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Halophytic Community of Foredunes in South-Central Coast of Vietnam

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Abstract: The halophytic communities on foredunes are increasingly threatened by global climate change and human activities, particularly in the South-Central Coast of Vietnam. The botanical surveys were conducted across 41 study sites in eight provinces along foredunes in this region. The results show that halophytic community comprises 195 native taxa, 64 families, and 165 genera. Fabaceae is the most diverse family, with the highest number of genera (20) and species (25), followed by Asteraceae (13 genera, 13 species), Malvaceae (10 genera, 12 species), and Poaceae (10 genera, 11 species), collectively representing 32.12% of total genera and 31.28% of total species. The ten most species-rich families contribute 55.38% of the total floristic diversity in the coastal foredunes while 37 families, comprising 57.8%, are represented by single genus and single species. The habit spectrum shows that the herbs predominate and constitute 23.59% (46 species) of the recorded taxa. These findings suggest that the halophytic composition of foredunes in the South-Central Coast of Vietnam exhibits similarities to tropical desert regions in Southwest Asia, China, Pakistan. The composition of habits indicates the distinguished vegetation between incipient dune (creepers, prostrate, herbs, rhizomatous, stoloniferous) and established foredune (shrubs, woody, climbers).

Keywords*:* Botanical survey, Flora, Halophytes, Vietnam

Halophytes are plant species adapted to survive in saline environments such as seawater, salt marshes, coastal shores, saline flats, and salt deserts. These plants are found across a wide range of climatic zones from cold temperate to subtropical to tropical ecosystems (Flowers and Colmer 2008, Cunningham and Jankowitz 2010, Grigore 2020). The eHALOPH database currently documented 1.457 records of salt-tolerant plants belonging to 123 families of flowering plants, with 75% concentrated in 24 families; Amaranthaceae accounting for nearly a quarter of all recorded halophytes (Santos et al.,., 2016). Grigore provided a definition and classification of halophytes as an ecological group of plants with specific salt tolerance mechanisms (Grigore 2020).

The floristic studies on coastal dune ecosystems in India, Kenia, South Australia, Southwest Assia, China, and Cape St Francis identified Poaceae, Fabaceae, Asteraceae, Euphorbiaceae, Rubiaceae, and Malvaceae as the dominant families (Abuodha et al., 2003, Khan and Qaiser 2006, Zhao et al., 2011, Ghazanfar et al., 2014). In these regions, psammophytes (sand-adapted plants), herbaceous form (grasses), and shrubs exhibit the highest species richness on the life-form spectra (Rodrigues et al., 2011, Cowling et al., 2019, Jr 2019, Valcheva et al., 2020, Grobler and Cowling 2021, Romeroso et al., 2021, Anbarashan et al., 2022).

In Vietnam, the concept of halophyte in relation to coastal sandy plant remains relatively new although the floristic studies have been conducted. Surveys on Truong Sa Archipelago flora documented its biodiversity (265 species) and analyzing lifeform proportions (Tran et al., 2022). Before that, research on species composition and biodiversity in Quang Tri province's sand dune region (311 species) and Doi Hong, Phan Thiet province (96 species) provided insights into coastal composition (Ho et al., 2018, Hoang et al., 2020). These authors indicate the dominant plant families are Fabaceae, Poaceae, Cyperaceae, Asteraceae, Euphorbiaceae, Apocynaceae, Verbenaceae, Rubiaceae, Myrtaceae, Malvaceae, Lauraceae, Convolvulaceae, Capparaceae, Connaraceae, Convolvulaceae and Sterculiaceae on coastal sand dune ecosystem. The findings are presentative for islands ecosystem as well as the north and the south coastal region of Central Vietnam (Ho et al., 2018, Hoang et al., 2020, Tran et al., 2022). However, these studies were conducted in relatively small scale and unmentioned to address the ecological aspects of salt resilience in the context of halophytes, as outlined in Grigore's (2020). The present research integrates ecological perspectives on foredune plant composition in the South-Central Coast of Vietnam.

The South-Central Coast is characterized by low rainfall and high temperature. So, plants growing in this environment are affected by multiple stressors including drought, saltwater intrusion into the soil, evaporation, and nutrient-poor sandy soil. Coastal salt-tolerant plant communities play a crucial role in stabilizing sandy ecosystems, serving as the first vegetation cover negatively impacted by climate change and human activities in the South-Central Coast of Vietnam, particularly sea level rise and tourism activities. Increasing risks of species loss, habitat degradation, and biodiversity decline highlight the need for comprehensive survey and assessment of halophytic plant communities in this region. Therefore, establishing databases of the halophytic community of the foredunes in the South of Central Coast of Vietnam is critical for evaluating the future impacts of climate change on foredune ecosystems and implementing effective conservation strategies.

MATERIAL AND METHODS

Study area: The South-Central Coast of Vietnam comprises eight provinces: Da Nang, Quang Nam, Quang Ngai, Binh Dinh, Phu Yen, Khanh Hoa, Ninh Thuan, and Binh Thuan (Fig. 1a, b). This study was conducted on foredune ecosystems (coastal dunes near seashores). The total of 41 study sites were established along the shoreline to assess plant taxa distribution (Fig. 1c). Global Positioning System (GPS) coordinates were recorded for each site at a local scale (Table 1). Sampling locations were selected to represent the foredunes, with monitoring sites randomly distributed at a minimum distance of 30 km from each other.

Source: Google Map 2024

Fig. 1. Surveyed zone a. Vietnam position, b. The South-Central Coast zone, c. Surveyed sites

Sampling and data analysis: Samples containing reproductive organs (flowers, fruits) and vegetative parts were collected on foredunes through five field trips. Diane Bridson and Leonard Forma's Herbarium Handbook (1999) employed drying samples and voucher preparation (Bridson and Forma 1999). The dried specimens made a checklist after identifying taxa using illustrated guides and literature (Pham 1999, Nguyen et al., 2003, 2005, Le et al., 2015, Ho et al., 2018, Hoang et al., 2020, Tran et al., 2022), and online available herbariums (VMN, DLU, K). The terms of habits are based on Henk Beentje (2010). The plant classification follows APG IV (https://www.mobot.org/mobot /research/apweb/). The figures were analyzed using Excel software, and survey map was created using Google Maps (2025).

RESULTS AND DISCUSSION

Diversity of flora: Halophytic plant identification was reported on incipient foredunes from the South-Central Coast of Vietnam after sampling had occurred comprises 195 native taxa, 64 families, and 165 genera (Fig. 2). All species were documented as belonging to Angiospermae (Table 4). The surveys of Ho et al.,.(2018) listed 96 plant species of 92 genus, 54 families of Magnoliophyta in Hong hill, a sand dune in Phan Thiet province while the other survey of Hoang et al. (2020). Quang Tri province's sand dunes, recorded a significantly higher diversity, with 311 species belonging to 226 genera, 94 families. The higher species richness observed in Quang Tri is likely due to its inland and landward sand dune ecosystems, which support greater biodiversity. In contrast, surveys conducted along the South-Central Coast focused on foredunes and sandy beaches near the shoreline, where halophytic species must adapt to extreme environmental conditions such as high salinity from seawater, salty winds, drought, loose sandy substrates, and nutrient-poor soils. These harsh conditions result in a lower number of recorded species compared to inland sand dune ecosystems.

Among the 64 plant families identified, 8 belong to Monocots (12.5%) and 56 to Eudicots (87.5%). Of the 165 genera recorded, 21 (12.73%) are Monocots, while 144 (87.27%) are Eudicots. In terms of species diversity, Monocots comprise 26 species (13.33%), whereas Eudicots account for 169 species (86.67%) (Fig. 2).

Fabaceae is the most diverse family, with the highest number of genera (20) and species (25), followed by Asteraceae (13 genera, 13 species), Malvaceae (10 genera, 12 species), and Poaceae (10 genera, 11 species), collectively representing 32.12% of total genera and 31.28% of total species (Table 2). Other families with notable richness

Table 1. Ethnobotanical and botany sites

Code	Latitude	Longitude	Localities
Vin1	11°59'20"N	109°12'45"E	Nguyen Chi Thanh Street, Cam Ranh City, Khanh Hoa Province
Vin2	12°00'27"N	109°12'30"E	Nguyen Tat Thanh Street, Cam Ranh City, Khanh Hoa Province
Vin3	12°44'16.7"N	109°22'27.2"E	Dam Mon port, Van Ninh district, Khanh Hoa province
Vin4	12°39'49"N	109°24'51"E	Dam Mon port, Van Ninh district, Khanh Hoa province
Vin5	12°05'22.1"N	109°11'41.8"E	Gac Ma Memorial Area, Cam Lam District, Khanh Hoa Province
Vin6	13°07'53.8"N	109°17'53.6"E	Nghinh Phong Tower, Tuy Hoa district, Phu Yen province
Vin7	13°34'56.7"N	109°15'54.9"E	Song Cau Town, Phu Yen Province
Vin8	13°30'28.6"N	109°17'23.7"E	Vinh Hoa, Song Cau Town, Phu Yen Province
Vin9	13°27'57.3"N	109°18'19.1"E	Tu Nham Beach, Song Cau Town, Phu Yen Province
Vin10	13°28'44.8"N	109°17'39"E	Tu Nham Beach, Song Cau Town, Phu Yen Province
Vin11	13°35'59"N	109°14'15"E	Song Cau Town, Phu Yen Province
Vin12	13°52'58"N	109°17'00"E	Quy Nhon City, Binh Dinh Province
Vin13	13°42'51"N	109°12'53"E	Science Avenue, Quy Nhon City, Binh Dinh Province
Vin14	12°33'44.5"N	109°13'54"E	Ninh Hoa Town, Khanh Hoa Province
Vin15	11°48'36"N	109°11'12"E	Binh Tien Beach, Cam Ranh City, Khanh Hoa Province
Vin16	11°45'35"N	109°12'38"E	Ninh Hai district, Ninh Thuan province
Vin17	11°48'36"N	109°11'32"E	Binh Tien Beach, Ninh Thuan Province
Vin18	10°54'57.7"N	108°17'19.2"E	Mui Ne beach, Phan Thiet city, Binh Thuan Province
Vin19	10°70'93"N	107°99'61"E	Ke Ga Beach, Tan Thanh Commune, Binh Thuan Province
Vin20	10°40'57.0"N	107°47'52.8"E	La Gi District, Binh Thuan Province
Vin21	10°56'46.4"N	108°16'02.9"E	Tien Thanh Commune, Phan Thiet City, Binh Thuan Province
Vin22	10°56'56.5"N	108°18'03.5"E	Mui Ne beach, Phan Thiet city, Binh Thuan Province
Vin23	90°61'25"N	107°66'60"E	Tan Thang Commune, Ham Tan District, Binh Thuan, Binh Thuan Province
Vin24	11°02'10.3"N	108°24'21.4"E	Tan Binh Commune, La Gi District, Binh Thuan Province
Vin25	11°03'47.8"N	108°27'26.6"E	Bac Binh district, Binh Thuan Province
Vin26	11°10'40.8"N	108°43'05.9"E	Bac Binh district, Binh Thuan Province
Vin27	11°00'32.4"N	108°21'03.7"E	Hon Rom, Phan Thiet City, Binh Thuan Province
Vin28	11°15'32.4"N	108°44'55.7"E	Phuoc The, Tuy Phong District, Binh Thuan Province
Vin29	11°22'56.6"N	108°59'43.6"E	Phuoc Dinh, Thuan Nam District, Ninh Thuan Province
Vin30	11°19'56.3"N	108°50'39.5"E	Vinh Tan, Tuy Phong District, Binh Thuan Province
Vin31	14°38'53.4"N	109°03'55.8"E	Sa Huynh, Pho Chau Commune, Duc Pho District, Quang Ngai Province
Vin32	15°14'25.0"N	108°56'23.9"E	Ba Lang An Beach, Tinh Ky Commune, Binh Son District, Quang Ngai Province
Vin33	15°11'19.3"N	108°53'41.7"E	My Khe Beach, Tinh Khe Commune, Son Tinh District, Quang Ngai Province
Vin34	15°15'47.4"N	108°53'36.1"E	Chau Tan Beach, Binh Chau Commune, Binh Son District, Quang Ngai Province
Vin35	15°37'19.6"N	108°31'40.6"E	Tinh Thuy village, Tam Thanh District, Quang Nam Province
Vin36	16°06'02.2"N	108°15'23.2"E	Hoang Sa Street, Tho Quang Ward, Son Tra District, Da Nang City
Vin37	16°08'17.2"N	108°07'23.9"E	Son Tra Peninsula, Tho Quang Ward, Son Tra District, Da Nang City
Vin38	16°06'16.4"N	108°17'58.1"E	South Beach, Son Tra Peninsula, Tho Quang Ward, Son Tra District, Da Nang City
Vin39	13°43'32.7"N	109°13'04.0"E	Science Avenue, Quy Nhon City, Binh Dinh Province
Vin40	13°01'24.2"N	109°21'53.2"E	Hiep Hoa Bac commune, Dong Hoa District, Phu Yen Province
Vin41	16°09'44.7"N	108°08'19.1"E	Van Village, Hai Van Pass, Hiep Hoa Bac District, Da Nang City

Table 2. Number of genera by family

Families	Number of genus	Percentage of genus	Number of species	Percentage of species
Fabaceae	20	12.12	25	12.82
Asteraceae	13	7.88	13	6.67
Malvaceae	10	6.06	12	6.15
Poaceae	10	6.06	11	5.64
Apocynaceae	9	5.45	9	4.62
Lamiaceae	8	4.85	8	4.10
Amaranthaceae	6	3.64	6	3.08
Euphorbiaceae	5	3.03	9	4.62
Rubiaceae	5	3.03	8	4.10
Cyperaceae	3	1.82	7	3.59
Phyllanthaceae	3	1.82	4	2.05
Annonaceae	3	1.82	3	1.54
Commelinaceae	3	1.82	3	1.54
Dipterocarpaceae	3	1.82	3	1.54
Rutaceae	3	1.82	3	1.54
Sapindaceae	3	1.82	3	1.54
Convolvulaceae	2	1.21	5	2.56
Combretaceae	2	1.21	3	1.54
Sapotaceae	2	1.21	3	1.54
Solanaceae	2	1.21	3	1.54
Aizoaceae	2	1.21	2	1.03
Capparaceae	2	1.21	2	1.03
Cucurbitaceae	2	1.21	2	1.03
Myrtaceae	2	1.21	2	1.03
Verbenaceae	2	1.21	2	1.03
Zygophyllaceae	2	1.21	2	1.03
Caryophyllaceae	1	0.61	3	1.54
Nyctaginaceae	1	0.61	2	1.03
Thymelaeaceae	1	0.61	2	1.03
Acanthaceae	1	0.61	1	0.51
Annacardiaceae	1	0.61	1	0.51
Arecaceae	1	0.61	1	0.51
Bignoliaceae	1	0.61	1	0.51
Boraginaceae	1	0.61	1	0.51
Cactaceae	1	0.61	1	0.51
Calophyllaceae	1	0.61	1	0.51
Celastreaceae	1	0.61	1	0.51
Colchicaceae	1	0.61	1	0.51
Connaraceae	1	0.61	1	0.51
Ebenaceae	1	0.61	1	0.51
Ericaulaceae	1	0.61	1	0.51
Gisekiaceae	1	0.61	1	0.51
Goodeniaceae	1	0.61	1	0.51

Cont...

Table 2. Number of genera by family

Families	Number of genus	Percentage of genus	Number of species	Percentage of species
Lecythidaceae	1	0.61	1	0.51
Linderniaceae	1	0.61	1	0.51
Melastomataceae	1	0.61	1	0.51
Molluginaceae	1	0.61	1	0.51
Moraceae	1	0.61	1	0.51
Moringaceae	1	0.61	1	0.51
Nepenthaceae	1	0.61	1	0.51
Oleaceae	1	0.61	1	0.51
Oxalidaceae	1	0.61	1	0.51
Pandanaceae	1	0.61	1	0.51
Passifloraceae	1	0.61	1	0.51
Plantaginaceae	1	0.61	1	0.51
Portulacaceae	1	0.61	1	0.51
Primulaceae	1	0.61	1	0.51
Restionaceae	1	0.61	1	0.51
Rhamnaceae	1	0.61	1	0.51
Salicaceae	1	0.61	1	0.51
Salvadoraceae	1	0.61	1	0.51
Simaroubaceae	1	0.61	1	0.51
Urticaceae	1	0.61	1	0.51
Xyridaceae	1	0.61	1	0.51
Total	165	100	195	100

include Apocynaceae, Lamiaceae, Amaranthaceae, Euphorbiaceae, Rubiaceae, Cyperaceae, and Phyllanthaceae. The ten most species-rich families contribute 55.38% of the total floristic diversity in the coastal foredunes. In contrast, 37 families within the halophytic community are represented by single genus and single species, comprising 57.8% of the total recorded families). This highlights the ecological specificity and limited distribution of many halophytic taxa in the region.

The first four families, Fabaceae, Asteraceae, Malvaceae, and Poaceae, are one of the most diversity genera and species of halophytes worldwide (Santos et al., 2016). This result also aligns with the halophytic families and genera reported in Southwest Asia, China, and Pakistan as well as global (Kefu et al., 2002, Khan and Qaiser 2006, Zhao et al., 2011, Ghazanfar et al., 2014, Santos et al., 2016). Furthermore, the dominant families are identified in this study are consistent with previous researches on coastal sand dune flora in Vietnam (Ho et al., 2018, Hoang et al., 2020, Tran et al., 2022), although some differences are noted, particularly in the presence of Capparaceae, Lauraceae, Sterculiaceae.

Amaranthaceae (including Amaranthaceae *s. str.* and Chenopodiaceae *s. str.*) is the most significant contributor to species richness worldwide and regionally of halophytes (Santos et al., 2016). It also is one of the most diverse families regarding the richness of genera and species on foredunes from the South-Central Vietnam. These results suggest that halophytic composition of the South-Central Coast's foredunes is resemble to tropical desert ecosystem (Southwest Asia, China, Pakistan and Vietnam).

Diversity of habit forms: The habit spectrum of foredune plants shows that herbs predominate and constitute 23.59% (46 species) of the recorded taxa, followed by tree (16.92%, 33 species), decumbent shrub (9.74%, 19 species), virgate shrub (8.21%, 16 species), ascending shrub (7.18%, 14

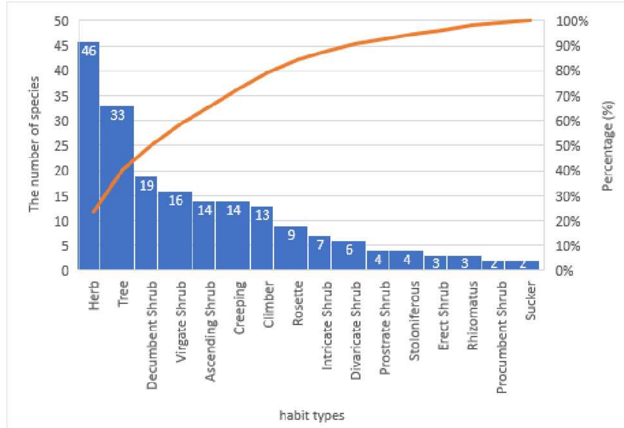

Fig. 3. The habit formed by numbers and accumulated percentage

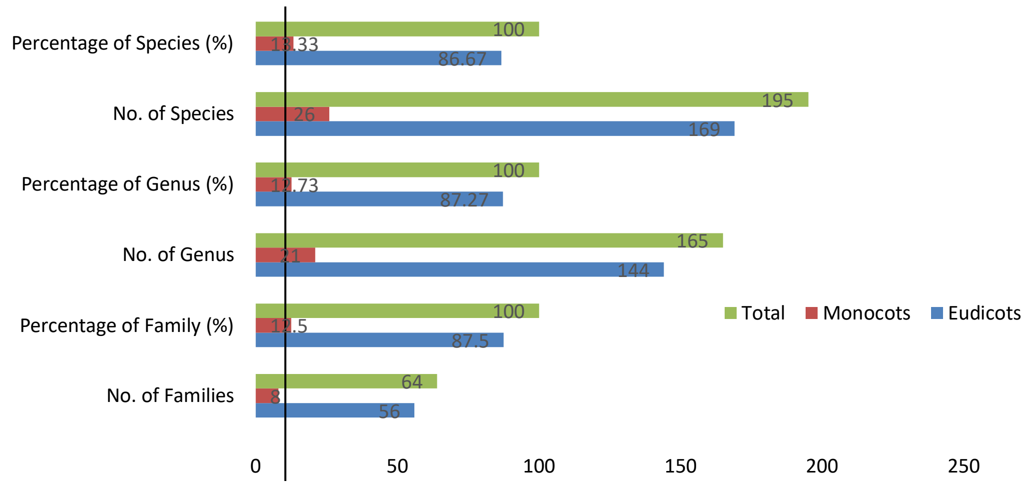

Fig. 2. Number and percentage by taxa

Fig. 4. Forms of foredunes

Table 3. Common plant and habit form on foredune type

Fore dunes	Habit forms	Common species
Incipient foredunes	Creepers, prostrate, herbs, rhizomatous, stoloniferous	*Ipomoea pes-caprae, Launaea sarmentosa, Spinifex littoreus, Vitex rotundifolia, Cyperus stoloniferus, Canavalia cathartica, Zoysia matrella*
Established foredunes	Shrubs, woody, climbers	*Canavalia cathartica, Zoysia matrella, Clerodendrum inerme, Scaevola taccada, Heliotropium foertherianum, Premna serratifolia, Severinia monophylla, Buchanania reticulata, Calophyllum inophyllum*

Table 4. Checklist of Halophytes in the south-central coast of Vietnam

Scientific names	Genus	Family	Group	Habits
Abrus precatorius L.	*Abrus*	Fabaceae	Eudicots	Climber
Abutilon indicum (L). Sweet.	*Abutilon*	Malvaceae	Eudicots	Virgate Shrub
Acalypha indica L.	*Acalypha*	Euphorbiaceae	Eudicots	Herb
Achyranthes aspera L.	*Achyranthes*	Amaranthaceae	Eudicots	Ascending Shrub
Adenanthera pavonina L.	*Adenanthera*	Fabaceae	Eudicots	Tree
Ageratum conyzoides L.	*Ageratum*	Asteraceae	Eudicots	Herb
Albizia corniculata (Lour.) Druce	*Albizia*	Fabaceae	Eudicots	Tree
Alternanthera sessilis (L.) A. DC.	*Alternanthera*	Amaranthaceae	Eudicots	Decumbent Shrub
Alysicarpus vaginalis (L.) DC.	*Alysicarpus*	Fabaceae	Eudicots	Procumbent Shrub
Amaranthus lividus L.	*Amaranthus*	Amaranthaceae	Eudicots	Herb
Antidesma cochinchinensis Gagnep.	*Antidesma*	Phyllanthaceae	Eudicots	Tree
Artabotrys sp.	*Artabotrys*	Annonaceae	Eudicots	Ascending Shrub
Arthraxon hispidus (Thunb.) Makino	*Athraxon*	Poaceae	Monocots	Rhizomatous
Atalantia buxifolia (Poir.) Oliv. ex Benth.	*Atalantia*	Rutaceae	Eudicots	Divaricate Shrub
Axonopus compressus (Sw.) Beauv.	*Axonopus*	Fabaceae	Eudicots	Rhizomatous
Azima sarmentosa (Blume) Benth. & Hook. f.	*Azima*	Salvadoraceae	Eudicots	Divaricate Shrub
Balanites roxburghii Planch.	*Balanites*	Zygophyllaceae	Eudicots	Tree
Barringtonia asiatica (L.) Kurz.	*Barringtonia*	Lecythidaceae	Eudicots	Tree
Bauhinia viridescens Desv.	*Bauhinia*	Fabaceae	Eudicots	Tree
Bidens pilosa L.	*Bidens*	Asteraceae	Eudicots	Herb
Blumea laevis (Lour.) Merr.	*Blumea*	Asteraceae	Eudicots	Decumbent Shrub
Boerhavia chinensis (L.) Rottb.	*Boerhavia*	Nyctaginaceae	Eudicots	Herb
Boerhavia diffusa L.	*Boerhavia*	Nyctaginaceae	Eudicots	Decumbent Shrub
Breynia fruticosa (L.) Hook. f.	*Breynia*	Euphorbiaceae	Eudicots	Virgate Shrub
Brucea javanica (L.) Merr.	*Brucea*	Simaroubaceae	Eudicots	Virgate Shrub
Buchanania reticulata Hance	*Buchanania*	Annacardiaceae	Eudicots	Decumbent Shrub
Cajanus scarabaeoides (L.) Thouars	*Cajanus*	Fabaceae	Eudicots	Climber
Callicarpa candicans (Burm.f.) Hchr	*Callicarpa*	Lamiaceae	Eudicots	Virgate Shrub
Calophyllum inophyllum L.	*Calophyllum*	Calophyllaceae	Eudicots	Tree
Calotis anamitica Merr.	*Calotis*	Asteraceae	Eudicots	Herb
Calotropis gigantea (L.) W.T.Aiton	*Calotropis*	Apocynaceae	Eudicots	Virgate Shrub
Canavalia cathartica Thouars	*Canavalia*	Fabaceae	Eudicots	Creeping
Canavalia rosea (Sw.) DC.	*Canavalia*	Fabaceae	Eudicots	Creeping
Capparis annamensis (Baker f.) M. Jacobs	*Capparis*	Capparaceae	Eudicots	Ascending Shrub
Catharanthus roseus (L.) G. Don	*Catharanthus*	Apocynaceae	Eudicots	Herb
Cenchrus brownii Roem. & Schult.	*Cenchrus*	Poaceae	Monocots	Herb
Centrosema pubescens Benth.	*Centrosema*	Fabaceae	Eudicots	Climber
Cerbera manghas L.	*Cerbera*	Apocynaceae	Eudicots	Tree
Christia pierrei (Schindl.) Ohashi	*Christia*	Fabaceae	Eudicots	Decumbent Shrub
Chromolaena odorata (L.) R.M.King & H.Rob.	*Chromolaena*	Asteraceae	Eudicots	Intricate Shrub
Cleome viscosa L.	*Cleome*	Capparaceae	Eudicots	Herb
Coccinia grandis (L.) Voigt	*Coccinia*	Cucurbitaceae	Eudicots	Climber
Colubrina asiatica (L.) Brongn.	*Colubrina*	Rhamnaceae	Eudicots	Ascending Shrub

Cont...

