CD (p=0.05)				2.86	

* average of three replications; ** at light intensity of 500-800 lux



**1. Mycelial aggregation 2. Pin head formation 3. Development of pin heads 4. Expansion of pinheads 5. Fruit body expansion 6. Mature fruit body

Plate I. Fruiting behaviour of *Ganoderma lucidum* after opening of the bags

wheat bran, whereas, in poplar and eucalyptus similar results were recorded with 20% and 30% wheat bran, respectively at light intensity of 500-800 lux. Average size of stipe and diameter of pileus was also better on these substrate combinations.

Biological efficiency (%) of 17.4, 8.7 and 10.2 was recorded on 10% wheat bran + 90% mango sawdust, 20% wheat bran + 80% poplar sawdust and 30% wheat bran +

70% Eucalyptus sawdust, respectively (Table 2). Among all the substrate combinations, maximum biological efficiency of *G. lucidum* was obtained with supplementation of 10% wheat bran in mango sawdust at light intensity of 500-800 lux. Rai (2003) reported biological efficiency of *G. lucidum* between 10-15 %. Mishra and Singh (2006) evaluated biological efficiency of *G. lucidum* on wheat straw supplemented with 5 % rice bran, and reported a biological efficiency between 15-17 %. Mango sawdust supplemented with 10 per cent wheat bran at light intensity 500-800 lux was best in terms of spawn run, primordial initiation, number of primordia, fruit body expansion and development, yield and biological efficiency.

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Fortification of Grape Juice with Probiotic *Lactobacilli*

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Abstract: The two probiotic isolates i.e. *Lactobacilli plantarum* and *L. acidophilus* were not only able to survive but utilize the fruit juices for their cell synthesis, as indicated by a decrease in sugar content and pH, and increase in the acidity of the juice and bacterial counts. Isolate *L. acidophilus* consumed the sugar at a faster rate than *L. plantarum*; although, the fall in sugar and pH and increase in acidity was faster during first 24 hr but these changes got little slower during the next 48 hrs.

Key Words: Acidity survival (cfu/ml), Changes in sugar concentration, Fortification, Grape juice, *Lactobacilli* isolates, pH

Probiotics are living microbial supplements, which affect the host beneficially by controlling intestinal infection and serum cholesterol levels; by influencing the immune system; improving lactose utilization in lactose maldigestors and having anticarcinogenic activity (Saarela *et al.* 2002; Rafter, 2003). Currently, industrial probiotic food products especially dairy products, such as yogurt and fermented milk has become very popular. Nevertheless, lactose intolerance and the cholesterol content in milk are two drawbacks related to the milk consumption by the consumers. On the contrary, fruit juice are healthy food product, and is currently consumed by a large percentage of the global population (Verbeke, 2005). Fruits are rich in antioxidants, vitamins, dietary fiber and mineral but do not contain any dairy allergens, such as casein and lactose (Luckow and Delahunty, 2004). Fruit juice was also suggested to serve as a good medium for probiotics (Tuorila and Cardello, 2002). Probiotic fortified fruit products could be used as a functional healthy beverage for better health and nutrition of the people, especially for those who are allergic, intolerant or reluctant to consume milk based products. In present investigation, an attempt has been made to prospect the fortification of grape juice with three isolates of probiotic *Lacto bacilli* with an aim to estimates their ability to utilize grape juice sugar, their tolerance to varying concentration of acids and lactic acid production without any nutrient addition and pH adjustment and their ability to survive and multiply in the juice.

The grape juice (100 ml) were separately inoculated using 1% of each of the following three *Lactobacilli* isolates i.e. *L. acidophilus* (*L.a.*), *L. plantarum* (*L.p.*) and *L.casei* (*L.c.*) under aseptic conditions in the flasks. No culture was added in the flasks labeled as control. All the flasks were then incubated at 37°C. 10 ml of juice from each flask was taken periodically (0, 24, 48 and 72 hrs after incubation) for

biochemical testing and was tested for various parameters.

Estimation of sugars in the form of glucose concentration in the grape juice samples was done in GOD/POD Kit-based method (Goldin and Gorbach, 1984).

Change in pH during fermentation of the juice was measured using pH meter (pH600, Milwaukee USA) after proper calibration.

Estimation of Lactic acid concentration (%) was done by using method of Pedrosa *et al.* (1995). A reduction in the sugar concentration was observed in case of all the microbial supplement cultures (Fig.1a). The initial sugar content of grape juice was 197.2 mg/dl, which was reduced to 175, 179 and 184 mg/dl by *L. acidophilus*, *L. plantarum* and *L. casei*, respectively after 72 hr incubation. The original pH 4.7 of the juice was reduced upto 3.5, 3.9 and 4.1 by *L. acidophilus*, *L. plantarum* and *L. casei*, cultures respectively (Fig. 1b). All the cultures survived at pH of 5.5 as well as 4.5 for upto 3hr; but at pH 3.5, only *L.acidophilus* survived for 3hr, while *L.plantarum* and *L.casei* could survive for only 2 hour. At pH 2.5, *L.plantarum* and *L.casei* showed no growth; but *L.acidophilus* was able to tolerate pH 2.5 for upto 1hr. The decrease in the pH was concurrent with the decrease in the sugar content of grape juice. The results of reduced pH conversely resulted in the increased acidity from 0.38 percent at 0hr to 0.45 - 0.58 per cent and after 72 hr of incubation with all the cultures of *Lactobacilli*. The present studies are supported by the earlier findings of Shah *et al.* (2000) who also reported that acid production ability by lactic acid bacteria, especially post-incubation (post-acidification), affected the cell viability of probiotic bacteria including *L. acidophilus* and *Bifidobacterium bifidum*. All the three cultures of *Lactobacilli* isolates were also tested for their ability to survive during lactic acid fermentation of fruit juice (Table 1). The initial cell count in the juice samples for *L.*

acidophilus, *L. plantarum* and *L. casei*, was 2.40×10^7 , 2.41×10^7 and 2.47×10^7 cfu/ml, respectively.

The bacterial cell increased in different cultures with lapse of time and after 72 hrs incubation, counts of *L. acidophilus*, *L. plantarum* and *L. casei* were found to be 2.89×10^7 , 2.84×10^7 and 2.80×10^7 , respectively (Table 1). Two cultures of *L. acidophilus* and *L. plantarum* were not only able to survive but also utilized is and fermented the fruit sugar for their cell synthesis and metabolism. The cell counts for *L. casei* after 72hr incubation indicated that growth of this probiotic was slower than those of *L. acidophilus* and *L. plantarum*.

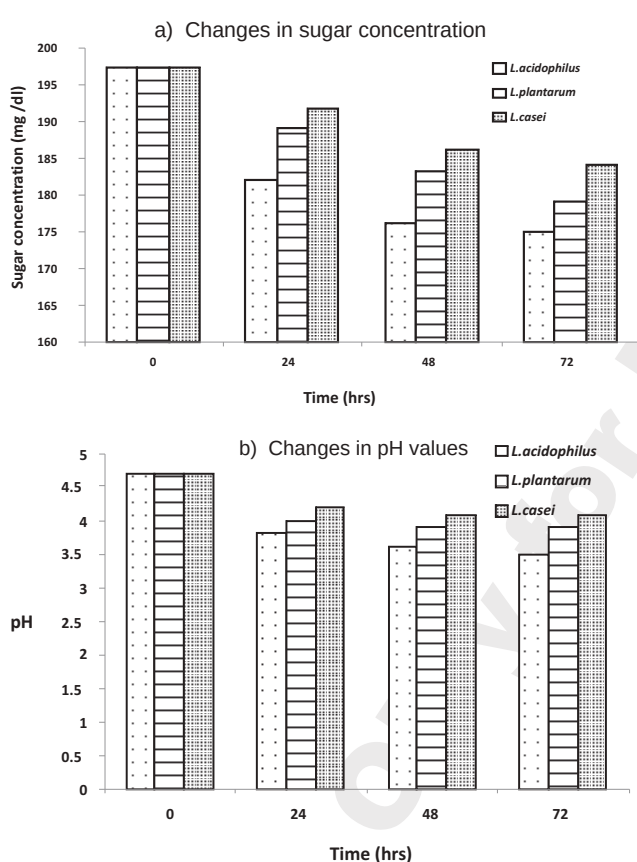


Fig. 1 (a). Changes in sugar concentration (b) pH during fermentation of grape juice (Values are means of three replicates)

Table 1. Mean cell viability of *Lactobacilli* during fermentation of the grape juice

<i>Lactobacilli</i> <i>Isolates</i>	Mean viable cell Counts ($\times 10^7$ cfu/ml) after hr of incubation			
	0 hr	24 hr	48 hr	72 hr
<i>L. acidophilus</i>	2.40	2.80	3.0	2.89
<i>L. plantarum</i>	2.41	2.78	2.96	2.84
<i>L. casei</i>	2.47	2.72	2.90	2.80

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Received 16 September, 2015; Accepted 5 January, 2016



Effect of Rooting Media and IBA on the Rooting Behaviour and Vegetative Growth of Kiwifruit (*Actinidia deliciosa* Chev.)

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Abstract: The rooting characteristics and vegetative growth responded significantly to the interactive effect of different rooting media and cultivars. The rooting medium comprising of forest soil + sand + soil (1:2:1) registered higher rooting (45.71 %) of cuttings in both the cultivars and resulted in highest number of main roots (15.64), secondary roots (11.47) and total root length (183.75cm). The vegetative growth was significantly influenced by various rooting media. Hayward had longest shoot length (7.85cm) in all the media except forest soil + sand + soil (1:2:1) in which the cultivar Abbott resulted in higher shoot length. Leaf number (6.48) was highest in cuttings planted in medium containing sand + soil (2:1), however, leaf area (92.25cm²) in cuttings planted in medium forest soil + sand + soil (1:2:1), which was significantly higher than all other rooting media under study.

Key Words: Abbott, Hayward, IBA, Kiwifruit, Rooting media,

The Chinese gooseberry or kiwifruit (*Actinidia deliciosa* Chev.) is a deciduous and dioecious fruiting vine native to south and central part of China. The availability of quality planting material is the first and foremost priority for commercialization of any fruit crop. To meet out the demand of kiwifruit plants for commercialization, the planting material is required on large scale which has necessitated the need for development of an easier, quicker and economic method of propagation. Although there are various methods of propagation for raising the fruit plants such as grafting, layering, budding and tissue culture, but most of these methods are expensive, time consuming, laborious and require skills. On the other hand, raising of kiwifruit plants by cuttings is quick, less expensive and require less space and skill. The application of growth regulators, rooting media also have profound influence on the ability of rooting of cutting in kiwifruit. Since the application of growth regulators and rooting media have a direct role in rooting and vegetative characters of kiwifruit cuttings, so the present study was carried out to find the combined effect of different rooting media and Indol-3-butyric acid (IBA) on the rooting behaviour and vegetative growth of kiwifruit.

The experiment was conducted during the month of January in the Kiwifruit Block of Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), located at 30°51'N latitude during 2013-14. The experiment was laid out in a randomized block design with two factors viz., cultivars and rooting media having three replications. The experiment comprised of seven treatments viz., T₁ (Sand), T₂ (Soil), T₃ (Sand + Soil, 2:1), T₄ (Sand + Soil + FYM, 2:1:1), T₅ (Banoak forest soil), T₆

(Banoak forest soil + Sand, 1:1) and T₇ (Banoak forest soil + Sand + Soil, 1:2:1). In this experiment, well matured dormant shoots i.e. hardwood cuttings of 25-30 cm length, 0.5-1.0 cm thickness and having atleast 3 healthy buds were selected and cuttings were prepared under shade. These cuttings were planted in the polybags filled with different rooting media prepared in different ratios according to the treatments mentioned above after treating them with recommended dose of IBA solution i.e. 5000 ppm and were kept under open condition. The observations on rooting and vegetative growth of hardwood cuttings planted in January were taken during October month on the various characters viz., rooting percentage, number of main roots, number of secondary roots, total root length, shoot length, number of leaves and total leaf area.

The experimental results revealed that different rooting media and cultivars have significant effect on the rooting of the cutting of the cultivars. The highest rooting (62.0%) were with cuttings planted in rooting medium containing forest soil + sand + soil (1:2:1), which was significantly superior to all other treatments, whereas, rooting was minimum (26.0%) in cuttings planted in medium containing soil only. The cv. Hayward recorded more rooting (45.71%) as compared to cv. Abbott, which had 42.29 per cent rooting (Table 1). When kiwifruit cutting treated with exogenous compounds i.e. IBA (Indole 3-butyric acid) at 5000 ppm resulted to encourage rooting of cutting (Rana and Jindal, 2001; Srivastava *et al.*, 2006). Saralch and Singh (2014) recorded good response of IBA in dek. Isfendiyaroglu *et al.* (2009) reported similar results in which he studied effect of 25 different substrates pure or mixtures and concluded that

sand-perlite (1:2 v/v) led to more than 90 per cent of rooting while pure sand, peat and peat-sand mixtures gave very low (5-28%) rooting percentages of Ayvalik olive cuttings. The highest callusing (23.50%) was recorded in sand medium, followed by the medium soil. Lowest callusing was in the cuttings planted in forest soil + sand + soil. The higher rooting percentage in forest soil has been attributed to the richness of forest soil in organic matter content and optimum moisture holding capacity which, in turn, promote root induction as well as increase the total length of roots (Loach, 1983). The results also showed that the cuttings planted in medium of forest soil + sand + soil resulted in highest number of main (15.64) and secondary roots (11.47) which was followed by forest soil + sand medium (Table 1). There was also significant variation between both the cultivars and highest numbers of main (10.27) and secondary roots (8.29) were in Hayward cultivar. The interaction was found to be significant for secondary roots.

This finding can be attributed to the fact that forest soil is rich source of organic matter and nutrients which provided better conditions and nutrient supply to the cuttings. Also, as indicated earlier (Rana *et al.*, 1999) that sand has good drainage and aeration properties which might have promoted the root induction in these cuttings. It is also evident from the (Table 1) that different rooting media and cultivars had significant effect on the total root length. Highest total root length (183.75cm) was recorded in the cuttings planted in the medium of forest soil + sand + soil. The poor performance of cuttings in the soil alone may be assigned to the fact that soil alone retain very high moisture content and has poor aeration than sand and forest soil, thus adversely affecting the rooting success and root growth of the cuttings.

Vegetative characters: Experimental findings showed that the vegetative growth was significantly influenced by various rooting media in both the cultivars under study. the largest shoot length (7.85 cm) was in the cuttings planted in the medium of forest soil + sand + soil, however, the least shoot length (3.08 cm) was with cuttings grown in soil medium (Table 2). There was also significant interaction between rooting media and cultivars. In general, Hayward had longest shoots in all the media except forest soil + sand + soil, leaf number (6.48) was maximum in cuttings planted in sand + soil (2:1) while, largest leaf area (92.25 cm²) was in cuttings sown in forest soil + sand + soil (1:2:1) which was significantly higher than the remaining media (Table 2). However, the minimum numbers of leaves (3.82) per cuttings and lowest leaf area (82.49 cm²) were in the cuttings planted in sand and soil medium, respectively. The interaction with respect to these characteristics between cultivars and rooting media were significant.