Table 4. Checklist of Halophytes in the south-central coast of Vietnam

Scientific names	Genus	Family	Group	Habits
Combretum deciduum Coll. & Hemsley	*Combretum*	Combretaceae	Eudicots	Tree
Combretum quadrangulare Kurz.	*Combretum*	Combretaceae	Eudicots	Tree
Commelina benghalensis L.	*Commelina*	Commelinaceae	Monocots	Decumbent Shrub
Connarus semidecandrus Jack	*Connarus*	Connaraceae	Eudicots	Tree
Conyza sp.	*Conyza*	Asteraceae	Eudicots	Herb
Corchorus aestuans L.	*Corchorus*	Malvaceae	Eudicots	Herb
Crotalaria anagyroides Kunth	*Crotalaria*	Fabaceae	Eudicots	Erect Shrub
Crotalaria retusa L.	*Crotalaria*	Fabaceae	Eudicots	Virgate Shrub
Croton dongnaiensis Pierre ex Gagnep.	*Croton*	Euphorbiaceae	Eudicots	Virgate Shrub
Croton hirtus L'Hér	*Croton*	Euphorbiaceae	Eudicots	Herb
Cucumis melo L.	*Cucumis*	Cucurbitaceae	Eudicots	Creeping
Cyanotis cristata (L.) D. Don	*Cyanotis*	Commelinaceae	Monocots	Rosette
Cynanchum hooperianum (Blume) Liede & Khanum	*Cynanchum*	Apocynaceae	Eudicots	Climber
Cyperus bulbosus Vahl	*Cyperus*	Cyperaceae	Monocots	Rosette
Cyperus radians Nees & Meyen ex Kunth	*Cyperus*	Cyperaceae	Monocots	Rosette
Cyperus sp2.	*Cyperus*	Cyperaceae	Monocots	Herb
Cyperus stoloniferus Retz.	*Cyperus*	Cyperaceae	Monocots	Stoloniferous
Dactyloctenium aegyptium (L.) Willd	*Dactyloctenium*	Poaceae	Monocots	Rhizomatous
Dapsilanthus disjunctus (Mast.) B.G.Briggs & L.A.S.Johnson	*Dapsilanthus*	Restionaceae	Monocots	Sucker
Decaspermum parviflorum (Lam.) A.J.Scott (Lam.) A.J.Scott	*Decaspermum*	Myrtaceae	Eudicots	Erect Shrub
Deeringia amaranthoides (Lam.) Merr.	*Deeringia*	Amaranthaceae	Eudicots	Ascending Shrub
Desmodium harmsii Schindl.	*Desmodium*	Fabaceae	Eudicots	Erect Shrub
Desmodium sp.	*Desmodium*	Fabaceae	Eudicots	Herb
Dimocarpus longan Lour.	*Dimocarpus*	Sapindaceae	Eudicots	Tree
Diospyros nhatrangensis Lecomte	*Diospyros*	Ebenaceae	Eudicots	Tree
Dodonaea viscosa Jacq.	*Dodonaea*	Sapindaceae	Eudicots	Virgate Shrub
Eclipta prostrata L.	*Eclipta*	Asteraceae	Eudicots	Herb
Epaltes australis Less.	*Epaltes*	Asteraceae	Eudicots	Creeping
Eragrostis zeylanica Nees & Meyen	*Eragrostis*	Poaceae	Monocots	Herb
Eriocaulon aff. *cinereum*	*Eriocaulon*	Ericaulaceae	Monocots	Herb
Euphorbia atoto Forst. & Forst. f.	*Euphorbia*	Euphorbiaceae	Eudicots	Decumbent Shrub
Euphorbia heterophylla L.	*Euphorbia*	Euphorbiaceae	Eudicots	Herb
Euphorbia hirta L.	*Euphorbia*	Euphorbiaceae	Eudicots	Decumbent Shrub
Euphorbia thymifolia L.	*Euphorbia*	Euphorbiaceae	Eudicots	Prostrate Shrub
Evolvulus alsinoides (L.) L.	*Evolvulus*	Convolvulaceae	Eudicots	Herb
Fimbristylis lasiophylla J. Kern	*Fimbristylis*	Cyperaceae	Monocots	Rosette
Fimbristylis sericea R. Br.	*Fimbristylis*	Cyperaceae	Monocots	Rosette
Gisekia pharnaceoides L.	*Gisekia*	Gisekiaceae	Eudicots	Creeping
Glinus oppositifolius (L.) Aug.DC.	*Glinus*	Molluginaceae	Eudicots	Decumbent Shrub
Gloriosa superba L.	*Gloriosa*	Colchicaceae	Monocots	Herb
Gmelina asiatica L.	*Gmelina*	Lamiaceae	Eudicots	Ascending Shrub
Gomphrena celosioides Mart.	*Gomphrena*	Amaranthaceae	Eudicots	Prostrate Shrub

Cont...

Table 4. Checklist of Halophytes in the south-central coast of Vietnam

Scientific names	Genus	Family	Group	Habits
Gynura lycopersicifolia DC.	*Gynura*	Asteraceae	Eudicots	Herb
Hedyotis auriculata L.	*Hedyotis*	Rubiaceae	Eudicots	Herb
Hedyotis crassifolia A. DC.	*Hedyotis*	Rubiaceae	Eudicots	Herb
Hedyotis sp.	*Hedyotis*	Rubiaceae	Eudicots	Herb
Heliotropium indicum L.	*Heliotropium*	Boraginaceae	Eudicots	Herb
Hexasepalum sarmentosum (Sw.) Delprete & J.H.Kirkbr.	*Hexasepalum*	Rubiaceae	Eudicots	Procumbent Shrub
Hibiscus tiliaceus L.	*Hibicus*	Malvaceae	Eudicots	Tree
Hopea cordata Vidal	*Hopea*	Dipterocarpaceae	Eudicots	Tree
Ichnocarpus frutescens (L.) W.T.Aiton	*Ichnocarpus*	Apocynaceae	Eudicots	Climber
Indigofera tinctoria L.	*Indigofera*	Fabaceae	Eudicots	Virgate Shrub
Ipomoea imperati (Vahl) Griseb.	*Ipomoea*	Convolvulaceae	Eudicots	Creeping
Ipomoea obscura (L.) Ker Gawl.	*Ipomoea*	Convolvulaceae	Eudicots	Climber
Ipomoea pes-caprae (L.) R. Br.	*Ipomoea*	Convolvulaceae	Eudicots	Creeping
Ipomoea pes-tigridis L.	*Ipomoea*	Convolvulaceae	Eudicots	Climber
Jasminum nervosum Lour.	*Jasminum*	Oleaceae	Eudicots	Ascending Shrub
Launaea sarmentosa (Willd.) Kuntze.	*Launaea*	Asteraceae	Eudicots	Stoloniferous
Leonotis nepetifolia (L.) R. Br.	*Leonotis*	Lamiaceae	Eudicots	Herb
Lepisanthes rubiginosa (Roxb.) Leenh	*Lepisanthes*	Sapindaceae	Eudicots	Tree
Leucaena leucocephala (Lam.) De Wit	*Leucaena*	Fabaceae	Eudicots	Tree
Leucas aspera (Willd.) Link	*Leucas*	Lamiaceae	Eudicots	Herb
Lycianthes biflora (Lour.) Bitter	*Lycianthes*	Solanaceae	Eudicots	Herb
Macroptilium atropurpureum (DC.) Urb.	*Macroptilium*	Fabaceae	Eudicots	Climber
Malvastrum coromandelianum (L.) Garcke	*Malvastrum*	Malvaceae	Eudicots	Ascending Shrub
Markhamia stipulata (Wall.) Seem. var. *pierrei* (Dop) Santisuk.	*Markhamia*	Bignoliaceae	Eudicots	Tree
Memecylon umbellatum Burm. f.	*Memecylon*	Melastomataceae	Eudicots	Ascending Shrub
Micromelum minutum (Forst.f.) Wight & Arn.	*Micromelum*	Rutaceae	Eudicots	Virgate Shrub
Microstachys chamaelea (L.) Müll. Arg.	*Microstachys*	Euphorbiaceae	Eudicots	Decumbent Shrub
Miliusa bangoiensis Ast.	*Miliusa*	Annonaceae	Eudicots	Tree
Moringa oleifera Lam.	*Moringa*	Moringaceae	Eudicots	Tree
Murdannia spectabilis (Kurz) Fade	*Murdannia*	Commelinaceae	Monocots	Rosette
Myrsine linearis (Lour.) S.	*Myrsine*	Primulaceae	Eudicots	Tree
Nepenthes mirabilis (Lour.) Druce	*Nepenthes*	Nepenthaceae	Eudicots	Herb
Ocimum basilicum L. var. *pilosum* (Willd.) Benth.	*Ocimum*	Lamiaceae	Eudicots	Herb
Oldenlandia corymbosa L.	*Oldenlandia*	Rubiaceae	Eudicots	Herb
Opuntia stricta (Haw.) Haw.	*Opuntia*	Cactaceae	Eudicots	Virgate Shrub
Oxalis barrelieri L.	*Oxalis*	Oxalidaceae	Eudicots	Herb
Pandanus odorifer (Forssk.) Kuntze	*Pandanus*	Pandanaceae	Monocots	Tree
Panicum repens L.	*Panicum*	Poaceae	Monocots	Stoloniferous
Passiflora foetida L.	*Passiflora*	Passifloraceae	Eudicots	Climber
Pennisetum alopecuroides (L.) Spreng.	*Pennisetum*	Poaceae	Monocots	Herb
Pentatropis pierrei Costantin	*Pentatropis*	Apocynaceae	Eudicots	Creeping
Phoenix humilis Royle	*Phoenix*	Arecaceae	Monocots	Rosette

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Table 4. Checklist of Halophytes in the south-central coast of Vietnam

Scientific names	Genus	Family	Group	Habits
Phragmites sp.	*Phragmites*	Poaceae	Monocots	Virgate Shrub
Phyla nodiflora (L.) Greene	*Phyla*	Verbenaceae	Eudicots	Creeping
Phyllanthus amarus Schumach. & Thonn.	*Phyllanthus*	Phyllanthaceae	Eudicots	Herb
Phyllanthus arenarius Beille in Lecomte	*Phyllanthus*	Phyllanthaceae	Eudicots	Decumbent Shrub
Pithecellobium dulce (Roxb.) Benth.	*Pithecellobium*	Fabaceae	Eudicots	Tree
Planchonella obovata (R. Br.) Pierre	*Planchonella*	Sapotaceae	Eudicots	Tree
Pleiospermium littorale (Miq.) Tanaka	*Pleiospermium*	Rutaceae	Eudicots	Intricate Shrub
Pluchea indica (L.) Less	*Pluchea*	Asteraceae	Eudicots	Intricate Shrub
Polycarpaea chungana V.T. Tran, H.T. Truong, N.V. Binh	*Polycarpaea*	Caryophyllaceae	Eudicots	Decumbent Shrub
Polycarpaea gaudichaudii Gagnep.	*Polycarpaea*	Caryophyllaceae	Eudicots	Herb
Polycarpaea vanana V.T. Tran, H.T. Truong, N.V. Binh	*Polycarpaea*	Caryophyllaceae	Eudicots	Herb
Pongamia pinnata (L.) Pierre	*Pongamia*	Fabaceae	Eudicots	Tree
Portulaca pilosa L.	*Portulaca*	Portulacaceae	Eudicots	Creeping
Pouzolzia zeylanica (L.) Benn	*Pouzolzia*	Urticaceae	Eudicots	Decumbent Shrub
Premna serratifolia L.	*Premna*	Lamiaceae	Eudicots	Intricate Shrub
Raphistemma pulchellum (Roxb.) Wall.	*Raphistemma*	Apocynaceae	Eudicots	Climber
Rhynchospora aff. *triflora*	*Rhynchospora*	Cyperaceae	Monocots	Rosette
Ruellia tuberosa L.	*Ruellia*	Acanthaceae	Eudicots	Herb
Sauropus bacciformis (L.) Airy Shaw	*Sauropus*	Phyllanthaceae	Eudicots	Decumbent Shrub
Scaevola taccada (Gaertn.) Roxb	*Scaevola*	Goodeniaceae	Eudicots	Intricate Shrub
Scleromitrion pinifolium (Wall. ex G.Don) R. J. Wang	*Scleromitrion*	Rubiaceae	Eudicots	Herb
Scolopia buxifolia Gagnep.	*Scolopia*	Salicaceae	Eudicots	Divaricate Shrub
Scoparia dulcis L.	*Scoparia*	Plantaginaceae	Eudicots	Herb
Sesuvium portulacastrum (L.) L.	*Sesuvium*	Aizoaceae	Eudicots	Creeping
Shorea falcata J. E. Vidal	*Shorea*	Dipterocarpaceae	Eudicots	Tree
Sida cordifolia L.	*Sida*	Malvaceae	Eudicots	Virgate Shrub
Sida rhombifolia L.	*Sida*	Malvaceae	Eudicots	Decumbent Shrub
Sida subcordata Span.	*Sida*	Malvaceae	Eudicots	Decumbent Shrub
Sindora siamensis Teysm. ex Miq. var. *siamensis* (Teysm. ex Miq.) Bake	*Sindora*	Fabaceae	Eudicots	Tree
Solanum americanum Mill.	*Solanum*	Solanaceae	Eudicots	Herb
Solanum torvum Sw.	*Solanum*	Solanaceae	Eudicots	Virgate Shrub
Spermacoce articularis L.f.	*Spermacoce*	Rubiaceae	Eudicots	Herb
Spermacoce setidens (Miq.) Boerl.	*Spermacoce*	Rubiaceae	Eudicots	Herb
Spinifex littoreus (Burm. f.) Merr.	*Spinifex*	Poaceae	Monocots	Stoloniferous
Stachytarpheta jamaicensis (L.) Vahl.	*Stachytarpheta*	Verbenaceae	Eudicots	Ascending Shrub
Sterculia foetida L.	*Sterculia*	Malvaceae	Eudicots	Tree
Streblus ilicifolius (S. Vidal) Corner	*Streblus*	Moraceae	Eudicots	Intricate Shrub
Streptocaulon juventas (Lour.) Merr.	*Streptocaulon*	Apocynaceae	Eudicots	Climber
Suaeda maritima (L.) Dumort.	*Suaeda*	Amaranthaceae	Eudicots	Sucker
Syzygium cumini (L.) Skeels	*Syzygium*	Myrtaceae	Eudicots	Tree
Tephrosia coccinea Wall.	*Tephrosia*	Fabaceae	Eudicots	Ascending Shrub

Cont...

Table 4. Checklist of Halophytes in the south-central coast of Vietnam

Scientific names	Genus	Family	Group	Habits
Tephrosia villosa (L.) Pers.	*Tephrosia*	Fabaceae	Eudicots	Ascending Shrub
Terminalia catappa L.	*Terminalia*	Combretaceae	Eudicots	Tree
Thespesia populnea (L.) Sol. ex Corrêa	*Thespesia*	Malvaceae	Eudicots	Tree
Torenia oblonga (Benth.) Hance	*Torenia*	Linderniaceae	Eudicots	Herb
Toxocarpus wightianus Hook. & Arn.	*Toxocarpus*	Apocynaceae	Eudicots	Climber
Trianthema portulacastrum L.	*Trianthema*	Aizoaceae	Eudicots	Decumbent Shrub
Tribulus terrestris L.	*Tribulus*	Zygophyllaceae	Eudicots	Decumbent Shrub
Tridax procumbens L.	*Tridax*	Asteraceae	Eudicots	Creeping
Tripogon aff. *thorelii*	*Tripogon*	Poaceae	Monocots	Herb
Triumfetta grandidens Hance	*Triumfetta*	Malvaceae	Eudicots	Creeping
Uvaria siamensis (Scheff.) L.L.Zhou, Y.C.F.Su & R.M.K.Saunders	*Uvaria*	Annonaceae	Eudicots	Tree
Vatica mangachapoi Blanco	*Vatica*	Dipterocarpaceae	Eudicots	Tree
Vitex rotundifolia L.f.	*Vitex*	Lamiaceae	Eudicots	Creeping
Volkameria inermis L.	*Volkameria*	Lamiaceae	Eudicots	Divaricate Shrub
Waltheria indica L.	*Waltheria*	Malvaceae	Eudicots	Virgate Shrub
Wikstroemia dolichantha Diels	*Wikstroemia*	Thymelaeaceae	Eudicots	Divaricate Shrub
Wikstroemia indica (L.) C.A. Mey. indica	*Wikstroemia*	Thymelaeaceae	Eudicots	Divaricate Shrub
Wollastonia biflora (L.) DC.	*Wollastonia*	Asteraceae	Eudicots	Ascending Shrub
Xantolis baranensis (Lecomte) P.Royen	*Xantolis*	Sapotaceae	Eudicots	Ascending Shrub
Xantolis maritima (Pierre) P. Royen	*Xantolis*	Sapotaceae	Eudicots	Decumbent Shrub
Xyris complanata R.Br.	*Xyris*	Xyridaceae	Eudicots	Rosette
Zoysia matrella (L.) Merr.	*Zoysia*	Poaceae	Monocots	Herb
Zoysia seslerioides (Balansa) Clayton & F.R.Richardson	*Zoysia*	Poaceae	Monocots	Herb

species), creeping (7.18%, 14 species), and climber (6.67%, 13 species), accounting for 79.49% (Fig. 3). Procumbent shrubs and suckers represent the least with two species per type, 1%, respectively These herbs and prostrate shrubs are predominantly distributed in sandbanks or drought foredunes near shorelines where the landscape resembles to the semi-desert in a much smaller scale (Fig. 4). These findings align closely with Ho et al. (2018) reported the most dominant growth forms in coastal dune vegetation are the herbaceous group (34.4%, 33 species), the shrubs (21.9%, 21 species) and the large trees (19.8%, 19 species). The climber plants and parasitic plants are the least contributing groups in Ho et al., 2018. In the other hand, in present study, climbing plant is ranked seventh among habit forms, while parasitic plants are absent from halophytic composition. In general, the shrub forms (decumbent shrub, virgate shrub, ascending shrub, intricate shrub, divaricate shrub, prostrate shrub, erect shrub, and procumbent shrub) comprise the most significant number of species, accounting for 71% of the shrub total.

Halophytic distribution on foredunes: The halophytic communities of coastal dunes contribute significantly to biomass production, sand surface stabilization, and dune integrity. Several halophytic species and habit forms widespread distribution and frequent occurrence across study sites (Table 3).

The incipient foredunes develop pioneer plant communities, predominantly consisting of herbaceous species, creepers, prostrate forms, rhizomatous plants, and stoloniferous species. Notably, creeping species are particularly dominant (Fig. 3) in this study. Hesp, 2002, and Martínez et al.,., 2008, observed that low-growing creepers are more diverse in tropical incipient foredunes, whereas taller grasses and sedges are more prevalent in temperate regions. This trend is evident in the South-Central Coast, *Ipomoea pes-caprae*, *Canavalia cathartica*, Apocynaceae predominant on the foredune flora. In contrast, temperate incipient foredunes are characterized by grasses and sedges such as *Ammophila arenaria*, *A. littoralis*, *Panicum* spp., *Spartina* spp., and *Sporobolus* spp. (Hesp and Walker 2013, Martínez et al., 2008). The findings of this study align with

Hesp's discussion, reaffirming the distinct vegetation patterns between tropical and temperate regions.

The established foredunes, in contrast, are predominantly composed of shrub forms, woody species, and climbers (Table 3). Some woody species, such as *Heliotropium foertherianum*, *Scaevola taccada*, *Calophyllum inophyllum*, *Guettarda speciosa*, and *Thespesia populnea*, are capable of surviving in wave-exposed zones, demonstrating their resilience to harsh coastal conditions. More stable foredunes, particularly in landward zones, support the development of taller shrubs (Fig. 4b). This landscape presents dwarf shrubs from sparse to dense vegetation in foredunes. The composition of habits indicates the distinguished vegetation between incipient dune and established foredune.

CONCLUSIONS

The halophytic composition on the foredunes of the South-Central Coast of Vietnam is characterized by high species diversity within the families Fabaceae, Asteraceae, Malvaceae, and Poaceae, followed by Apocynaceae, Lamiaceae, Amaranthaceae, Euphorbiaceae, Rubiaceae, Cyperaceae, and Phyllanthaceae. These families grow in the harsh conditions, particularly in drought and saline environments. In these extreme habitats, ecological features often limit adaptation to only a few taxa, frequently represented by a single genus and single species, emphasizing the narrow ecological amplitude and restricted distribution of many halophytic plants in the region. The habit spectrum of foredune flora reveals that herbaceous species predominate among recorded taxa. However, the shrub forms (decumbent shrub, virgate shrub, ascending shrub, intricate shrub, divaricate shrub, prostrate shrub, erect shrub, and procumbent shrub) exhibits the most diversity of the growth forms, where the stable foredunes support to develop the higher shrubs. The foredunes landscape is characterized by a gradient of vegetation structure, ranging from sparse herbaceous and prostrate forms in the shoreline to dense dwarf shrubs in landward zone. These findings suggest that the halophytic composition of foredunes in the South-Central Coast of Vietnam exhibits similarities to tropical desert regions in Southwest Asia, China, Pakistan. The composition of habits indicates the distinguished vegetation between incipient dune (creepers, prostrate, herbs, rhizomatous, stoloniferous) and established foredune (shrubs, woody, climbers). Given the ecological significance of these plant communities support further conservation efforts to mitigate the impacts of climate change and human activities on coastal biodiversity.

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Stand Structure, Species Richness, and Diversity of Community Forests with Reference to the Soil Characteristics in Western Himalaya, India

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Abstract: The present study examines the vegetation structure, density, basal area, species richness, diversity, and soil parameters of community forests in the Almora district of Uttarakhand, located in the Western Himalayas. Each community forest was divided into three sub-sites to achieve concrete, real-time vegetation data. Vegetation data for different parameters were collected using 30 quadrats in each sub-site of each community forest. Soil samples were taken from a depth of 0-30 cm at each site using a soil corer. Tree density, basal area, diversity, and richness of these forests varied significantly, ranging from 373.3 to 1200.0 ind. ha^{-1}, 25.0 to 31.9 m^2 ha^{-1}, 0.1 to 2.5, and 0.2 to 1.4, respectively. Shrub density, basal area, diversity, and richness of these forests varied significantly, ranging from 1026.7 to 3789.7 ind. ha^{-1}, 0.1 to 0.4 m^2 ha^{-1}, 0.4 to 1.3, and 0.33 to 1.0, respectively. Herb density, diversity, and richness of these forests varied significantly, varying from 9.2 to 14.6 ind. m^2, 0.3 to 2.5, and 0.15 to 3.3, respectively. The Important Value Index (IVI) of dominant tree species in community forests ranged from 119.5 (*Quercus leucotrichophora)* to 289.64 (*Pinus roxburghii*). Forests are facing significant depletion of natural resources due to unsustainable resource extraction, inadequate management, and increasing impacts of climate change. The study suggests implementing an integrated approach to conservation and management for the biodiversity conservation and sustainable development of the western Himalayan forests in the country.

Keywords*:* Species composition, Species diversity, Soil characteristics, Community Forest, Western Himalaya

Forest species composition and diversity are essential indicators of the stability and sustainability of forest communities. The assessment of these parameters plays a crucial role in planning the conservation and management of forest ecosystems (Ahmad et al., 2020). To understand the negative and positive impacts of each factor on vegetation, effective conservation and management measures must be implemented to mitigate these effects. Additionally, new policies must be adopted to enhance the sustainability of Himalayan forests. Himalayan forests provide diverse ecological services to hill communities in various ways, yet these services gradually lose their sustainability over time (Naidu and Kumar 2016, Dhyani and Dhyani 2016, Manral et al., 2018, Negi et al., 2018, Joshi et al., 2023) stated that the high community dependence on forest resources has increased the vulnerability of forests with respect to human interference. Assessing species composition, diversity, and structure has been a significant focus of ecological studies (Bhat et al., 2020). The forest area of Uttarakhand accounted for 71.05% (38,000 sq km) of the state's total geographical area (FSI 2023). Of this, 20% of the forest area has been managed by village communities. Approximately 12,089 community forests exist in Uttarakhand, with 2,199 located in the Almora district (Nagahama et al., 2016). All these community forests in the Himalayan state of Uttarakhand have played a vital role in the development of village communities by providing domestic livelihood needs and income, as well as supporting the farm activities of small landholders. Apart from these, community forests are also considered and have been utilized to mitigate the recent problems caused by human disturbances and climate change. However, the growing diversity of impacts on these community forests means that the existing conditions have not been able to meet the increasing livelihood needs of villagers in the coming years; therefore, they need adequate support to recover and manage them sustainably. Himalayan forests not only conserve local biodiversity and stabilize the climate but also play a vital role in preserving global biodiversity and the carbon cycle. The primary objective of this study is to examine the vegetation structure, density, basal area, species richness, diversity, and soil parameters of community forests in the Almora district of the Western Himalayas.

MATERIAL AND METHODS

Description of the study area*:* This study was conducted in the community forests of the western Himalayas in the state of Uttarakhand, India. These community forests are dominated by oak forest (Gairar-barati-bhainar Community Forest, GCF site-1), pine forest (Soangaon Community Forest, SCF site-2), and oak-pine forest (Bhatkot Community Forest, BCF site-3). These forests are in the Almora district, at altitudes ranging from 981 to 2149 meters, and are situated at 29.8150°N and 79.2902°E. The following map indicates the location of the community forest sites (Table 1, Fig. 1).

Meteorological information: The annual average rainfall of the study area was 1180.3 mm. The maximum and minimum rainfalls were 342.4 mm in September and 0.32 mm in March 2022, respectively. The highest temperature was 33.23 °C in June 2022, while the lowest was -3.45°C in February 2022. The annual relative humidity was 63.56%. The relative humidity was highest in September (85.75%) and August (85.19%), whereas the lowest was 30.25% in April. This meteorological data, i.e., rainfall, temperature, and relative humidity, reflects the yearly climatic changes in the study area (https://power.larc.nasa.gov, 2022) (Fig. 2).

Vegetation analysis: Species composition, density, basal area, diversity, richness and importance value index (IVI), as well as provenance value (PV) of community forests, were determined using the quadrat method (Misra 1968, Saxena and Singh 1982). Each community forest was divided into three sub-sites to enable accurate assessment during data collection. Each sub-site contained 30 random quadrats for assessing trees, shrubs, and herbs. Quadrat sizes of 10 × 10 m for trees, 5 × 5 m for shrubs, and 1 × 1 m for herbs were used. The girth of the trees was measured at a height of 1.37 m. Species diversity, evenness, and richness of community forests were assessed using the Shannon diversity index (Shannon and Wiener, 1963), Pielou's evenness index (Pielou 1966), and Margalef's richness index (Margalef 1958), respectively. 'Forest Flora of Kumaon' (Osmaston 1927) was used to identify forest species. To confirm the accepted binomial name of plants, the Royal Botanical Gardens Kew, Plants of the World online site was utilized (https://powo.science.kew.org, 2023)

Soil analysis: Soil samples were collected at a depth of 0-30 cm using a soil corer during the summer of 2022. A total of 81 samples were collected randomly from each forest site. A composite mixture was prepared for soil testing. The soil samples were analysed for soil moisture content (Jackson 1973, Misra 1968), water holding capacity (Piper 1950), soil texture (Misra 1968), bulk density ((Misra 1968), soil pH, soil porosity (Kumar 2000), soluble salts (EC) content, organic matter content Misra, 1968), available nitrogen alkaline $KMnO_4$ method (Subbiah and Asija 1956), available phosphorus (Jackson 1973) and available potassium (Jackson 1973).

Statistical analysis: MS Excel was used to create graphs and tables, while RStudio was employed for statistical analysis. Indicator species analysis was done to identify specific species significantly associated, PCA was applied to determine relationships between vegetation and soil parameters, and a Venn diagram was prepared to analyse the beta diversity of plants across the community forest. The map of the study area was prepared using QGIS software.

RESULTS AND DISCUSSION

A total of 67 plant species from 35 families were recorded in the studied community forest. Trees, shrubs, and herbs comprise 14.9%, 22.4%, and 62.7% of the total, respectively. Among these species, the distribution of various plant groups was as follows: Angiosperms (95.5%) (dicotyledons 76.1% and monocots 19.4%) > Ferns (3%) > Gymnosperms (1.5%) (Table 2). The results were similar to those of previous studies on Indian and Central Himalayan forests (Khera et al., 2001, Mandal et al., 2014, Haq et al., 2024). A Venn diagram was utilized to understand the biodiversity distribution of plants in forests at the beta level (Fig. 3). For a

Table 1. Geographical features of community forests in the Almora district of the Western Himalaya

Parameter	Name of the community forest site		
	Oak forest (GCF site-1)	Pine forest (SCF site-2)	Oak pine forest (BCF site-3)
Elevation (m)	1613-2149	1049-1282	981-1103
Aspect	West facing	South facing	North East
Latitude	29°48'36.0"N	29°53'38.4"N	29°52'05.3"N
Longitude	79°26'09.6"E	79°19'08.4"E	79°20'42.4"E

GCF site-1 (Gairar-bairati-bhainar community), SCF site-2 (Soangaon community forest), and BCF site-3 (Bhatkot community forest).

Table 2. Plant species of different groups in the community forests of the Western Himalaya

Component	No. of species	Angiosperms	Monocots	Dicots	Gymnosperm	Ferns
Tree	10	9	0	9	1	0
Shrub	15	15	0	15	0	0
Herb	42	40	13	27	0	2
Total	67	64	13	51	1	2

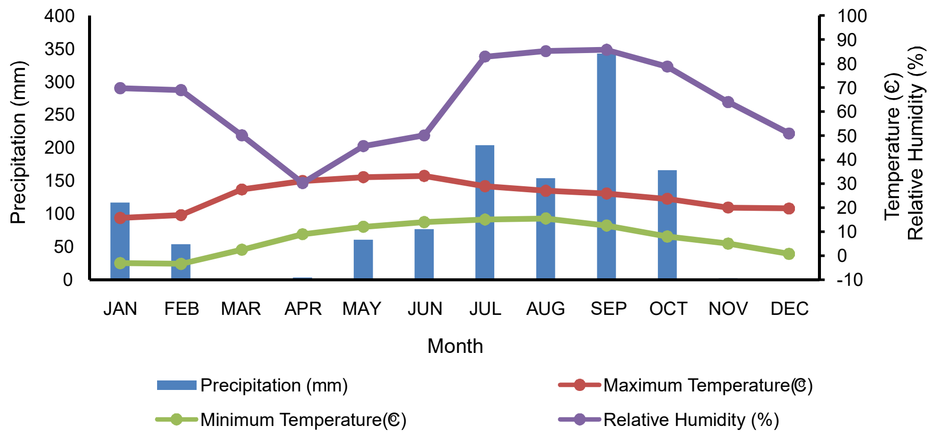

Fig. 1. Meteorological depiction of temperature, rainfall, and relative humidity of the study sites in 2022 (Source: power.larc.nasa.gov).

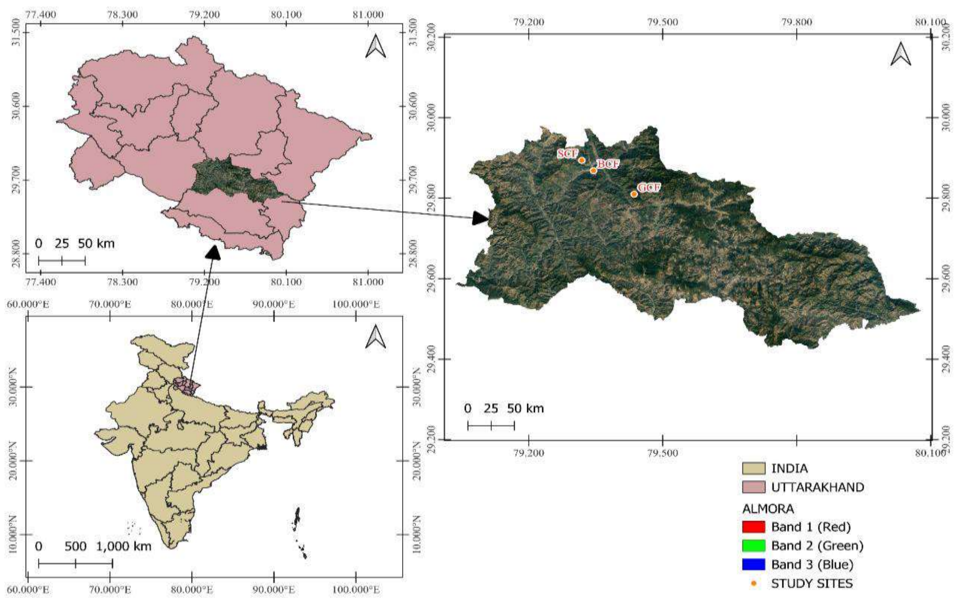

Fig. 2. GPS locations of the studied community forests

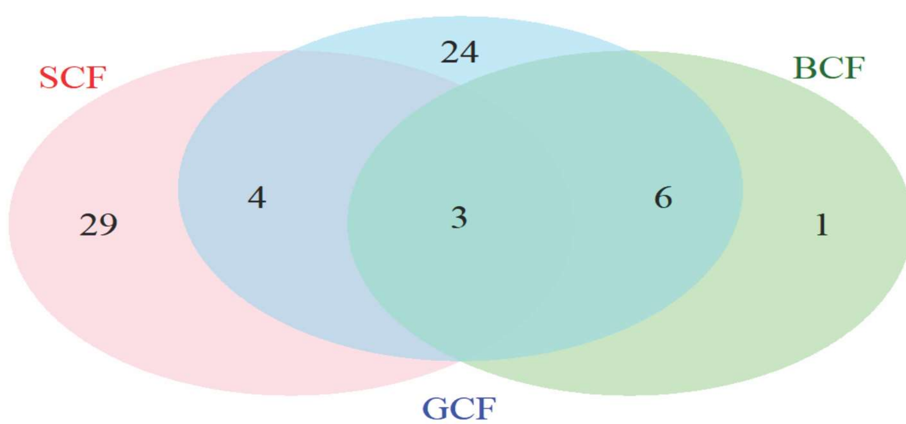

Fig. 3. Venn diagram of plant species in the community forests of the Western Himalaya

more comprehensive analysis of this forest, hierarchical clustering has been conducted to provide a graphical representation of the 67 plant species (Fig. 8).

Vegetation Characteristics

Tree layer: Among tree species, the indicator value of tree species was highest for *Lyonia ovalifolia* in the oak forest, while *Syzygium cumini* and *Pinus roxburghii* were dominant indicators in the pine forest. In the Oak-Pine forest, the maximum indicator value for tree species *Pinus roxburghii is* followed by *Quercus leucotrichophora*. In tree species, the maximum IVI was 119.5 recorded for *Quercus leucotrichophora,* followed by *Myrica esculent*a in oak forest. In the pine forest, *Pinus roxburghii,* showed the highest IVI 289.6, followed by *Syzygium cumini* 10.36. In the oak-pine forest, *Quercus leucotrichophora* had the maximum IVI of 152.9 followed by *Pinus roxburghii* 138.6 (Table 4). The density of trees ranged from 373.3 - 1200 ind. ha^{-1} (Fig.4). The tree density was somewhat similar to central and Kumaun Himalaya forests (360 - 1140 ind. ha^{-1}) (Pandey et al., 2022, Joshi et al., 2023, Haq et al., 2024), while was higher than 153 to 182 ind. ha^{-1} (Kaushal and Baishya 2021, Panday et al., 2022).

The basal area of trees ranged from 25.01 - 31.9 $m^2 ha^{-1}$ (Fig.4). The estimated tree basal area falls within the range of 28.02 - 29.50 m^2 ha^{-1} reported for central and Kumaun Himalayan forests (Kaushal and Baishya, 2021 Pandey et al., 2022, Haq et al., 2024). Area was higher than 12.6 -18.8 m^2 ha^{-1} reported for western and central Himalayan forests (Gurarni et al., 2010, Pandey et al., 2022) and was lower than (33.4-43.6 m^2 ha^{-1}) reported for central Himalayan forests (Joshi et al., 2023).

The diversity, evenness and richness of trees ranged from 0.1 - 0.8, 0.2 – 0.8 and 0.2 – 1.4, respectively (Fig. 4). The diversity of tree species was close to 0-0.5 for western and Kumaun Himalayan forests (Kharkwal et al., 2009, Gurarni et al., 2010, Pandey et al., 2022) but the present finding was lower than 1.2-3.13 was compared to Kumaun and western Himalayan forests (Khan and Arya, 2017, Joshi et al., 2023, Haq et al., 2024) (Table 5). This variation was attributed to differences in tree species composition and abundance within the forests, influenced by external factors such as anthropogenic activities, summer fires, and the regular felling of tree species for fuel and fodder.

Shrub layer: For shrubs, the indicator value was highest for *Indigofera tinctoria* in the oak forest, while *Carissa spinarum*, *Woodfordia fructicosa,* and *Ziziphus nummularia* were dominant indicators in the pine forest and *Berberis aristata,* followed by *Himalrandia tetrasperma* in oak-pine forest. In the oak forest, the maximum IVI of 184.2 was for *Berberis aristata* among shrubs*,* followed by *Himalrandia tetrasperma,* while in the pine forest, the highest IVI of 128.7 was estimated for *Carissa spinarum,* followed by *Woodfordia fructicosa* .. In the oak-pine forest, the maximum IVI 197.7 for shrubs was observed for *Berberis aristata,* followed by *Rubus ellipticus* 61.7 (Table 4). The density of shrub species ranged between 1026.7 -3789.6 ind. ha^{-1} (Fig. 4) which falls within the range (760-8520 ind. ha^{-1}) reported for Kumaun Himalayan forests (Gurarni et al., 2010, Khan and Arya 2017, Joshi et al., 2023). The value is higher than 40 - 540 ind. ha^{-1} as observed for central Himalayan forests (Kharkwal et al., 2009, Khan and Arya, 2017), and shrub density was on the lower side than (26107-28546 ind. ha^{-1}) r for Himalayan forests of Uttarakhand (Arya and Ram, 2016). The basal area of shrub species ranged between 0.1 - 0.4 $m^2 ha^{-1}$ (Fig. 4) and was lower than (4.7 m^2 ha^{-1}) reported for Himalayan forests (Gurarni et al., 2010).

Shrubs diversity, evenness and richness ranged from 0.4 - 1.3, .5 - 0.8 and 0.3 - 1.0, respectively (Fig. 4). The diversity of shrubs falls within the range of 0.9-1.7 reported for Himalayan forests (Kharkwal et al., 2009, Khan and Arya 2017) (Table 5). However, were lower than 1.5-4.6 reported for Himalayan forests (Arya and Ram, 2016, Khan and Arya 2017, Joshi et al., 2023). The variation was observed due to changes in soil and climate change at the site. Additionally, the opening up of the forest site, and frequent forest fires influence the density and diversity of shrub species.

Herb layer: In the herb layer, the maximum indicator was recorded for *Galium aparine*, *Paeonia emodi,* and *Thalictrum foliolosum* in oak forest and *Chaerophyllum tainturieri*, *Chrysopogon aciculatus*, *Euphorbia hirta*, *Evolvulus alsinoides*, *Flemingia procumbens*, *Imperata cylindrica*, *Lepidagathis incurve,* and *Oxalis corniculate* for pine forest. For oak-pine forest, the maximum indicator value was for *Ageratina Adenophora,* followed by *Themeda anathera* (Table 3). The maximum PV in oak forest was 63.8 observed for *Ageratina Adenophora,* followed by *Themeda anathera*. In the pine forest, the highest PV of 39.43 was for *Chrysopogon aciculatus* followed by *Themeda anathera* . In the oak-pine forest, *Ageratina Adenophora* shows the highest PV of 179.0*,* followed by *Themeda anathera* for oak-pine forest (Table 5).

The herb density ranged between 9.2 - 14.6 ind. m^{-2} (Fig.4). The estimated herb density was somewhat similar to Kumaun Himalayan forests(9.4-15.8 ind. m^{-2}) (Kharkwal et al., 2009, Arya and Ram 2016). But was lower than 81-234.8 ind. ha^{-1} for central and Kumaun Himalayan forests (Gurarni et al., 2010, Joshi et al., 2012, Arya and Ram, 2016, Joshi et al., 2023). The value is higher than the value 4.7 - 5.14 reported for Himalayan Forest (Gurarni et al., 2010, Rawal et al., 2012).

The herb diversity, evenness and richness ranged

Table 3. Indicator value of plant species in the community forests of Western Himalaya

Forest plant species	Oak forest GCF, Site-1	Pine forest SCF, Site-2	Oak-pine forest BCF, Site-3
Adiantum capillus-veneris L.	0.00	0.58	0.00
Ageratina Adenophora Spreng.	0.69	0.11	0.69
Ajuga parviflora Benth.	0.00	0.58	0.00
Anaphalis adnata DC.	0.82	0.00	0.00
Anaphalis contorta D Don Hook f.	0.92	0.22	0.00
Apluda mutica L.	0.61	0.54	0.00
Arundinella nepalensis Trin.	0.82	0.00	0.00
Asparagus racemosus Willd.	0.89	0.00	0.26
Barleria cristata L.	0.00	1.00	0.00
Berberis asiatica Griff.	0.68	0.00	0.74
Boenninghausenia albiflora Hook...Rchb..ex	0.58	0.00	0.00
Carissa spinarum L.	0.00	1.00	0.00
Caryopteris odorata D Don BL Rob.	0.58	0.00	0.00
Chaerophyllum tainturieri Hook Arn.	0.00	1.00	0.00
Chrysopogon spp.	0.00	1.00	0.00
Chrysopogon fulvus Spreng Chiov.	0.58	0.00	0.00
Cirsium wallichii DC.	0.00	0.58	0.00
Cotinus coggygria Scop.	0.58	0.00	0.00
Daphne papyracea Wall ex Steud.	0.58	0.00	0.00
Dioscorea bulbifera L.	0.00	0.58	0.00
Erigeron canadensis L.	0.00	0.58	0.00
Eulaliopsis binata Retz	0.77	0.18	0.00
Euphorbia hirta L.	0.00	1.00	0.00
Evolvulus alsinoides L.	0.00	1.00	0.00
Flemingia procumbens Roxb.	0.00	1.00	0.00
Flemingia strobilifera L W T Aiton	0.00	0.82	0.00
Galinsoga parviflora Cav.	0.58	0.00	0.00
Galium aparine L.	1.00	0.00	0.00
Glochidion velutinum Wight	0.58	0.00	0.00
Grevillea robusta A Cunn ex R Br.	0.00	0.00	0.58
Grona heterophylla Willd H Ohashi K Ohashi	0.00	0.82	0.00
Heteropogon contortus LP Beauv ex Roem Schult.	0.00	0.82	0.00
Himalrandia tetrasperma Wall ex Roxb T Yamaz.	0.70	0.00	0.42
Imperata cylindrica L Raeusch.	0.00	1.00	0.00
Indigofera tinctoria L.	0.82	0.00	0.00
Indigofera cassioides Rottler ex DC.	0.42	0.70	0.00
Jacobaea nudicaulis Buch Ham ex D Don B Nord.	0.00	0.82	0.00
Jasminum nervosum Lour	0.00	0.82	0.00
Lantana camara L.	0.58	0.00	0.00
Lepidagathis incurve Buch Ham ex D Don	0.00	1.00	0.00
Leucas lanata Benth.	0.00	0.58	0.00
Lyonia ovalifolia Wall Drude	1.00	0.00	0.00
Micromeria biflora buch Ham ex D Don Benth	0.00	0.58	0.00

Cont...

Table 3. Indicator value of plant species in the community forests of Western Himalaya

Forest plant species	Oak forest GCF, Site-1	Pine forest SCF, Site-2	Oak-pine forest BCF, Site-3
Myrica esculenta Buch Ham ex D Don	0.88	0.00	0.27
Myrsine africana L.	0.58	0.00	0.00
Onychium lucidum D Don Spreng.	0.82	0.00	0.00
Oplismenus hirtellus L P Beauv.	0.58	0.00	0.00
Oplismenus undulatifolius Ard P Beauv.	0.00	0.58	0.00
Origanum vulgare L.	0.82	0.00	0.00
Oxalis corniculate L.	0.00	1.00	0.00
Paeonia emodi Royle	1.00	0.00	0.00
Pinus roxburghii Sarg.	0.36	0.62	0.70
Pleurolobus gangeticus L J St Hil ex H Ohashi K Ohashi	0.00	0.82	0.00
Prunus cerasoides D Don	0.58	0.00	0.00
Pyrus pashia Buch Ham ex D Don	0.82	0.00	0.00
Quercus leucotrichophora A Camus	0.81	0.00	0.59
Rhododendron arboreum nilagiricum Zenker Tagg	0.82	0.00	0.00
Rubus ellipticus Sm.	0.62	0.00	0.37
Scutellaria scandens D Don	0.58	0.00	0.00
Senna tora L Roxb.	0.00	0.82	0.00
Syzygium cumini L Skeels	0.00	0.82	0.00
Teucrium quadrifarium Buch Ham ex D Don	0.00	0.58	0.00
Thalictrum foliolosum DC.	1.00	0.00	0.00
Themeda anathera Nees ex Steud Hack.	0.58	0.43	0.40
Viburnum mullaha Buch Ham ex D Don	0.58	0.00	0.00
Woodfordia fruticose L Kurz	0.00	1.00	0.00
Ziziphus nummularia Burm f Wight Walk Arn.	0.00	1.00	0.00

See Table1 for details

between 0.3 - 2.5, 0.2 – 0.7, and 0.15 – 3.3 respectively (Fig. 4). The observed herb diversity was close to (2.2-2.7) reported for Himalayan forests (Arya and Ram, 2016, Khan and Arya, 2017, Joshi et al., 2023), but was lower than central Himalayan forests (3.0 – 4.0) (Kharkwal et al., 2009; Joshi et al., 2023) (Table 5). The variations in herb density and diversity in the present study forest site resulted from seasonal climate variations, existing topographical features, anthropogenic pressures and disturbances caused by humans and animals, accumulation of herbs and grasses, frequent summer forest fires, and alteration in the microclimate of the forest sites, which affected the density, and diversity of the herbs.

Soil physicochemical characteristics: The soil texture, consisting of sand, silt, and clay, ranged from 17.5 - 29.9, 35.8 -59.1, and 23.3- 42.1%, respectively. The soil moisture, bulk density, porosity, and water holding capacity ranged between 2.8 - 10.85%, 0.9 – 1.2 g cm^{-3}, 53.3 - 64.5%, and 31.2 - 58.3%, respectively. The estimated soil moisture level was lower than the 19.7- 26.5% reported for the Western Himalayan and Shiwalik forests (Lodhiyal et al., 2016, Joshi et al., 2023). The bulk density falls within the range of value 0.56-1.24g cm^{-3} reported for the central Himalayan, Shiwalik subtropical coniferous and mixed forests (Lodhiyal et al., 2016, Bargali et al., 2018, Khan et al., 2025). This was higher than of 0.42-0.65 g cm^{-3} reported for the central Himalayan Forest (Bargali et al., 2018). The water-holding capacity falls within the range between 41.5-51.50% of the Western and central Himalayan Forest (Bargali et al., 2018, Manral et al., 2020, Joshi et al., 2023). The chemical properties of the soil, including soil pH, electrical conductivity (EC), organic matter, and total nitrogen, ranged from 5.6 - 6.1, 0.8 - 1.1 dS m^{-1}, 1.2 - 1.3%, and 0.06 - 0.07%, respectively. The phosphorus and potassium levels were 28.3 – 34.7 and 285.3 – 442.3 kg ha^{-1}, respectively across the forest sites (Fig. 5). The soil moisture level was 10.85%, which was lower than the 25.3% reported for the Western Himalayan forest (Joshi et al., 2023). The soil pH was lower than 6.91 in the coniferous forest and 7.24 in the mixed forest of the Himalayan region (Khan et al., 2025). The electrical conductivity is somewhat similar to of

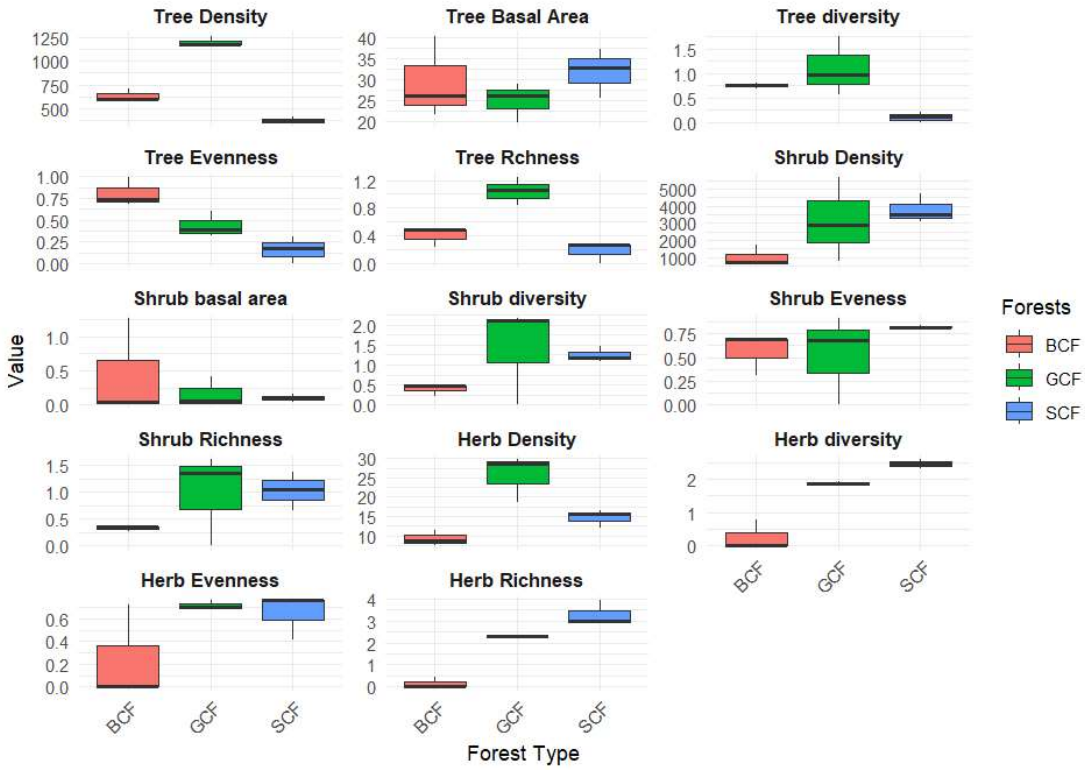

Fig. 4. Vegetation characteristics of the community forests of the Western Himalaya.Tree and shrub density are in (ind. ha^{-1}), herb density is in (ind. m^{-2}), and Tree basal area and shrub basal area are in ($m^2 ha^{-1}$)

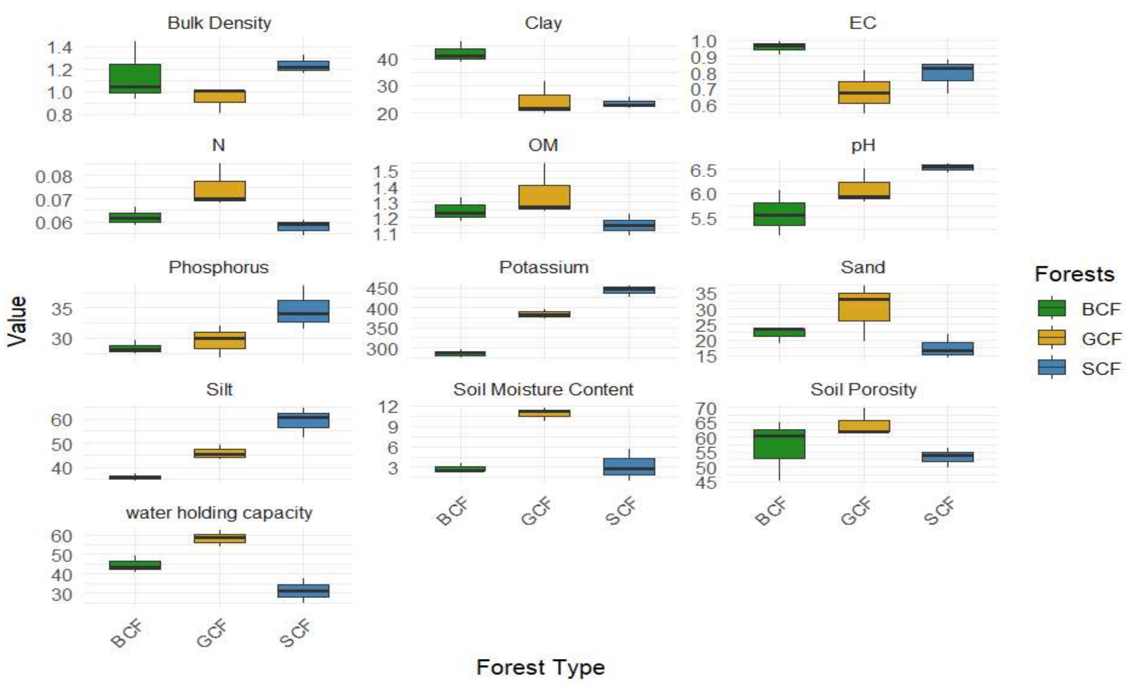

Fig. 5. Physico-chemical properties of soils of the community forests in Western Himalaya (Sand, silt, clay, soil moisture, soil porosity, water holding capacity, Organic matter, and Nitrogen (N) in (%), bulk density in (g cm^{-2}), EC in (dS m^{-1}), Phosphorus (P) and Potassium (K) are in (Kg ha^{-1})

Table 5. Provenance value of herb species in community forest of Western Himalaya

Herb species	Oak forest GCF, Site-1	Pine forest SCF, Site-2	Oak-pine forest BCF, Site-3
Adiantum capillus-veneris L.	-	3.56	-
Ageratina adenophora (Spreng.)	178.99	2.85	63.78
Ajuga parviflora Benth.	-	1.28	-
Anaphalis adnata DC.	-	-	7.92
Anaphalis contorta (D.Don) Hook.f.	-	1.07	13.92
Apluda mutica L.	-	2.76	4.96
Arundinella nepalensis Trin.	-	-	7.56
Asparagus racemosus (Willd.)	5.43	-	7.04
Barleria cristata L.	-	11.80	-
Boenninghausenia albiflora (Hook.) Rchb. ex	-	-	2.45
Chaerophyllum tainturieri Hook. & Arn.	-	9.43	-
Chrysopogon spp	-	39.43	-
Chrysopogon fulvus (Spreng.) Chiov.	-	-	5.40
Cirsium wallichii DC.	-	2.55	-
Dioscorea bulbifera L.	-	1.07	-
Erigeron canadensis L.	-	2.14	-
Eulaliopsis binata (Retz.)	-	1.70	14.34
Euphorbia hirta L.	-	12.21	-
Evolvulus alsinoides (L.) L.	-	11.57	-
Flemingia procumbens Roxb.	-	1.28	-
Flemingia prostrata Roxb.Junior ex Roxb.	-	4.48	-
Galinsoga parviflora Cav.	-	-	7.71
Galium aparine L.	-	-	6.39
Grona heterophylla (Willd.) H.Ohashi & K.Ohashi	-	3.95	-
Heteropogon contortus (L.) P.Beauv. ex Roem. & Schult.	-	2.64	-
Imperata cylindrica (L.) Raeusch.	-	17.75	-
Curcuma longa L.	-	-	5.54
Jacobaea nudicaulis (Buch. -Ham. ex D.Don) B.Nord.	-	4.27	-
Lepidagathis incurva Buch. -Ham. ex D.Don	-	11.17	-
Leucas lanata Benth.	-	1.49	-
Micromeria biflora (buch.-Ham. ex D.Don) Benth	-	1.27	-
Onychium lucidum (D.Don) Spreng.	-	-	2.53
Oplismenus hirtellus (L.) P.Beauv.	-	-	3.12
Oplismenus undulatifolius (Ard.) P.Beauv.	--	0.96	-
Origanum vulgare L.	-	-	7.84
Oxalis corniculata L.	-	9.19	-
Paeonia emodi Royle	-	-	5.07
Pleurolobus gangeticus (L.) J. St.-Hil. ex H. Ohashi & K. Ohashi	-	5.51	-
Scutellaria scandens D.Don	-	-	7.22
Teucrium quadrifarium Buch.-Ham. ex D.Don	-	3.82	-
Thalictrum foliolosum DC.	-	-	5.94
Themeda anathera (Nees ex Steud.) Hack.	15.58	28.78	21.26

See Table1 for details

0.9 dS m^{-1} reported for mixed forests (Khan et al., 2025). The soil organic matter in the present forest site was significantly lower than the value 2.91 - 3.87% reported for mixed forests and coniferous forests (Khan et al., 2025). The soil nitrogen concentration was somewhat close to the values of 0.05 - 0.06% reported for Western Himalayan forests (Manral et al., 2020). However, it is significantly lower than the value of 0.10 - 0.38 0.21% for Western and central Himalayan forests (Manral et al., 2020, Joshi et al., 2023).

Based on the soil properties studied in the community forests, these forests contain very small quantities of organic matter due to various internal and external reasons, such as collection of litter, burning of litter organic matter in summer season, surface run-off during the rainy season, collection of fodder and grasses. Apart from these, erratic weather and climatic conditions prevail in the forest areas. The study forest sites were in a warm climatic zone, resulting in the rapid decomposition of litter present in the forest, which was washed out due to runoff. This is why the addition of organic nutrients could not penetrate the forest soils. Thus, it is essential to conserve and manage the organic litter present in the forest so that the soil structure and fertility of these forest sites can be maintained for the health of the soil and the growth of forest stands.