Table 1. Effect of rooting media and IBA on the callusing, rooting, root and shoot length percentage in hardwood cuttings of Kiwifruit

Treatments	Callusing (%)			Rooting (%)			Root length (cm)			Shoot length (cm)		
	Abbott	Hayward	Mean	Abbott	Hayward	Mean	Abbott	Hayward	Mean	Abbott	Hayward	Mean
T ₁ :Sand	25.00	22.00	23.50	32.00	37.00	34.50	152.00	176.80	164.40	3.98	5.33	4.66
T ₂ :Soil	18.00	15.00	16.50	24.00	28.00	26.00	104.00	136.00	120.00	2.12	4.03	3.08
T ₃ :Sand + Soil (2:1)	11.00	10.00	10.50	36.00	40.00	38.00	137.00	157.00	147.00	4.73	6.94	5.84
T ₄ :Sand + Soil + FYM (2:1:1)	13.00	11.00	12.00	42.00	44.00	43.00	160.00	184.80	172.40	7.15	8.23	7.69
T ₅ :Ban oak Forest soil	12.00	9.00	10.50	50.00	53.00	51.50	162.00	170.00	166.00	5.64	6.62	6.13
T ₆ :Ban oak Forest soil + Sand (1:1)	8.00	6.00	7.00	52.00	56.00	54.00	148.00	173.50	160.75	6.24	7.01	6.63
T ₇ :Ban oak Forest soil + Sand + Soil (1:2:1)	5.00	4.00	4.50	60.00	62.00	61.00	168.00	199.50	183.75	8.58	7.12	7.85
Mean	13.14	11.00		42.29	45.71		147.29	171.09		5.49	6.47	

CD (p=0.05)

Cultivars NS 2.18 2.16 0.12

Rooting media 6.35 4.10 2.20 1.27

Rooting media x Cultivar NS NS NS 1.34

Table 2. Effect of rooting media and IBA on the main root, secondary roots, number of leaves and total leaf area in hardwood cuttings of Kiwifruit

Treatments	Main roots			Secondary roots			Number of leaves			Total leaf area (cm ²)		
	Abbott	Hayward	Mean	Abbott	Hayward	Mean	Abbott	Hayward	Mean	Abbott	Hayward	Mean
T ₁ : Sand	5.42	6.22	5.82	3.35	5.08	4.22	4.12	3.52	3.82	84.54	86.40	85.47
T ₂ : Soil	3.44	5.18	4.31	2.18	4.44	3.31	6.35	4.82	5.59	81.98	83.00	82.49
T ₃ : Sand + Soil (2:1)	5.92	7.12	6.52	2.50	6.25	4.38	7.82	5.10	6.48	84.50	86.20	85.35
T ₄ : Sand + Soil + FYM (2:1:1)	12.20	13.58	12.89	9.67	10.08	9.88	5.07	5.22	5.15	86.12	88.50	87.31
T ₅ : Ban oak Forest soil	7.42	9.52	8.47	7.44	8.77	8.11	6.38	5.63	6.01	85.67	87.00	86.34
T ₆ : Ban oak Forest soil + Sand (1:1)	13.50	14.22	13.86	9.78	11.19	10.49	5.66	3.26	4.46	87.00	88.98	87.99
T ₇ : Ban oak Forest soil + Sand + Soil (1:2:1)	15.20	16.08	15.64	10.74	12.19	11.47	3.02	5.52	4.27	91.25	93.24	92.25
Mean	9.01	10.27		6.52	8.29		5.49	4.72		85.87	87.62	

CD (p=0.05)

Cultivars 12.62 0.12 2.16 0.12

Rooting media 12.22 0.21 2.20 1.27

Rooting media x Cultivar NS 0.26 NS 1.34

The reason for more vegetative growth in forest soil + sand + soil may be ascribed to the fact that forest soils generally hold optimum level of moisture, have more organic matter and contain more number of beneficial soil micro-organisms than the common soil. The higher amount of nitrogen due to more organic matter tends to increase the vegetative and succulent growth of the plant.

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Received 11 November, 2015; Accepted 2 February, 2016



Effect of Plant Growth Retardants on Flowering and Presentability of *Primula malacoides* Franch

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Abstract: An experiment was conducted with the objective to ascertain an effective growth retardant dose on flowering and presentability of pot grown *Primula malacoides* Franch. Spray with paclobutrazol (Paclobutrazol – (2RS, 3RS) - 1- (4- Chlorophenyl)- 4,4- dimethyl-2- (1 H- 1, 2, 4- trizol- 1-yl)-pentan-3-ol) (10ppm, 15ppm and 20ppm) and B-nine (N-(dimethylamine) succinamic acid) (500ppm, 1000ppm and 1500ppm) was done after transplanting of 30 days (single spray), 45 and 90 days (double spray) and 30, 60 and 90 days (triple spray). Paclobutrazol when applied as triple spray (5ppm) resulted in increased days to first flowering, duration of flowering, number of inflorescence open per plant, Vase life but decreased the inflorescence stalks length. B-nine treated plants resulted in increase the inflorescence stalk length. However, the most presentable pots were obtained when the plants were sprayed with a triple application of Paclobutrazol as compared to control.

Key Words: B-nine, Flowering, Growth, Paclobutrazol, Pot presentability, Primula

Floriculture has been identified as the fast emerging sector and is now branded as an industry in domestic as well as export market. In the quest for diversification in agriculture production system, floriculture has emerged as a prominent and an attractive sector in view of high returns per unit area. Amongst the traditional floricultural products, apart from cut flowers, pot plants occupy a major share as with ever increasing standard of living the demand for pot plant have increased manifolds. Besides increasing the aesthetic value of the location where they are placed, pot plants also reduce indoor air pollution of harmful gases and microbes (Euisuk *et al.*, 1997 and Wolverton and Wolverton, 1996). Pot plants are generally valued for their foliage and flowering with Primula being an important flowering.

Primula malacoides belonging to the family Primulaceae is a native of China, is a species generally grown in pots as an annual. The compact and dwarf growth habit with profuse flower production of primula makes it desirable for growing in pots/containers. Primula when grown as a pot plant, requires strong, straight stem, dense crown, prolific and early flowering for its presentability. Such form is achieved by pinching, but this is labour intensive activity and also sometimes delays flowering. Therefore, to achieve the desired shape, size and form of the plants use of growth retardants is advocated (Jin *et al.*, 2010). Retardants can delay cell division and elongation in shoot tissues resulting in influential effects. Most of commercially available retardants are primarily used to retard plant height, regulate flowering and stimulate lateral or basal branching. They work by inhibiting the synthesis of gibberellins, although others modes of action may exist (Henry, 1985). Growth retardants

like Cycocel (CCC) and Ancymidol (A-Rest) have been widely used in Primula for many years. However, paclobutrazol, developed triazole, is quite effective in checking growth and manipulating flowering of Primula. Time of application also has a huge impact on the effectiveness of growth retardants on the crop can greatly influence the growth pattern thereby, modifying its presentability.

The present investigation was undertaken at the experimental farm of department of Floriculture and Landscaping, Nauni, Solan, Himachal Pradesh (H P). Seedlings of *Primula malacoides* were planted in shade net house on 1st September, 2012 (one seedling per pot) in plastic pots of 15 cm size. Potting media mixtures of sand: soil : vermicompost in equal ratio. After 30 days of transplanting, plants were treated with single spray of Paclobutrazol (10, 15 & 20 ppm) and B-9 (500, 1000 & 1500 ppm). Two weeks thereafter single and double spray of B-nine and Paclobutrazol were given and subsequent sprays were given at an interval of two weeks thereafter. Before the application of paclobutrazol and B-nine, all pots were irrigated thoroughly. The pots were kept under 75% shade net house to fulfill the light requirement and to maintain the health and presentability of pots, Standard plant protection measures were adopted which include fortnightly drenching with Mancozeb @ 2g l⁻¹ and Carbendazin @ 1g l⁻¹, alternatively.

Preparation of paclobutrazol and B-nine solution: A stock solution of 50ppm and 10,000 ppm was prepared by dissolving 50mg and 10,000 mg of paclobutrazol and B-nine in 1 litre of distilled water but before making solution there is need to dissolve in alcohol otherwise precipitation take place.

Further dilutions were made as follows:

$$\text{Volume to be used} = \frac{\text{Concentration required}}{\text{Concentration given}} \times \text{Volume to be prepared}$$

Therefore, for preparing 10ppm of paclobutrazol = $10/50 \times 1000 = 200\text{ml}$ of stock solution + 800 ml of distilled water. Similarly, 15, 20ppm of paclobutrazol and 500, 1000, 1500 ppm of B-nine solutions was prepared.

Pot presentability: (Score out of 100) Quality of the pot plants was evaluated on the basis of point system modified after Conover (1986). The parameters studied and points allotted to each parameter out of maximum of 100, were as follows:

Parameter	Description	Max Points
Condition	Fresh appearance, no indication of senescence, any mechanical damage, insect, or disease damage on flowers and stems.	20
Number of flowers	Scoring of the pots at the time of peak flowering (Number of flowers per inflorescence open at a time)	
(I)	i) 30 and above	10
	ii) 24 - 30	7
	iii) < 24	3
(II)	Inflorescence stalk length	
	i) Large (> 15 cm)	3
	ii) Medium (10 - 15 cm)	7
	iii) Small (< 10 cm)	10
Form	Plants in balance with pot, neither too large nor too small, generally 1.5 times the height of container.	20
Colour	Flowers / inflorescence with clear colours, no fading.	20
Stem and foliage	Plant self supporting with strong stems, foliage without chlorosis or necrosis.	20

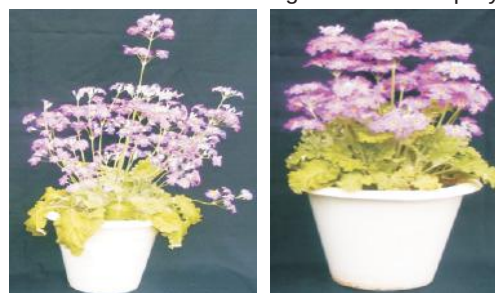
Both the growth retardants delayed flowering in *Primula* and the maximum delayed flowering (115.1 days) was obtained when the plants were sprayed with triple application paclobutrazol (@ 5ppm each), which was at par with the treatments of B-nine. while earliest flowering (104.3 days) was obtained when no growth retardants was applied. The delay in flowering with the use of growth retardants is due to the prolonged vegetative phase and reduced gibberellic acid biosynthesis with the application of paclobutrazol. Similar results have been reported by Farthing and Ellis (1990).

The longest inflorescence stalks were (23.7 cm) in control plants and these were reduced approximately to half (41.44 %) with triple spray of paclobutrazol (@ 5 ppm each). This might be as consequence of reduced GA_3 bio- synthesis with the paclobutrazol application. These findings are in consonance with the results of Pathak and Sharma (1996) who recorded reduction in the length of inflorescence stalk in *Begonia x tuberhybrida* and *Primula obconica*. The

applications of paclobutrazol have proved to be beneficial for induction of more inflorescences and maximum inflorescences open per plant (12.7) were with triple application of paclobutrazol (@ 5ppm each). This may be due to the fact that paclobutrazol produced more leaf area, which might have generated more flowering shoots. Latimer and Baden (1994) also reported similar results in geranium.

The number of inflorescence open per week increased significantly with all the paclobutrazol and B-nine treatments. However, maximum increase (44.72%) over control was with triple application of paclobutrazol (@ 5 ppm each). Growth retardants indirectly influence flowering by retarding vegetative growth in many floricultural crops (Larson, 1985; Stuart, 1962). Bazzocchi *et al.* (2001) also reported higher flower production in *Fuchsia* cv. Beacon when the medium was enriched with paclobutrazol @ 0.5 mg plant⁻¹. The maximum increase in duration of flowering (26.23 %) over control was achieved with triple spray of paclobutrazol (@ 5ppm each). The increased duration of flowering by paclobutrazol may be attributed to more number of flowers produced by paclobutrazol application. Andrade *et al.* (1991) reported that growth retardants cause increase in the duration of flowering. Similar results were obtained in all treatments with paclobutrazol.

In general, the application of paclobutrazol and B-nine enhanced the presentability of potted primula as compared to control. The treated plants were dwarf, compact, and uniform bearing more number of inflorescence which contributed to the increased presentability score. These results are in close agreement with the findings of Pathak and Sharma (1996) in *Begonia x tuberhybrida* and *Primula obconica* and Sullhan *et al.* (2005) in geranium. Thus for popularizing primula as a flowering pot plant; triple spray of paclobutrazol @ 5ppm each at 30, 60 and 90 days of transplanting is effective in producing most presentable pots as compared to control. The effect of any growth retardant is not very persistent and in single spray it lasts only for few months. However, growth retardants in split dosages can control growth more effectively. Thus, the application of triple spray was more effective than single and double spray.



Control

T₆ Paclobutrazol
(triple spray @ 5 ppm each),

Table 1. Effect of Paclobutrazol (PBZ) and B-nine (B-9) on reproductive growth of *Primula malacoides* Franch

Treatment	Days to first Flowering (days)	Length of inflorescence stalk (cm)	Number of inflorescence open per plant	Duration of flowering (days)	Pot presentability (%)
T ₀ (Control)	104.3	23.7	6.8	40.3	77.0
T ₁	106.0	17.2	8.6	52.4	83.0
T ₂	105.7	16.5	8.1	53.6	82.6
T ₃	111.1	17.5	9.0	54.0	82.0
T ₄	105.1	18.4	9.2	52.5	86.1
T ₅	110.5	18.0	11.8	54.2	86.0
T ₆	115.1	13.9	12.7	54.6	88.5
T ₇	105.6	17.8	8.5	52.4	87.1
T ₈	108.9	17.9	9.5	49.6	87.0
T ₉	106.2	17.3	9.1	51.6	86.3
Mean (PBZ)	108.2	17.2	9.6	52.8	85.4
T ₁₀	105.6	18.6	8.5	49.7	80.0
T ₁₁	104.2	19.0	9.4	54.6	81.0
T ₁₂	107.5	17.4	8.2	50.8	82.0
T ₁₃	107.3	17.4	11.5	53.8	81.0
T ₁₄	107.3	18.7	11.8	52.8	79.0
T ₁₅	104.7	21.2	8.4	51.6	81.0
T ₁₆	111.8	17.1	8.1	51.2	80.6
T ₁₇	111.8	18.4	9.6	50.5	79.0
T ₁₈	104.5	17.4	9.5	52.6	82.0
Mean (B-9)	107.0	18.4	9.4	51.9	80.6
CD (p=0.05)					
(Control and Retardants)	3.0	2.5	0.96	4.7	2.9
Treatments	4.1	3.4	1.2	6.4	3.9

T₀: control, T₁: PBZ (single spray @ 10 ppm), T₂: PBZ (double spray @ 5 ppm each), T₃: PBZ (triple spray @ 3, 3 & 4 ppm), T₄: PBZ (single spray @ 15 ppm), T₅: PBZ (double spray @ 7.5 ppm each), T₆: PBZ (triple spray @ 5 ppm each), T₇: PBZ (single spray @ 20 ppm), T₈: PBZ (double spray @ 10 ppm each), T₉: PBZ (triple spray @ 6, 7 & 7 ppm), T₁₀: B-9 (single spray @ 500 ppm), T₁₁: B-9 (double spray @ 250 ppm each), T₁₂: B-9 (triple spray @ 150, 150 & 200 ppm), T₁₃: B-9 (single spray @ 1000 ppm), T₁₄: B-9 (double spray @ 500 ppm each), T₁₅: B-9 (triple spray @ 300, 300 & 400 ppm), T₁₆: B-9 (single spray @ 1500 ppm), T₁₇: B-9 (double spray @ 750 ppm each), T₁₈: B-9 (triple spray @ 500 ppm each)

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Response of W. Murcott Mandarin to Wax Coating under Ambient Conditions

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Abstract: Fully mature, uniform, healthy and disease free fruits of W. Murcott mandarin were individually waxed with different wax formulations and kept at ambient temperature (11.8-15.3°C) and analyzed for physiological loss in weight, organoleptic rating, spoilage, TSS and total acids after 7, 14 and 21 days of storage. Fruits coated with waxes retained their general appearance and taste after storage. The spoilage was also found to be less in wax treated fruits as compared to the uncoated fruits. Citrosol emerged as best treatment with minimum physiological loss in weight, spoilage and maximum organoleptic rating after 14 days of storage followed by the Citrashine wax. Total soluble solids increased up to 14 days of storage interval however acidity decreased with increase in storage interval.

Key Words: Mandarin, Organoleptic rating, Shelf life, Spoilage, Wax, Weight loss

Wax coating is known to reduce chilling injury, rind staining and physiological loss in weight in mandarins, which limit post harvest shelf life and reduce quality of mandarins (Bajwa and Anjum, 2007). Wax coatings also improve the gloss and appearance of Tangerines than control fruits (Seehanam and Boonyakiat, 2010). However, very limited information is available with respect to effect of different waxes on shelf life of W. Murcott mandarin, a new mandarin cultivar of Punjab. Therefore, the present study was conducted with the objective to retain quality and to extend the post-harvest shelf life of W. Murcott mandarin by the use of wax coatings.

The present study on the physico-chemical changes during ambient storage of W Murcott mandarin was undertaken in the Department of Fruit Science, Punjab Agricultural University, Ludhiana in 2014-15. Fully mature W. Murcott fruits having uniform size and disease free were obtained from College Orchard, Punjab Agricultural University, Ludhiana. The bruised, punctured and damaged fruits were sorted out and washed with 0.01% chlorinated water. These fruits were partial shade-dried and fresh fruits were treated with different wax coatings with the help of foam pad. The experiment consisted of three treatments viz. edible

coatings of citrashine, citrosol and uncoated fruits as control along with six replications. The fruits were stored at ambient temperature (11.8-15.3°C) in well ventilated room for 7, 14 and 21 days. The physiological loss in weight (PLW) was determined by subtracting final weight from initial weight of fruits. Spoilage percentage was calculated by counting the fruits that had rotten during the studies. The total soluble solids (TSS) were recorded with hand refractometer and temperature correction applied. The acidity was determined by titrating one ml of juice against 0.1 N sodium hydroxide using *Phenolphthalein* as an indicator. The results were expressed as percentage of anhydrous citric acid. The fruits were rated organoleptically by a panel of judges on 0-10 scale.