Ordination analysis: Principal Component Analysis (PCA) was conducted using 29 parameters to assess the relationships between vegetation, soil, and topography associated with community forests. Of these, 14 were vegetation parameters and 15 were environmental parameters. Vegetation parameters include tree density, shrub density, herb density, tree basal area, shrub basal area, tree diversity, shrub diversity, herb diversity, tree richness, shrub richness, herb richness, tree evenness, shrub evenness, and herb evenness. The environmental

Table 4. Important Value Index of tree and shrub species in the community forest of Western Himalaya

Tree species name	Oak forest GCF, Site-1	Pine forest SCF, Site-2	Oak-pine forest BCF, Site-3
Glochidion velutinum Wight	-	-	3.27
Grevillea robusta A. Cunn. ex R. Br.	3.41	-	-
Lyonia ovalifolia (Wall.) Drude	-	-	17.01
*Myrica esculent*a Buch. -Ham. ex D. Do	5.14	-	83.63
Pinus roxburghii Sarg.	138.58	289.64	36.5
Prunus cerasoides D. Don	-	-	3.48
Pyrus pashia Buch. -Ham. ex D. Don	-	-	9.88
Quercus leucotrichophora A. Camus	152.87	-	119.53
Syzygium cumini (L.) Skeels	-	10.36	-
Rhododendron arboreum nilagiricum (Zenker) Tagg	-	-	26.69
Shrub species name			
Berberis aristata DC.	197.67	-	184.18
Carissa spinarum L.	-	128.66	
Caryopteris odorata (D.Don) B.L.Rob.	-	-	12.52
Cotinus coggygria Scop.	-	-	2.65
Daphne papyracea Wall. ex Steud.	-	-	1.74
Himalrandia tetrasperma (Wall. ex Roxb.) T.Yamaz.	40.62	-	40.04
Indigofera cassioides Rottler ex DC.	-	17.71	21.65
Jasminum nervosum Lour.	-	12.98	-
Lantana camara L.	-	-	3.56
Myrsine africana L.	-	-	1.80
Rubus ellipticus Sm.	61.71	-	14.26
Senna tora (L.) Roxb.	-	32.60	-
Viburnum mullaha Buch. -Ham. ex D.Don	-	-	17.59
Woodfordia fruticosa (L.) Kurz	-	66.35	
Ziziphus nummularia (Burm. f.) Wight & Walk. -Arn.	-	41.70	-

See Table1 for details

parameters included one topographical parameter (i.e., elevation) and thirteen soil parameters (i.e., sand, silt, clay, bulk density, soil moisture, soil porosity, water holding capacity, soil pH, soil electrical conductivity, organic matter, nitrogen, phosphorus, and potassium). The bi-plot reveals that tree richness, density, basal area, diversity, and

Table 6. Summary of PCA plot

Dimension	Eigenvalue	Variance	Cumulative variance (%)
Dim. 1	15.396	54.99	54.99
Dim. 2	12.604	45.01	100.00
Dim. 3	~0	~0	100.00

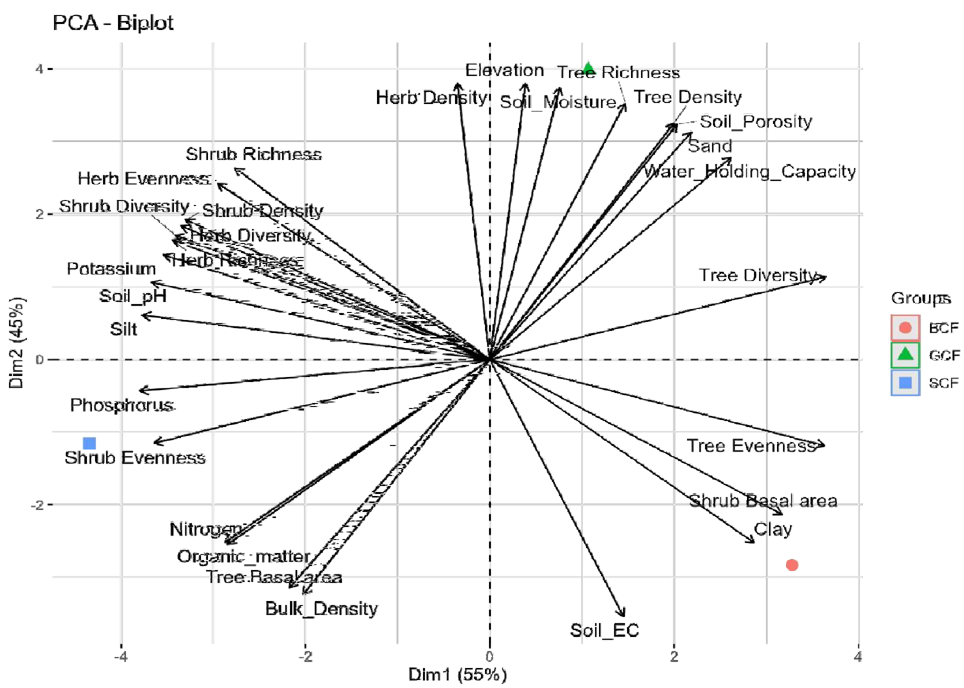

Fig. 6. PCA biplot of vegetation, soil, and topographic characteristics of the community forests in Western Himalaya

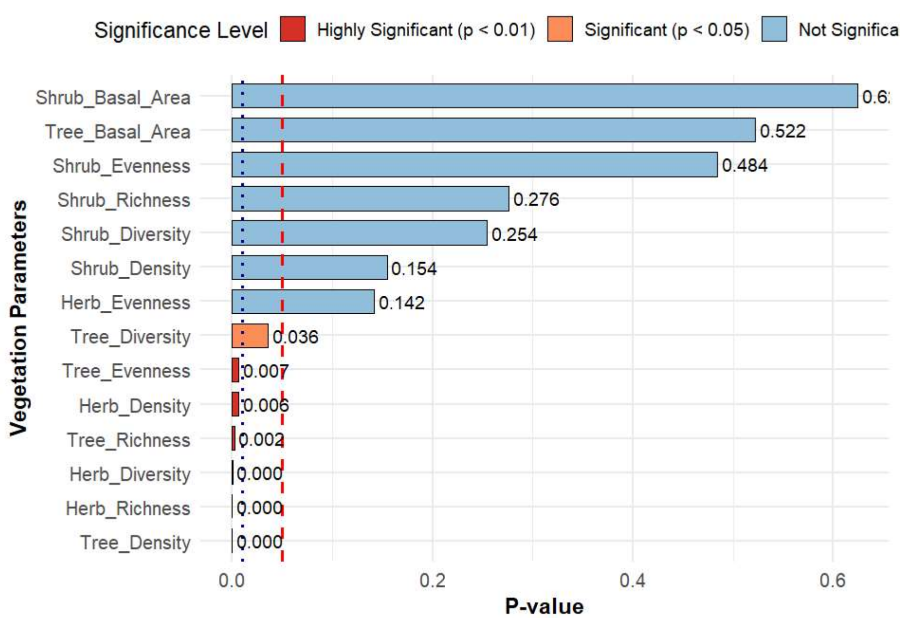

Fig. 7. Tukey HSD test for vegetation characteristics of the community forests in Western Himalaya

Table 7. Comparative study of different vegetation parameters of trees, shrubs, and herbs of forests in the regions of the country

Forest type	Density (ind. ha^{-1})		Density (ind.m^{-2})	Basal area (m^2ha^{-1})		Diversity			Reference
	Tree	Shrub	Herb	Tree	Shrub	Tree	Shrub	Herb	
Oak forest	1200	3120	11.9	25.0	0.2	0.8	1.0	1.9	Present study
Oak forest	400-933	995-1404	4.8-5.0	29.50-40.16	-	-	-	-	(Rawal et al., 2012)
Oak forest	390	540	-	28.02		1.2	1.5	-	(Khan and Arya 2017)
Oak forest	652	1700	234.8	51.58	-	2.0	3.0	3.3	(Joshi et al., 2023)
Oak forest	670-1140	40-100	15.8-42.9	-	-	0.2-0.4	1.8-2.8	3.0-4.2	(Kharkwal et al., 2009)
Oak forest	360	50.43	-	-	-	-	-	-	(Kaushal and Baishya 2021)
Ban oak forest	381-930	22.0-35.05	-	-	-	0.9-1.7	-	-	(Pandey et al., 2022)
Oak forest	640±140.9	-	-	-	-	3.31±0.13	-	-	(Haq et al., 2024)
Pine forest	373.3	3789.7	14.6	31.9	0.1	0.1	1.3	2.5	Present study
Pine forest	307.8-376.5	26107-28546	8.1-9.4	28.4-33.2	-	2.1-2.2	4.5-4.6	2.5-2.7	(Arya and Ram 2016)
Pine forest	485	1460	4.7	12.6	-	0.5	2.1	2.2	(Gurarni et al., 2010)
Pine forest	300-560	760-8,520	-	28.7 -34.4	-	0.4-1.5	0.9-1.7	-	(Khan and Arya 2017)
Pine forest	672	6160	119.9	43.6	-	1.1	2.3	4.1	(Joshi et al., 2023)
Pine forest	470-850	200-1360	13.9-44.0	-	-	0-0.3	0.5-1.5	2.4-4.5	(Kharkwal et al., 2009)
Pine forest	153	-	-	37.40	-	-	-	-	(Kaushal and Baishya 2021)
Pine forest	182	-	-	18.8	-	0.3	-	-	(Pandey et al., 2022)
Pine forest	707.5± 148.2	-	-	-	-	2.99±0.26	-	-	(Haq et al., 2024)
Oak-pine mixed forest	633.3	1026.7	9.2	29.4	0.4	0.8	0.4	0.3±	Present study
Pine-oak forest	1000	1660	5.14	14.55	4.69	1.26	2.42	2.45	(Gurarni et al., 2010)
Pine-oak forest	-	-	67.2	-	-	-	-	-	(Joshi et al., 2012)
Oak-pine mixed forest	160	-	-	39.17	-	-	-	-	(Joshi et al., 2013)
Oak pine mixed forest	640-930	-	-	21.0-32.7	-	--	-	-	(Verma and Garkoti 2019)
Mixed-oak Forest	884	3504	152.5	33.4		2.3	3.3	2.5	(Joshi et al., 2023)

Fig. 8. Cluster analysis of plant species of the community forests in Western Himalaya

evenness are positively correlated with certain soil components, including moisture, porosity, water-holding capacity, and organic matter. Shrub and herb parameters show a positive correlation with nitrogen, phosphorus, and elevation. Additionally, clay and EC are negatively correlated with tree parameters. (Fig. 6 and Table 4). The P-value indicated that these forests differed significantly in terms of tree density, tree diversity, tree richness, herb density, and herb diversity. However, these forests did not vary considerably in terms of tree basal area, shrub density, shrub basal area, shrub diversity, and herb diversity (Fig. 7).

CONCLUSION

The community forests are home to a diverse variety of flora and fauna, and also considered one of the livelihood options for village communities, particularly in the Himalayan region of Uttarakhand, India. However, with the growing human population and its interference, these forest areas are highly affected by fragmentation, summer forest fires, and the uncontrolled extraction of forest resources, which has also led to a decline in biodiversity in the forest. Apart from these disturbing factors, the community forest faces poor productivity, a declining growing stock, and a lower CO2 mitigation potential., The findings of vegetation and soil characteristics of community forests varied from one forest to another, indicating that the forests were not in good condition. This assumes that several factors, including unscientific resource extraction, fires, anthropogenic pressure, and climate-related variations, have influenced the forests. This inadequate management inputs have consequently led to a poor and degraded condition of community forests in the studied sites. The soil properties, such as moisture, porosity, water-holding capacity, and organic matter, favour the tree layer. In contrast, the shrub and herb layers were found to be correlated with nitrogen, phosphorus, and elevation. The high clay content and soil electrical conductivity (EC) limit the abundance of plants in the forest.

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Traditional Phytotherapies with Medicinal Plants in Outer Seraj Area of the North Western Himalayas

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Abstract: In the current study, survey was carried out on ethnic uses of therapeutic plants in the Outer Seraj area of Kullu distract of Himachal Pradesh. The purpose of the study was to gather information related to therapeutic plant species commonly used in that area to cure common diseases. The data was gathered through interviews with traditional healers, Vaidyas, and other well-informed individuals. The study lists 39 plants from 32 different families. The highly represented families were Asteraceae, Rosaceae, Polygonaceae, Apiaceae, Saxifragaceae, and Fabaceae. Most of the plants from the region are used to cure multiple ailments and used in the form of paste, powder and decoctions. However, the overexploitation of these therapeutic plants has posed a threat to their extinction. Seven plant species from the region were reported to be endangered. Therefore, the need of the hour is to document the precious knowledge of indigenous practices and take steps for the *in-situ* and *ex-situ* conservation of these plants. Strict biodiversity conservation laws needs to be enforced along with community participation for the sustainable utilization of the natural resources including plant wealth.

Keywords: Medicinal plants, Conservation, Indigenous uses, *in-situ, ex-situ,* Sustainable utilization

The Indian Himalayan Region is an extensive and ecologically vital mountainous area, encompassing around 16% of the nation's total land area, and has been recognized as a key biological diversity hotspot (Myers et al., 2000). The IHR is characterized by diverse ecosystems, rich biodiversity, and cultural uniqueness. The 1748 therapeutic plants in IHR have cultural, folk, and modern therapeutic applications. Himachal Pradesh is one of the most biologically diverse areas within the Indian Himalayan landscape (Badola and Pal 2003). The vegetation of Himachal Pradesh is diverse ranging from subtropical to alpine due to its varied agro-climatic conditions. Kullu district is well known for rich plant wealth and source of natural remedies for various illnesses for the local populations of the region. Numerous studies have been conducted on the therapeutic plants of the Indian Himalayan Region (Singh and Rawat 1998, Samant et al., 1998, Dhaliwal and Sharma 1999, Singh 2004, Kala 2006, Kumar et al., 2021). However, the studies on the therapeutic plants of Outer Seraj (District Kullu, H.P.) are fragmentary. Hence, the current research focused on examining the distribution, variety, utilization patterns, and indigenous usages of therapeutic plants employed by the local population in their traditional healthcare practices in this area.

MATERIAL AND METHODS

Study area: The area of the present study comprised of local villages of Outer Seraj of District Kullu. The geographical coordinates of the study area lie between 31^0 58'88" N latitude and 77^0 25' 4" E longitudes. The altitudinal range varies from 700 - 4000 m above sea level. The climate of the region is subtropical to temperate with a temperature range of 25 -30^0 C.

Ethnobotanical survey: Comprehensive field surveys were carried out across various seasons to get maximum information on the therapeutic herbs of the study area. The survey area included different localities covering Jalori, Khanag, Bhainal, Chawai, Kandugad, Anni, Luhri, and Dalash. Structured questionnaires and participatory observation were employed to collect data on the traditional uses of therapeutic plants. Several local knowledgeable persons from each village were consulted to authenticate the information on indigenous uses of significant plants (Table 1). The respondents were interviewed in their local language to make them comfortable and understand easily. The information regarding vernacular names, parts used, diseases cured, and mode of use was provided by local healers/Vaidyas. Fresh samples were collected and identified with the help of local flora (Collett 1902; Aswal and Mehrotra 1994; Dhaliwal and Sharma 1999).

Quantitative Analysis

Use value: It reflects the relative significance of locally known plants. It was determined using the following formula (Phillips et al., 1994):

$$UV = \sum U/n$$

Where UV represents the use value of a species, where 'U' stands for the number of use reports provided by each informer for a specific plant species, and 'n' refers to the total number of informers interviewed about that plant. The UV is used to identify the plants most commonly cited for treating specific ailments, while the use report (U) records the usage for each species.

RESULTS AND DISCUSSION

Diversity and distribution pattern: The total of 39 therapeutic plant species belonging to 38 genera and 32 families were recorded which are usually found and used in the study area. Amongst the different families, the highly represented families are Asteraceae, Rosaceae, Polygonaceae, Apiaceae, Saxifragaceae, and Fabaceae (Table 1) The 17 plant species were herbs, 11 plant species were shrubs, 10 plant species were trees and 1 species were climbers. The highest number of therapeutic herbs (39) was at an altitude of 1000-2000 meters, followed by some plants in the 2000-3000 meter range, while only 1 plant was found above 4000 meters. In terms of habitat-wise distribution, maximum plant diversity (20 sps.) was in cool, shaded, and moist areas, followed by sunny areas (12 sps.), dry hillsides (4 sps.), open slopes (2 sps.) with the lowest diversity (1 sps. each) in forest edges, rocky surfaces, and roadsides.

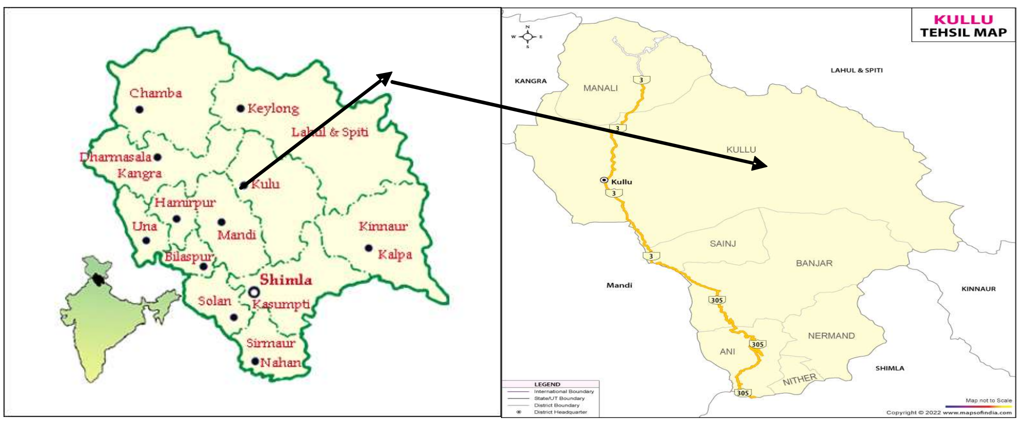

Fig. 1. Study area

Fig. 2. Some important plants 1. *Acorus calamus* 2. *Angelica glauca* 3. *Bauhinia variegata* 4. *Berberis lycium* 5. *Bergenia ciliata* 6. *Bombax ceiba* 7. *Cirsium wallichii* 8. *Cuscuta reflexa* 9. *Hypericum perforatum* 10. *Oxalis corniculata* 11. *Pistacia integerrima* 12. *Prinsepia utilis* 13. *Rhododendron arboreum* 14. *Rubus ellipticus* 15. *Rumex hastatus* 16. *Rumex nepalensis* 17. *Withania somnifera* 18 *Zanthoxylum armatum*

Table 2. Ethnobotany of some important medicinal plants of Outer Seraj, District, Kullu

Botanical name	Family	Habit	Altitudinal range	Habitat	Part (s) used	Threat status	Indigenous uses and practices	UV value
Abies pindrow (Royle ex D.Don) Royle	Pinaceae	Tree	2000-3500 m	Cool, Moist areas	Bark		Middle layer of bark is removed and ground with oil or ghee to make a paste. This paste is applied with a bandage for one month for the setting of dislocated joints.	0.037
Acorus calamus L.	Araceae	Herb	1000-1500 m	Near water sources	Roots		Rot paste is applied on the forehead, nose, and chest for common cough and cold in children. Decoction of roots is made and gargles are taken to get relief from toothache.	0.104
Aesculus indica (Wall. ex Cambess.) Hook.	Sapindaceae	Tree	1500-2700 m	Moist area	Bark and fruits		The bark paste is employed at dislocated joints. Fruits are dried and washed in water several times to eliminate bitter taste, ground into flour, and used for curing stomachaches and dysentery.	0.08
Ainsliaea aptera DC.	Asteraceae	Herb	2000-3500 m	Moist areas	Roots	Endangered	Decoction of dried roots is taken for stomachache.	0.022
Ajuga parviflora Benth.	Lamiaceae	Herb	1000-2500 m	Moist area ,especially near water sources	Leaves		Leaves are ground with ghee to make a paste. One spoonful of this paste is taken at bedtime for one month for treatment of Hernia. Paste of leaves is used for curing boils and sores.	0.044
Angelica glauca Edgew.	Apiaceae	Herb	2000-3600 m	Moist areas	Roots	Endangered	Used for flavoring of dishes. Paste of roots relieves leg pain.	0.052
Arnebia benthamii (Wall. ex G.Don) I.M.Johnst.	Boraginaceae	Herb	3000-4500 m	Open slopes	Roots	Endangered	Dried roots are immersed in mustard oil for a week until the oil turns reddish pink. This oil is then massaged into the scalp and hair to help reduce hair fall.	0.074
Bauhinia variegata (L.) Benth.	Fabaceae	Tree	1100- 1500 m	Sunny areas	Bark and flowers		Floral buds are eaten as a vegetable. Decoction of bark is used for controlling diarrhea.	0.059
Berberis lycium Royle	Berberidaceae	Shrub	1200-2400 m	Dry hillsides	Root and leaves		Leaves are chewed to control high Blood pressure. Roots are boiled and kept overnight. The extract is filtered and 2-3 drops of it is drippled into eyes twice a day for about one week to get rid of eye troubles. Decoction of roots is taken at bed time for treating Piles.	0.126
Bergenia ciliata (Haw.) Sternb.	Saxifragaceae	Herb	1900-2600 m	Moist rocks and under forest shade	Roots		The dried roots are simmered in water, and this decoction is administered in the early morning to help dissolve kidney and bladder stones.	0.089
Bombax ceiba L.	Malvaceae	Tree	1200- 1600 m	Dry open places	Bark		Bark paste is applied for joint pains and piles.	0.111

Cont...

Table 2. Ethnobotany of some important medicinal plants of Outer Seraj, District, Kullu

Botanical name	Family	Habit	Altitudinal range	Habitat	Part (s) used	Threat status	Indigenous uses and practices	UV value
Calotropis procera (Aiton) W.T.Aiton	Apocynaceae	Shrub	700- 1200 m	Dry areas	Milky Latex		Milky latex is applied on the scalp to treat the baldness.	0.067
Cotoneaster microphyllus Wall. ex Lindl.	Rosaceae	Shrub	1200- 2000 m	Sunny areas	Fruits		Fruits are eaten to replenish the blood in case of deficiency and it also has anthelmintic properties.	0.029
Cirsium wallichii DC.	Asteraceae	Herb	1400-3500 m	Moist areas	Roots		One spoonful of Root powder is given orally with lukewarm water to relieve cough. Decoction of roots is given to children once a day for about 1 week for the treatment of abdominal worms.	0.067
Cuscuta reflexa Roxb.	Convolvulaceae	Herb	1000-2000 m	Dry areas	Stem		Paste of the stem is utilized to cure skin disorders.	0.052
Dalbergia sissoo Roxb.	Fabaceae	Tree	800-1200 m	Sunny areas	Leaves		Leaves powder is mixed with geru to form a paste and applied for relieving sprains.	0.022
Dicliptera bupleuroides Nees	Acanthaceae	Herb	1500-2000 m	Moist semi evergreen forests	Stem and leaves		Decoction of stem and leaves is taken for relieving pain, fever, and cough.	0.097
Euphorbia hirta L.	Euphorbiaceae	Shrub	1000-2000 m	Dry areas	Latex		Latex is used for curing toothache.	0.141
Ficus palmata Forssk.	Moraceae	Small tree	1000-1600 m	Open sunny areas	Latex from stem		Latex of the plant has antiseptic property and when applied to thorn pricked area it results in automatic removal of the thorn. 3-4 drops of the latex when applied relieve aching gums and teeth.	0.186
Hypericum perforatum L.	Hypericaceae	Shrub	1000- 2200 m	Moist areas	Flowers and Leaves		Flowers and leaves are boiled in mustard oil till the color of oil changes. This oil alone or in combination with olive oil acts as good analgesic and is also applied on boils and wounds	0.082
Jasminum officinale L.	Oleaceae	Herb	1200-3000 m	Moist areas	Leaves		Juice of leaves is effective for fistulas.	0.022
Juglans regia L.	Juglandaceae	Tree	1000-2000 m	Moist areas	Bark		Bark of the tree is used for brushing of teeth and also for toothache.	0.164
Melia azadirachta L.	Meliaceae	Tree	1000-2100 m	Dry Sunny places	Fruit		Fruit powder is mixed with warm water to form a paste and is employed to the skin in the form of a pack for skin diseases.	0.029
Oxalis corniculata L.	Oxalidaceae	Herb	1200-2300 m	Common lawn and garden weed	Leaves	Endangered	Juice of leaves is extracted and dropped into the eyes twice a day for eye troubles.	0.194
Pistacia integerrima J.L.Stewart ex Brandis	Anacardiaceae	Tree	800-2000 m	Sunny areas	Pod		Pod is roasted and the ash formed is mixed with honey uniformly to make a paste. One spoon of this paste is administered orally once daily to treat cough.	0.208
Potentilla argyrophylla Wall. ex Lehm.	Rosaceae	Shrub	2200-4000 m	Moist and cool areas	Leaves		Leaves are chewed for toothache and also used as a toothbrush for cleaning of teeth.	0.067
Prinsepia utilis Royle	Rosaceae	Shrub	1800-2500 m	Sunny open places	Seeds		Oil extracted from seeds is massaged on joints at bed time in rheumatism. Oil is applied on forehead at night for cough and cold.	0.134

Cont...