The physiological loss in weight was significantly higher in uncoated fruits at all the storage intervals as compared to citrosol and citrashine coated fruits (Table 1). The fruits coated with wax retained their weight up to 14 days of ambient storage, but uncoated fruits were highly shrivelled and 14.10 per cent loss in physiological weight was recorded up to 21 days of storage. After twenty one days of storage, uncoated fruits became unmarketable (PLW> 10%). On an average, Citrosol emerged as best treatment with minimum

Table 1. Effect of different wax coating treatments on physiological loss in weight and Spoilage in W Murcott mandarin in the year 2014-15

Treatments	Physiological loss in weight (%)				Spoilage (%)			
Storage period [days]	7	14	21	Mean	7	14	21	Mean
Citrashine	4.05	4.84	7.67	5.52	0.00	0.00	6.25	2.08
Citrosol	3.94	4.06	5.51	4.50	0.00	0.00	4.16	1.39
Control	4.70	9.50	14.10	9.43	0.00	4.16	18.70	7.62
CD (p=0.05)	0.36	2.56	3.89	2.27	-	2.09	6.85	2.98

Table 2. Effect of different wax coating treatments on Organoleptic rating, TSS and Acidity in W Murcott mandarin in the year 2014-15

Treatments	Organoleptic Rating				TSS Brix				Acidity (%)			
Storage period [days]	7	14	21	Mean	7	14	21	Mean	7	14	21	Mean
Citrashine	8.40	7.60	6.20	7.40	9.40	9.50	9.00	9.30	0.50	0.49	0.46	0.48
Citrosol	8.50	8.00	6.50	7.67	9.30	9.50	9.20	9.33	0.54	0.50	0.48	0.51
Control	7.10	5.40	3.20	5.23	9.00	8.70	8.00	8.57	0.52	0.48	0.43	0.48
CD (p=0.05)	0.68	1.22	1.59	1.16	0.18	0.4	0.56	0.38	0.02	0.01	0.02	0.02

physiological loss in weight (4.5%) followed by Citrashine. The reduction in weight loss was probably the result of the effects of these coatings as a semipermeable barrier against O₂, CO₂, moisture, and solute movement, thereby reducing respiration, water loss, and oxidation rates (Baldwin *et al.*, 1999). Pal *et al.* (1997) made similar observations on the effect of wax emulsions on weight loss in Kinnow mandarin. Chahal *et al.* (2012) also observed reduction in physiological loss in weight by the use of pre harvest chemicals in Kinnow mandares, which helped to enhance the shelf life of fruits

No difference was observed among different treatments for spoilage after 7 days of storage. But after 14 days of storage both the wax treatments significantly reduced spoilage of fruits due to decay as compared to control (Table 1). The average difference in spoilage among different treatments was non-significant even after 21 days of storage, however, spoilage was significantly lower in all the wax treatments as compared to control with minimum in citrosol followed by citrashine. Citrosol wax on an average reduced spoilage by approximately five times as compared to control after 21 days of storage. Wax coating of fruits prevent fresh inoculation during storage periods and contaminations from the rotting fruits, thus total spoilage was reduced.

At the termination of each storage period, the fruits were rated for quality on the scale of 0-10 based on appearance, taste and development of off flavour. Uncoated fruits rapidly loose their taste after harvesting. Considerable reduction in organoleptic rating was observed in uncoated fruits after 14 days of storage as compared to wax coated fruits (Table 2). The organoleptic rating of wax coated fruits was significantly higher as compared to control at all the storage intervals and maximum was observed in citrosol followed by citrashine. After 21 days of storage organoleptic rating of uncoated fruits was significantly lower with maximum rating in citrosol which did not show any significant difference with citrashine wax. The observed superior organoleptic rating may be due to the better retention of quality parameters. These results are in confirmation with the findings of Mahajan and Singh (2014). Untreated fruits were severely shrivelled and also showed unacceptable market appearance.

The TSS of wax coated and uncoated fruits differ significantly. There was non-significant difference in TSS among both the wax treatments at all storage intervals. However, the highest TSS was recorded in citrosol treated fruits after 21 days of storage (Table 2).

The acid content was significantly higher in citrosol treated fruits. The lowest acidity was recorded in control after 14 and 21 days of storage. The decrease in titratable acids during storage may be attributed to utilization of organic acid in pyruvate decarboxylation reaction occurring during the ripening process of fruits (Pool *et al.*, 1972). When the fruits were coated, the lowering of acidity was delayed, which might be due to the effect of coatings in delaying the respiratory and ripening process (Mahajan and Singh, 2014)

The present study clearly indicated that fruits treated with citrosol prior to storage under ambient conditions can retain their post-harvest quality for about two weeks which could help in extending the marketing period.

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Comparative Toxicity and Susceptibility of Commonly used Insecticides against Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* in Punjab

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Abstract: The toxicity of five insecticides viz., cypermethrin, deltamethrin, fenvalerate, triazophos and quinalphos was evaluated against second instar larvae of brinjal shoot and fruit borer, *Leucinodes orbonalis* using the leaf disc dip bioassay technique for the populations collected from three different locations of Punjab. Based on the LC₅₀ values, cypermethrin was most toxic against all the tested populations with LC₅₀ values ranging from 0.002 to 0.008% except against Malerkotla where toxicity of triazophos (0.001%) was more. Quinalphos was least effective against all the tested populations with LC₅₀ values 0.006 to 0.047%.

Key Words: Brinjal, Insecticides, *L. orbonalis*, Susceptibility, Toxicity

Brinjal (*Solanum melongena* L.) is one of the widely used vegetable crops by most of the people and is popular in many countries viz., Central, South and South East Asia, some parts of Africa and Central America (Harish *et al.*, 2011). This crop is regularly and simultaneously attacked by several insect pests like leafhopper (*Amrasca bigutulla bigutulla* Ishida), whitefly (*Bemisia tabaci* Gennadius) and brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee. Brinjal shoot and fruit borer is regarded as one of the most destructive pest attacking brinjal crop right from nursery stage to harvesting and has proved devastating to the crop. The yield losses caused by this pest have been estimated upto 70-92% (Chakraborti and Sarkar, 2011). Larvae of pest bore into tender shoots, make zig zag feeding tunnels in fruits, which are clogged with frass that make fruits unfit for consumption and marketing (Atwal and Dhaliwal, 2007). Several chemical insecticides including different organophosphates, carbamates and synthetic pyrethroids dominate other means of control of this pest in the field. The indiscriminate use of synthetic pesticides has created several problems to the ecosystem resulting in environmental pollution, pest resistance, pest resurgence, residual toxicity, health hazards, etc. (Dhawan *et al.*, 2013; Gill and Garg, 2014). Although the resistance is a natural mechanism for survival, its development has been accelerated in recent years due to excessive dependence upon chemical pesticides. Insecticides resistance in brinjal shoot and fruit borer especially to pyrethroids is now widespread in many brinjal producing countries (Rahman and Rahman, 2009). In view of the reports of field control failure, it is considered appropriate to undertake studies to

assess the toxicity of recommended insecticides against brinjal shoot and fruit borer.

The relative toxicity and susceptibility status of different insecticides against *L. orbonalis* populations collected from three different locations viz., Amritsar, Hoshiarpur and Malerkotla of Punjab were determined by using leaf disc dip technique (Busvine, 1957). These populations were reared on natural diet consisting of brinjal fruits in B.O.D. incubator maintained at $25 \pm 1^\circ\text{C}$ and 65 ± 5 per cent relative humidity. Commercial formulations of test insecticides viz., quinalphos (Ekalux 25 EC), triazophos (Hostathion 40 EC), cypermethrin (Ripcord 10 EC), deltamethrin (Decis 2.8 EC), fenvalerate (Sumicidin 20 EC) were diluted in water to obtain a range of test concentrations. The dilution range of test concentrations of insecticides viz., quinalphos (0.0031-1.6 %), triazophos (0.00078-0.2%), cypermethrin (0.00032-0.16%), deltamethrin (0.00056-0.287%) and fenvalerate (0.00063-0.2%) were prepared. Fresh brinjal leaves were collected from unsprayed plots and dipped into the required concentrations of test insecticide for 30 seconds and then air-dried by hanging. The treated leaves were cut into disc (3.5 cm diameter) and leaf disc was kept in a plastic cups (3.5 cm diameter). Minimum five of 2nd instar larvae of first generation were released at each concentration in three replications. After releasing larvae, open top of plastic cups were covered with muslin for proper aeration. Larvae in control were treated with water only. Data on mortality were recorded after 12 and 24-hr exposure period. A larva was considered dead if it failed to move in co-ordinated manner. The log concentration-mortality regression was estimated by probit analysis using the POLO-

Table 1. Susceptibility levels of three different field strains of brinjal shoot and fruit borer *L.orbonalis* to different insecticides

Population	Insecticide	d.f.	Slope± S.E.	χ^2	LC ₅₀ (%)	Fiducial limits of 95% C. I	Toxicity ratio
Amritsar	Triazophos	6	1.574±0.251	0.9403	0.009	0.006– 0.013	5.22
	Cypermethrin	6	1.500±0.240	0.8907	0.008	0.005– 0.012	5.87
	Deltamethrin	6	1.035±0.176	2.2883	0.010	0.005– 0.018	4.70
	Fenvalerate	6	1.543±0.248	0.7907	0.028	0.018– 0.042	1.67
	Quinalphos	7	1.240±0.195	1.6753	0.047	0.028– 0.075	1.00
Malerkotla	Triazophos	5	1.645 ± 0.300	1.4952	0.001	0.001– 0.002	6.00
	Cypermethrin	7	1.244 ± 0.195	1.6913	0.002	0.001– 0.004	3.00
	Deltamethrin	6	1.515 ± 0.246	0.7471	0.006	0.004– 0.009	1.00
	Fenvalerate	6	1.545 ± 0.248	0.7891	0.004	0.002– 0.005	1.50
	Quinalphos	7	1.189 ± 0.189	2.1265	0.006	0.004– 0.010	1.00
Hoshiarpur	Triazophos	5	1.661 ± 0.299	0.5651	0.003	0.002– 0.004	8.67
	Cypermethrin	6	1.542± 0.243	1.5301	0.002	0.001– 0.003	13.00
	Deltamethrin	4	1.255 ± 0.243	3.8978	0.008	0.004– 0.015	3.25
	Fenvalerate	6	1.543 ± 0.248	0.7907	0.007	0.004– 0.011	3.71
	Quinalphos	6	1.228 ± 0.192	1.3861	0.026	0.014–0.043	1.00

PC. The toxicity ratios were worked out by dividing the highest LC₅₀ value of the insecticide with the LC₅₀ value of the insecticide in question.

The larval mortality of *L.orbonalis* populations collected from Amritsar, Malerkotla and Hoshiarpur areas ranged from 0 to 100 %. The LC₅₀ of triazophos, cypermethrin, deltamethrin, fenvalerate and quinalphos against pest populations from Amritsar area were worked out to be in the range of 0.009-0.047%. The respective toxicity ratios were worked out to be 5.22-1.00. The corresponding LC₅₀ values of triazophos, cypermethrin, deltamethrin, fenvalerate and quinalphos against the pest population of Malerkotla area were observed to be in the range of 0.001-0.006%, whereas the LC₅₀ values against pest population of Hoshiarpur were 0.003-0.026% (Table 1). Based on LC₅₀ values obtained, the order of toxicity of these insecticides against insect pest populations of Amritsar was found to be cypermethrin > triazophos > deltamethrin > fenvalerate > quinalphos, whereas, order of toxicity of insecticides for Malerkotla population was triazophos > cypermethrin > fenvalerate while deltamethrin and quinalphos were at par with each other. However in Hoshiarpur population, the order of toxicity was cypermethrin > triazophos > fenvalerate > deltamethrin > quinalphos (Table 1).

Cypermethrin was found to be most toxic to Amritsar and Hoshiarpur populations of *L.orbonalis* except Malerkotla (0.002) where it ranked after triazophos. The results are in agreement with the findings of some scientists

who have reported the highest toxicity of cypermethrin against *L. orbonalis* under field conditions (Kumar *et al.*, 2012; Tiwari *et al.*, 2011; Deshmukh and Bhamare, 2006). However, Latif *et al.* (2010) reported cypermethrin as less effective than flubendiamide and carbosulfan that were more effective against fourth instar larva of *L.orbonalis*. Triazophos ranked as second highly toxic compound, whereas in Malerkotla toxicity of triazophos was more than cypermethrin. Deltamethrin was more toxic than fenvalerate against Amritsar population, whereas, the difference between the LC₅₀ values of Hoshiarpur and Malerkotla populations are not much apparent. The population from Amritsar was least susceptible against fenvalerate but comparative susceptibility was observed against populations of Malerkotla and Hoshiarpur. Quinalphos was the least effective against *L. orbonalis* populations collected from Amritsar and Hoshiarpur with LC₅₀ values 0.047 and 0.026%, respectively but Malerkotla population showed susceptibility against quinalphos with LC₅₀ value of 0.006 and also at par with deltamethrin.

This study has shown that among the recommended insecticides, cypermethrin with minimum LC₅₀ values (0.002-0.008%) holds promise to control this pest in Punjab. Quinalphos with LC₅₀ values 0.047 and 0.026% was the least effective against populations collected from Amritsar and Hoshiarpur, respectively, whereas Malerkotla population showed susceptibility against quinalphos with LC₅₀ value of 0.006%.

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Received 21 August, 2015; Accepted 26 October, 2015



Efficacy of Tyre Bait Station for Rodenticide Application in Rice Crop Fields during Monsoon Season

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Abstract: The zinc phosphide bait consumption was more on paper bait stations as compared to tyre bait stations but the control success was found to be more with tyre bait stations (85.94-87.93%) as compared to paper bait stations (65.59-77.90%). The cut tillers ($0.70 \pm 0.06\%$) and yield loss ($98.27 \pm 11.08 \text{ kg ha}^{-1}$) and total post-harvest burrow count (25.85 ± 6.67) were found lowest in fields treated with zinc phosphide kept in tyre bait stations.

Key Words: Bait stations, Rice, Rodents, Rodenticide, Zinc phosphide

In paddy cultivation, besides insect pests and diseases, attack by rodents also causes significant yield losses (Srinivasa Rao and Nanda Kishore, 2010). The rodents attack rice plants throughout their growth periods *i.e.* from seedling to harvesting and the attack intensifies during maximum tillering (Buckle *et al.*, 1985). In India, on an average, rodents have been estimated to cause 0.44 to 60% tiller damage, which accounts for 5-10 % of the total grain yield loss in pre-harvested rice (Singla and Babbar, 2010; Borah and Bora, 2012; Gogoi and Borah, 2013). The irregular rodent outbreaks are sometimes responsible for extreme crop losses of 30-100%, occasionally leading to localized or widespread famine (Douangboupchal *et al.*, 2003).

A number of rodent control practices are available but the use of rodenticides is the most common and expedient method (Singla *et al.*, 2012). Farmers often leave the poisonous bait material on field bunds in a paper or leaf and such improper application of rodenticides results in under control of the rodent pest species. Bait stations are the containers, in which baits are placed safely for effective delivery to the target rodent species as these devices prevent accidental poisoning among non-target species and also protect the baits in rainy season. Studies on such bait stations were also carried out earlier to control rodent pest of agriculture (Srinivasa Rao and Nanda Kishore, 2013; Sakthivel *et al.*, 2013). The present study was carried out to compare the efficiency of paper bait and tyre bait stations for better delivery of poison baits for control of rodents in rice crop fields.

The experiment was conducted in 4.8 ha area of rice fields at Ladhawal University Seed farm, Ludhiana during August to October, 2014 at $30^{\circ}58' \text{ N}$ latitude and $75^{\circ}47' \text{ E}$ longitude. The area was divided into four blocks: I,

II, III and IV with each block consisting of three replications of 0.4 ha area. Paper bait stations were kept in blocks I and II and tyre bait stations in blocks III and IV.

Prior to treatment of blocks, pre-census was carried out in each field of all blocks for two days by placing 1 kg ha^{-1} of plain bait. The bait consisted of a loose mixture of cracked wheat, powdered sugar and vegetable oil in 96: 2: 2. The bait consumption was recorded to determine the pre-treatment consumption pattern. After taking pre-census, blocks I and III were treated with 2% zinc phosphide bait @ 1 kg/ha and blocks II and IV were kept as untreated control. Paper and tyre bait stations (made from pieces of discarded scooter tyre) were installed @ 50 ha^{-1} with 20g of zinc phosphide bait in each station. Remaining bait was collected after two days, brought to laboratory and weighed to determine the acceptance of poison bait. After 15 days of treatment, in fields of all the blocks, post-census was carried out again for two days by placing 1 kg ha^{-1} of plain bait. The bait consumption was recorded to determine the post-treatment consumption pattern and control success.

Pre-harvest rodent damage: A survey of rodent damage to rice crop at pre-harvest was conducted from all the fields. From each field, five samples of 1 m^2 were taken covering all the four geographical sides and the centre of the field. From each sample, total number of healthy as well as cut tillers were counted to determine rodent damage. For calculating yield loss due to rodents, ten tillers of rice were taken from each field at random and their grain yield per tiller (g) was calculated. Yield loss (g/m^2) was determined by multiplying cut tillers/ m^2 by yield/tiller. The data was extrapolated to calculate yield loss in kg/ha .

Post harvest burrow count: Survey of rodent burrows was conducted after harvesting of rice crop in the fields of all the blocks. Burrows of different rodent species were identified on

the basis of characteristic burrow entrances (Dubey, 2001; Neelanarayanan, 2004). The data was interpreted as mean $\pm$ S.E of three replications and subjected to paired Student's t-test and one way ANOVA to draw the conclusions.