Table 2. Ethnobotany of some important medicinal plants of Outer Seraj, District, Kullu

Botanical name	Family	Habit	Altitudinal range	Habitat	Part (s) used	Threat status	Indigenous uses and practices	UV value
Rhododendron arboreum Sm.	Ericaceae	Tree	3000-3800 m	Moist areas	Flowers		Used for making Chutneys. Juice of flowers is used for nose bleeding.	0.104
Rubus ellipticus Sm.	Rosaceae	Shrub	1000-2400 m	Dry areas	Leaves and shoots		Decoction of leaves and shoots is given to children to treat fever and dysentery. Fruits are edible and relished by children.	0.216
Rumex hastatus D.Don	Polygonaceae	Herb	1800-3600 m	Hillsides	Leaves		Used for making chutneys and brushing of teeth. Juice of leaves is applied for itchy skin.	0.126
Rumex nepalensis Spreng.	Polygonaceae	Herb	1200-3500 m	Grassy slopes	Roots and petioles		Decoction of roots and petioles is given orally once a day for 4-5 weeks for curing piles.	0.097
Saussurea lappa (Falc.) Lipsch.	Asteraceae	Herb	2500-4000 m	Cool moist area	Roots	Endangered	Decoction of roots is taken orally twice a day to control dysentery. Root paste is applied on body by ladies after delivery to keep body warm.	0.022
Tinospora cordifolia (Thunb.) Miers	Menispermaceae	Climbing shrub	800- 1000 m	Sunny places	Stem		Stem is cut into small pieces and grinded to make paste along with leaves of tulsi. This mixture is added to 1 litre water and put on stove at low flame till water reduces to 250 ml. The decoction prepared is taken 3 times a day for fever.	0.231
Trillium govanianum Wall. ex D.Don	Trilliaceae/ Melanthiaceae	Herb	2500-4000 m	Moist areas	Roots	Endangered	Decoction of roots is taken to control diarrhea and also treat asthma.	0.089
Urtica dioica L.	Urticaceae	Shrub	1200-3000 m	Dry areas	Leaves and Shoot		Paste of leaves and shoot is used to cure sprain and swelling.	0.119
Valeriana jatamansii Jones ex Roxb.	Valerianaceae/ Caprifoliaceae	Herb	1500-3500 m	Cold and shady places	Leaves and roots	Vulnerable	Leaves are grinded and small tablets are made of it (about 10 leaves are ground for one pill). One tablet is given once a day for about 25 days for the treatment of joint pains and fractures.	0.164
Viola odorata L.	Violaceae	Herb	1500-2500 m	Roadsides,shaded woody edges	Leaves		Leaves are used for curing the common cold.	0.156
Withania somnifera (L.) Dunal	Solanaceae	Shrub	700- 1500 m	Dry open places	Roots and leaves		Leaf Paste is employed on the skin for various skin diseases. Root powder is used to decrease asthma.	0.141
Zanthoxylum armatum DC.	Rutaceae	Shrub	1200-2000 m	Dry Forests and Roadsides	Stem and seeds	Endangered	Twigs of plants are utilized as toothbrushes. Bark of the plant is removed and small pieces of it are chewed for 4-5 minutes in case of toothache.	0.179

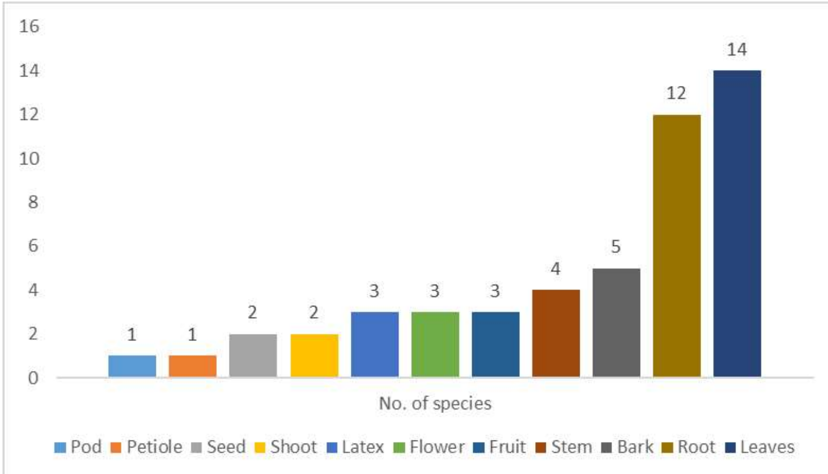

Fig. 3. Statistics of plant parts used

Utilization pattern: The therapeutic plants reported from the study area are utilized to cure diverse ailments such as fever, cough, common cold, dislocated joints, asthma, skin infections, eye troubles, diarrhea, piles, etc. Among these reported plant species, the most commonly used plant species are *Berberis aristata, Prinsepia utilis, Valeriana jatamansii, Zanthoxylum armatum, Ajuga parviflora, Abies pindrow, and Angelica glauca.* Some of the reported herbs are utilized in the management of a single illness while many possess multiple therapeutic uses. Different parts of these plants i.e. roots (12 sps.), leaves (14 sps.), bark (5 sps.), stem (4 sps.), fruits (3 sps.), seeds (2 sps.), pod (1 sps.), latex (3 sps.) and flowers (3 sps.) possess medicinal values and are utilized by the local individuals in the preparation of medicines (Fig. 3). These herbs are used as whole or their parts are used in the form of paste, powder, and decoction.

Rarity: The present study indicates that the overexploitation of these therapeutic plants has posed a threat to their extinction. Seven plants namely *Oxalis corniculata, Zanthoxylum armatum, Arnebia benthamii, Angelica glauca, Trillium govanianum, Saussurea lappa,* and *Ainsliae aptera* are endangered and 1 plant species namely *Valeriana jatamansii* is vulnerable. This data highlights the need to take steps towards the conservation and sustainable utilization of these plants.

CONCLUSION

The present paper offers detailed information on the diversity, distribution patterns, traditional uses and mode of administration of the therapeutic plants employed by the local inhabitants of the Outer Seraj area for curing various ailments. The total of 39 plant species belonging to 32 families have been listed. Most of the reported plant species possess multiple therapeutic uses. Different parts of these plants viz roots, leaves, bark, stem, fruits, seeds, flowers etc. possess medicinal values and are used by the local individuals in the form of powder, paste and decoctions. *Tinospora cordifolia, Pistacia integerrima, Rubus ellipticus, Oxalis corniculata, Juglans regia, Withania somnifera, Euphorbia hirta* and *Berberis lycium* are some of the plants which possess very high UV value indicating their high significance for the local people. However, unscientific and over- exploitation of these plants is resulting in imminent danger of extinction of these plants. Seven plants species have become endangered and 1 plant species is vulnerable facing the threat of extinction. Efforts to conserve and sustainably manage medicinal plant resources are crucial to ensure their continued availability for the future generations. There is also an urgent need to take steps for the *in-situ* and *ex-situ* conservation of these plants.

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Biomass and Carbon Stock at Various Habitats of Alpine Meadows in the Kumaun Himalaya, India

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***Abstract*:** This study investigates biomass and carbon stock across six alpine habitats (Moist Habitat, Gentle Slope Habitat, Upland Mountain Habitat, Open Grassy Habitat, Stream Bank Habitat, Dry Rocky Habitat) in the Kumaun Himalaya, examining species richness, aboveground biomass, belowground biomass, total biomass, and associated carbon stocks. Species richness varied from 22 to 35. Among all habitats the species richness was highest in Stream Bank Habitat with 35 and lowest in Dry Rocky Habitat with 22. Aboveground biomass was highest in Moist Habitat 89.16 g/m², while the lowest was observed in Gentle Slope Habitat 64.1 g/m². Belowground biomass was highest in Stream Bank Habitat 97.55 g/m², while the lowest was observed in Dry Rocky Habitat 78.94 g/m². Total biomass was highest in Moist Habitat (183.4 g/m²) and lowest in Dry Rocky Habitat (149.7 g/m²). Significant differences were observed for Above Ground Biomass, Total Biomass, Above Ground Carbon, Total Carbon, and species richness across habitats. Below Ground Biomass and Below Ground Carbon did not vary significantly.

***Keywords*:** Alpine, Meadows, Biomass, Carbon, Biodiversity

Alpine grasslands play a crucial role in supporting the livelihoods of over one billion people worldwide while providing significant ecological benefits (Buisson et al., 2022, Lopez et al., 2022). The typical mosaics of alpine micro-habitats, which show a significant deal of heterogeneity among the plant communities and survival strategies among individual species, are more pronounced on steeper mountain slopes and terrain with different topographic structure (Liang et al., 2016). Alpine environments are distinguished by their nutrient-poor soils, chilling winds, winter precipitation in the form of snow, and abrupt seasonality pattern. Above ground biomass also provides key information for understanding the responses of vegetation to climate change and resilience (Liang et al., 2016) and can be used to directly estimate grassland productivity (Lopez et al., 2022). Overgrazing, poor management of natural grasslands and biodiversity loss have all been linked to changes in plant community structure and declining productivity in some grasslands (WenJun et al., 2014).

Biomass serves as a fundamental ecological parameter, influencing species diversity, productivity, and carrying capacity in rangelands and pasturelands (Rawat et al., 2012). Grassland biomass is influenced by various topographical factors, including altitude, slope characteristics, as well as biodiversity, all of which contribute to ecosystem stability and productivity (Bhandari and Zhang 2019, Yang et al., 2021, Liu et al., 2022). The grasslands are a vital component of global vegetation, storing approximately one-third of the terrestrial carbon pool (Liang et al., 2016). Alpine and subalpine grasslands primarily occur at high elevations where extreme environmental conditions prevent tree growth, typically forming low stature vegetation (Padalia et al., 2019). Furthermore, plant biomass allocation patterns, particularly between above- and belowground components, reflect species adaptation to environmental constraints such as low temperatures and nutrient availability (Ma et al., 2010). Accurate estimation of aboveground biomass and belowground biomass is critical for understanding the role of alpine grasslands in the global carbon cycle and for informing sustainable management strategies (Yang et al., 2010).

Plant habitats are the specific environmental conditions in which species grow, survive, and reproduce. These conditions include both abiotic factors (such as soil type, moisture, temperature, elevation, and light availability) and biotic interactions (such as competition, herbivory, and mutualism), all of which influence plant distribution and community composition (Sandel et al., 2011). The diversity of plant habitats ranging from tropical forests and grasslands to alpine meadows and arid deserts reflects the variety of ecological niches occupied by different species (Scheiter et al., 2013). In the face of global environmental change, understanding plant habitat preferences is critical for predicting shifts in species distributions, conserving biodiversity, and managing ecosystems sustainably (Rumpf et al., 2019).

Alpine plants exhibit specialized traits such as dwarfism, deep-root systems, and physiological resilience to cope with extreme conditions (Choler 2015). Although limited in spatial extent, these ecosystems support a high proportion of endemic species and serve as sensitive indicators of climate change impacts (Rumpf et al., 2019). Understanding plant adaptation and community dynamics in alpine habitats is essential for biodiversity conservation and ecosystem management under ongoing environmental change. Habitat degradation, fragmentation, the invasion of alien species, over-exploitation, and an ever-increasing human population are among the critical factors contributing to species loss across the globe (Barnosky et al., 2012), placing approximately one-fifth of plant species at risk of extinction. Species habitat recovery is recognized as one of the most effective ecological engineering strategies for species rehabilitation and habitat conservation (Polak & Saltz 2011 and Rawat et al., 2021).

The several studies have been made on the Himalayan alpine grasslands among these are (Rawat et al., 2012, Namgail et al., 2012, Padalia et al., 2019, Joshi et al., 2020, Chandra et al., 2021, Barman et al., 2021, Rawat et al., 2021 and Kumar 2024). The alpine climate formed a diverse habitat for the growth and development of plants. The habitat may be different in species composition and community structure as well as biomass. The conservation and management of different habitat condition would be of paramount importance. There is little or no information is available on the biomass and carbon stock in the Byans valley of Kumaun Himalaya. Thus, the present study aims to measure the biomass in different habitats of the Byans valley in central Himalayan region.

MATERIAL AND METHODS

Study area: The Study area is located between 30°10´ and 30°20´ N latitude and 80° 20´and 80°50´ E longitude between 3000-3500m elevation in high elevation area of Byans valley Kumaun Himalaya, India. The Byans valley is the last valley of the Indian Himalayan region before the bordering with Tibet. It runs along a North West to South East axis, formed by the Kuti yankti river, which is one of the headwaters of the Kali River that forms the boundary between India and Nepal in this region. The study area was selected considering various geographical attributes along with environmental coordinates such as latitude, longitude, altitude, and slope using a global positioning system (Garmin model 2000).

Climate: The area covered with snow during early winter season to late spring (November-April). The climatic data were collected from tehsil Dharchula, nearest climatic station in 2023, the mean maximum temperature was 13.9°C and mean minimum temperature was -2.3°C. The rainy season (summer monsoon) extends from mid-June to September, and maximum rainfall (about one third of the annual) occurs during this period (1520mm) (Fig. 2). The snowfall, begins from October and continue until the first week of April. Snow melt begins around mid-April which provide sufficient moisture to the growth of herbaceous vegetation. Geologically the study area situated in greater Himalayan region. The soil in the study area has diverse nature and showed close relationship with vegetation pattern. Texture of soil is sandy loam and clay loam varies in depth across the altitudinal gradients and slightly reddish in colour.

The area is situated at high elevation where climatic conditions do not favour the growth of tree vegetation and shows the presence of small-structured woody vegetation,

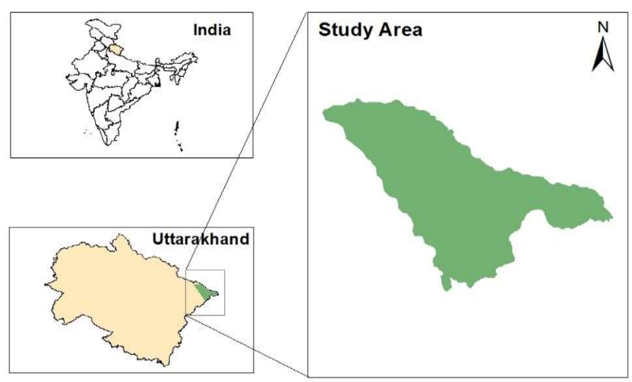

Fig. 1. Map of study area

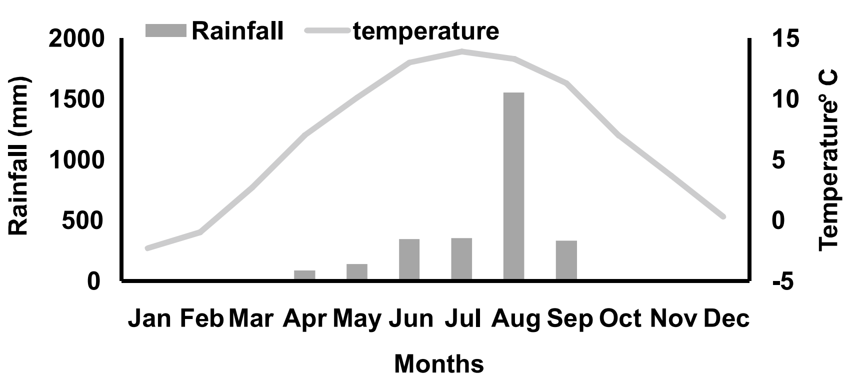

Fig. 2. Monthly variation in rainfall (mm) and temperature (°C)

grasses, and herbs. The plant communities exhibit a diverse distribution, because of their extremely modest spatial extents, many of these communities can be seen in small patches (<1 to 2 m^2) at a single place.

Methods: The study was conducted during 2023-2024 at an elevation of 3000-3500m above sea level in Byans valley of Kumaun Himalaya. After through survey six different habitats were identified based on topography, moisture, and vegetation types (Table 1). In each habitat, three plots of 1 × 1 m were randomly selected and used to harvest for the aboveground biomass. The fresh weight of the aboveground material was measured in the field. All harvested aboveground plant material was packed in polyethylene bags, and brought to the laboratory. Then oven dried at 60° C until the constant weight and weighed. Similarly, the belowground plant material was collected using a monolith (25 × 25 × 30 cm) from each harvest plot. The roots were washed and free from soil particles using a thin jet of water and fresh weight was taken in the field, then brought to the laboratory, oven-dried at 60°C until a constant weight and weighed. The biomass carbon stocks for herbs were determined following the IPCC guidelines, assuming a carbon content of 47.5% of the aboveground and belowground biomass (IPCC 2006; Joshi et al., 2021).

$C = Y \times 0.475$

Where, C = carbon stocks, Y = Biomass

The habitat was identified based on the field conditions like soil moisture, slope, and landscape and the habitat characteristics are given in (Table 1).

Statistical analysis: The statistical data were analysed, using MS Excel 2019, IBM-SPSS 16.0 version.

RESULTS AND DISCUSSION

Among all six habitats, the number of species ranged between 22 to 35. It was minimum in Dry Rocky Habitat and maximum in Stream Bank Habitat. Biomass are also varied

Table 1. Habitat types and its characteristics in high altitude alpine meadow

Habitat	Site	Slope	Elevation (m)	Soil moisture	Site characteristics
Moist Habitat (MH)	Budi	7.5%	3005	65.7	Slopes that are shaded and mostly covered with herbaceous plants, moderate slope, a fair amount of moisture, and an open canopy of blue pine and silver fir trees.
Gentle Slope Habitat (GSH)	Challekh 01	10.2%	3155	56.3	Gentle, slopes are undulating landscape. Exposed and dominated by a variety of herbaceous species.
Dry Rocky Habitat (DRH)	Challekh 02	33%	3267	23.7	Dry mountain slopes are well-exposed but generally less solid, with many small loose rocks and dominated by sparsely growing *Juniperous communis* with few patches of grasses on and around it.
Stream Banks Habitat (SBH)	Champhunala	13.4%	3378	78.6	A seasonal stream flows through this narrow, shaded mountain ravine and several small rocky outcrops along the sides.
Open Grassy Habitat (OGH)	Champhunala	44.3%	3428	53.8	Steep slopes with few small scattered boulders and dominated mainly by grasses herbaceous plants.
Upland Mountain Habitat (UMH)	Challekh 03	8.7%	3502	37.6	Elevated mountain tops an exposed, tableland with sporadic tiny rocks scattered throughout. It contains a flat vegetation mat and dominated by *potentilla argyrophylla, bistorta affinis*, *Sibbaldia parviflora, Primula denticulata, Geranium wallichianum*, and *Trachydium roylei.*

Statistical analysis: The statistical data were analysed, using MS Excel 2019, IBM-SPSS 16.0 version

Table 2. Biomass, and carbon stock in different habitats

Parameters	OGH	GSH	SBH	MH	UMH	DRH	F	P value
No. of species	30	24	35	33	32	22	6.8	0.003
AGB (g/m^2)	87.2	64.1	80	89.16	79.18	70.75	41.2	0.00
BGB (g/m^2)	86.9	89.23	97.55	94.23	85.81	78.94	0.99	NS
TB (g/m^2)	174.1	153.3	178.3	183.4	165	149.7	3.94	0.02
AGC (g/m^2)	41.41	30.46	38.3	42.35	37.6	33.6	41.1	0.00
BGC (g/m^2)	41.27	42.38	46.3	44.7	40.7	37.4	0.99	NS
TC (g/m^2)	82.69	72.84	84.7	87.11	78.3	71.1	3.9	0.02

AGB; Above ground biomass, BGB; Belowground biomass, TB; Total biomass, AGC; Above ground carbon, BGC; Belowground carbon, TC; Total carbon across the habitat and support the findings of earlier studies (Padalia et al., 2019, Aziz et al., 2019, Wan et al., 2024). The Aboveground biomass ranged between 70.75 - 89.16 g/m^2 in present study, maximum in Moist Habitat (89.16 g/m^2) followed by Open Grassy Habitat (87.2 g/m^2), and minimum in Gentle Slope Habitat (70.75 g/m^2). The belowground biomass ranged between 78.94 - 97.55 g/m^2 in present study, maximum in Strem Bank Habitat (97.55 g/m^2) followed by Moist Habitat (94.23 g/m^2), and minimum in Dry Rocky Habitat (78.94 g/m^2). The total biomass ranged between 149.7 - 183.4 g/m^2 in present study, maximum in Moist Habitat (183.4 g/m^2) followed by Strem Bank Habitat (178.3 g/m^2) and minimum in Dry Rocky Habitat (149.7 g/m^2) (Table 2). The ANOVA indicates that the species richness (F = 6.8, P < 0.005), Aboveground biomass (g/m^2) (F = 41.2, P = < 0.0001), Total biomass (F = 3.94, P = < 0.05) showed significant variation among habitats (Table 2). The LSD test revealed that biomass in Moist Habitat was significantly higher than in Gentle Slope Habitat, Open Grassy Habitat, Stream Bank Habitat, Dry Rocky Habitat, and Upland Mountain Habitat. A similar trend of carbon stock was also observed in each habitat (Table 2). Wan et al. (2024) reported 70.1 - 122.8 g/m^2 aboveground biomass and 666.3 - 1385.4 g/m^2 for belowground biomass. The total biomass was 745.2 - 1493.5 g/m^2 in Tibetan Plateau. Similarly, Aziz et al., (2019) reported 1.03 – 92.12 t/ha aboveground biomass, and 0.2 – 18.42 t/ha belowground biomass. The total biomass 1.23 – 110.6 t/ha in Kashmir western Himalaya.

The alpine zone of the Himalaya experiences a short growing season (May to September), and many plant species are completed their growth cycle within three to five months. Consequently, peak community biomass tends to be lower than peak species biomass, as different species reached their peak biomass in different periods of growing season (Wan et al., 2024). The availability of abundant sunlight and moisture throughout the growing season makes it possible to grow and survive greater number of species in the Stream Bank Habitat and Moist Habitat. Thus, the habitats will be one of the important aspects to regulate the community structure and composition. The Himalayan alpine vegetation formed a mosaic in different ecological and topographical conditions. The moisture is never a limiting factor for the growth and development of plant species. The relationship between soil moisture and aboveground

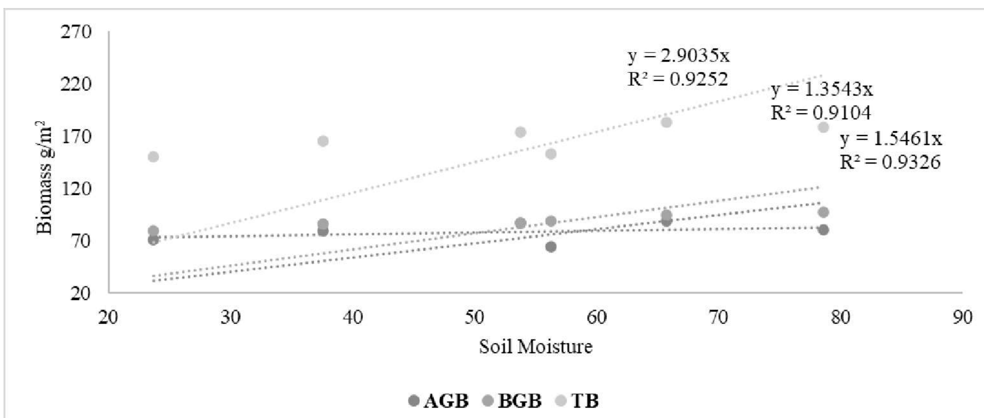

Fig. 3. Relation between soil moisture and biomass

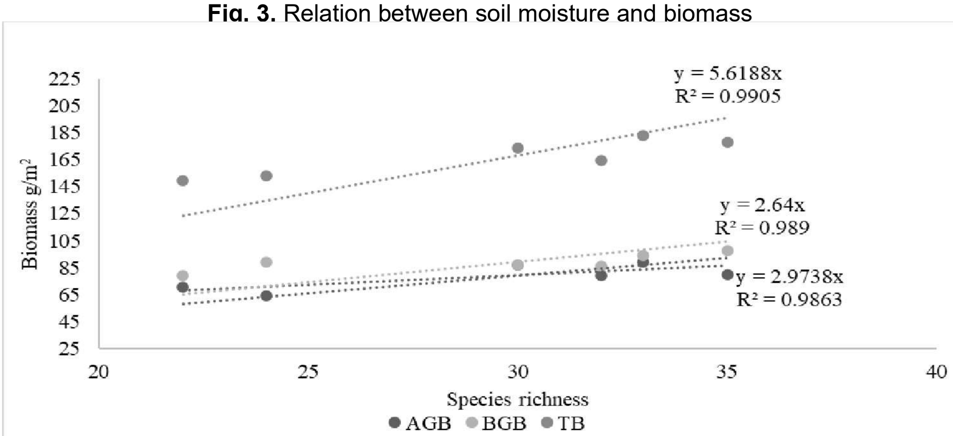

Fig. 4. Relation between species richness and biomass

biomass, belowground biomass and total biomass showed positive relationship (Fig. 3).

Among all the studied habitats, Moist Habitat exhibited the highest belowground biomass, may be due to more consistent soil moisture, deeper rooting zones, and prolonged growing seasons compared to drier sites. The saturated conditions in Moist Habitat supports a dense growth of graminoids and forbs, which are efficient in biomass accumulation under favourable moisture regimes. This result aligns with previous studies in alpine and subalpine systems which report higher productivity in hydrologically stable sites (Zhang et al., 2022). The species richness, and biomass were low as compare to other studies reported for similar vegetation type (Padalia et al., 2019, Wan et al., 2024) because this can be attributed to a combination of ecological constraints such as steeper slopes, shallow or poorly developed soil, and potentially less favourable microclimatic conditions. The dominance of unpalatable species such as *Anaphilis, Primula, Rumex, Iris* and *Potentilla* indicates the presence of high grazing pressure in this area. These factors significantly limit plant growth, leading to lower aboveground and belowground biomass, species richness, and carbon stock values. Moreover, anthropogenic pressures such as grazing, tourism may further stress these fragile alpine ecosystems, inhibiting vegetation development and biomass accumulation. Despite these limitations, the study area still supports considerable species richness and diversity, indicating the ecological resilience of alpine plant communities under harsh environmental conditions. The relationship between species richness and aboveground biomass, belowground biomass and total biomass showed strong positive relationship (Fig. 4).

CONCLUSION

To conclude this study indicated that aboveground biomass and total biomass varied significantly along the habitat. The belowground biomass was comparatively high in all habitats except Open Grassy Habitat. This may be due to the growth ceased earlier for aboveground vegetation and food material translocated too belowground. The belowground biomass was not significantly varied among the habitats, because the moisture and other factor regulate the belowground biomass. Thus, the variations in habitat conditions govern the accumulation of above and belowground biomass. Therefore, the conservation of habitats will be important for the species diversity as well as for biomass and production of these alpine meadows.

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Carbon Dynamics in Community-managed Forests across Altitudinal Gradient in Central Himalaya, India

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Abstract: The present study investigates the variation in carbon stock distribution across different carbon pools along elevational gradients in community-managed forests (Van Panchayats) of the Kumaun Central Himalaya. The forests were categorized into three altitudinal zones: low, mid, and high-elevation sites. Among the various pools, tree biomass carbon accounted for more than 80 percent of the total carbon stock, highlighting its dominant role in carbon sequestration. Soil organic carbon contributed between 14.06 and 17.58 percent, whereas forest floor carbon contributed between 1.19 and 1.81 percent to the total carbon pool. The herb layer biomass carbon showed the least contribution. The strong positive correlation was observed between tree and shrub carbon stocks and elevation ($R^2 > 0.84$), suggesting an elevation-driven pattern in above-ground carbon accumulation. The overall total carbon stock ranged from 169.51 to 185.62 Mg C ha^{-1}. This study emphasizes the necessity of adopting site-specific conservation and management practices to sustain and enhance the carbon stock potential of these forests across elevational gradients.

Keywords: Carbon pools, Community managed forests, Forest floor carbon, Soil organic carbon, Elevational gradient

Forests play a vital role in carbon sequestration, serve as a significant reservoir of biomass, and act as a carbon sink. These forest ecosystems are the largest carbon reservoirs, holding over 80% and 40% of the Earth's terrestrial carbon pools above ground and below ground, respectively (Pan et al., 2011). Plant biomass (both above and below ground), woody debris, litter, and soil are the major carbon pools in forest ecosystems (Sharma et al., 2016). Globally, forests hold more than 650 gigatons of carbon in total. Out of this, around 44 percent is stored in forest biomass, about 11 percent is found in deadwood and litter, and nearly 45 percent is held in the soil. Specifically, forest biomass alone contains nearly 298 gigatons of carbon, showing how vital it is in the global carbon cycle. Carbon stocks have been declining worldwide due to deforestation and land use change (FAO 2020). The estimated carbon stock is 3 billion metric tonnes, accounting for around 40% of the total carbon pool in India's forests (Rawal et al., 2021). The Indian Himalayan forests sequester around 65 million metric tonnes of carbon per year (Tolangay and Moktan 2020). The estimated total forest carbon stock in Uttarakhand across all carbon pools is 378.16 million tonnes.

Based on management, Uttarakhand forests are classified into three categories: Reserve Forests, Community-managed Forests, and Civil Soyam Forests. The community forests, known as Van Panchayat forests and managed by village-level institutions in collaboration with State Forest and Revenue departments (Mukherjee 2004, Nagahama et al., 2022). This decentralized forest governance approach came into existence in 1931 and is a participatory conservation initiative that aids in resource conservation and sustainable utilization while providing livelihoods to rural communities. There are 12,089 Van Panchayats, which are more than 16% (544,964 hectares) of the forest area in Uttarakhand (Agrawal and Ostrom 2008, Negi et al., 2012). These forests not only support the livelihoods of local people but also play an important role in climate change mitigation and biodiversity conservation. As local people and forests are interlinked, they provide a strong incentive for both forest management and conservation efforts (Joshi et al., 2011, Chakraborty et al., 2018). In recent years, global climate change has become a major concern, and forests are considered natural mitigators of climate change as they absorb a significant amount of CO_2 from the atmosphere (Rawat and Singh 2016). These forests offer numerous social, environmental, and economic benefits to the communities residing near forest areas. In community-managed forests, the assessment of carbon stock is crucial to initiate climate change mitigation programs such as Reducing Emissions from Deforestation and Forest Degradation (REDD+), conserving carbon stock, sustainable forest management, and enhancing carbon stock (UNFCCC 2016).

In the past few years, forest ecosystems have been deteriorating due to climatic factors and various human activities (fodder and fuelwood collection, grazing, forest fires, etc. which ameliorate the climate change forces. Several other studies have been conducted on biomass and carbon stock dynamics in the reserved forests in Uttarakhand regions (Gairola et al., 2011, Joshi et al., 2011, Dar et al., 2017). In addition, some research has also been carried out in the community-managed forests of the Kumaun region (Rawat 2012, Vikrant and Chauhan 2014, Bagri et al., 2022, Pimoli et al., 2024). Keeping in view the increasing human interference and poor engagement of community owned forests, the aim of the present study is to assess the variation in carbon pool dynamics along elevational gradients in community-managed forests of the Nainital district in the Central Himalaya.

MATERIAL AND METHODS

The study area is situated between 29°19'19.5"N and 29°25'54.9"N latitudes and 79°27'29.3"E and 79°35'40.1"E longitudes at altitude ranging from 1200 to 2400 m in the central Himalayas (Fig. 1). The mean minimum temperature varied between 0.02°C (January) and 18.7°C (July), while the mean maximum temperature ranged between from 19.3°C (January) and 36.5°C (June). The average monthly rainfall was 0.3 mm (November) and 297.0 mm (September) and the total annual rainfall was 1128.4 mm (Fig. 2). The climate data was taken from the website https://power.larc.nasa.gov in 2022. The study area was selected considering various geographical attributes along with environmental coordinates such as latitude, longitude, altitude using a global positioning system.

The rocks of the study are composed of limestone, pyritic, and carbonaceous. The forest soils are loamy and silty clay (Table 1). The community managed forests include *Quercus leucotrichophora* A. Camus, *Cupressus torulosa* D. Don ex Lamb., *Pyrus pashia* Buch. -Ham. ex D. Don, *Syzygium cumini* (L.) Skeels and *Bauhinia racemosa Lam at* low-elevation VP forest. The mid-elevation VP forest includes *Pinus roxburghii* Sarg., *Quercus leucotrichophora* A. Camus, and *Glochidion velutinum* Wight, while the high-elevation VP forest characterized by *Quercus floribunda* Lindl. ex A. Camus, *Rhododendron arboreum* Sm., *Lyonia ovalifolia* (Wall.) Drude, and *Ilex dipyrena* Wall.

After a thorough reconnaissance of the study area, three community-managed forests (VPFs) were identified at different elevations: Thapalia Mahara Gaon VPF located at low elevation (1200m-1500m), Bhudhalakote VPF at mid-elevation (1500m-1800m), and Satbunaga VPF at high elevation (2100m-2400m). The detailed site characteristics

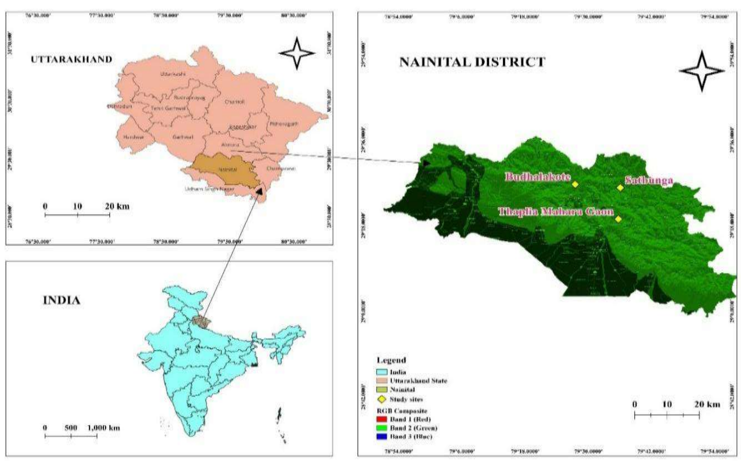
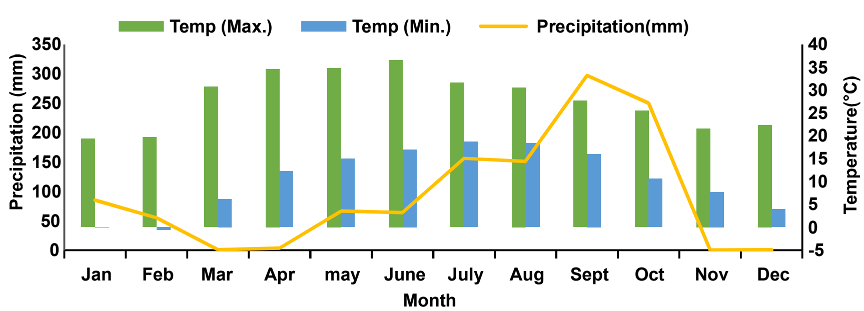


Fig. 1. Location map of the study sites



Source: https://power.larc.nasa.gov.

Max.= maximum; Min.= minimum; temp= temperature

Fig. 2. Mean monthly maximum and minimum temperature (°C) and precipitation (mm)

of these forests are given in Table 1.

In each VPF, three composite random soil samples were collected in three different depths: 0–10 cm, 10–20 cm, and 20–30 cm. Walkley and Black's titration method was used to estimate the SOC content in the collected soil samples (Walkley and Black 1934).

SOC = 0.1 × BD × D × C

Where, SOC: organic carbon storage in the soil horizon (Mg ha^{-1}),

D: depth of soil horizon (cm),BD= Bulk density(cm^{-3}),C= Organic carbon concentration ($g.kg^{-1}$)

Total organic carbon storage in the soil profile was calculated as the sum of the carbon content in each soil horizon. The total carbon stock was estimated by summing the individual carbon pools, following the method of Pearson et al. (2007).

Tree biomass was measured using a non-harvesting method. All individual trees with a circumference greater than 30 cm were recorded, and the circumference was measured at breast height (1.37 m above the ground) in the sampled quadrats. For shrubs, CBH was taken at the collar height. To estimate the biomass of different tree and shrub components (bole, bole bark, branches, twigs, leaves, stump roots, lateral roots, and fine roots) previously established allometric equations,

$\ln Y = a + b \ln X$

where Y denotes the dry weight of the component (kg), X is the circumference at breast height (CBH in cm), a is the intercept, b is the slope and ln refers to the natural logarithm, was used. The values of *a* and *b* were adopted from previously established regression models (Rawat 1983, Chaturvedi and Singh 1987, Rawat and Singh 1988, Rana et al., 1989, Adhikari et al., 1995). For herbs, above ground biomass was determined by placing quadrats of 1 x 1 m in each forest. The below ground biomass was collected from the monolith of 25 cm ×25 cm × 30 cm from each harvest plot and washed with fine jat remove all foreign material. The fresh weight was taken in the field and then the samples were placed in perforated paper bags, brought to the laboratory, oven-dried at 60° C till constant weight and weighted.

The biomass carbon stocks for trees, shrubs and herbs were determined following the IPCC guidelines, assuming a carbon content of 47.5% of the above ground and below ground biomass (Magnussen and Reed 2004, IPCC 2006)

C = B ×0.745

Where, C=carbon content and B= biomass

Forest floor litter was collected from 1 m × 1 m permanent quadrats, randomly placed in the forest. Litter from each quadrat was placed in a separate polyethylene bag and brought to the laboratory. The samples were then separated into fresh leaves, partially decomposed leaves, wood, and miscellaneous litter components. These were oven-dried at 60 °C to a constant weight. The dry biomass obtained was multiplied by the corresponding carbon fraction of 47.5%.

Statistical analysis: The collected data in each Van Panchayat forests were analysed using SPSS (version 16), Microsoft Excel 2019, and R-Studio (version 12.1) software. Regression analysis was conducted to assess the relationships between vegetation and soil attributes, while principal component analysis (PCA) was used to explain the variance in these attributes.

RESULTS AND DISCUSSION

More than 80 percent of total carbon was stored in the living biomass of the tree layer across all sites, while the shrub and herb layers made only minor contributions. Soil organic carbon, comprising over 12 percent of total stock, represented the second-largest pool. Both shrub biomass and soil carbon varied significantly with elevation. The relatively higher carbon storage at high elevation may reflect

Table 1. Site characteristics of community-managed forests

Parameter	Low-elevation	Mid-elevation	High-elevation
Van Panchayat	Thapalia Mahara Gaon	Bhudhalakote	Satbunga
Altitude (m)	1200-1500	1500-1800	2100-2400
Latitude	N 29º 19′	N 29º 26′	N 29º 25′
Longitude	E 79º 35′	E 79º 27′	E 79º 36′
Forest area (ha.)	380	44	103
Dominant Tree Species	*Quercus leucotrichophora* A. Camus	*Pinus roxburghii* Sarg. *Quercus leucotrichophora* A. camus	*Quercus floribunda* Lindl. ex A. Camus
pH	6.57	6.5	6.6
SOC %	0.7	0.87	0.81
Soil type	Loam	Loam	Clay loam

SOC= Soil organic carbon

differences in forest structure, stand age, and management practices, consistent with elevational patterns reported in other Himalayan studies (Simegn and Soromessa 2015, Vikrant et al., 2021).

Soil organic carbon (SOC) in the 0–30 cm layer was significantly higher in mid-elevation Van Panchayat forest (VPF) compared to low- and high-elevation sites. The mean SOC stock per hectare, estimated based on soil depth, bulk density, and carbon content, declining trend with increasing depth across all sites.

The highest mean SOC was recorded in mid-elevation VPFs (29.93 Mg ha^{-1}), while the lowest was observed in high-elevation VPFs (26.11 Mg ha^{-1}) (Fig. 3). These SOC values are notably lower than those reported for other Himalayan forest ecosystems, such as the 39.1-91.4 Mg ha^{-1} range observed by Dar and Sundarapandian (2015) and the 47.61–55.20 Mg ha^{-1} by Pawar and Gupta (2013). The comparatively lower SOC in high-elevation VPFs may be attributed to continuous open access for grazing and intensive leaf-litter collection over a three-month period by local communities, which likely disrupts nutrient cycling and reduces organic matter accumulation.

The high-elevation Van Panchayat Forests (VPFs) recorded the greatest total carbon storage (185.62 Mg ha^{-1}), followed by mid-elevation (170.24 Mg ha^{-1}) and low-elevation VPFs (169.51 Mg ha^{-1}) (Table 2). These values lie within the range reported for temperate forests in the Himalaya, such as 107.94-230.12 Mg ha^{-1} (Joshi et al., 2021), 272.52 Mg ha^{-1} (Kaushal and Baishya 2021), and 137.10 Mg ha^{-1} (Sharma et al., 2010).

The tree layer contributed the highest proportion to the vegetation carbon pool, ranging from 80.34 to 82.80 percent, indicating that trees are the dominant component of carbon sequestration across all elevation sites. Tree carbon storage ranged from 136.78 to 153.69 Mg ha^{-1}, with the highest value at the high-elevation Van Panchayat Forest (VPF) and the lowest at the mid-elevation VPF. These values fall within the range documented by Joshi et al. (2021) for temperate Himalayan forests (107.93–274.15 Mg ha^{-1}), yet remain lower than the estimates of 120.23 Mg ha^{-1} and 157.47–203.57 Mg ha^{-1} provided by Mbaabu et al. (2014) and Gwal and Lodhiyal (2019), respectively, for similar forest types. Tree-layer carbon was highest at the high-elevation site, where *Quercus floribunda* dominates, suggesting the presence of dense and mature oak forests capable of accumulating high levels of biomass.

The carbon stock in the shrub layer ranged from 0.95 to 1.98 Mg ha^{-1}, while in the herb layer varied between 0.55 and

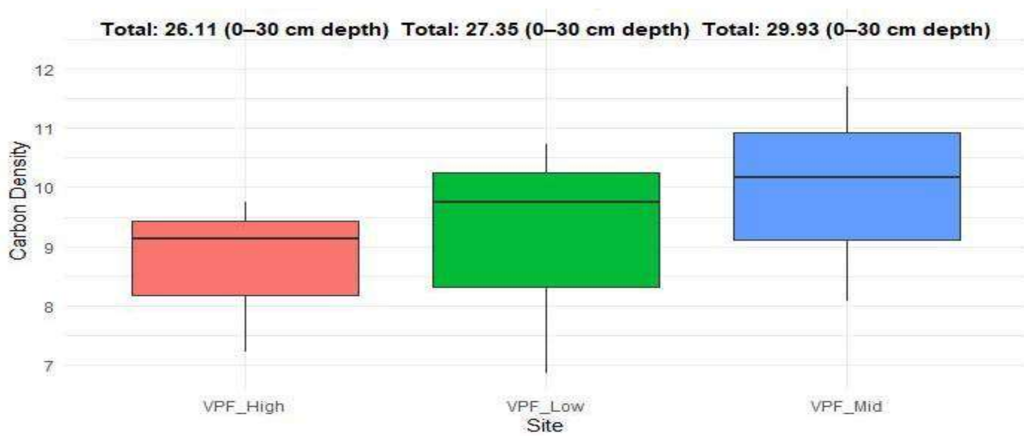


Fig. 3. Soil carbon stock at different elevations in Van Panchayat Forests

Table 2. Carbon pools across elevational gradients in Van panchayat Forests

Parameter	Low-elevation (1200-1500m)	Mid-elevation (1500-1800m)	High-elevation (2100-2400m)
Carbon stock of tree layer (Mg C ha^{-1})	137.22	136.78	153.69
Carbon stock of shrub layer (Mg C ha^{-1})	1.13	0.95	1.98
Carbon stock of herb layer (Mg C ha^{-1})	0.74	0.55	0.71
Total vegetation carbon (Mg C ha^{-1})	182.3	138.28	156.39
Soil organic carbon (Mg C ha^{-1})	27.34	29.93	26.11
Forest floor carbon stock (Mg C ha^{-1})	3.07	2.03	3.11
Total carbon (Mg C ha^{-1})	169.51	170.25	185.62

0.74 Mg ha^{-1} (Table 2). These are significantly higher for shrubs and significantly higher for herbs compared to those reported by Thakur et al. (2024), recorded 1.31 Mg ha^{-1} and 0.37 Mg ha^{-1}, respectively, in similar temperate forest conditions. In the lower elevation Van Panchayat (VP) forest, dominated by *Quercus leucotrichophora* and characterized by a relatively young forest structure, the higher carbon stock in the herb layer may be attributed to limited extraction of fodder and fuelwood, along with improved regeneration. These factors likely supported enhanced herbaceous growth and soil restoration.

The carbon stock in forest floor litter across Van Panchayat Forests (VPFs) ranged from 2.03 to 3.11 Mg ha^{-1} (Table 2). These were higher than the estimates reported by Krishan et al. (2017), which ranged from 0.84 to 1.44 Mg ha^{-1}. In contrast, these values were lower than the range of 4.21 to 5.97 Mg ha^{-1} reported by Gosain et al. (2015), and the 2.5 to 3.1 Mg ha^{-1} recorded by Dar and Sundarapandian (2015) in temperate Himalayan and tropical dry evergreen forests. The mid-elevation VPF also experiences occasional forest fires, although these are actively suppressed by community members, the combination of fire events and pine needle accumulation likely inhibits understory vegetation growth and reduces litter retention, thereby limiting overall carbon storage. Interestingly, soil organic carbon was found to be highest in this forest, possibly due to the slow decomposition rate of pine needles and minimal disturbance to the forest floor.

Principal component analysis (PCA) correlation variance of carbon stock of tree, shrub, herb, forest floor, and soil organic carbon stock showed PC 1 accounting for 62.6% (Eigen value = 6.26) and Principal Component 2 accounting for 37.4% (Eigen value = 3.74) of the total explained variance in relation to elevation. Variables such as total carbon stock (TCS), tree carbon stock (Tr_c), shrub carbon stock (Sh_c), and herb carbon stock (H_c) showed strong positive associations with PC1. Elevation (Ele) and shrub carbon stock (Sh_c) showed stronger associations with PC2. The high-elevation site (VPF_high) is closely associated with elevation, shrub carbon stock, and tree carbon stock. The mid-elevation site (VPF_mid) shows moderate association with soil and organic carbon properties. The low-elevation site (VPF_low) is located away from major carbon stock vectors, indicating weaker association with total and tree carbon stock (Fig. 4).

The variation in carbon stocks along various elevational gradients is influenced by a number of factors such as forest type, soil, stand structure, and environmental conditions, which determine the carbon storage capacity and ecological dynamics of forest ecosystems. However, elevational changes increase carbon storage and nutrient cycling, which improves ecosystem functioning (Kumar et al., 2013). The variation in carbon storage across different elevational Van Panchayat Forests (VPFs) appears to be influenced not only by altitude and dominant vegetation type but also by forest use patterns and community interactions. However, Singh et al. (2023) observed that carbon stock potential may increase with elevation, depending on site-specific ecological conditions. In contrast, Thakur et al., (2024) reported a peak in carbon stock at mid-altitudes, with a subsequent decline at higher elevations. Among the different carbon pools, tree

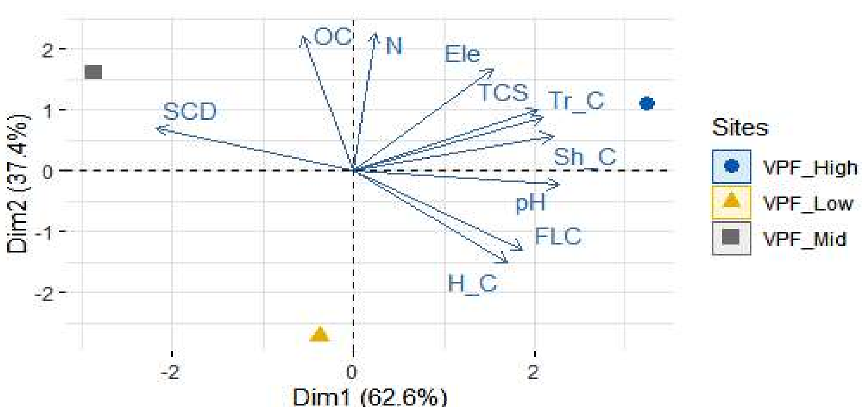


Fig. 4. Principal component analysis correlation (percent variance) diagram based on vegetation biomass, different carbon pools and soil properties in three different elevational community managed forests (where TCS: total carbon stock; Tr_C: tree carbon stock; Sh_C: shrub carbon atock; H_C: herb carbon stock; FLC: floor litter carbon; SCD: soil carbon density; OC: total soil organic carbon percent; N: total nitrogen %; Ele: elevation. Low-elevation VPF site= yellow tringle shape; mid-elevation VPF site = grey square shape; high-elevation VPF site = blue circle shape)

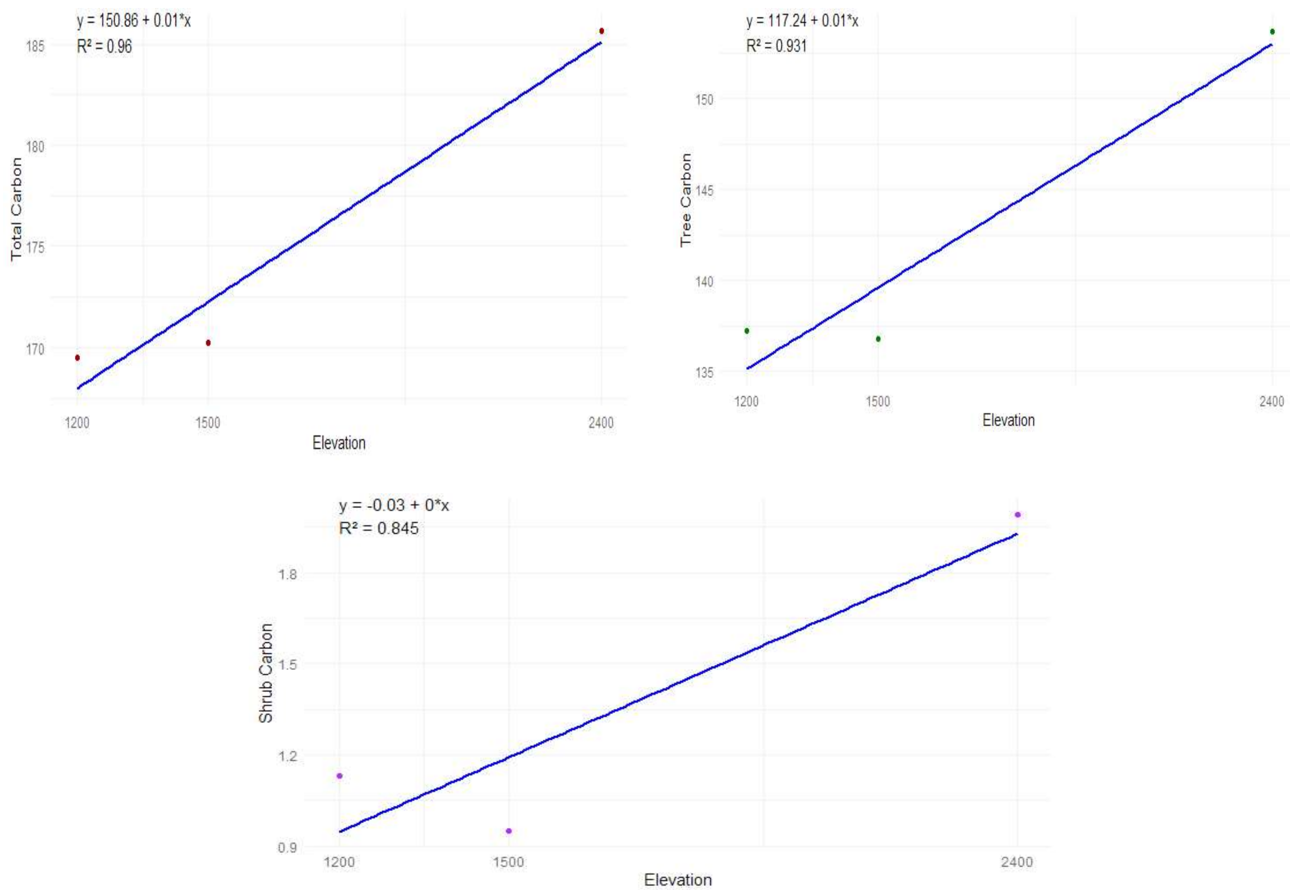


Fig. 5. Relationship Between Carbon Pools (Tree, shrub, and total carbon) and Elevational Gradient in Van Panchayat Forests

carbon, shrub carbon, and total carbon stock exhibited a strong positive correlation with elevation ($R^2 > 0.84$), while soil organic carbon showed a moderately negative correlation ($R^2 = 0.32$). In contrast, forest floor and herb layer carbon stocks demonstrated negligible relationships with elevation ($R^2 < 0.10$) (Fig. 5). These results suggest that elevation influences carbon pools through distinct ecological processes, with aboveground carbon components being more sensitive to altitudinal variation.

CONCLUSION

Community-managed forests are an essential part of the lives of rural people residing in nearby areas. They not only provide livelihoods but also play a crucial role in ecosystem sustainability and carbon sequestration. There is a need to adopt site-specific conservation and management practices to sustain and enhance the carbon stock potential of these forests across different elevation gradients.

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Intra-Population Variation in Mahua (*Madhuca longifolia* var. *latifolia* Roxb. A. Chev.) for Fruit, Seed and Germination Traits

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Abstract: Experiment was designed to understand the intra-population variation for fruit, seed and germination attributes of *Madhuca longifolia* var. *latifolia* (mahua) - a high value tropical tree species. Study was carried out in two years (2015 and 2016). Seed lots of identified open pollinated mother trees (10) were segregated and used for study. Fruit and seed traits like length, thickness and weight showed significant variation among open pollinated mother trees. Trees coded as MLLMG-3, MLLMG-4 and MLLMG-9 comparatively recorded with large seeds. Germination percentage, germination rate index, mean daily germination, peak value of germination, germination value mean germination time showed significant variation. Pooled analysis showed that there was no significant effect of collection period (year to year) on fruit, seed and germination traits except fruit length. Further, there was an auto correlation among various fruit and seed traits. Strong correlation observed for fruit traits (weight and thickness) with germination attributes. Experimental outcomes revealed a strong intra-population variation for studied attributes in mahua. Therefore, further selection and improvement work can be done on this species for production of quality seeds in large quantity.

Keywords: Correlation, Germination, Intra-population, Mahua, Selection, Tropical tree

Non-Timber Forest Products (NTFPs) play an important role in sustainable livelihood of the tribes as well as other forest dwellers living in and fringe forest areas. NTFPs are the backbone to the Indian forest economy contributing over 50 per cent to the forest revenues (Patel and Naik 2010). These products also serve as an important source of food, nutrition, medicine *etc*. *Madhuca longifolia* var. *latifolia* (mahua) is one of such resource that contributes greatly to the forest revenue. Mahua is one of the ecologically and economically important species having wide distribution in South Asian countries including India, Nepal, Burma, Myanmar and Sri Lanka. In India, this species is distributed in northern, central and southern part of peninsular India (Mishra and Pradhan 2013, Akshatha et al 2013). Tree has great value for its flowers and seeds in Gujarat state, India. Products of mahua play an important role in income and livelihood of tribal areas of the country and considerable part of their total income is obtained from sale of forest products. At local, the forest dwellers and tribal people use products from mahua regularly and use full of its extent. There is a great demand for seeds and flowers of mahua for commercial purpose and required raw materials come from natural sources. Large scale plantation of this species is required to fulfill the sustainable utilization of mahua resources. On other hand, genetic variation helps to identify individuals as well as species to withstand against the extreme environmental conditions and biotic pressures. Intra specific variation leads trees to show better phenotypic characters, resistance against the harsh conditions and biological agents (Anonymous 2013). These characters would help to screen the best genotypes for the specific purpose. The basis for any breeding programme is to understand the intra and inter-specific/population variation for traits of interest. Furthermore, scientific information regarding the variability and superiority of the individuals growing within the population are also important for the conservation of genetic diversity as well as improvement and multiplication of species. Moreover, genetic diversity is needed in order to ensure that forest trees can survive, adapt and evolve under changing environmental conditions (Whitham 2014). Review shows that there is a little work on tree improvement aspects of this species (George et al 2003, Wani and Ahmad 2013, Divakara and Das 2014). There is no report of intra-population variation for morphometric distinction in fruit, seed and germination attributes in mahua and their correlation. Therefore, to fulfill some of these gaps in intra-population, variability studies in this high value tropical tree species was undertaken.

MATERIAL AND METHODS

Site location: To study the intra-population variation, a mahua population was identified as a focal population and it is situated in Makadban forest of Valsad district, Gujarat state in India and between 20° 26' 38" N and 73°13' 53" E with an altitude of 41 m above MSL. The study area located in the northern part of Western Ghats comprising the laterite and medium black soils and experiences the typical tropical climatic conditions. The maximum daily temperature during the year ranges from 32.2°C in August to 41.2°C in April, while minimum temperature ranges from 9.9°C in January to 23.3°C in May. The average annual normal rainfall of the area is 2422 mm. Approximate extent of this population is 10 ha that consists of good number of mahua trees and well distributed in the study area. The fruit and seed characterization as well as germination experiment was undertaken during the year 2015 and 2016 at Navsari Agricultural University, Gujarat, India. Navsari Agricultural University, Navsari (20° 55′ 38″ N 72° 53′ 54″ E with an altitude of 9 m above MSL). The location experiences a typical tropical warm climate characterized by fairly hot summer, moderately cold winter and warm humid monsoon. Generally, monsoon in this region commences in the second week of June and ends in September. Most of the precipitation is received from South west monsoon, concentrating in the months of July and August. Average annual rainfall of this region generally ranges from 1200 to 1500 mm. April and May months recorded highest maximum temperature. December, January and February months recorded the lowest minimum temperature.

Fruit collection and germination study: Total ten healthy matured good fruit bearing open pollinated mother trees were selected randomly and marked for fruit collection. Growth observation such as tree height, GBH, commercial bole height and crown diameter of selected trees were recorded using standard procedure (Cheturvedi and Khanna 2011) (Table 1). Fruits were collected during June from selected Open Pollinated Mother Trees (OPMTs) and individual identity of OPMT was maintained. For each OPMT, total five hundred seeds *i.e.*, five samples containing 100 seeds each, were used to assess the variation for fruit and seed traits along with seed germination among different populations (ISTA 2017). Data on various quantitative traits of fruits and seeds like length, thickness and weight were recorded. Germination experiment was carried out in the nursery under shade-net. Daily germination count was made up to 30 days. Emergence of the shoot just above the sand level was considered as germination. Germination per cent and its various parameters such as germination rate index, mean daily germination, peak value of germination, germination value and mean germination time were calculated as per standard procedure following Czabtor (1962), Orchard (1977) and Esechie (1994).