Poison bait acceptance and control success: The 2% zinc phosphide bait consumption was non-significantly more on paper bait stations ($81.58 \pm 8.04\%$) as compared to tyre bait stations ($59.50 \pm 7.53\%$) (Table 1). However, in contrast to this, Kaur and Parshad (2001) reported that baits were accepted more from ice-cream cones, scooter tyre pieces and wooden traps in comparison to commonly used paper pieces. Also, Sakthivel *et al.* (2013) reported that bait consumption was poor and rats showed neo-phobia when baits were offered openly. On the other hand, control success was found more with tyre bait stations (85.94-87.93%) as compared to paper bait stations (65.59-77.90%), which indicates the efficacy of these bait stations for better rodent control in rice crop. According to Srinivasa Rao and Nanda Kishore (2013) open baiting had many disadvantages like; complete exposure of poisonous bait to non-target organisms in the field, no protection from rain and other weathering and in the present studies high consumption of bait on paper bait stations might be due to consumption of bait by the non-target organisms and the high control success in fields with tyre bait stations was correlated with efficient delivery of poison bait to control the target rodent pests.

Pre-harvest rodent damage and post- harvest burrow count: Rodent fauna in rice crop fields in present study

consisted of lesser bandicoot rat, *Bandicota bengalensis* followed by field mouse, *Mus* spp, soft furred field rat, *Millardia meltada* and Indian gerbil, *Tatera indica*. The burrow of *B. bengalensis* consisted of loose heap of fresh soil at the entrance of burrow. Singla and Babbar (2010) also reported similar rodent fauna in wheat and rice crop fields of Punjab state. Rodent damage was found to be in the form of a characteristic slant cut on the tiller. Rodents hoarded the cut tillers towards their burrows. The pre-harvest rodent damage and post-harvest burrow count was significantly much lower in treated blocks I and III as compared to control blocks II and IV due to rodent population control by poison baiting in treated blocks (Tables 2). The per cent cut tillers (0.70 ± 0.06), yield loss ($98.27 \pm 11.08 \text{ kg ha}^{-1}$) and total post-harvest burrow count (25.85 ± 6.67) were found to be lowest in block III which was treated with 2% zinc phosphide using tyre bait stations, thus highlighting the efficacy of tyre bait stations for rodent control in rice crop. The post-harvest burrow count of different rodent species significantly varied among the treated and untreated blocks. The post-harvest burrow count of *B. bengalensis* and *Mus* spp. in block III treated using tyre bait station was found to be significantly low than that of control block IV. Earlier, Borah and Bora (2012) reported the role and economic importance of various indigenous bait stations over the open baiting in effective delivery of poison baits for successful rodent control.

Although the bait consumption was found to be more on paper bait stations but more control success and

Table 1. Relative consumption of zinc phosphide bait and percent control success in fields with different bait stations

Block	Bait station	Treatment	Pre-census bait consumption (%)	Poison bait consumption (%)	Post-census bait consumption (%)	Control success (%)	
						w.r.t. same field	w. r. t. control field
I	Paper	Treated	79.75 ± 11.0	81.58 ± 8.04	16.67 ± 6.92	77.90 ± 10.09	65.59 ± 15.72
II	Paper	Control	96.0 ± 0.61	-	55.83 ± 6.01	-	-
III	Tyre	Treated	64.5 ± 1.10	59.50 ± 7.53	9.17 ± 4.30	85.94 ± 6.42	87.93 ± 5.47
IV	Tyre	Control	57.0 ± 5.28	-	65.5 ± 3.20	-	-

Values are mean $\pm$ S.E.

Table 2. Pre-harvest rodent damage and post-harvest burrow count in different blocks

Block	Bait	Treatment	Pre-harvest rodent damage		Post-harvest burrow count/ha				
			Percent cut tillers	Yield loss (kg ha^{-1})	<i>Bb</i>	<i>M</i> spp.	<i>Mm</i>	<i>Ti</i>	Total
I	Paper	Treated	0.81 ^a	105.87 ^a	15.00 ^a	4.18 ^a	4.18 ^a	5.82 ^a	29.18 ^a
II	Paper	Control	2.06 ^b	252.47 ^b	65.82 ^b	25.00 ^a	11.67 ^a	3.33 ^a	105.83 ^b
III	Tyre	Treated	0.70 ^a	98.27 ^a	19.17 ^a	6.67 ^a	0.00 ^a	0.00 ^a	25.85 ^a
IV	Tyre	Control	2.51 ^b	342.53 ^b	58.33 ^b	20.00 ^b	14.17 ^a	2.50 ^a	95.00 ^b

Values with different superscripts a-b in a column differ significantly at $P \leq 0.05$.

Bb- *Bandicota bengalensis*, *M* spp.- *Mus* spp., *Mm*-*Millardia meltada*, *Ti*- *Tatera indica*

minimum cut tillers, yield loss and post-harvest burrow count was obtained when zinc phosphide bait was applied using tyre bait stations which indicates the role of these devices in better delivery of rodenticide bait to target rodent species in rice fields.

ACKNOWLEDGEMENT

We wish to thank Indian Council of Agricultural Research, New Delhi, India for financial support and Department of Zoology, Punjab Agricultural University, Ludhiana, India for providing necessary facilities for undertaking research work.

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Monitoring of Mosquito Larvae from Temporary Water Collections of Ludhiana, Punjab

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Abstract: Water samples collected from different temporary water collections at Ludhiana district of Punjab revealed the mosquito larval density (LD/300ml) ranging from 9.67 ± 1.34 to 39.83 ± 19.93 . Three types of mosquito larvae observed during the present study showed the habitat preference as *Culex* and *Anopheles* were found to be present in the water samples collected from paddy fields, road side ditches and gardens, while *Aedes* were found in the desert coolers and earthen pots.

Key Words: Larval population, Mosquito larvae, Temporary water collections

Different standing water bodies like ponds, swamps, marshes, paddy fields and man-made reservoirs act as breeding grounds of mosquitoes. Different genera of mosquitoes show specific habitat and breeding preference i.e., *Anopheles* species are associated with fresh water habitats, whereas, *Culex* species may also be found in polluted conditions including septic tanks and *Aedes* species breed in peridomestic and other small water collections including desert coolers (Parthiban and David, 2007). Evaluation of larval mosquito habitats in terms of their species composition and population level is of paramount importance for their control. Therefore, the present study was planned to determine the mosquito type and population count of larvae in various temporary water collections under local environmental conditions.

Water samples were collected from 212 sites like road side ditches, gardens, desert coolers and earthen pots of urban areas of Ludhiana and paddy fields adjoining to the city from June to November 2013. These samples were brought to the laboratory for the identification and counting of total mosquito larval density. Fourth instar larvae (preserved in 5% formaldehyde) were identified up to generic level on the basis of morphological features observed under microscope by following the standard keys (Becker *et al.*, 2010). Total mosquito larval density (LD) i.e. number of mosquito larvae/300ml of water sample were counted by manual picking of each larva with the help of dropper. Number of specific type of mosquito larvae were counted from the water samples and their relative abundance in terms of larval density index (LDI %) was calculated by using the formula:

$$\text{LDI (\%)} = \frac{\text{Number of larvae of one specific type}}{\text{Total number of larvae}} \times 100$$

The presence of mosquito larvae in terms of larval positivity was found to be 67.86, 61.76, 62.96, 62.50 and

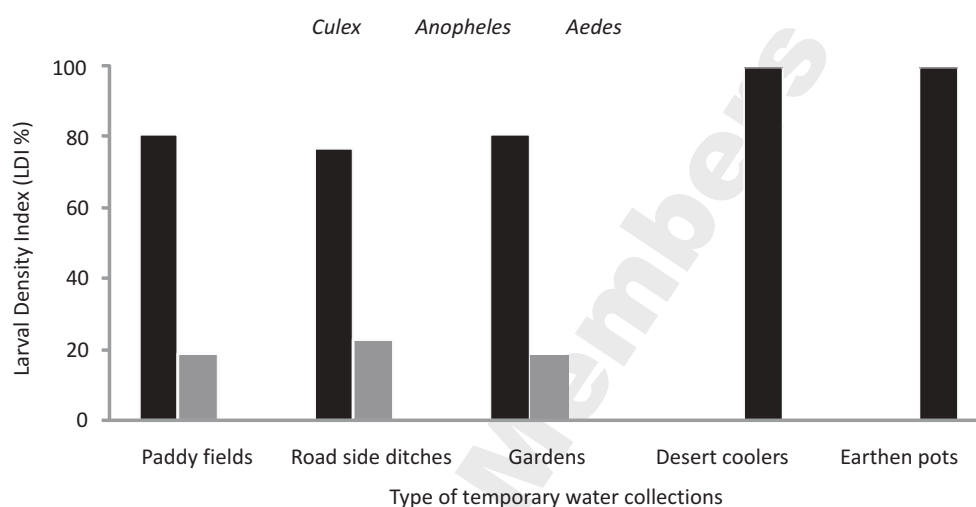
71.42 per cent from the paddy fields, road side ditches, gardens, desert coolers and earthen pots respectively (Table 1). Average larval density (LD/300ml) was highest i.e., 39.83 in water samples collected from road side ditches followed by paddy fields. The high population count of mosquito larvae in road side ditches and paddy fields may be due to the fact that these habitats possess a wide range of salinity, dissolved organic and inorganic matter, air, water temperature and sunlight (Muturi *et al.*, 2008). Vegetation in paddy fields also provides optimal breeding conditions, as it creates stagnant water by decreasing water flow. In other water collections, average larval density/300ml was found to be lower than paddy.

Out of the three types of mosquitoes larvae i.e., *Culex*, *Anopheles* and *Aedes* found in various selected water collections, *Culex* and *Anopheles* were observed in the water samples collected from paddy fields, road side ditches and gardens. *Culex* was predominant type with larval density index of 80.89, 77.01 and 81.33 per cent while *Anopheles* showed LDI 19.11, 22.99 and 18.67 per cent, respectively in paddy fields, road side ditches and gardens (Fig. 1). This is in accordance with the earlier studies of Kirti and Kaur (2013) who described *Culex* as very common type of mosquito found abundantly in Punjab and prevalent throughout the year. *Culex* can tolerate various degrees of water pollution, salinity level and temperature with low level of dissolved oxygen in breeding sites (Ibrahim *et al.*, 2011). *Culex* has been known to thrive in habitats with higher densities of other aquatic invertebrates and is more tolerant to interspecific association among invertebrates as compared to any other type of mosquito larvae, which make this group most abundant at various sampling sites (Muturi *et al.*, 2008). Even stagnant polluted water bodies are a favoured breeding habitat of *Culex* (WHO, 1996). In nature, *Anopheles* larvae

Table 1. Population count of mosquito larvae in samples collected from temporary water collections

Temporary water collections	Number of sites surveyed	Larval positivity (%)	Average larval density (LD/300ml)*
Paddy fields	56	67.86	31.58±3.67
Road side ditches	68	61.76	39.83±19.93
Gardens	27	62.96	9.67±1.34
Desert coolers	40	62.50	19.43±0.50
Earthen pots	21	71.42	28.42±0.71

*LD Value is Mean ±S.E.

**Fig. 1.** Larval density index (LDI %) of mosquitoes in samples collected from temporary water collections

inhabit clean or semi-polluted water with their less count throughout the year (Adeleke *et al.*, 2008), this can be the reason for the lower population count of *Anopheles* larvae observed from the selected sites during the present study. *Aedes* mosquito larvae were found only in the water samples collected from desert coolers and earthen pots because it is a freshwater type mosquito, which prefers egg laying in artificial containers (Parthiban and David, 2007).

The monitoring carried out under the present study indicated that mosquitoes show habitat preference as *Culex* and *Anopheles* were found in paddy fields, road side ditches and gardens. However, *Aedes* preferred the desert coolers and earthen pots as their habitat.

ACKNOWLEDGEMENT

Authors are thankful to Department of Science and Technology, Ministry of Science, New Delhi for providing the financial assistance to carry out this research work and to Head, Department of Zoology, Punjab Agricultural University, Ludhiana for providing other facilities.

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Occurrence and Morphometric Variations of Lesion Nematodes (*Pratylenchus* Species) Infesting Ginger (*Zingiber officinale* Rosc.) in Major Cultivation Belts of Himachal Pradesh

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Abstract: *Pratylenchus coffeae* species was recorded from most of the surveyed localities. The frequency of occurrence of this species varied from as low as 20% in Nohradhar to as high as 88.8% in Naineti, both located in district Sirmour. In Solan, the nematode varied from 37.5 to 80%. The average maximum population of this species was recorded in Sirmour (67.12%) and minimum in Solan (38.09%). Besides, other plant parasitic nematodes associated with ginger in the area under survey were *Meloidogyne incognita*, *Tylenchorhynchus mashoodi*, *Helicotylenchus dihystra* and *Paratylenchus curvatus*. Out of these, the most abundant was *Pratylenchus coffeae* with 78% frequency of occurrence followed by *Tylenchorhynchus mashoodi* (58%), *Helicotylenchus dihystra* (47%) *Meloidogyne incognita* (33%) and *Paratylenchus curvatus* (20%) in order of their frequency of occurrence. Morphometric studies revealed that the most reliable diagnostic characters for identification were number of head annules, shape of spermatheca, structure of lateral field and shape of female tail.

Key Words: Ginger, *Helicotylenchus dihystra*, *Pratylenchus coffeae*, *Meloidogyne incognita*, *Paratylenchus curvatus*, *Tylenchorhynchus mashoodi*

Ginger (*Zingiber officinale* Rosc.) is an important commercial crop belonging to the family Zingiberaceae grown for its aromatic rhizomes which are used both as spice and medicine. In India, ginger is grown in an area of 149.1 thousand hectare with a production of 702.0 thousand metric tonnes contributing about 50% of the world production with productivity of 4.7 metric tonnes per hectare (Anonymous, 2011). Himachal Pradesh is one of the ginger producing states of the country where it is grown on 2040 hectare of land in low and mid hills. The nematodes like *Meloidogyne incognita*, *Pratylenchus zeae* and *P. coffeae* were widely distributed in ginger growing tracts in mid-hill regions of Himachal (Makhnotra and Khan, 1997) and are known to cause 11-80 per cent yield losses (Thakur and Sharma, 2015). It was thus thought pertinent to study the occurrence of plant parasitic nematodes in ginger and identify the major nematode parasite infecting ginger in the state so that appropriate management practices could be adopted.

A systematic survey of ginger growing tracts of Solan and Sirmour, districts in Himachal Pradesh was conducted to find out the prevalent nematode genera associated with the crop. The frequency of occurrence of the important genera was worked out.

The nematode recorded from most of the areas under survey with highest frequency of occurrence was selected as test nematode for identification and morphometric variations. For morphometric analysis, 10-20 specimens of mature female and 5-10 male specimens (if present) from different localities were subjected to detailed

morphology and morphometric. Characters (body length, a value, b value, b' value, c value, stylet length and position of vulva was recorded for female specimens). Spicule length and gubernaculum length was recorded for males. Based upon CV values, the characters were rated as stable, variable and highly variable.

Identification of *Pratylenchus* species was done by using the polytomous/dichotomous key provided by Loof (1990). *Pratylenchus* species was present in 67 out of 115 samples examined (58.26%). From all the locations surveyed for the prevalence of *Pratylenchus* species, *P. coffeae* was most prevalent (Fig.1). Identification keys for the species of the genus *Pratylenchus* have been published by Handoo and Golden (1989), Loof (1991), Siddiqi (2000), Ryss (2002) and Castillo and Vovlas (2007).

Number of head annules: All specimens of *P. coffeae* populations from both districts examined have two lip annules. Román and Hirschmann (1969) also observed that in *Pratylenchus* species characterized by the presence of two head annules, such as *P. coffeae*, individuals were sometimes found with three annules on one side and two annules on the other side. Corbett and Clark (1983) remarked that in the genus *Pratylenchus* the number of head annules varied between species and somewhat within species but that the variability was never so large as to invalidate the number of head annules as a reliable diagnostic character. Therefore, the number of head annules was included as a diagnostic morphological character in the multi-entry keys of Ryss (2002).

Table 1. Occurrence of *Pratylenchus* species associated with ginger in Solan and Sirmour district

Solan			Sirmour		
Localities**	No. of samples	Nematode population/200cc of soil	Localities**	No. of samples	Nematode population/200cc of soil
Pandah (1200 m)	07	34-180 (57.14)*	Narag (1552 m).	08	32-45 (32.1)
Chail (2250 m)	05	45-165 (80)	Daron-Deveria (1350 m)	05	38-240 (75)*
Dharja (1300 m)	08	14-38 (37.5)	Doon-Deria (1340 m)	06	32-63 (40)
Nauni (1275 m)	05	33-62 (40)	Bhuira (1520 m)	06	16-75 (33.33)
Deothi (1502 m)	07	18-53 (42.85)	Shillai (1900 m)	07	15-32 (66.66)
Berthi (1500 m)	05	0 (0)	Jajjar (1500 m)	10	146-500 (42.85)
Kudhar (1440 m)	05	0 (0)	Nainatikka (1435 m)	05	132-600 (90)
			Nohradhar (1520 m)	05	26-58 (40)
			Nai-neti (1550 m)	09	0-29 (20)
			Sarahan (1552 m)	07	450-6000 (88.88)
			Lanacheta (1530 m)	05	95-835 (57.14)
Total	42			73	28-97 (80)

*Figures in parenthesis denote the per cent frequency of occurrence calculated on per cent basis

**Altitude above main sea level in parentheses

Shape of spermatheca: In *P. coffeae* populations from Sirmour and Solan the shape of the spermatheca varied from small rounded to large oval, usually filled with sperms.