RESULTS AND DISCUSSION

Fruit, seed and germination studies: Upshot of the study shows significant and strong intra-population variation for fruit traits like length, thickness, weight as well as seed traits like length, thickness, weight, dry biomass, volume and density (Table 2, 3 and 4). Pooled analysis showed non-significant influence of collection period on fruit, seed and germination parameters except fruit length. In the present study, significant intra-population variation was recorded in fruit length (36.60-51.90 mm & 31.70-49.70 mm), fruit thickness (32.08-41.47 mm & 27.20-38.07 mm) and fruit weight (18.19-37.54 g & 13.94-27.95 g), in 1st and 2nd year of study, respectively. Similarly, intra-population variation was also recorded in seed length (25.08-33.26 mm & 25.30-32.90 mm), seed thickness (14.70-18.95 mm & 11.35-15.49 mm) and seed weight (2.84-5.26 g & 2.96-5.09 g), in first and second year, respectively. OPMTs namely MLLMG-3, MLLMG-4 and MLLMG-9 recorded with heavier and bigger fruits and seeds than other individuals. Germination percentage, germination rate index, mean daily germination, peak value of germination, germination value mean germination time showed significant variation among OPMTs selected within a population (Table 5, 6). The germination per cent ranged from 56.2 to 93.0 in first year (2015) and 66.0 to 98.0 in second year. Interestingly, OPMTs *viz.,* MLLMG-1, MLLMG-9 and MILLMG-10 showed better germination per cent to the tune of 93.00 & 90.00 per cent, 84.00 and 96.00

Table 1. Growth attributes of open pollinated mother trees selected for intra-population variation study

Open pollinated mother trees	Tree height (m)	Girth at breast height (cm)	Commercial bole height (m)	Crown diameter (m)
MLLMG-1	21.50	280.00	8.00	14.05
MLLMG-2	19.00	178.00	7.20	13.95
MLLMG-3	21.00	232.00	8.50	13.85
MLLMG-4	24.50	309.00	7.50	16.50
MLLMG-5	18.00	232.00	6.50	15.35
MLLMG-6	19.50	261.00	6.00	13.45
MLLMG-7	17.50	130.00	4.50	13.85
MLLMG-8	18.50	172.00	6.50	11.95
MLLMG-9	20.50	270.00	7.00	14.40
MLLMG-10	22.50	340.00	7.50	16.90

per cent and 85.00 and 98.00 respectively for 2015 & 2016.

Correlation studies: There was an auto correlation among fruit and seed traits (Table 7). The fruit length showed positive correlation with fruit thickness, fruit weight, seed thickness and seed dry biomass. Similarly, fruit thickness also showed positive correlation with fruit weight. Seed weight showed positive correlation with seed length and seed thickness. Such significant association was also observed between seed dry biomass and seed thickness. Such strong and positive association was also observed among germination attributes. Seed germination showed significant and positive correlation with Germination rate index. However, none of the fruit and seed traits does not show significant correlation with germination. Further, GRI also showed positive correlation with MDG and GV. Moreover, fruit weight positively correlated with GRI, MDG and GV.

Table 2. Fruit traits variation in *Madhuca longifolia* var. *latifolia* (Roxb.) A. Chev.

Open pollinated mother trees	Fruit length (mm)			Fruit thickness (mm)			Fruit weight (g)		
	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled
MLLMG-1	40.52	39.60	40.06	33.49	34.10	33.80	21.61	21.45	21.53
MLLMG-2	36.60	40.00	38.30	36.41	27.20	31.81	22.89	13.94	18.42
MLLMG-3	46.56	46.70	46.63	41.47	32.36	36.92	37.54	19.32	28.43
MLLMG-4	43.14	37.20	40.17	36.58	32.48	34.53	26.31	17.78	22.05
MLLMG-5	48.20	48.90	48.55	40.68	38.07	39.38	33.78	27.95	30.87
MLLMG-6	38.32	35.50	36.91	32.08	29.64	30.86	18.19	14.08	16.14
MLLMG-7	40.26	31.70	35.98	37.11	30.77	33.94	25.97	14.14	20.06
MLLMG-8	46.78	39.70	43.24	34.54	34.07	34.31	23.57	19.52	21.55
MLLMG-9	51.88	49.70	50.79	34.30	33.11	33.71	27.23	24.98	26.11
MLLMG-10	43.66	43.50	43.58	36.30	33.25	34.78	26.90	24.07	25.49
Mean	43.60	41.20	42.40	36.30	32.50	34.40	26.40	19.72	23.06
CD @ 5%	1.10	1.20	6.67	1.00	1.36	NS	1.51	1.57	NS
CV (%)	1.97	2.24	2.10	2.15	3.25	2.70	4.47	6.18	5.20
CD @ 5% (YxT)	-	-	1.12	-	-	1.17	-	-	1.51

Table 3. Seed traits variation in *Madhuca longifolia* var. *latifolia* (Roxb.) A. Chev.

Open pollinated mother trees	Seed length (mm)			Seed thickness (mm)			Seed weight (g)		
	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled
MLLMG-1	30.56	29.50	30.03	17.06	12.19	14.63	4.60	3.44	4.02
MLLMG-2	26.42	32.60	29.51	14.70	15.18	14.94	2.84	5.05	3.95
MLLMG-3	33.26	32.90	33.08	18.95	14.03	16.49	5.26	5.09	5.18
MLLMG-4	29.64	31.70	30.67	17.87	14.37	16.12	5.14	4.91	5.03
MLLMG-5	25.08	29.30	27.19	16.65	13.48	15.07	3.74	3.72	3.73
MLLMG-6	26.00	31.60	28.80	16.25	11.35	13.80	3.69	3.56	3.63
MLLMG-7	25.66	25.30	25.48	17.93	11.40	14.67	4.55	2.99	3.77
MLLMG-8	28.10	31.40	29.75	15.22	13.02	14.12	3.59	3.79	3.69
MLLMG-9	29.92	25.40	27.66	16.46	15.27	15.87	3.98	2.96	3.47
MLLMG-10	28.30	29.60	28.95	16.06	15.49	15.78	3.95	3.28	3.62
Mean	28.29	29.93	29.11	16.72	13.58	15.15	4.13	3.88	4.01
CD @ 5%	0.86	1.24	NS	0.48	0.90	NS	0.20	0.51	NS
CV (%)	2.35	3.24	2.85	2.23	5.17	3.63	3.78	10.18	7.49
CD @ 5% (YxT)	-	-	1.06	-	-	0.70	-	-	0.38

Understanding intra-population variation for reproductive traits would be fundamental steps for domestication and conservation of species. Information generated through such studies help in selection and improvement of species. In the present study, significant intra-population variation was for fruit and seed traits. Among 10 OPMTs selected, MLLMG-3, MLLMG-4, MLLMG-5 and MLLMG-9 showed superiority for fruit and seed traits, which produces bigger and wider fruits and heavy seeds than other mother trees. However, such tree to tree variation within a population recorded in *Dysoxylum binectariferum* (Gunaga et al 2015), *Mammea suriga* (Gunaga and Vasudeva 2009), *Nothapodytes nimmoniana* (Hareesh et al 2008), *Garcinia talbotii* (Bansude et al 2013) and *Sterculia urens* (Bhuva 2016).

Variations in the fruit and seed sizes could be due to genetic potential among the selected individuals or it may be

Table 4. Seed dry biomass and moisture content variation in *Madhuca longifolia* var. *latifolia* (Roxb.) A. Chev.

Open pollinated mother trees	Dry biomass of seed (g)			Moisture content (%)		
	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled
MLLMG-1	2.02	0.88	1.45	55.62	74.44	65.03
MLLMG-2	1.33	1.43	1.38	53.24	71.79	62.52
MLLMG-3	2.10	1.06	1.58	60.18	78.98	69.58
MLLMG-4	1.80	1.81	1.81	64.92	62.61	63.77
MLLMG-5	1.40	1.14	1.27	62.64	69.28	65.96
MLLMG-6	1.68	1.17	1.43	54.25	66.71	60.48
MLLMG-7	1.94	0.44	1.19	57.21	85.17	71.19
MLLMG-8	1.58	1.18	1.38	56.00	68.45	62.23
MLLMG-9	2.37	1.81	2.09	40.41	37.80	39.11
MLLMG-10	1.90	1.36	1.63	51.93	58.55	55.24
Mean	1.81	1.23	1.52	55.64	67.38	61.51
CD @ 5%	0.32	0.14	NS	8.17	6.26	NS
CV (%)	13.72	8.54	9.77	11.45	7.24	7.09
CD @ 5% (YxT)	-	-	0.18	-	-	5.62

Table 5. Seed germination, GRI and MDG variation in *M. longifolia* var. *latifolia*

Open pollinated mother trees	Seed Germination (%)			GRI			MDG		
	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled
MLLMG-1	93.00 (79.25)	90.00 (78.47)	91.50	5.74	6.10	5.92	4.43	3.82	4.13
MLLMG-2	73.00 (59.12)	94.00 (81.00)	83.50	4.59	6.13	5.36	3.20	4.11	3.66
MLLMG-3	79.00 (62.84)	66.00 (54.61)	72.50	4.66	3.87	4.27	2.83	2.70	2.77
MLLMG-4	81.67 (64.88)	84.00 (72.00)	82.84	5.28	5.54	5.41	3.73	3.69	3.71
MLLMG-5	87.50 (72.48)	80.00 (66.51)	83.75	5.77	5.58	5.68	3.63	3.72	3.68
MLLMG-6	56.25 (48.89)	88.00 (74.36)	72.13	3.07	5.08	4.08	2.14	3.72	2.93
MLLMG-7	90.00 (71.86)	84.00 (69.04)	87.00	5.38	5.33	5.36	3.79	3.37	3.58
MLLMG-8	87.50 (75.06)	90.00 (75.69)	88.75	5.87	5.84	5.86	4.73	4.11	4.42
MLLMG-9	84.00 (66.94)	96.00 (82.63)	90.00	4.85	6.67	5.76	3.23	4.64	3.94
MLLMG-10	85.00 (67.54)	98.00 (86.31)	91.50	5.54	5.93	5.74	3.75	4.36	4.06
Mean	81.69 (66.88)	87.00 (74.06)	84.35	5.08	5.61	5.34	3.54	3.82	3.69
CD @ 5%	10.71	17.63	NS	0.85	1.11	NS	0.86	0.76	NS
CV (%)	12.48	18.55	16.13	13.00	15.46	14.43	18.96	15.49	17.19
CD @ 5% (YxT)	-	-	14.53	-	-	0.97	-	-	0.79

GRI =Germination Rate Index; MDG= Mean Daily Germination; : Figures in the parenthesis are arc–sine transformed values due to the differences in microsite environmental as well as edaphic factors in which the mother trees grow (Xu et al 2015, Zang et al 2016, Kołodziejek 2017). On other hand, the character of maternal and paternal parent also influences fruit and seed traits due to the nature of cross pollination in forest species (Kahn et al 2003, Lim and Lauders 2009). The mahua is highly cross-pollinated species and many pollinating agents are involved in pollination. It could be one reason of wide variability occurrence in fruit and seed characters in mahua. Forest tree species are typically long-lived, highly heterozygous organisms that have developed natural mechanisms to maintain high levels of intraspecific

Table 6. PV, GV and MGT variation in *M. longifolia* var. *latifolia*

Open pollinated mother trees	Seed Germination (%)			GRI			MDG		
	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled	Year -1 (2015)	Year-2 (2016)	Pooled
MLLMG-1	2.22	2.40	2.31	9.74	9.16	9.45	16.50	15.35	15.93
MLLMG-2	1.51	2.08	1.80	4.96	8.52	6.74	16.46	16.04	16.25
MLLMG-3	1.43	1.33	1.38	4.04	3.60	3.82	17.73	17.88	17.81
MLLMG-4	2.37	1.85	2.11	9.40	7.31	8.36	16.22	16.02	16.12
MLLMG-5	2.27	2.78	2.53	8.26	10.37	9.32	15.77	15.00	15.39
MLLMG-6	0.84	1.83	1.34	1.79	6.84	4.32	18.95	17.85	18.40
MLLMG-7	2.27	1.96	2.12	8.84	6.60	7.72	17.14	16.68	16.91
MLLMG-8	2.50	2.12	2.31	12.56	8.68	10.62	15.33	15.97	15.65
MLLMG-9	1.88	2.45	2.17	6.06	11.20	8.63	17.86	14.81	16.34
MLLMG-10	1.48	1.27	1.38	5.54	5.59	5.57	15.69	17.30	16.50
Mean	1.87	2.01	1.94	7.11	7.79	7.45	16.75	16.29	16.53
CD @ 5%	0.59	0.82	0.52	3.75	3.67	NS	1.20	1.74	NS
CV (%)	24.30	32.01	28.69	41.05	36.78	38.82	5.54	8.34	7.40
CD @ 5% (YxT)	-	-	NS	-	-	3.64	-	-	1.48

PV= Peak Value; GV= Germination Value and MGT = Mean Germination Time

Table 7. Influence of fruit and seed traits on seed germination parameters in *M. longifolia* var. *latifolia*

Traits	FL (mm)	FT (mm)	FW (g)	SL (mm)	ST (mm)	SW (g)	SDW (g)	MC (%)	GRI	MDG	PV	GV	MGT
FL: Fruit length	-												
FT: Fruit thickness	0.534**												
FW: Fruit weight	0.809**	0.863**											
SL: Seed length	-0.038	-0.165	-0.235										
ST: Seed thickness	0.562**	-0.028	0.330	0.177									
SW: Seed weight	0.019	-0.225	-0.281	0.754**	0.363*								
SDW: Seed dry weight	0.432*	0.034	0.273	0.215	0.691**	0.273							
MC: Moisture Content	-0.511	-0.164	-0.467	0.250	-0.493	0.317	-0.806						
GRI	0.288	0.280	0.439*	-0.410	0.207	-0.267	0.239	-0.377					
MDG	0.331	0.278	0.437*	-0.189	0.228	-0.290	0.482**	-0.622	0.679**				
PV	0.236	0.337	0.298	-0.318	-0.107	-0.173	-0.095	0.009	0.572	0.215			
GV	0.330	0.397*	0.416*	-0.320	0.013	-0.212	0.117	-0.215	0.730**	0.545	0.925**		
MGT	-0.504	-0.469	-0.492	0.187	-0.181	0.026	-0.257	0.292	-0.597	-0.489	-0.598	-0.685	
Seed germination (%)	-0.047	-0.023	0.155	-0.306	0.106	-0.325	0.126	-0.275	0.741**	0.532	0.183	0.341	0.078

GRI =Germination Rate Index; MDG= Mean Daily Germination; PV= Peak Value; GV= Germination Value and MGT = Mean Germination Time variation such as a high rate of out-crossing, and dispersal of pollen and seeds over wide areas (Anonymous 2013).

Variation in seed germination reflects adaptations to site conditions which strongly influence the seedling growth and survival. Seed germination capacity is one of the ecological important parameters and forest regeneration is highly depends upon seed germination, viability and seed set (Khurana and Singh 2001). Further, all these parameters along with ecological condition shape the forest structure and composition (Bhadouria et al 2016). Variability in the germination response of seeds from different provenances, populations, individuals of the same population, or from different locations in the crown of the same individual tree is well known (Mamo et al 2006). The seed source and individual trees have more influence on seed germination (Vasav et al 2011). Review shows that there are many studies pertaining to inter-population or provenance or seed source variation for seed germination attributes among forest tree species; however, intra-population variation for germination and its attributes is sparse. In fact, such studies are very important for a breeder to understand the genetic base of the population and information generated through this study would help in further selection and improvement of species.

In present study, year to year variation for seed germination and its attributes was not significant among mother plants within a population. In contrast, a significant intra-population variation was for seed germination parameter for each year of study among 10 OPMTs. Absolutely no report on intra-population variation for seed germination parameters in mahua , however, report on tree to tree variation for this trait has been recorded in mahua (Divakara et al 2011). Similar intra-population variation has been documented in other forest species (Gunaga & Vasudeva 2009, Gunaga et al 2015, Bansude et al 2013, Bhuva 2016, Smita et al 2015). Intra-population variability in germination is also a common occurrence in forest trees as a result of genetic factors (Van der Vegte 1978) and climate variability during seed ripening (Meyer and Allen 1999). Further, variability in seed germination may also be attributed owing to other maternal provisioning during seed development like hormones, proteins and nutrients.

Correlation study helps breeder to choose multiple traits for selection of superior individuals, especially during indirect selection. In many genetic studies, phenotypic and genotypic correlations are done for identifying suitable traits for tree selection. Study shows that bigger/heavier seeds speedup the germination and it may also produce higher seedling biomass (Gunaga et al 2010, Souza and Fagundes 2014). Therefore, selection of seedlot with heavier seeds may resulted in higher and speeder germination (Selvan and Guleria 2012, Sofi and Singh 2013, Mishra et al 2014). In the present study, germination parameters such as GRI, MDG and GV is highly influenced by the fruit weight in mahua. Furthermore, dry weight of seeds also resulted in positive association with MDG, indicating bigger seeds speedup the germination. Therefore, fruit weight and seed biomass may be considered while selection of genotypes in mahua.

CONCLUSION

The study indicates strong intra-population variation for fruit and germination traits; however, no significant variation observed in most of the traits studied for the period of seed collection (year to year), which suggest that, fruit, seed and germination characters of mahua seed are sturdily controlled by genetic factors. Therefore, there is a lot of scope for selection of genotypes within a population. Perhaps, this would be the first report on intra-population variation in mahua. Traits such as fruit weight and thickness resulted in strong correlations with germination attributes; therefore, one should consider such fruit parameters while selecting better genotype as well as further breeding programme. The screening of superior genotypes within a population would help foresters and tree breeders to identify climate resilient individuals to grow without disturbance in the era of climate variation and recognize the reliable source of viable seeds for further programmes.

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AUTHOR's CONTRIBUTION

Dr. H.T. Hegde - Conceptualization, investigation, original draft preparation; Dr. R.P. Gunaga-Methodology, formal analysis and supervision of the study; Dr. N. S. Thakur-Validation of data,review & editing; Dr. J.B. Bhusara, Dr. R.L. Soundarva & amp; D.C. Bhuva – Assistance in data collection, compilation & field management activities.

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Aggregate Stability in Relation to Hydrolysable Organic Carbon in Humid Tropical Ultisol under Manure-Fertilizer Amendments

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***Abstract*:** Improving soil aggregate stability (AS) and soil organic carbon (SOC) through manure and fertilizer management is a well-established agricultural practice. This study investigates the impact of poultry manure (PM) and inorganic fertilizers (IFs) applied at varying rates to hybrid maize on AS and soil organic carbon fractions (SOCFs) in Nsukka, Nigeria. Both PM and IF, whether separately or together, enhanced mean weight diameter (MWD) in 2013, notably in PM-treated plots in subsequent years, supported by positive potential structural enhancement index (PSEI). PM decreased clay dispersion index (CDI) and dispersion ratio (DR) while increasing aggregated silt plus (ASC), especially with higher PM rates. Aggregate density (AD) decreased with increasing PM rates, while non-hydrolysable soil organic carbon (NHC) and hydrolysable soil organic carbon (HOC) increased. Both NHC and HOC showed significant positive correlations with MWD and PSEI, with only HOC exhibiting a negative association with AD. The SOCFs correlated negatively with CDI and DR, and positively with ASC, except for the non-significant association of NHC with DR. Overall, the positive effects of manure-fertilizer applications on AS indices, and SOCFs, highlighted the importance of enhancing HOC and NHC through organic inputs for soil structural stability and long-term SOC and labile C stabilization.

Keywords: Aggregate stability, Non hydrolysable carbon, Hydrolysable carbon, Poultry manure inorganic fertilizer

Tropical soils, known for their high weathering and fragility, often suffer from low soil organic carbon (SOC) and poor aggregate stability due to factors such as climate change, continuous cultivation, and inadequate agronomic practices (Zhang et al., 2016, Guo et al., 2019). Soil aggregates, which are crucial for soil health, consist of macro-aggregates (>250 µm) formed from smaller micro-aggregates (<250 µm) bound together by organic materials. The stability of these aggregates impacts soil functions, including erosion resistance and compaction and high organic carbon storage (Berhe and Kleber 2014, Wang et al., 2015).

Fertilization practices, particularly the application of organic manure and inorganic fertilizers, show influence on SOC and aggregate stability. Studies indicate that organic manure can improve micro-aggregate stability and overall SOC in fragile tropical soils and demonstrated that sustainable soil management that allow restoration of SOM have the potential to reduce DR and CDI while increasing ASC and CFI (Udom and Anozie 2018). The addition of organic manure alone or combined with inorganic fertilizers has been reported to enhance macro-aggregate stability, as opposed to the application of inorganic fertilizers alone. (Sadiq et al., 2018) However, other research suggests that organic amendments may not always improve aggregate stability (Gardenas et al., 2011). The contribution of fertilization to increased SOM in bulk soil is well researched (Xu et al., 2016), but few have evaluated the effect of fertilization on the soil organic carbon fractions (SOCFs). Chemical fractionation methods separate SOC into hydrolysable (labile) and non-hydrolysable (passive) fractions, with the latter being more resistant to decomposition and critical for long-term SOC storage (Sanni et al., 2020). Understanding how these SOC fractions affect aggregate stability can inform better soil management practices. Thus, this study aims to evaluate the effects of poultry manure and inorganic fertilizers on aggregate stability and SOC fractions in tropical Ultisols of Nigeria, addressing gaps in the current understanding of how SOC fractions influence soil aggregate stability.

MATERIAL AND METHODS

Site description: The experiment was conducted at Nsukka situated in the derived Savannah ago-ecological zone of Southeastern Nigeria. It is located on latitude 06° 51' N and longitude 07° 29' E and at an altitude of about 447 masl. It has a bimodal annual rainfall pattern with annual precipitation of about 1600 mm, mean annual temperature of 28 °C and average relative humidity is rarely below 60%. Soils in Nsukka are Ultisol, formed from Nsukka formation (Ezeaku et al., 2015) and derived from false bedded sandstone. The soil is classified as Typic Kandiustults in soil Taxonomy. The experimental site is in the University of Nigeria Teaching and

Research Farm and was under cultivation for 20 years. It was under fallow for eight years prior to the commencement of the field experiment. Common crops grown were maize (*Zea mays* L), garden egg (*Solanum* spp.) and cowpea (*Vigna unguiculata*).

Experimental design and treatment layout: The study was a field experiment laid as randomized complete block design (RCBD) replicated four times with twelve fertilizer treatment combinations. The treatment combinations were derived from three rates of poultry manure (0, 5 and 10 t ha^{-1}; represented as PM_0, PM_5 and PM_{10}, respectively) combined with four rates of inorganic fertilizer (0, 50, 100 and 150%, coded IF_0, IF_{50}, IF_{100} and IF_{150}, respectively) being the recommended dose of IF for hybrid maize (400 kg ha^{-1} NPK 20-10-10, containing 80 kg N - 40 kg P_2O_5 - 40 kg K_2O ha^{-1}) plus 150 kg ha^{-1} Urea, containing 69 kg N ha^{-1}). Sample of the air-dry poultry manure, sourced from deep litter poultry houses in the study location, was analyzed for their contents of N, P, K, Ca, Mg, Na and organic C.

The site was cleared and demarcated into plots. Each plot size was 1.5 m x 3.6 m. Plots and blocks were separated by1m buffer strip amounting to total area of 30 m x 18.4 m. Stipulated PM rates were applied in 2013 and 2014 to plots one week before planting, and hybrid seeds (*Zea mays* L. var. M9211 Yellow) from Manoma Seed Company Abuja, Nigeria were sown on 2nd September, 23rd May and 3rd June in 2013, 2014 and 2015, at two seeds/hole at an inter row spacing of 60 cm, and plant- to-plant distance of 25 cm. Inorganic fertilizers were applied in three splits. The stipulated dose of 400 kg/ha NPK 20:10:10 at the rate of 0, 50, 100 and 150%, of 400 kg ha^{-1} NPK 20-10-10 was applied at one week after planting, while 150 kg ha^{-1} Urea, containing 69 kg N ha^{-1} at the rate of 0, 50, 100 and 150% was applied in two equal splits, at four (4) weeks after planting and at tasseling. The quantity of fertilizer per plant (NPK and Urea) was calculated and placed 5-8 cm away from each plant, and at 2-3 cm depth below the soil surface. In 2015, the residual effects of the fertilizer treatments were evaluated, hence there was no re-application of the treatments.

Soil sampling: Prior to bed preparation for planting, soil samples were taken at random from 20 points at 0-20 cm depth and mixed homogeneously to get a composite sample. The samples were air dried and passed through a 2mm sieve for the determination of available phosphorous, exchangeable calcium (Ca^{2+}), exchangeable magnesium (Mg^{2+}), exchangeable sodium ((Na^{+}), exchangeable potassium (K^{+}), soil organic carbon (SOC), total nitrogen, pH and particle size distribution. Ten core samplers were used to take samples at random for determination of soil bulk density. At the end of maize maturity in each planting season, soil samples were taken within the net plot of each bed at three points in a zig-zag pattern and mixed to get a composite sample. They were air dried and a part was passed through an 8 mm sieve for the evaluation of aggregate density while another part was passed through a 4.75mm sieve for determination of aggregate stability. The remaining part was passed through a 2-mm sieve for the determination of total soil organic carbon, non-hydrolysable organic carbon (NHC) and hydrolysable organic carbon (HOC).

Soil analysis: Soil particle size distribution was determined by the hydrometer method (Kalra and Maynard, 1982). The core method, by Blake and Hartge (1986) was used in the determination of bulk density.Soil pH was measured in 0.10N potassium chloride solution at ratios of 1:2.5 soil/ water suspension (Mclean, 1982). The soil organic carbon was determined by the Walkley- Black wet-oxidation as modified by Nelson and Sommers (1982). Total N was determined using the micro Kjeldahl digestion-distillation method (Bremner, 1996). The available P was determined by Mehlich-3 extractant method (Mehlich, 1984). The exchangeable bases (K^{+}, Ca^{2+}, Mg^{2+} and Na^{+}) were extracted by leaching the soil using ammonium acetate (NH4OAc) solution after neutralizing this reagent (pH 7) to standardize the analysis. The K^{+} and Na^{+} in the extract were determined using a flame photometer while Ca^{2+} and Mg^{2+} were determined using Atomic Absorption Photo-spectrophotometer.

Determination of SOC and SOC fractions: The soil organic carbon was determined by the Walkley and Black method as modified by Nelson and Sommers (1986). The method of Silveira et al. (2008) was used to fractionate SOC into NHC and HOC. In this procedure, 2 g of soil (< 2.00 mm) was mixed with 6 M HCl and heated at 105 °C for 3 h, washed three times with distilled water and then centrifuged for 10 min. to remove the HCl. The residue was dried and analyzed for organic carbon which represents the NHC. The HOC concentration was calculated as the difference between SOC in < 2.00 mm soil and NHC concentration (Mchlauchlan et al., 2004).

Bulk density and aggregate density: The core method of Blake and Hartge (1986) was used in the determination of bulk density. Aggregate density (AD) was measured using 5-8 mm aggregates by the clod method (Grossman and Reinsch 2002). Each aggregate was weighed (Ma), coated with wax and weight taken (Ma+w). Coated aggregate was immersed in distilled water at 20 ^{0}C and weight taken Ma1. The loss (Ma - Ma1) was equal to the volume of wax coated aggregate. All weights were expressed in Mg and the density (Mgm^{-3}) was computed as

$$AD = \frac{Ma}{\left(\frac{\mathrm{Ma}}{\mathrm{Pw}}\right) - \frac{(Ma - Ma + w)f}{Ps}} \tag{1}$$

Where, Pw is density of water at 20 ^{0}C and Ps is density of wax coating (0.93 Mgm^{-3}).