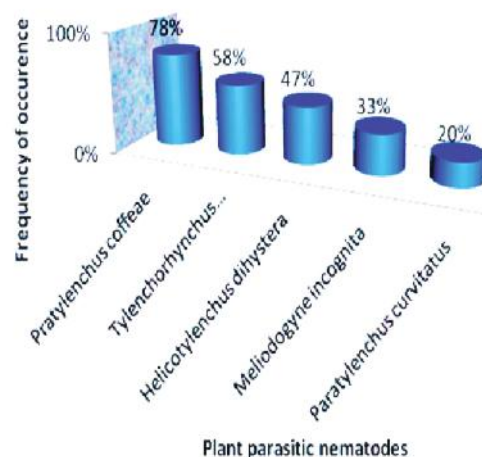
Shape of female tail and tail tip: In some *Pratylenchus* species, including *P. coffeae*, the intraspecific variability in tail and, especially, in tail tip is very high (Frederick and Tarjan, 1989; Corbett and Clark, 1983; Bajaj and Bhatti, 1984). The present study revealed that, *P. coffeae* populations have smooth terminus, truncated and obliquely truncated tail shape which is based on a combination of the tail tip and tail tip shape follows the descriptions by above authors.

Based on the coefficient of variation (CV), body length in the Sirmour populations was important variable morphometrical character while in Solan populations this was stable morphometrical character (Table 1 and Table 2). The stylet length was also variable morphometrical character in both populations. Male are similar to female in general morphology. The best morphometrical male characters for the separation of the populations are body length, ratio and distance from the anterior end to the excretory pore.

In the last few years, production of ginger in the state has declined due to various biotic and abiotic factors, among those, the role of nematodes in general is considered to be very important as they are known to cause 11-80% yield loss to this crop. The population of *Pratylenchus coffeae* was found in all the localities of Sirmour district to the maximum (67.12%) and minimum in Solan (38.09%).

Table 2. Morphometric variations in *Pratylenchus coffeae* (n = 73 ♀♀) from Sirmour and (n = 19 ♀♀) from Solan district

Measurements	Mean±Sd (Sirmour)	Mean±Sd (Solan)	C.V.%
L (mm)	605.32±65.9	607.21±46.8	10.88
a (µm)	28.25±4.59	28.20±3.04	16.28
b (µm)	5.98±0.94	5.17±0.60	15.87
c (µm)	21.21±3.7	19.77±3.39	17.44
V(%)	77.95±2.51	75.47±1.34	3.22
Stylet (µm)	17.62±2.26	19.67±3.11	12.82
Tail (µm)	33.55±4.32	31.5±2.8	12.87

Frequency of occurrence of plant parasitic nematodes**Fig.1.** Occurrence of plant parasitic nematodes

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Received 21 November, 2015; Accepted 14 April, 2016



Esterase Polymorphism for Testing Hybridity in Okra [*Abelmoschus esculentus* (L.) Moench]

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Abstract: Nine hybrids of okra were subjected to hybridity test using esterase, which showed totally ten isozyme bands ranging from 0.099 to 0.596 relative mobility (Rm). Hybridity of $H_2(L_2 \times T_4)$, $H_4(L_4 \times T_4)$ and $H_6(L_6 \times T_4)$ hybrids were confirmed by esterase. H_6 is being a genic male sterility (GMS) based hybrid its proven hybridity using esterase is having great significance in determining the hybridity of GMS based hybrid seed lots.

Key Words: *Abelmoschus esculentus*, Esterase, Genic male sterility, Grow out test, Hybridity, Isozyme markers

India ranks first in okra production (73% of world's production) with productivity of 12 Metric tons ha^{-1} (Anon., 2013). Marked heterosis found in okra for yield and its components has lead to the development of several hybrids in okra. Genetically Controlled male sterility is an important genetic mechanism for hybrid seed production in several crops (Kaul, 1998). In okra, Genic Male Sterility (GMS) has been induced by gamma radiation and GMS system has made hybrid seed production more easy, economical and commercially feasible (Pitchaimuthu *et al.*, 2012). But there is an additional risk of obtaining sibs due to improper rouging of male fertile plants occurring as a result of segregation in GMS line. Therefore, identifying breeding lines and determining hybrid purity will be the major requirements in hybrid seed production of okra. Therefore, present investigation was carried out to assess the potential of esterase (EST) in okra for confirming the F_1 hybridity.

In the present study, nine hybrids viz., $H_1(L_1 \times T_4)$, $H_2(L_2 \times T_4)$, $H_3(L_3 \times T_4)$, $H_4(L_4 \times T_4)$, $H_5(L_5 \times T_4)$, $H_6(L_6 \times T_4)$, $H_7(L_7 \times T_4)$, $H_8(L_1 \times T_5)$ and $H_9(L_2 \times T_5)$ were analyzed for electrophoretic banding pattern of esterase isozyme. Seeds of hybrids and parental lines showing radical emergence were selected for isozyme extraction and grounded thoroughly with liquid nitrogen by adding a pinch of PVP, 400 μ l of extraction buffer, 40 μ l of Tris, and 20 μ l of EDTA and extract was incubated at 4°C for two hours. Later transferred to -20°C over night. Then samples were centrifuged at 12,000 rpm for 10 minutes at 4°C. Supernatant was collected and 10 μ l of loading dye was added. The prepared samples were loaded on to PAGE gel @ 50 μ l/well for electrophoresis. A current of 1.5 mA per well with a voltage of 200 volts was maintained till tracking dye reached the

bottom of the resolving gel. Staining was done with specific substrate to identify isozyme banding pattern. The zymogram (Fig. 1) of the gel was prepared by measuring the distance travelled by each band from the point of loading. Then Rm value was calculated as given below:

$$Rm = \frac{\text{Distance travelled protein/ isozyme}}{\text{Distance travelled by the tracking dye}}$$

Bands were numbered on the basis of increasing Rm values, the presence or absence of specific bands or combination of different bands.

The esterase profile (Plate 1) showed totally ten isozyme bands with the relative mobility (Rm) values ranging from 0.099 to 0.596 (Table 1). Esterase was able to confirm the hybridity of three hybrids viz., $H_2(L_2 \times T_4)$, $H_4(L_4 \times T_4)$ and $H_6(L_6 \times T_4)$. All these three hybrids could be distinguished by observing extra band at Rm value of 0.298 (band no. 4). Band no. 4 was transferred from male parent T_4 and absent in

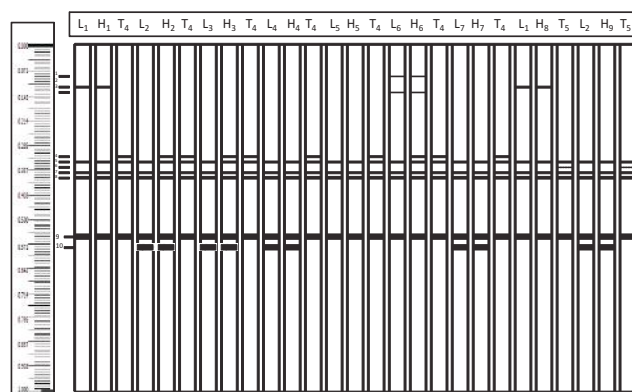


Fig. 1. Zymogram of esterase profile

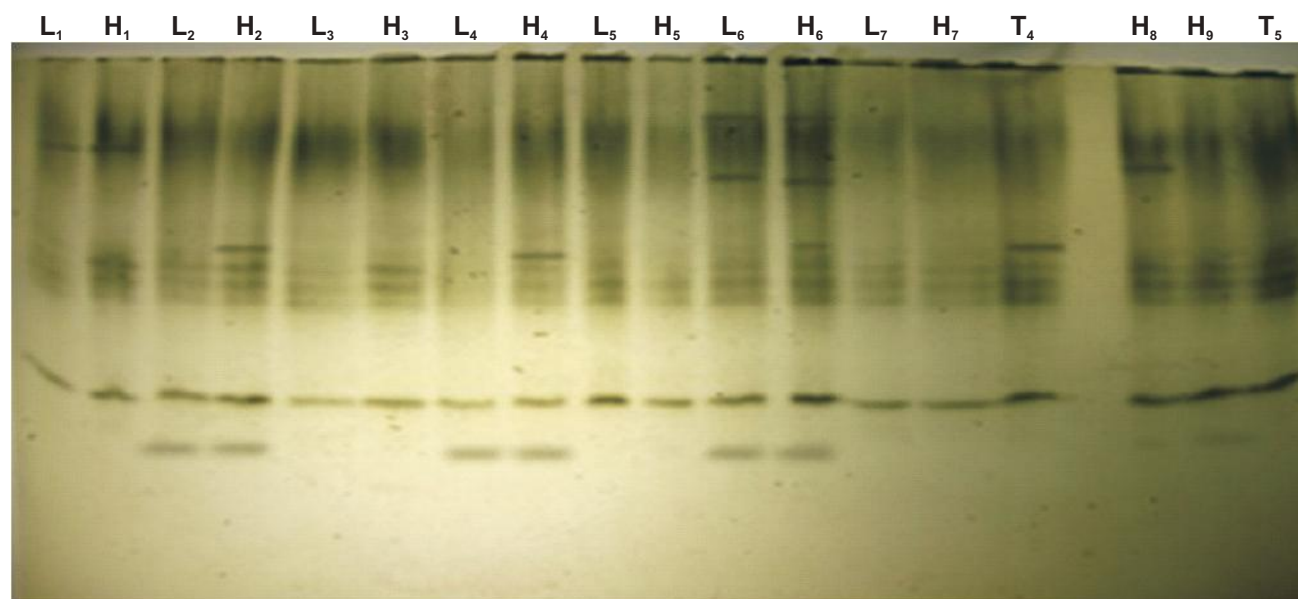
Table 1. Relative mobility of Esterase (EST) isozyme of okra hybrids and their parental lines

Band No.	Rm value	L ₁ (IIHR-285)	H ₁	T ₄ (JNDO-5)	L ₂ (IIHR-291)	H ₂	T ₄ (JNDO-5)	L ₃ (IIHR-294)	H ₃	T ₄ (JNDO-5)
1	0.099	--	--	--	--	--	--	--	--	--
2	0.127	++	++	--	--	--	--	--	--	--
3	0.138	--	--	--	--	--	--	--	--	--
4	0.298	--	--	**	--	**	**	--	--	**
5	0.337	++	++	++	++	++	++	++	++	++
6	0.348	--	--	--	--	--	--	--	--	--
7	0.37	++	++	++	++	++	++	++	++	++
8	0.403	++	++	++	++	++	++	++	++	++
9	0.552	++	++	++	++	++	++	++	++	++
10	0.596	--	--	--	++	++	--	++	++	--
Band No.	Rm value	L ₄ (IIHR-296)	H ₄	T ₄ (JNDO-5)	L ₅ (IIHR-299)	H ₅	T ₄ (JNDO-5)	L ₆ (GMS-1)	H ₆	T ₄ (JNDO-5)
1	0.099	--	--	--	--	--	--	++	++	--
2	0.127	--	--	--	--	--	--	--	--	--
3	0.138	--	--	--	--	--	--	++	++	--
4	0.298	--	--	++	--	--	**	--	**	**
5	0.337	++	**	**	++	++	++	++	++	++
6	0.348	--	--	--	--	--	--	--	--	--
7	0.37	++	++	++	++	++	++	++	++	++
8	0.403	++	++	++	++	++	++	++	++	++
9	0.552	++	++	++	++	++	++	++	++	++
10	0.596	++	++	--	--	--	--	--	--	--
Band No.	Rm value	L ₇ (GMS-4)	H ₇	T ₄ (JNDO-5)	L ₁ (IIHR-285)	H ₈	T ₅ (V.Uphar)	L ₂ (IIHR-291)	H ₉	T ₅ (V. Uphar)
1	0.099	--	--	--	--	--	--	--	--	--
2	0.127	--	--	--	++	++	--	--	--	--
3	0.138	--	--	--	--	--	--	--	--	--
4	0.298	--	--	**	--	--	--	--	--	--
5	0.337	++	++	++	++	++	++	++	++	++
6	0.348	--	--	--	--	++	**	--	++	**
7	0.37	++	++	++	++	++	++	++	++	++
8	0.403	++	++	++	++	++	++	++	++	++
9	0.552	++	++	++	++	++	++	++	++	++
10	0.596	++	++	--	--	--	--	++	++	--

++ : Female specific band; ** : Male specific band; -- : No band present

their female parents. T₄ is common male parent for these three hybrids. Mean while they also possessed a female specific band from their respective female parents. Out of these three hybrids confirmed their hybridity, H₆ (L₆ x T₄) is a GMS based hybrid. GMS system is having great commercial significance as it has made hybrid seed production more easy, economical and commercially feasible at the risk of

getting sibs due to improper rouging of male fertile segregants. This problem can be solved by employing esterase for testing hybridity of commercial seed lots. However, esterase profile failed to confirm the hybridity of remaining six hybrids viz., H₁, H₃, H₅, H₇, H₈ and H₉. Hybrids H₁, H₃, H₇, H₈ and H₉ possessed only female specific bands. H₅ did not possess neither male nor female specific bands,



L₁ : (IIHR-285)
 L₂ : (IIHR-291)
 L₃ : (IIHR-294)
 L₄ : (IIHR-296)

L₅ : (IIHR-299)
 L₆ : (GMS-1)
 L₇ : (GMS-4)

T₄ : (JNDO-5)
 T₅ : (V. Uphar)

H₁, H₂, H₃,
 H₄, H₅, H₆,
 H₇, H₈, H₉

Hybrids

Plate 1. Esterase profile

suggesting that the other enzyme systems like peroxidase, alcohol dehydrogenase, malleic dehydrogenase or DNA markers can be explored for testing hybridity of these hybrids.

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Received 26 February, 2016; Accepted 22 April, 2016



Effect of Crop Geometry and Drip Irrigation Levels on Growth, Yield and Water Use Efficiency of Pearl Millet (*Pennisetum glaucum*) in Irrigated Western Arid Rajasthan

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Abstract: An increase in yield attributes, seed, straw and biological yield with increase in irrigation levels from 40% to 80% ETc was evident. However, 60% and 80% ETc gave at par yield but both were superior to surface irrigation. The highest water use efficiency ($6.00 \text{ kg ha}^{-1} \text{ mm}^{-1}$) was with drip irrigated at 60% ETc as compared to surface irrigated crop ($2.82 \text{ kg ha}^{-1} \text{ mm}^{-1}$). Highest seed (21.70 q ha^{-1}), straw (98.97 q ha^{-1}), biological (120.66 q ha^{-1}) yield and water use efficiency ($5.44 \text{ kg ha}^{-1} \text{ mm}^{-1}$) was with normal sown crop (60 cm row spacing) at 60 cm drip line spacing which was at par with paired row sown crop (45cm x 75cm paired row) at 120 cm drip line spacing.

Key Words: Crop geometry, Drip irrigation, Pearl millet, Seed yield, Water use efficiency

Pearl millet (*Pennisetum glaucum*) is an important coarse grain cereal generally grown as rainfed crop on marginal lands under low input management conditions. It is adapted to drought and poor soils, but responds very well to irrigation and fertilizer. It is generally cultivated in area where rainfall ranges from 150 to 600 mm. Rajasthan ranks first in area (46 lakh ha) and production (2.8 m tonnes) with productivity 400 kg ha^{-1} (Anonymous, 2010). Western region of Rajasthan comes under hyper arid zone with very scarce water resource and scanty and erratic rainfall. The arid climate is quite extreme with temperature as high as near about 49°C and as low as 0°C . Thus, productivity of any crop is very poor in this climatic condition. However, by maintaining adequate moisture content (nearer at field capacity), crops can be grown successfully. Drip irrigation maintains moisture content at near about field capacity in one hand and eliminates water losses on other hand and this system would be the most effective tool in increasing yield as well as water use efficiency in even close growing crop (Yang *et al.*, 2010; Gupta and Chattoo, 2014). Further, drip irrigation proved to be effective in improving yield and water use efficiency compared to level-basin irrigation method even under condition of deficit water (Dlong *et al.*, 2013; Brar *et al.*, 2015). However, modifying crop geometry and using wider spacing between two drip lines can make pearl millet growing under drip system economically feasible. Change of crop geometry brings about the change in growth and yield of wheat under drip system (Ahmad *et al.*, 2009, Kato and Osawa, 2013). Keeping all those in view the experiment has been conducted to optimize drip irrigation level for pearl millet under different crop geometry.

Field experiments were conducted on pear millet

during *kharif* season of 2012 to 2014 for consecutive three years at SK Rajasthan Agricultural University, Bikaner situated in arid western hyper arid zone of Rajasthan. The soil was sandy loam in nature, having field capacity 6.60%, PWP 1.92%, bulk density 1.52 g/cc , pH (1:2) 8.3, electrical conductivity (1:2) 0.2 dS m^{-1} . The soil is very low in organic carbon (0.13%), medium in available P (32.6 kg ha^{-1}) and high in available K (352 kg ha^{-1}). The experiment was laid out in randomized block design with three replications. The treatments consist of three irrigation levels viz. 40%, 60% and 80% ETc; three crop geometry levels viz. normal sowing (60 cm row spacing) in 60 cm drip line spacing, normal sowing (60 cm row spacing) in 120 cm drip line spacing and paired row sowing (45cm x 75cm paired row) in 120 cm drip line spacing) and a control (surface irrigation). Irrigation was applied from July to October and number of irrigation events was 25 during the crop growing period. The total irrigation water provided were 361.69, 398.84 and 436.00 mm at 40%, 60% and 80% ETc including rainfall, respectively (Table 1). Pearl millet variety "RHB173" was sown on 24th, 16th and 24th July using seed rate of 4 kg ha^{-1} and harvested on 5th November, 28th October and 5th November in 2012, 2013 and 2014, respectively. All the cultural operations were carried out as per recommendations (Anonymous, 2010).

Irrigation levels: Increasing irrigation levels from 40% to 80% ETc under drip system increased plant height, dry matter accumulation and yield attributes viz., ear length, number of tiller and test weight (Table 2). Increased plant height and yield attributes with increasing irrigation levels through drip irrigation thus, enhanced grain, straw and biological yield of pearl millet and highest grain (25.33 q ha^{-1}), straw (111.37 q ha^{-1}) and biological yield (136.70 q ha^{-1}) were

Table 1. Irrigation events and irrigation water applied in mm (mean of three years)

Month	Irrigation events	Irrigation levels (ETc)		
		40%	60%	80%
July (15 days)	4	6.26	9.38	12.51
August	6	22.30	33.44	44.59
Sept	10	38.16	57.24	76.33
Oct. (10 days)	5	7.59	11.39	15.19
Total	25	74.31	111.46	148.61
Irrigation immediately after sowing		25	25	25
Rainfall		262.38	262.38	262.38
Total		361.69	398.84	436.00

recorded at 80% ETc. However, drip irrigation at 60% ETc gave at par grain, straw and biological yield with 80 % ETc (Table 3). This is in confirmatory with Godara *et al.* (2013), who reported maximum plant height, dry matter of plant, seed yield and biological yield of fennel with 1.0 ETc under drip irrigation.

Drip irrigation levels from 40% to 80% ETc saved water by 80.69 to 6.38 mm over surface irrigation which used 442.38 mm water. Hence, increased yield coupled with less water use in drip irrigation, recorded higher water use efficiency (WUE) of 3.57, 6.00 and 5.81 kg ha⁻¹ mm⁻¹ at 40%, 60% and 80% ETc, respectively against 2.82 kg ha⁻¹ mm⁻¹ in surface irrigation. Kharrou *et al.* (2011) reported that drip irrigation applied to wheat was more efficient and saved 20% of water in comparison to surface irrigation. Rajbir *et al.* (2009) also reported that water use efficiency was highest under drip irrigation as compared to surface irrigation. Lower water use efficiency in surface irrigation (absolute control) may be due to loss of irrigation water from sandy loam soil through deep percolation resulted in higher water use but lowered grain yield.

Crop geometry; The plant height, dry matter accumulation and yield attributes viz. number of tiller and ear length increased in normal sown crop (60 cm row spacing) at 60 cm drip line spacing, which was at par with paired row sown crop (45cm x 75cm paired row) at 120 cm drip line spacing (Table 2). Increased plant height and yield attributes with normal sown crop (60 cm row spacing) at 60 cm drip line spacing, thus, enhanced grain, straw and biological yield of pearl millet. The highest grain (21.70 q ha⁻¹), straw (98.97 q ha⁻¹) and biological (120.66 q ha⁻¹) yields were recorded at normal sown crop (60 cm row spacing) at 60 cm drip line spacing which was at par with paired row sown crop (45cm x 75cm paired row) at 120 cm drip line spacing (Table 3). This is due to the fact that, proper crop geometry facilitates sufficient interception of sunlight and satisfactory absorption of nutrients and water from the soil due to proper development of root system (Annadurai *et al.*, 2009).

Among different crop geometries water use efficiency showed significant variation. Highest water use efficiency was recorded with normal sown crop (60 cm row spacing) at 60 cm drip line spacing (5.44 kg ha⁻¹ mm⁻¹). It may be due to increased availability of water due supply of water to each crop rows by a drip line. Thus, the narrow row spaced crop improved crop water use, which in turn recorded improved water use efficiency (Yin *et al.*, 2010).

Interaction effect of crop geometry and drip irrigation: Irrigation x crop geometry interaction was significant. The study 60 cm drip line spacing with 40, 60 and 80% ETc levels gave yield of 15.04, 24.40 and 25.62 q ha⁻¹, respectively. However, paired row sown crop (45cm x 75cm paired row) at 120 cm drip line spacing with 60% and 80% ETc were at par with normal sown crop (60 cm row spacing) at 60 cm drip line

Table 2. Effect of drip irrigation levels and crop geometry on yield attributes of pearl millet (pooled of three years)

Treatment	Height (cm)	No. Tiller plant ⁻¹	Ear length (cm)	Test weight (g)	Dry matter (g plant ⁻¹)		
					30DAS	55DAS	80DAS
Irrigation levels							
40% ETc	166.96	3.55	28.40	9.14	3.01	18.40	120.59
60% ETc	199.11	5.65	32.85	11.40	3.76	32.10	202.33
80% ETc	205.59	5.72	34.33	11.64	4.06	33.74	204.03
CD (p=0.05)	8.23	0.43	2.50	NS	0.32	3.43	6.52
Crop geometry							
60 cm drip line (60 cm row spacing)	197.51	5.13	33.10	11.10	3.88	28.98	178.26
120 cm drip line (45cm x 75cm paired row)	191.29	4.97	31.35	10.98	3.67	28.33	174.74
120 cm drip line (60 cm row spacing)	182.85	4.82	31.02	10.77	3.28	27.58	173.96
CD (p=0.05)	8.23	0.50	2.88	NS	0.31	3.37	5.78
Surface irrigation (60 cm row spacing)	158.11	2.99	28.66	8.72	3.20	18.73	102.30
CD (p=0.05)	6.51	0.23	1.38	NS	0.23	2.19	4.18

Table 3. Effect of drip irrigation levels and crop geometry on yield, water use and water use efficiency of pearl millet (pooled of three years)

Treatment	Grain yield (q ha ⁻¹)	Biological yield (q ha ⁻¹)	Straw yield (q ha ⁻¹)	Harvest index (%)	Water use (mm)	WUE (kg ha ⁻¹ mm ⁻¹)
Irrigation levels						
40% ETc	12.90	79.66	66.76	16.19	361.69	3.57
60% ETc	23.93	132.31	108.38	18.08	398.84	6.00
80%ETc	25.33	136.70	111.37	18.53	436.00	5.81
CD (p=0.05)	1.45	6.75	5.31	NS	-	-
Crop geometry						
60 cm drip line (60 cm row spacing)	21.70	120.66	98.97	17.98	398.84	5.44
120 cm drip line (45cm x 75 cm paired row)	20.43	118.45	98.02	17.25	398.84	5.12
120 cm drip line (60 cm row spacing)	20.03	109.55	89.52	18.28	398.84	5.02
CD (p=0.05)	1.45	6.75	5.31	NS	-	-
Surface irrigation	12.45	84.44	71.99	14.75	442.38	2.82
CD (p=0.05)	1.13	5.31	4.21	NS	-	-

Table 4. Interaction effect of irrigation levels and crop geometry on grain yield (q ha⁻¹) of pearl millet (pooled of three years)

Irrigation level	60 cm drip line (60 cm row spacing)	120 cm drip line (45cm x 75 cm paired row)	120 cm drip line (60 cm row spacing)
40% ETc	15.04	12.17	11.07
60% ETc	24.40	24.00	23.55
80%ETc	25.62	25.30	25.50
CD (p=0.05)	2.55		

spacing at 60% and 80% ETc. It is thus concluded that irrigation level of 80% ETc + 60 cm drip line spacing gave maximum yield (25.62 q ha⁻¹) as compared to other interaction effects (Table 4).

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Performance of Front Line Demonstrations on Sunflower (*Helianthus annuus* L.) in Kalaburagi Region of Northern Karnataka

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Abstract: The constraints in the production of sunflower crop were identified with a major emphasis against the pest and diseases in the sunflower. The critical inputs required were identified from the recommended package of practices of Karnataka and their usages were discussed in practicing farmer's trainings at the farmer's field and KVK campus. The average three years data revealed that an average yield of demonstrated plot was obtained 11.66 q ha^{-1} , local check (9.56 q ha^{-1}) and the average percentage increase in yield over local check was 18.84 per cent. The average extension gap was found to be 2.09. The hybrid RSFH – 130 performed better at front line demonstrated plot as compared to local check. Further, benefit: cost ratio was recorded to be higher under demonstrated plot against check during the part of the study.

Key Words: Economic impact, Extension gap, FLD, Sunflower, Yield

Sunflower (*Helianthus annuus* L.) belongs to *Asteraceae* family is one of the important edible oilseed crops cultivated in different parts of the world and has become the fourth most important oilseed crop in India. Sunflower cultivation in India was started with varieties introduced from outside the country and subsequently the hybrids were developed and are being grown widely covering nearly 95 % of the cultivated area. Generally, hybrids have higher yield potential compared to varieties (Kumar *et al.*, 2013). At present, the oilseeds production in India is not meeting the domestic demands and now dependent on imports. The continuous increase in import of oilseed is a matter of great concern today (Katare *et al.*, 2011). A wide gap exists in sunflower production with the use of available techniques and its actual application by the farmers and the higher incidence of pest attack leads to further reduction in yield, reflected through poor yield of sunflower crop on farmer's field. There is a tremendous opportunity for increasing the productivity of sunflower crop by adopting the improved technologies. To demonstrate the scientific cultivation of sunflower front line demonstrations should be laid out at farmer's field. The basic objective of FLDs is to demonstrate the proven technology at farmer's field through KVKs (Verma *et al.*, 2014).

The experimental study area, Kalaburagi district of Karnataka state is situated between $76^{\circ}.04'$ to $77^{\circ}.42'$ east longitudes and $17^{\circ}.12'$ to $17^{\circ}.46'$ north latitude with 454 MSL elevations covering an area of 10,951 km². The present study was conducted for three years (2011-12 to 2013-14). In total, 48 demonstrations were conducted in different villages of Kalaburagi district of Karnataka. The traditional practices were maintained in case of local checks. The data output were collected from both FLD plot as well as check plots and finally the extension gap (Katare *et al.*, 2011) along with the benefit cost ratio were worked out (Samui *et al.*, 2000).

In general the soils under study was sandy loam in texture with a pH ranging between 7.1 -7.5. The sowing of the sunflower seeds should be done by the end of January as delayed sowing of crop in the second fortnight of February or in the month of March lead to increases in the incidence of pests and diseases. The 5 kg seed were sown per hectare. Ridge sowing were done and the seed were placed about 6-8 cm below the ridge top. Irrigation to ridge sown crop was done 2-3 days after sowing and care was done that water level in the ridges well below the seed placement line.. The data on average cost of cultivation, average gross return, average net return and benefit: cost ratio were collected from frontline demonstrations plots for working out the economic feasibility of sunflower hybrid RSFH – 130. The recommended packages of practices were followed at the farmer's field. The difference between the demonstration package and existing farmers practices are given in Table 1.

The average yield of three years in demonstrated plot was 11.66 q ha^{-1} against local check (9.57 q ha^{-1}). The per cent increase in yield was 18.84 per cent over local check (Table 1). Further, average higher benefit- cost ratio (3.0) was recorded under demonstrated plot against check (2.31). The results clearly indicate the positive effects of FLDs over the existing practices towards enhancing the yield of the RSFH – 130 in Kalaburagi (Karnataka). The reason for the higher yield in FLDs is due to recommended dosages of fertilizers and modules for pest management against the pest and diseases were followed properly. However, yield of the hybrid RSFH – 130 were varied in different years which might be due to the variation in climatic conditions and incidence of disease/ pest attack.

The technological gap *i.e.* the difference between potential yield and yield of demonstration plot were 13.20, 12.81 and 14.00 during the year 2011-12, 2012-13 and 2013-

Table 1. Details of sunflower hybrid RSFH – 130 growing under existing farmer's practices and improved practices adopted in frontline demonstrations at farmer's field in Kalaburagi (Karnataka)

Operations	Existing farmer's practices	Improved/Recommended* practices adopted in demonstrated plot(FLDs)
Farming situation	Irrigated	Irrigated
Variety	Local	Hybrid RSFH – 130
Time of sowing	Second fortnight of February or March	January end or February (first week)
Seed treatment	Not done	Imidacloprid 60FS @ 5 gm kg ⁻¹ seeds
Method of sowing	Broadcasting	Line sowing
Fertilizers Dosages	Non-adoption of recommended package of practices. Usually more emphasizes were done on the higher dosages of Urea and application of Muriate of Potash (MOP) is omitted due to higher cost.	Recommended dose fertilizer + Application of Boron (0.5 %) @ 1 kg ha ⁻¹ + Application of ZnSO ₄ @ 10 kg ha ⁻¹
Weed control	No hoeing and application of stomp 30 EC (Pendimethalin) @500 ml/acre	Application of Stomp 30 EC (pendimethalin) @500 ml acre ⁻¹
Plant protection measures	Non-adoption of recommended package of practices and injudicious use of pesticides and spray Dursban 20 EC(Chloropyriphos)@500 ml/acre	Spray of imidacloprid 17.8 SL @ 0.3 ml l ⁻¹ of water at 45 and 60 DAS @ 300 ml ha ⁻¹ + Spray of hexaconazole @1 ml l ⁻¹ of water at 45 and 60 DAS @ 1 lit ha ⁻¹ + Hand picking of grown up larvae + application of HaNPV @ 250 LE ha ⁻¹

Table 2. Performance of front line demonstrations (FLD) of sunflower hybrid 64A57 in Kalaburagi (Karnataka)

Year	Area (ha)	No. of farmers	Seed yield (q ha ⁻¹)		% increase over control	Technology gap	Extension gap	Technology Index (%)	B. C. ratio	
			Demonstration	Control					Demonstration	Local check
2011-12	10	24	11.80	9.60	18.64	13.20	2.20	52.80	2.81	2.18
2012-13	5	12	12.19	9.80	19.60	12.81	2.39	51.24	3.20	2.41
2013-14	5	12	11.00	9.30	18.28	14.00	1.70	56.00	3.00	2.35
Average			11.66	9.57	18.84	13.34	2.10	53.35	3.00	2.31

14, respectively. The average technology gap in all the years was 13.33. Technology gap imply researchable issues for realization of potential yield, while the extension gap imply what can be achieved by the transfer of existing technologies. The technological index revealed the feasibility of the demonstration technology. As such variation in technology index (54.24 to 56 %) during the study period in certain area may be attributed to dissimilarity in the soil fertility condition, pest-diseases attack, non availability and poor quality of irrigation water and weather condition. The extension gap showed the trend ranged from 1.70 to 2.39 q ha⁻¹ during the period of study (Table 2). The percentage increases in yield over local check were recorded constantly year wise during the period of study. In Kalaburagi district of Karnataka, the low productivity of sunflower is because of farmers are not adopting recommended package of practices.

The above findings inferred that the usage of recommended package of practices leads to control the pest and diseases attack below the Economic Threshold level (ETL), thus led to increased productivity of hybrid RSFH – 130

in the Kalaburagi (Karnataka). To reduce the technological gap and to reverse the trend of extension gap, emphasizes is required to conduct more number of practicing farmer's training at farmer's field and KVK campus regarding recommended package and practices.

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Correlation and Path Coefficient Analysis among Different Characters in Genotypes of Brinjal (*Solanum melongena* L.)

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Abstract: Marketable yield per plant, average fruit weight, fruits per plant, fruit circumference and primary branches per plant were significantly and positively associated with fruit yield both at phenotypic as well as genotypic levels, while negatively correlated with fruit length and total soluble solids. Inter relationship study revealed that for improvement of brinjal, marketable yield per plant, average fruit weight, fruits per plant, fruit circumference and primary branches per plant to be given due consideration. Path coefficient analysis revealed that marketable fruit yield per plant followed by unmarketable yield per plant exerted high positive direct effect at phenotypic level.

Key Words: Brinjal, *Solanum melongena*, Correlation and path analysis, Fruit yield

Brinjal or egg plant (*Solanum melongena* L.) is one of the most important solanaceous vegetable crop having diploid chromosome number $2n=2x=24$. It is grown in the tropics and subtropics of India and other parts of the world. It is widely grown in India, Japan, Indonesia, China, Bulgaria, Italy, France, USA, Pakistan, Bangladesh, Philippines, and several African countries. It is also known as the poor man's crop because of its high productivity and wide adoptability. It is undoubtedly of Indian origin and has been cultivated for long time though Vavilov (1951) suggested China as the centre of origin. In India the area, production and productivity of brinjal in the year 2013 was 772 ('000 ha), 13444 ('000 MT), and 18.6 MT ha⁻¹, respectively (Anonymous, 2013). Phenotypic variability changes under different environmental conditions while genetic variability remains unchanged and more useful to a plant breeder for exploitation in selection or hybridization, consequently estimates of heritability and genetic advance are useful for selection. Yield is very complex characteristics controlled by several yield contributing components and significantly influenced by environmental factors. Estimation of correlation co-efficient among the yield contributing characters is necessary to understand the direction of selection and maximize yield in the shortest period. Path co-efficient provides an effective means of entangling direct and indirect causes of association of characters and measures the relative importance of each causal factor.

The experiment was conducted in Randomized Complete Block Design with 34 genotypes of brinjal collected from different places in India and being maintained at Main Experiment Station in the Department of Vegetable Science, N.D. University of Agriculture & Technology, Kumarganj, Faizabad (U.P.) during autumn-

winter season in 2013. Twenty plants were maintained in each replication at a spacing of 60 cm between row to row and 45 cm plant to plant. All the recommended agronomic package of practices and plant protection measures were followed to raise a good crop. Observations were recorded on randomly selected five plants from each genotype in each replication for various parameters (Table 1 and 2). The correlations between different characters at genotypic (g) and phenotypic (p) levels were worked out between characters including path-coefficient analysis.

In the present study, correlations between ten characters were worked out in all possible combinations at phenotypic and genotypic levels. In general, the magnitude of genotypic correlation coefficients were higher than the corresponding values of the phenotypic correlation coefficients. This indicated a strong genetic association between the traits and the phenotypic expression, which was suppressed due to environmental influence. The present study also suggested that both genotypic and phenotypic correlation were similar in direction. A perusal of data in Table 1 revealed that most important traits of total fruit yield per plant had highly significant and positive association with marketable fruit yield per plant, average fruit weight, fruits per plant, and fruit circumference at both phenotypic and genotypic levels. Thus, these characters emerged as most important associates of fruit yield in egg plant. The available literature has also indicated positive correlation between total fruit yield per plant and character mentioned above in egg plant.

Marketable fruit yield per plant, average fruit weight, number of fruits per plant, fruit circumference and total fruit yield per plant were found significantly and positively correlated among themselves (Table 1). Average fruit weight

Table 1. Direct and indirect effect of ten characters on fruit yield per plant (kg) at phenotypic and genotypic level in brinjal

Character	Days to 50% flowering	Plant height (cm)	Primary branches	Fruit length (cm)	Fruit circumference (cm)	Fruits plant ⁻¹	Average fruit weight(g)	T.S.S.	Marketable yield plant ⁻¹	Unmarketable yield plant ⁻¹	Total fruit yield plant ⁻¹
Days to 50% flowering	0.030	0.007	0.006	0.004	0.000	0.003	0.001	0.000	0.005	-0.004	0.145
Plant height (cm)	-0.013	-0.052	-0.007	-0.015	0.007	-0.010	0.017	-0.003	0.002	-0.015	0.013
Primary branches	-0.003	-0.002	-0.015	0.001	0.000	-0.002	-0.001	0.004	-0.004	-0.002	0.262
Fruit length cm	0.010	0.021	-0.003	0.071	-0.037	0.005	-0.034	0.004	-0.021	-0.008	-0.288
Fruit circumference (cm)	0.001	-0.007	0.001	-0.027	0.052	-0.016	0.039	0.006	0.014	0.001	0.281
Fruits per Plant	-0.011	-0.018	-0.011	-0.006	0.028	-0.094	0.026	0.009	-0.045	-0.014	0.432
Average fruit weight(g)	-0.001	0.014	-0.002	0.022	-0.034	0.013	-0.045	0.000	-0.021	0.003	0.461
T. S. S.	-0.001	-0.002	0.011	-0.002	-0.005	0.004	0.000	-0.040	0.007	0.001	-0.194
Marketable yield plant ⁻¹	0.180	-0.039	0.248	-0.300	0.260	0.482	0.481	-0.164	1.005	-0.069	0.919
Unmarketable yield plant ⁻¹	-0.047	0.091	0.034	-0.035	0.009	0.047	-0.024	-0.011	-0.022	0.322	0.216

R² = 0.9342, Residual effect = 0.2565; *Genetic level values in parentheses**Table 2.** Direct and indirect effect of ten characters on fruit yield per plant (kg) at genotypic level in brinjal

Character	Days to 50% flowering	Plant height (cm)	Primary branches	Fruit length (cm)	Fruit circumference (cm)	Fruits plant ⁻¹	Average fruit weight(g)	T.S.S.	Marketable yield plant ⁻¹	Unmarketable yield Plant ⁻¹	Total fruit yield plant ⁻¹
Days to 50% flowering	0.037	0.010	0.015	0.007	0.000	0.005	0.001	0.001	0.007	-0.006	0.148
Plant height (cm)	-0.022	-0.084	-0.021	-0.029	0.012	-0.016	0.029	-0.014	0.006	-0.027	-0.015
Primary branches	-0.013	-0.008	-0.031	0.007	-0.001	-0.009	-0.007	0.014	-0.021	-0.002	0.656
Fruit length cm	0.027	0.052	-0.033	0.149	-0.083	0.012	-0.079	0.030	-0.053	-0.018	-0.337
Fruit circumference (cm)	0.001	-0.015	0.004	-0.055	0.099	-0.031	0.078	0.020	0.026	0.005	0.296
Fruits per plant	-0.020	-0.030	-0.048	-0.013	0.050	-0.159	0.048	0.023	-0.078	-0.022	0.432
Average fruit weight(g)	-0.004	0.035	-0.024	0.054	-0.079	0.031	-0.101	0.000	-0.052	0.010	0.497
T. S. S.	-0.002	-0.012	0.032	-0.015	-0.015	0.010	0.000	-0.072	0.026	-0.005	-0.375
Marketable yield plant ⁻¹	0.207	-0.079	0.736	-0.396	0.295	0.538	0.566	-0.405	1.104	-0.096	0.934
Unmarketable yield plant ⁻¹	-0.062	0.116	0.026	-0.045	0.018	0.051	-0.038	0.028	-0.032	0.365	0.203

R² = 0.9776, Residual effect = 0.1497

is highly significant but negatively correlated with plant height and fruit length while positively and significantly correlated with fruit circumference. Primary branches per plant was significantly and positively correlated with days to 50% flowering suggesting that, selection for marketable yield per plant, average fruit weight, number of fruits per plant and fruit circumference will be helpful for yield improvement.

Path coefficient analysis: The present study on path coefficient analysis revealed that the highest magnitude of positive direct effect on yield per plant was exerted by marketable fruit yield per plant followed by unmarketable fruit yield per plant at both phenotypic and genotypic levels (Table 2). This indicates that direct selection based on marketable fruit yield per plant and unmarketable yield per plant would result in an appreciable improvement of yield per plant. Marketable fruit yield per plant was not only found to have maximum direct effect on total fruit yield per plant but it also contributed substantial positive indirect effect for fruits per plant followed by average fruit weight and negative indirect effect for fruit length and plant height. Therefore during

selection, these characters should also be taken into consideration. Similar inference had also been reported earlier by Naliyadhara *et al.* (2007); Thangamani and Jansirani (2012); Paramappa *et al.*, (2014).

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Effect of Region and Herd Size on Dairy Herd Performance Parameters in Punjab

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Abstract: A field study was conducted on 45 dairy farms of Punjab for one year to assess the dairy herd performance parameters. The average lactation yield was not significantly different among regions; however it was significantly higher (5062 litres) at large herd size farms (>99 milch animals) and lowest (4232 litres) at small herd size farms (20-49 animals). The wet average varied significantly among different herd size farms whereas the herd average differed significantly among regions. The herd size had significant effect on days open as the small farms were having the maximum days open 112 days followed by the medium and large farms. The region as well as herd size had no effect on the somatic cell count in milk. Income over feed ratio was not significantly different among regions and farm sizes, however, value was highest for Majha region and large size herds. It may be concluded that the large herd size had significant effect on average lactation yield, wet average, days open, somatic cell count and average annual income where as region significantly affect the herd average and price of milk only.

Key Words: Dairy farming, Herd performance, Herd size, Income over feed ratio

Punjab is one of the leading state in dairying and milk production (Kumar, 2009), contributing 9.7 MT (NDDB, 2015) towards national milk grid with 3% of total bovine population of India. There is ample scope to improve productive potential of dairy animals through adoption of scientific practices and efficient management system. Efficient management utilise commercial technologies to improve herd performance as it closely related to the economical viability of a dairy farm. But the management practices may differ in different regions and also vary with variation in herd size. There are certain dairy management parameters that are helpful to check the farm viability i.e. days open, milk production, price of milk, feed cost, income over feed ratio and somatic cell count (Aulakh *et al.*, 2013). Keeping in view, the study was designed to assess the effect of herd size and region on herd performance parameters at commercial dairy farms of Punjab.

A field study was conducted on 45 commercial dairy farms of Punjab for one year to assess the dairy herd performance parameters. The dairy farms were selected with stratified random sampling method from all three regions of Punjab (Majha, Malwa and Doaba). From each region five small sized farms (20-49 milch animals), five medium sized farms (50-99 milch animals) and five large sized farms (above 100 milch animals) were selected randomly. The information regarding days open, milk production, price of milk, feed cost, income over feed ratio and somatic cell count were collected. The data was analyzed with standard statistical methods to draw the results.

The average lactation yield was not significantly different among regions; however value was highest (4851

liters) for Malwa region (Table 1). The average lactation yield was significantly higher for large herd size farms (5062 litres), whereas, lowest (4232 litres) for small herd size farms, which may be due to the better management practices followed at large dairy farms as compared to small dairy farms.

The wet average was highest in Malwa region (16.11 liters) followed by the Majha and Doaba region however the differences were non-significant (Table 1). The wet average differed significantly ($P < 0.05$) with herd size, as the wet average of the large dairy farms was highest (16.87 liters) followed by the medium and small dairy farms. Oleggini *et al.* (2001) had also reported similar results. Higher wet average may be due to elite breeds and better management practices followed at large dairy farms lead to higher milk production.

The herd average differed significantly among regions, being highest (7.11 liters) followed by Majha and Doaba region. The findings are in agreement with Oleggini *et al.* (2001). The herd averages of the various categories revealed that the small farms were having the least herd average of 5.68 liters followed by the medium farms 6.62 liters and the large farms 6.92 liters. The herd average of the different group of the farmers didn't show any significant difference ($P < 0.05$).

The farmers of Malwa region were having the lowest days open i.e. 93 days followed by Majha region and the maximum days was of Doaba region (Table 1). The days open of three regions did not show any significantly difference among regions ($P < 0.05$). The herd size has significant effect on days open as the small farmers were having the maximum days open 112 days followed by the

Table 1. Effect of region and herd size on dairy herd performance parameters

Parameter	Average lactation yield (L)	Wet average (L)	Herd average (L)	Days open, (days)	Somatic cell (count μL^{-1})	Price of milk (Rs. kg^{-1})	Average feed cost (Rs. yr^{-1})	Average income (Rs. yr^{-1})	Income over feed ratio
Effect of region									
Majha	4653 ^a	15.46 ^a	7.11 ^b	101 ^a	647 ^a	23.46 ^b	33,25,578 ^a	5453648 ^a	1.64 ^a
Malwa	4851 ^a	16.11 ^a	6.8 ^a	93 ^a	451 ^a	20.94 ^a	26,33,466 ^a	4160876 ^a	1.58 ^a
Doaba	4362 ^a	14.54 ^a	5.38 ^a	105 ^a	723 ^a	22.02 ^{ab}	30,52,100 ^a	4944402 ^a	1.62 ^a
Effect of herd size									
Small size farm (20-49)	4232 ^a	14.1 ^a	5.68 ^a	112 ^b	640 ^a	21.96 ^a	15,35,400 ^a	2395224 ^a	1.56 ^a
Medium size farm (50-99)	4613 ^{ab}	15.37 ^{ab}	6.62 ^a	97 ^a	609 ^a	22.96 ^a	21,00,600 ^a	3423978 ^a	1.63 ^a
Large size farms (>99)	5062 ^b	16.87 ^b	6.92 ^a	92 ^a	575 ^a	21.84 ^a	55,90,000 ^b	9279400 ^b	1.66 ^a

medium (97 days) and large farmers (92 days), which indicating good breeding and management practices at the larger farms. The findings are in line with Sorensen and Ostergaard (2003). The somatic cell count, which was lowest (451/ μL) for Malwa region and highest (723/ μL) for Doaba region (Table 1) but differences were statistically non-significant. Similarly, herd size also had no significant effect on the somatic cell count. The findings are in line with the Oleggini *et al.* (2001).

The average annual feed cost per farm was highest for Majha region whereas lowest for Malwa region, however the difference was statistically non-significant among different regions (Table 1), however, feed cost varies significantly among different herd sizes, as the feed cost at large farms was higher than that of medium and small farms because they had elite milch animals with high production potential; hence required more quality feed.

Income over feed ratio (IOFR), didn't significantly differ among regions (Table 1). Herd size also has non-significant effect on income over feed ratio. The findings are in line with Sahu *et al.* (2012). The higher IOFR of the Majha region may be due to the milk price which varied significantly among different regions and it was highest in the Majha region due to private sale of milk.

The study conclusively revealed that the herd performance parameters like average lactation yield, wet average, days open, feed cost, income over feed ratio and somatic cell count didn't varied significantly among regions except price of milk, which was highest for Majha region, however, herd size has significant effect on most of the herd performance parameters and the values were highest for large size herds. But the variation in income over feed ratio was non-significant among different herd size farms however increase in ratio was observed with increase in herd size.

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Efficacy of Plant Extracts against Tomato Late Blight under Net House Conditions

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Abstract: Aqueous extracts of three plants i.e. *Eucalyptus camaldulensis*, *Allium sativum*, and *Azadirachta indica* were tested against *Phytophthora infestans* on detached tomato leaves and under net house condition. All the treatments significantly reduced the severity of late blight over untreated control. On detached leaves, the most effective treatment was clove extract of *A. sativum* followed by *E. camaldulensis* and *A. indica* at 20% concentration. In net house also clove extract of *A. sativum* showed the best results followed by *E. camaldulensis* and *A. indica* in reducing late blight severity of tomato. All treatments, plant extracts and fungicide (Ridomil Gold), significantly reduced the late blight disease of tomato compared to infected control under net house.

Key Words: Disease, Pesticides, *Phytophthora infestans*, Plant extracts, Tomato

Tomato (*Solanum lycopersicum* L.) is an important vegetable grown world wide (FAO, 2014). Late blight caused by the Oomycetes *Phytophthora infestans* (Mont) de Bary is the major disease affecting its global production (Kapsa and Koodziejczyk, 2005). Late blight causes severe loss of tomato production. This disease is currently controlled by growing resistant varieties or spraying fungicide (Cao and Bruggen, 2001). However, resistant varieties are rather rare. Fungicide being chemical have a wide range of side effects. Therefore, the use of fungicide in agriculture is being gradually reduced.

In recent years, there has been an increased interest in using natural substances for pest management in agriculture (Mansingh, 2004). In most cases, it would appear that natural products derived from plants are safer than fungicides because they have low acute toxicity and are readily biodegradable into non-toxic products (Tripathi and Dubey, 2004; Manisha *et al.*, 2014). Consequently, these compounds can be considered as substances of reduced risk of environment and human health (Duke *et al.*, 2003).

The aim of this study was to evaluate the efficacy plant extracts of *Eucalyptus camaldulensis*, *Allium sativum*, and *Azadirachta indica* for the management of late blight, in the hope of finding an alternative to chemical pesticides.

The causal fungus *P. infestans* was isolated from naturally infected tomato leaves and fruits showing blight symptoms. Pathogenicity tests of *P. infestans* isolates were carried on one month old tomato plants grown in earthen pots. The inoculum was prepared by scraping *P. infestans* colony surface in sterilized water. The resulting conidial and mycelial fragment suspension was adjusted to 1×10^6 cfu / ml using a haemocytometer and used for inoculation of 15 tomato plants in pots in net house. After inoculation, pots

were covered with polythene bags for 48 hrs to maintain high humidity conditions. After 48 hrs, polythene was removed. The disease severity was recorded two weeks after inoculation using 0-5 rating scale based on leaf area, stem and fruit covered by blight symptoms following the rating scale described by Pandey *et al.* (2003). The mean value of the (PDI) (from ten individual plants) was calculated.

Preparation of extracts per cent disease index: Leaves of two plants namely *Eucalyptus camadulensis* and *Azadirachta indica* and bulbs of *Allium sativum* were collected from field/campus of Punjab Agricultural University, Ludhiana, Punjab and used for preparing extracts. Plant leaf extract was prepared according to Sallam (2011). Ten grams of fresh leaf/clove material of each plant species was collected, washed with water and crushed in a grinder by adding sterile distilled water at the rate of 10 ml gm⁻¹ of plant tissue and the homogenates were centrifuged at 10,000 x g for 15 min and the supernatant solutions were collected. The plant extract was diluted further to have 10 and 20% concentration (v/v).

Efficacy evaluation of plant extracts on detached tomato leaves: Detached leaves were collected from the tomato plants eight weeks after planting. Five to six compound leaves were put into a plastic tray (45 cm x 35 cm x 8 cm) with two pieces of wet filter paper. The trays were used to maintain high humidity adding distilled water. Leaf extracts of *E. camadulensis* and *A. indica* and clove extracts of *A. sativum* were tested at 10 and 20% concentration in the plastic tray. Ridomil Gold @ 0.25% was used as standard treatment. Untreated control was maintained with distilled water. Each treatment involved three replicates. All the trays were incubated at 17°C in the dark. Sporangial suspensions of *P.*

Table 1. Effect of plant extracts against *P. infestans* in a detached leaf assay under lab and net house condition

Treatments	Concentration (%)		Disease severity (%)		% Reduction	
	Lab	Net House	Lab	Net House	Lab	Net House
<i>Eucalyptus camadulensis</i>	20	20	35.3	30.3	50.5	54
<i>Allium sativum</i>	20	20	32.3	26.7	58.6	59.5
<i>Azadirachta indica</i>	20	20	43.1	38.5	39.6	41.6
Ridomil Gold	0.25	0.25	15.8	14.8	77.8	77.5
Control			71.3	65.9		
CD (p=0.05)			-	-	6.8	5.6

infestans (1×10^4 sporangia/ml) were inoculated on the surface of the leaflets 24 hrs after treatment. The entire trays were reincubated at 17°C in the dark. Disease measurement was done seven day after inoculation and disease incidence and disease severity were calculated.

Efficacy evaluation of plant extraction on net house grown tomato plants: Treatments with plant extracts at 20% concentrations were performed as foliar application on 12 weeks old tomato plants and every seven days up to 60 days after planting. After two days from the second spraying, tomato plants were inoculated with *P. infestans* suspension containing 1×10^4 cfu/ml. After inoculation, plants were kept in a climate chamber at a daily temperature of 18°C with 85% relative humidity for 24 hrs. Disease development was recorded 15 days after inoculation. Disease severity was recorded as described before. Phytotoxicity effect of the treatments was also recorded.

All the plant extracts significantly reduced the late blight severity. The most effective treatments with plant extracts were *A. sativum* followed by *E. camadulensis* and *A. indica* at 20% concentration (58.6, 50.5 and 39.6% disease reduction, respectively). All the treatments with plant extracts also significantly reduced the late blight disease under net house conditions. The maximum reduction of disease severity was achieved by *A. sativum* and least reduction was obtained when tomato plants were treated with *A. indica* (59.5% and 41.6%, respectively). The highest reduction of disease severity 77.5% was achieved by Ridomil Gold. There were no phytotoxic effects of plant extracts observed in the net house.

Other workers have also reported that extract of garlic cloves completely inhibited zoospore formation (Cao and Van Bruggen 2001; Wang *et al.*, 2001) and colony growth of *P. infestans*. Abd-El- Khair and Wafaa (2007) observed that cold water extracts of basil leaves (*Ocinum bacilicum*), eucalyptus leaves (*Eucalyptus globulus*) and garlic bulbs (*Allium sativum*), reduced the spores germination (%) of *P.*

infestans from 30 to 56%, 41 to 72%, and 58 to 81% at concentrations of 2.5%, 5.0 and 10.0%, respectively. Sunita and Rai (2008) showed that garlic has antifungal effect. Katooli *et al.* (2011) found that oil of eucalypt (*E. camaldulensis*) showed complete inhibition of mycelia growth *P. ultimum* and *R. solani*.

ACKNOWLEDGEMENT

The authors are thankful to Department of Science and Technology, Government of India, New Delhi, for sponsoring the study under WOS B Scheme.

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Received 4 December, 2015; Accepted 8 May, 2016

Oh y for Members



African Catfish, *Clarias gariepinus*: An Eco-terrorist Destroying Delicate Ecological Balance in Aquatic Ecosystems of National Parks and Wildlife Sanctuaries in India

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Abstract: African Catfish, *Clarias Gariepinus* (Bruchell, 1822) - an omnivores fish was illegally introduced in India in late 1980s. But soon it became invasive and started eating native fish species and other aquatic fauna. After receiving number of complaints from environmentalists, farmers and fisheries research organizations, Govt. of India, Ministry of Agriculture banned its culture in 1997. However, its culture, sale and purchase still continue illegally. It has invaded almost all inland aquatic ecosystems of the country and is particularly posing a big threat to aquatic ecosystems of national parks and wildlife sanctuaries of India. The devastation of the delicate aquatic ecological balance being caused by this eco-terrorist including loss of habitat for migratory birds by eating phytoplankton, snails, slugs, gastropods, arthropods, crustaceans, amphibians, prawns, shrimps, reptiles, fish and small birds in village ponds, lakes, rivers and other water bodies has been discussed in the paper. The legal framework for dealing with this invasive alien species in India has been described and some management measures have been suggested in the paper. The cause of worry however is that many forest and wildlife managers are not familiar with the invasive and destructive nature of *Clarias gariepinus*

Key Words: African Catfish, *Clarias gariepinus*, Invasive Alien Species, National Parks, Wildlife Sanctuaries

While travelling around the globe, the human beings were fascinated by nature, plants and animals for various reasons like aesthetic beauty and satisfaction of the taste buds. So, they carried with them exotic species of plants/animals and introduced them in new habitats. Some of these species like maize, rice, wheat, potato, soybean, sugarcane, etc. became so dear that they came under cultivation and are today the backbone of human food security. But some of these alien species escaped from the controlled conditions in the new location, became invasive in due course of time and are now altering the ecosystems by wiping out indigenous species of the area. Some of the invasive alien species (IAS) are now harming human livelihood and aggravating poverty by altering/reducing the ecosystem services and the economy of the nation. Aquatic ecosystems are the world's most important ecosystems and their ecological and economic importance has been felt the world over. They are the repositories of aquatic wealth and directly or indirectly support a variety of tangible benefits like food, shelter and water. The intangibles like educational and aesthetic/recreational benefits are also very useful to the society. In terms of the ecological value, they perform many important environmental functions. They recycle nutrients, purify water, control floods, recharge ground water and provide habitats for variety of flora and fauna. The wetlands, which are a form of aquatic ecosystem are regarded as the kidneys of water bodies as they regulate the flow of water.

The importance of aquatic systems for the environment can be judged from the fact that Indian wetlands alone support 47

per cent of India's flora and fauna. It has been estimated that 25 per cent of the global aquatic flora is harbored by Indian wetlands, which includes 1500 species of angiosperms, 3000 species of algae and 200 species of mosses and lichens. This humble flora becomes food for the lowest organism in the food chain on which a number of higher organisms thrive. So, there are variety of faunal species in the aquatic ecosystems, which include starfish, snails, slugs, gastropods, arthropods, amphibians, pisces, waterfowls, reptiles and mammals whose survival depends upon the well being of the aquatic ecosystems. Aquatic ecosystems are very important for maintaining the delicate ecological balance that the future of globally threatened species like Gangetic dolphin, (*Platanista gangetica*), Irrawaddy dolphin (*Orcaella brevirostris*) & dugong (*Dugong dugon*), Siberian crane (*Leucogeranus leucogeranus*), The Manipuri brow-antlered deer or dancing deer or the sangai deer (*Rucervus eldii eldii*), wild buffalo (*Bubalus arnee*), Indian one horned rhinoceros (*Rhinoceros unicornis*), etc. depends upon the survival of the aquatic ecosystems only.

Ecologically, the aquatic ecosystems are delicate and with a bit of disturbance the whole food chain gets disturbed. Global warming, climate change, overexploitation leading to habitat destruction, invasion by invasive alien species (IAS) and pesticides draining into them, soil erosion, and landfills are some of the reasons which are proving fatal to the Indian aquatic ecosystems. However, after habitat destruction, it is the invasion by the IAS which is posing serious threat to the ecosystems. Water hyacinth (*Eichhornia*

crassipes) is an example of aquatic IAS. Recent in the list is a fish called African catfish (*Clarias gariepinus*). The cause of the worry is that unfortunately, its invasive problems are not yet known to most of the forest managers.

African Catfish : an introduction: African Catfish also called saw toothed fish, walking catfish and African magur/catfish, *Clarias gariepinus* (Burchell 1822). is omnivores fish that can eat anything that is available in the aquatic system. So, it eats phytoplankton, snails, slugs, gastropods, arthropods, crustaceans, amphibians, prawns, shrimps, reptiles, fish, small birds, skin of mammals, dung, dead animals' visceral organs, hairs, feathers and even leather. It is a large scaleless, eel-like nostril fish with wide mouth, having dark gray or black coloration on the back which fades to a white belly. The maximum recorded length is 170 cm (Froese *et al.*, 2014). with an average length of 90cm. It can weigh upto 60 Kgs. It prefers quiet shallow waters of lakes, ponds, swamps, dark and dirty waters and muddy substrates, which are subject to seasonal drying but it can also survive in fast flowing rivers (FAO). It is potamodromous, which means that it migrates within rivers and streams (Teugels, 1986).

It is a nocturnal bottom feeder (but adaptable to surface feeding) and comes out of water during night for feeding with the help of strong pectoral fins to feed on a variety of living organisms as well as dead animal matter. With its wide mouth, it is able to swallow big organisms like birds as well (Anoop *et al.*, 2011). Infact African catfish possesses a pair of suprabranchial chambers located in the dorsal-posterior part of the bronchial cavity having extensions from the upper parts of the second and fourth gill arches, forming the arborescent organs. This structure is an air-breathing organ and allows aerial breathing (Belão *et al.*, 2011). That is why they are able to live out of water for a long time. Its maximum life is fifteen years.

Illegal introduction of African Catfish and ban by Govt. of India: The efforts for the culture of this catfish in Africa started in 1960s and by 1970s its rearing practices were standardized. By late 1970s, its introduction started in other countries. It was introduced in Thailand in 1980s and Bangladesh in 1989. It was illegally introduced in India as well from Bangladesh and this fish was first cultured in West Bengal in 1991. The fish farmers soon observed that the seed of the fish species other than African catfish that they had put in the pond no longer existed and they saw only African catfish everywhere in their pond. They realized that cat fish devours other species and what remains in the pond is monoculture, which is financially non-remunerative. After receiving a number of complaints from environmentalists, fish farmers and various fisheries research organizations, the

Govt. of India, Ministry of Agriculture constituted a national committee to overview the regulation of exotic aquatic species (Fisheries). The committee studied all aspects of *Clarias gariepinus* and recommended that the exotic magur, which has been introduced in country without official sanctions be destroyed immediately. All hatcheries may be identified and destroyed by concerned states and not be allowed to establish in natural environment. It should not cross with endemic species *Clarias batrachus* and strict control measures should be taken to avoid spread of diseases from *C. gariepinus* to endemic fishes. The Fisheries and Aquaculture Department of the Food and Agriculture Organization (FAO) of the United Nations, in their assessment of Magar, had stated that 'this alien, carnivorous, and predatory species is a threat to native fish varieties. Govt. of India, Ministry of Agriculture also wrote to all states Fisheries Development Commissioner (December 1997) to immediately destroy the *Clarias gariepinus* in their respective states. Since then, the farming of this cat fish has been officially banned in India.

African catfish, has escaped from fish ponds and has invaded virtually all the aquatic ecosystems across the length and breadth of the country. In Haryana, fisheries department has removed it from its aquaculture but its culture continues in the areas adjoining the national capital, New Delhi as it is highly remunerative. In many states, at the time of the auction of the village ponds, the bidder has to give an undertaking that he will not use the fish pond for African catfish culture but there is no monitoring thereafter. Infact those who are involved in its illegal trade just manage to get its fry/fingerlings through illegal means and then throw them in canals, ponds and even stagnant water bodies found here and there. In the case of running canals, when the canal is closed for routine cleaning purpose or the water supply is stopped to such canals or the seasonal water body gets dried up, fish is collected and sold illegally. Fish farmers are more interested in this variety as it gives much profit in a short period. However, the fish sellers do not openly sell this fish to anybody. Infact for the sale purposes, the local sellers display other fishes. The drum containing African catfish is concealed underneath. They will sell the catfish only to their regular and routine customers. In Chittur (Kerala) alone, the fish variety is being farmed on 1,000 acres (Prabhakaran, 2014).

African Catfish : an ecological nuisance: African catfish has already invaded the natural ecosystems of the country and is wrecking havoc on the native flora and fauna. The incidents of ecological disturbances have been noticed in a number of national parks, wildlife sanctuaries and reserve forests. No predator of African catfish exists in India though it is predated

upon by some birds but that happens during fry stage only. In the absence of any predator, it is flourishing and many endangered species of water bodies in the wildlife sanctuaries and national parks are already perceiving extinction threat of native flora and fauna due to the disturbance of food chain. It is feared that *Clarias gariepinus* may cross breed with local endemic *Clarias batrachus*, which has already been placed in the list of endangered species and may transmit diseases like argulosis to it. In village ponds and lakes where this fish has virtually taken control of the ponds at the cost of other fauna, it has already become a nuisance even in the rural water bodies. The village people call it as teat eating fish. The African catfish with its sharp teeth bites the animals and specially injures teats of buffaloes and cows in village ponds.

Lessons learned from Sultanpur National Park: Sultanpur National Park in Haryana is regarded as a heaven for migratory water fowls and a number of resident terrestrial birds. It is located at a distance of about 40 km from Delhi. Due to the mushrooming farm houses activity in its catchment area, the natural flow of water to the lake of this national park has been blocked. To fill this lake with water, the requirement is met from Gurgaon canal. In 2010, it was noticed that the migratory birds are not landing up in the lake. At the same time, the park authorities noticed that the lake water has been dominated by very big fishes of one kind only. They also noticed that other fishes like carps (*Catla catla*, *Cirrhinus cirrhosus*, etc.) and rohu (*Labeo rohita*), whose fry they used to add every year as food supplement to feathered guests were not seen. The managers were caught unawares as they were not familiar with the fish. The fish was later identified as African catfish and ichthyologists revealed that this fish was the reason for the disappearance of other fishes and aquatic life from the lake. They also revealed that feathered guests were not landing in the park because of the fear of this fish. The lake was dried in the following summer and had to be kept so during the winters as well. The result was that no feathered guests landed up during next winter. Rarely the environmentalists, journalists and resource managers had correct knowledge about the fish. Dark dirty water provides effective camouflaging. It is able to survive even under low oxygen industrial effluent waste. Its omnivorous habit, strong digestive system and ability to live without food for months make it to survive under odd conditions as well. It survives on anything from organic matter like dung to living organism. Strong digestive system helps it to digest even leather. Also, being the cheap source of proteins, both consumers and sellers have craze for live fish. There is fairly good demand as a result of cheap price (100 Rs. kg⁻¹) in market, and no practical ban on culture and

transport, is encouraging fish mafia and aquaculturists to go ahead with green signal.

What do the existing Indian environmental laws say about invasive alien species (IAS)?: Existing laws like Wildlife Protection Act, 1972, Indian Forest Act, 1927, and Environment Protection Act, 1986 are silent about IAS. As per the provisions of section (29) of Wildlife Protection Act-1972, Chief Wildlife Warden of the state can take any action for the habitat improvement in wildlife sanctuaries and national parks including killing of the fish as well. But the killed fish (or any other wildlife) has to be burnt and buried and it cannot be sold as it amounts to trade. Drying can be another alternative but drying of rivers and big water bodies is virtually impossible. Drying of the shallow water bodies is possible but not deep water bodies. It would lead to death of other faunal wealth. Further, due to prolific egg laying nature, ability to survive without oxygen and hide itself in mud, even this method is not full proof. Even one fish, which is able to survive in mud can produce thousands of eggs (Plate 1) and invade the ecosystem afresh. Being an exotic species, it does not have any predator in India nor has any animal become its predator so far in India. In its natural home, leopard predares upon it. There is probably no legal frame work to deal with this pest.



Plate 1. Prolific reproducer (thousands of eggs)

The need of the hour is to recognize the severity of the problem and the ecologists, environmentalists and wildlife managers have to agree to the fact that African catfish is an obnoxious pest and it has to be eradicated at all costs. Scientists will have to find solution through biological control, which may selectively kill only African catfish and not other useful aqua fauna. Public awareness is also required to be generated among all sections of the society along with strict punishment to the offenders involved in its culture and trade may safeguard the aquatic ecosystem from this invasive alien species.

ACKNOWLEDGEMENTS

The author sincerely thanks Dr. S.K. Chauhan, Professor Forestry, Punjab Agriculture University, Ludhiana, India and IUFRO Coordinator Short Rotation Forestry, for his constant encouragement for ecological studies and support in improving the manuscript. Govt. of Haryana, Principal Chief Conservator of Forests, Haryana, Dr Amrinder Kaur, Forest Officers of Haryana and the staff of Research Circle, Pinjore have provided platform for conducting this study. The author thanks them all for their constant support.

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Received 29 April, 2015; Accepted 2 May, 2016