The value of f was taken as 0.3 for 5 to 8 mm aggregates (Munkholm and Kay 2002).

Assessment of macro-aggregate stability of the soil: The distribution of water stable aggregates was estimated by the wet sieving technique described in detail by Kemper and Rosenau (1986). To separate water stable aggregate, 25 g samples of the > 4.75 mm air dried aggregates were placed on top of a nest of four sieves measuring 2 mm, 1 mm, 0.25 mm and < 0.25 mm and pre-soaked for 10 minutes in water. The sieves and their content were oscillated vertically, once per second in water 20 times using an amplitude of 4cm. Care was taken to ensure that the soil particles on the topmost sieve were always below the water. The resistant aggregates on each sieve were oven dried at 105 °C for 24 h and weighed. The mass of < 0.25 mm was obtained by difference between the initial sample weight and the sum of sample weight collected on the 2.00, 1.00, 0.50 and 0.25 mm sieve nests. Coarse fraction was corrected on each size fraction by soaking in sodium hexametaphosphate and washing back through the same sieve size and the sample retained on the sieve was oven dried, weighed and recorded as the coarse fraction (Hillel 1998). The mean weight diameter (MWD), was calculated.

$$MWD = \sum_{i=1}^{n} W_i X_i \qquad (2)$$

where Wi is weight of aggregate in the ith aggregate size range as fraction of dry weight of sample and Xi is mean diameter of any particular size range of aggregates separated by sieving.

The potential structural enhancement index (PSEI) is used to measure the effect of the treatments on aggregate stability (Mbagwu and Bazzoffi 1989), and was computed as follows:

$$PSEI = 1 - \left(\frac{MWDc}{MWDt}\right) x\,100 \qquad (3)$$

where MWDc is mean weight diameter for control and MWDt is mean weight diameter of treated soil.

Assessment of particle size distribution and micro-aggregate stability of the soil: Particle size distribution of less than 2-mm fine earth was measured by the hydrometer method as (Kalra and Maynard 1991) using sodium hexa-metaphosphote (calgon) as the dispersing agent. To determine the micro-aggregate stability indices the same procedure was followed but water was used in place of calgon.

The micro-aggregate stability indices were computed as follows:

DR = % clay + % silt (water) /% clay + % silt (calgon) x 1004

CDI = % clay (water) / % clay (calgon) 5

ASC = % clay + % silt (calgon) - % clay + % silt water) 6

where DR is Dispersion Ratio, CDI is Clay Dispersion Index and ASC is Aggregated Silt plus Clay.

Statistical analysis: The data generated were subjected to analysis of variance using GENSTAT Release 7.2 DE statistical software. Treatment means were separated using Fisher's least significant difference at 5% probability level. Simple linear correlation analysis between soil organic carbon fractions and aggregate stability indices were carried out for each year using Spearman correlation co-efficient (Rs).

RESULTS AND DISCUSSION

Initial soil properties in experimental location and chemical properties of poultry manure: The soil was sandy clay loam and highly acidic (4.1), which may result to nutrient fixation. The bulk densities were below the critical limit for root growth based on Morris and Lowery (1988) on critical limits for different soil textures (Table 1, 2). Hence soils of the study location may support root growth and development. Soil nitrogen, soil organic carbon, exchangeable K, exchangeable sodium and magnesium were all below critical limit for maize production except available phosphorus that was above the critical limit (FMANR, 1990). The results apparently depicted the need for external sources of nutrient to support optimum maize yield and soil sustainability.

Effect of poultry manure and inorganic fertilizers on soil properties: In 2013, all amended plots showed significant increases in mean weight diameter (MWD), with the highest was with 5 t/ha of poultry manure (PM5+ IF0(2.45 mm)), compared to 1.56 mm in the control (PM0+ IF0) (Table 3). This increase may be attributed to the effects of iron and aluminum oxides and root biomass, as these oxides are known to stabilize soil in tropical and semi-tropical regions (Igwe and Obalum 2013). The addition of artificial fertilizers also enhances soil structural stability by increasing soil organic carbon (SOC) (Sainju et al., 2003, Álvaro-Fuentes et al., 2012, Plaza-Bonilla et al., 2013). In 2014, MWD declined in plots with only inorganic fertilizers (IF) due to increased cultivation but remained stable with PM. The highest MWD was in PM10+ IF100 (1.42 mm), similar to other PM treatments. This suggests that higher manure inputs improve aggregate stability. Residual MWD was highest in PM10+ IF50, reflecting the role of microbial decomposition of organic material in macro aggregation (Mikha et al., 2010, Rasoulzadeh and Yaghoubi 2010, Mandal et al., 2013, Sadiq 2018). The potential structural enhancement index (PSEI) was positive in most treatments, indicating improved macro

Table 1. Initial soil properties at the experimental station

Parameter	Unit	Nsukka
Soil organic C	g/kg	9.13
Total N	%	0.09
Mehlich available P	mg/kg	18.43
Exchangeable K	cmol/kg	0.12
Exchangeable.Ca	cmol/kg	0.38
Exchangeable Mg	cmol/kg	0.13
Exchangeable Na	cmol/kg	0.14
pH	,	4.1
Bulk density	Mg/cm^3	1.53
Sand	g/kg	630
Silt	g/kg	101.
Clay	g/kg	269.
Texture		Sandy clay loam

Table 2. Chemical properties of poultry manure at Nsukka

Parameter (%)	2013	2014
Organic carbon	32.42	30.80
Nitrogen, N	2.01	1.99
Phosphorus, P	0.81	0.77
Potassium ,K	1.44	1.56
Calcium, Ca	1.56	1.49
Magnesium, Mg	0.48	0.55
Sodium, Na	0.16	0.16
pH	7.70	7.50

aggregation, except in PM0+ IF150 in 2014, where excessive IF application led to dispersion due to increased soil acidity (Mikha et al., 2010, Guo et al., 2019). Even after amendment withdrawal in 2015, structural stability was maintained, highlighting that it is not solely dependent on organic matter. For both years, PM reduced aggregate density (AD), with the best reductions observed in PM5+ IF0 (1.43 Mg/m³) in 2013 and PM10+ IF50 (1.51 Mg/m³) in 2014. The lack of residual effect from fertilizers on AD suggests it relies on SOC input (Munkholm and Kay 2002, Selvi et al., 2005, Karami et al., 2012). Enhanced aggregate properties improve soil aeration, hydraulic properties, and nutrient retention (Udom et al., 2018).

PM_0, PM_5, PM_{10} is poultry manure at 0, 5, 10 t ha^{-1}, respectively; IF_0, IF_{50}, IF_{100}, IF_{150} is inorganic fertilizer at 0%, 50% 100% and 150% recommended fertilizer dose, respectively, being 400 kg ha-1 NPK-20:10:10 plus 150 kg ha-1 urea

Effect of poultry manure and inorganic fertilizers on soil microaggregates stability indice: In 2013, fertilizer treatments did not significantly affect CDI, DR, or ASC compared to the control, likely due to the experiment's short duration of one year(Table 4). Nweke et al. (2013) also found no significant impact of manure and inorganic fertilizers (IF) on CDI, while Ogunwole et al. (2010) suggested that other stabilizing substances might influence aggregate stability differently.In 2014 and 2015, poultry manure (PM) generally exhibited lower CDI values (37.2% and 37.8%, respectively)

Table 3. Effect of poultry manure and inorganic fertilizers on aggregate properties at Nsukka

Year	2013			2014			2015		
Parameter	MWD	PSEI	AD	MWD	PSEI	AD	MWD	PSEI	AD
Fertilizer. Treatment	Mm		Mgm^{-3}	Mm		Mgm^{-3}	mm		Mgm^{-3}
$PM_0 + IF_0$	1.56	0.00	1.63	0.82	0.00	1.64	0.57	0.00	1.67
$PM_5 + IF_0$	2.45	0.36	1.43	0.94	0.13	1.57	0.73	0.19	1.66
$PM_{10} + IF_0$	2.20	0.25	1.47	1.24	0.31	1.58	0.79	0.24	1.58
$PM_0 + IF_{50}$	2.10	0.21	1.61	1.03	0.09	1.70	0.82	0.11	1.68
$PM_5 + IF_{50}$	2.27	0.31	1.50	1.12	0.23	1.57	0.67	0.28	1.64
$PM_{10} + IF_{10}$	2.12	0.26	1.47	1.28	0.29	1.51	0.75	0.19	1.59
$PM_0 + IF_{100}$	1.95	0.21	1.64	1.01	0.11	1.59	0.72	0.2	1.64
$PM_5 + IF_{100}$	2.37	0.34	1.52	0.99	0.16	1.58	0.84	0.29	1.61
$PM_{10} + IF_{100}$	2.22	0.29	1.48	1.42	0.35	1.57	0.68	0.16	1.57
$PM_0 + IF_{150}$	1.99	0.22	1.64	0.81	0.08	1.71	0.71	0.20	1.68
$PM_5 + IF_{150}$	2.14	0.26	1.52	0.98	0.13	1.59	0.61	0.06	1.65
$PM_{10} + IF_{150}$	2.27	0.31	1.52	1.23	0.27	1.58	0.84	0.32	1.57
LSD (0.05)	0.34		0.13	0.24		0.09	0.13	0.13	NS

MWD, Mean weight diameter, PSEI, potential structural enhancement index, AD, Aggregate density
PM_0, PM_5, PM_{10} is poultry manure at 0, 5, 10 t ha-1, respectively; IF_0, IF_{50}, IF_{100}, IF_{150} is inorganic fertilizer at 0%, 50% 100% and 150% recommended fertilizer dose, respectively, being 400 kg ha-1 NPK-20:10:10 plus 150 kg ha-1 urea

compared to the control, except for T11 in 2015. The highest CDI reduction in 2014 was with PM10+ IF100 (25.46%), and the best residual CDI in 2015 was in PM10+ IF50 (25.59%). These results indicate that soil organic matter (SOM), either alone or with IFs, can effectively reduce clay dispersion. Furthermore, PM, whether alone or combined with IFs, reduced DR and increased ASC in 2014 and 2015. This supports findings by Mba et al. (2010) and Ogunwole et al. (2010) of improved DR and ASC, respectively. Nweke et al. (2013) observed similar benefits with PM and NPK 20:10:10. IFs, however, showed minimal impact on stability and might exacerbate soil acidity, increasing dispersion. Enhanced micro-aggregate stability, linked to organo-mineral interactions, reduces water erosion vulnerability (Udom and Anozie 2018, Osakwe et al., 2021).

Soil organic carbon, non-hydrolysable and hydrolysable soil organic carbon: In 2013, SOCb values ranged from 8.97 g/kg (PM0+ IF0) to 15.25 g/kg (PM10+ IF50), and in 2014, from 8.95 g/kg (PM0+ IF0) to 13.9 g/kg (T12: PM10+ IF150) (Table 5). Residual SOCb varied between 6.3 g/kg

Table 4. Effect of poultry manure and inorganic fertilizers on micro-aggregate stability indices at Nsukka

Year	2013			2014			2015		
Parameter/Fertilizer	CDI (%)	DR (%)	ASC (g/kg)	CDI (%)	DR (%)	ASC (g/kg)	CDI (%)	DR (%)	ASC (g/kg)
$PM_{0+}IF_0$	23.5	33.1	249.2	37.2	43.2	206.8	37.8	44.5	190.5
$PM_{5+}IF_0$	22.9	35.6	241.0	26.7	40.4	217.2	26.7	40.0	213.6
$PM_{10+}IF_0$	22.2	35.1	246.4	28.1	37.6	236.4	26.9	39.1	227.4
$PM_{0+}IF_{50}$	26.4	35.2	244.6	35.9	41.7	209.3	37.8	44.6	189.6
$PM_{5+}IF_{50}$	23.8	34.8	249.6	27.8	36.6	225.6	27.9	39.8	215.4
$PM_{10+}IF_{10}$	22.0	34.7	251.0	26.1	36.7	233.0	25.6	39.6	217.2
$PM_{0+}IF_{100}$	23.8	36.5	233.4	30.5	39.2	217.4	36.1	41.5	204.8
$PM_{5+}IF_{100}$	22.5	35.3	245.0	29.9	38.9	231.4	30.7	39.6	210.0
$PM_{10+}IF_{100}$	22.5	34.6	251.4	25.5	36.2	241.9	26.2	38.5	233.6
$PM_{0+}IF_{150}$	24.1	36.3	234.0	38.8	42.5	206.4	42.8	43.3	204.0
$PM_{5+}IF_{150}$	23.5	36.0	236.4	29.1	40.2	226.4	33.7	41.2	213.6
$PM_{10+}IF_{150}$	23.5	35.9	236.4	29.1	40.2	226.4	29.6	39.6	217.2
LSD (0.05)	1.70	NS	NS	3.01	2.39	10.6	4.50	2.40	9.80

See Table 3 for treatment details

Table 5. Effect of poultry manure and inorganic fertilizers on soil organic carbon in bulk soil and soil organic carbon fractions (g/kg)

Year	2013			2014			2015		
Treatment	SOCb	NHC	HOC	SOCb	NHC	HOC	SOCb	NHC	HOC
$PM_{0+}IF_0$	8.97	1.53	7.45	10.1	2.03	8.08	6.49	0.75	5.65
$PM_{5+}IF_0$	12.52	3.33	9.2	10.42	2.49	7.93	8	1.65	6.35
$PM_{10+}IF_0$	13.85	4.25	9.6	12.38	3.4	8.98	9.2	2.18	7.02
$PM_{0+}IF_{50}$	9.53	1.78	7.75	8.95	0.91	8.04	6.13	0.65	5.48
$PM_{5+}IF_{50}$	14.3	4.4	9.9	12.75	3.15	8.6	8.2	1.68	6.52
$PM_{10+}IF_{10}$	15.25	5.2	10.1	13.3	4.63	8.67	10.23	2.38	7.85
$PM_{0+}IF_{100}$	9.45	0.93	8.52	9.13	0.6	8.53	6.4	0.66	5.74
$PM_{5+}IF_{100}$	12.2	3.53	8.68	11.75	3.37	8.38	7.68	1.58	6.1
$PM_{10+}IF_{100}$	15.2	5.95	9.25	13.75	4.81	8.94	10.28	2.55	7.74
$PM_{0+}IF_{150}$	9.4	0.9	8.5	9.15	0.55	8.6	6.3	0.88	5.42
$PM_{5+}IF_{150}$	13.95	3.95	10	12.8	4.08	8.73	7.88	1.75	6.13
$PM_{10+}IF_{150}$	15.1	5.75	9.35	13.9	4.88	9.02	8.33	2.23	6.1
LSD (0.05)	1	0.85	1.12	0.7	0.53	0.83	0.78	0.46	0.42

See Table 3 for treatment details

Table 6. Correlation of soil organic fractions with aggregate stability indices

Year	2013		2014		2015	
	NHC	HOC	NHC	HOC	NHC	HOC
MWD	0.64*	0.56*	0.59*	0.75**	0.13ns	0.13ns
PSEI	0.75**	0.64*	0.55ns	0.80***	0.17ns	0.25ns
AD	-0.47ns	-0.60*	-0.37ns	-0.74**	0.76**	-0.85***
CDI	-0.63*	-0.56ns	-0.36ns	-0.75**	-0.76**	-0.86***
DR	0.21ns	-0.16ns	-0.47ns	-0.61*	-0.71**	-0.87***
ASC	0.15ns	0.43	0.62*	0.82***	0.73**	0.90***

NHC, non-hydrolysable carbon, HOC, hydrolysable organic carbon, * Significant at p= 0.05, **Significant at p= 0.01

(PM0+ IF0) and 10.28 g/kg (PM10+ IF100). SOC generally increased with higher poultry manure (PM) rates, but inorganic fertilizers (IF) alone (PM0+ IF50, PM0+ IF100, PM0+ IF150) did not improve SOC compared to the control. The highest SOCb was consistently observed with 10 t/ha PM combined with 50% IF, showing no additional benefit from higher IF levels. SOC decreased with subsequent cultivation. Previous studies also report enhanced SOC with manure and crop residues combined with inorganic fertilizers (Mbah et al., 2017, Alishar Sharma et al., 2023, Mbah et al., 2023). Continuous PM application is crucial for maintaining soil quality, as loss of SOC can reduce soil structure, microbial activity, and nutrient availability.

In 2013, NHC increased in all poultry manure (PM) treated plots, with PM10 + IF50 showing the highest contribution at 10.1 g/kg, similar to other PM treatments (except PM5 + IF100). This increase is likely due to the recalcitrant nature of PM and root biomass, as NHC is influenced by the organic material's inherent properties and the decomposition process (Six et al., 2002, Rovira and Vallejo 2002). In 2014, NHC rose with 10 t/ha PM, while other treatments were comparable to the control. Ding et al. (2012) also found lower NHC in control and NPK plots compared to those with NPK + manure. Residual NHC was highest in PM10 + IF50 (7.83 g/kg), indicating a synergy between PM and IF, though IF alone did not improve NHC across the three years, suggesting it does not contribute to stabilized SOC sequestration (Six et al., 2002, Wiesenberg et al., 2010).

PM consistently improved hydrolysable organic carbon (HOC) in 2013, 2014, and 2015, with greater increases observed with higher manure rates (Table 5). Combinations of PM and IF were more effective than sole applications, supporting findings by Adeniyan et al. (2005), Ojo et al. (2015), and Soremi et al. (2017). IF alone did not significantly affect HOC compared to the control and may deplete labile organic carbon. HOC supports microbial activity and nutrient availability.

Correlation of soil organic fractions with aggregate stability indices: The correlation between soil organic fractions and aggregate stability indices. In 2013 and 2014, non-hydrolysable carbon (NHC) and hydrolysable organic carbon (HOC) was positively correlated with mean weight diameter (MWD) and percentage stable aggregates (PSEI) (r = 0.56-0.80), though NHC had a non-significant relationship with PSEI in 2014 (Table 6). Fertilization significantly influenced both carbon fractions and aggregate stability, but correlations were non-significant when amendments were removed in 2015. HOC showed a negative correlation with aggregate density (AD) in 2013, 2014, and 2015 (r = -0.60 to -0.85), with the relationship strengthening over time, indicating changes in soil organic carbon (SOC) quality. Conversely, NHC had a positive relation with AD in 2013 and 2014, suggesting that increased NHC might reduce aggregate porosity and affect soil aeration and hydraulic properties. In 2013, micro-aggregate stability indices showed no significant correlations with either carbon fraction, except a negative correlation of NHC with clay dispersion index (CDI) (r = -0.68). In 2014, NHC positively related to aggregated silt plus clay (ASC), while HOC negatively related to CDI and dispersion ratio (DR), but positively related to ASC. In 2015, both fractions negatively correlated with CDI and DR (r = -0.71 to -0.87) and positively with ASC (r = 0.73 to 0.90). HOC exhibited a stronger relationship with aggregate stability indices compared to NHC.

CONCLUSION

Manure-fertilizer applications affected aggregate stability and soil organic carbon fractions at Nsukka. Both IF and PM applied alone or combined, improved the MWD and PSEI. The increase by only IF application may suggest other background agents of stabilization other than SOC from PM. Furthermore, the micro aggregate stability indices, hydrolysable carbon and non-hydrolysable carbon significantly improved at the application of poultry manure and with added benefit when in combination with IFs. But with regards to treatment combinations and the control, PM alone at 10 t/ha (PM_{10}+IF_{0}) or in combination with IF especially at reduce IF rate (PM_{10}+IF_{50}) indicated best positive contribution, contrary to sole application of IF. Furthermore, NHC and HOC significantly correlated with aggregate stability indices, hence continuous application of PM in combination with 50 % IF will ensure both long term C storage and labile C in the tropical ultisol at Nsukka.

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Analysis of Soil Organic Carbon Functional Groups by FTIR Method among Land Use Types in Semi-arid Region of Kadapa, Andhra Pradesh

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Abstract: Soil organic carbon is an important component of soils as it determines the soil fertility and soil carbon sequestration potential. The SOC comprises of components of plant and animal litter present at varied decomposition stages. FTIR Spectroscopy was used to identify different functional groups of soil organic carbon components across the four land use types. FTIR method measures the absorbance of IR radiation (4000 cm^{-1} - 400 cm^{-1}) by which functional groups (C-O, C=O, C=C, C-H, N-H) associated with soil organic carbon. A total of 72 soil samples were randomly collected from the four selected study areas and composite soil sample was prepared. Aliphatic -CH stretching was found in all land use types at 2925 - 2300 cm^{-1}. The presence of aromatic phenolic compounds 1410-1380 $cm^{-1}$with (-CO, OH) groups of humic acid, Si-O mineral groups 798-779cm^{-1}were reported in all four land use types. While the band 779-798 cm^{-1} which corresponded to the stretching vibrations of the Si-O mineral group is observed in all the four land use types. The results suggest that although SOC content is varied across the four study sites, the decomposition products featured more or less same re-synthesized products showing similar peaks in the FTIR spectral graphs.

Keywords: Soil organic carbon, FTIR, Humic substances, Aliphatic components, spectroscopy, persistence of SOC

Soil allows plants to live and grow by providing suitable substratum, water and essential nutrients. It is mainly possible by the presence of an important constituent - soil organic carbon in the soil, which comprises of plant, animal and microbial origin components at different stages of decomposition (Rao and Reddy 2023). The organically-binded carbon and nutrients are released as inorganic nutrients by soil microorganisms, fungi and soil fauna present in the soil through decomposition and mineralization processes (Ramana and Reddy 2021). Further, SOC possess binding sites for essential nutrients to be made available for plants in solution. In addition SOC consists of humic substances that are usually adsorbed on clay mineral surfaces by different mechanisms and become less accessible to microbial decomposition and thus exhibits high persistence nature (Kaushal et al., 2021). This kind of varied stability nature of SOC will influence the global carbon cycle (Schmidt et al., 2011). Thus, the amount of SOC content strongly influence soil fertility as well as the soil properties like water holding capacity, soil structure nutrient availability and carbon sequestration (Denef et al., 2009).

The decomposing material of the soil organic carbon resulting from the un-decayed plant and animal tissues comprises of diverse organic compounds such as carbohydrates, polysaccharides, proteins-polypeptides, lipids and nucleic acids and recalcitrant materials like waxes, cutins, suberin (Huang and Hardie 2009). Each type of organic matter along with resynthesized polymers of fulvic acid and humic acids components present in SOC possess functional groups like carbonyl - C=O, alcoholic C-OH, phenolic –OH, amino = NH and amine - NH_2 hydroxyl -OH, methyl-CH_3, phosphate, sulphydryl –SH (Schnitzer and Monreal 2011). The objective of the study is to identify the functional groups associated with soil organic components in soil organic carbon across the four land use types and test the hypothesis that there are no differences in functional groups across the four land use types.

MATERIAL AND METHODS

Study area: The analysis of soil organic carbon functional groups was conducted in four land use types: Guvvalacheruvu forest (GF;14˚16' 28.3" N 78˚51'57.6" E) belonging to Palakonda hill ranges, Miyawaki plantation in Yogi Vemana University campus (YVU) (plantation;14˚28 10.8 N, 78˚42' 43.56,' E), agricultural crop land (AG;14˚2810.8' N, 78˚42' 43.56' E) and barren land with sparse vegetation in the YVU campus (barren land; 14°28' 10.8” N, 78°42' 43.56”E;). Total of six soil samples in three replicates were collected randomly from each of the four land use types, resulting in 72 soil samples, from 0-30 cm soil depth by randomly laying quadrats in each of the four land use types for the quantification of soil organic carbon. The soil samples of each site are air dried and thoroughly mixed to form a composite mixture and was passed through 2mm sieve for the estimation process. The same soil samples are used for the identification of functional groups.10 mg of soil sample was mixed with 40 mg of KBr FTIR grade, the mixture was finely ground in a mortar and pestle. The milled sample mixture was transferred to the sample holder. By using a vacuum pump at ten metric tons per minute pressure, the KBr pellet is prepared. The pellet was collected on the microscopic glass slide and subjected to the characterization work mid-infrared region on FTIR spectroscopy (Reddy et al, 2018). The FTIR spectroscopy technique tries to measure these molecular vibrations of organic compounds supposed to present in the different land use type soils. (Silverstein et al, 2014).

RESULTS AND DISCUSSION

Among the four land use types forests have high SOC average of 13.9g/kg followed by Miyawaki plantation soils (7.1 g/kg) the lowest mean SOC (4.8 g/kg) was in barren land. The infrared spectrum contains specific absorption bands for different functional groups, like aliphatic compounds, aromatic compounds and carboxylic acid groups. The chemical bonds associated with these compounds exhibit vibrational movements like stretching and bending specific to each molecule by which the functional groups can be identified (Thabit et al., 2024). Comprehensive bands that correspond to compounds, functional groups of humic substances present in the SOC was prepared based on the past selective studies on SOC (Carthy 2001, Silverstein et al., 2014, Nuzzo et al., 2020). Mainly include C-H stretching vibrations associated with bands between 2950 and 2800 cm^{-1} corresponds to aliphatic saturated hydrocarbons (Table 1). The spectral region between 1750 and 1660cm^{-1} is attributed to the stretching vibrations of the C=O bond corresponding to carbonyl functional groups such as ketone,

Table 1. Band positions in the FTIR ATR spectra observed among soil samples (up to 30cm) of four land use types

Peak position (cm^{-1})		Assignment
Band-A	Present study	
3200 – 3700 cm^{-1}	3784 cm^{-1}	Stretching of -OH, NH components of humic acids of Soil Organic Carbon
	3711 cm^{-1}	
	3738 cm^{-1}	
Band-B	Present study	Assignment
2300 – 2925 cm^{-1}	2349 cm^{-1}	Vibration of C-H and (CH_2-CH) components of soil Organic Carbon
	2353 cm^{-1}	
	2349 cm^{-1}	
	2353 cm^{-1}	
	2349 cm^{-1}	
Band-C	Present study	Assignment
1630 - 1660 cm^{-1}	1639 cm^{-1}	Amide C=O stretching of Humic Acid in SOC
Band-D	Present study	Assignment
1380 – 1410 cm^{-1}	1419 cm^{-1}	The presence of Aromatic phenolic compounds C-O stretch and -OH deformation of Humic Acid in Soil Organic Carbon
	1427 cm^{-1}	
1500 – 1590 cm^{-1}	1519 cm^{-1}	Aromatic C-H deformation,Amide N-H bending and C=N stretch (Amide) components of Soil Organic Carbon
	1597 cm^{-1}	
Band-E	Present study	Assignment
1000 – 1050 cm^{-1}	1002 cm^{-1}	Saturated aliphatic compounds of Soil Organic Carbon
	1018 cm^{-1}	Saturated aliphatic compounds of Soil Organic Carbon
	1010 cm^{-1}	Saturated aliphatic compounds of Soil Organic Carbon
	1006 cm^{-1}	Saturated aliphatic compounds of Soil Organic Carbon
1000 – 1160 cm^{-1}	1033 cm^{-1}	Ester, phenol C-O-C, C-OH stretch, attributed to polysaccharides of Soil Organic Carbon.
Band F	Present study	Assignment
779 - 798 cm^{-1}	775 cm^{-1}	Symmetric Si-O stretching in quartz of clay minerals
	779 cm^{-1}	
	783 cm^{-1}	

esters or carboxylic acids. In addition aromatic compounds can be associated with bands observed from 1600-1490 cm^{-1}. The region < 900cm^{-1} was attributed to quartz and silicates.

The mid IR spectrum is divided into six regions A, B, C D E and (Fig. 1, 2, 3 and 4). The band regions 3629-3618 cm^1 and 3401-3430 cm^1 recorded in the spectra in all the four land use types correspond to the -OH groups associated with clay mineral structure. Similarly band 779-798 cm^{-1} which may identify to the stretching vibrations of the Si-O mineral group is observed in all the four land use types. In the same line, the band B (2925-2300 cm^{-1}) having a prominent peak (–CH stretching) suggest the presence of aliphatic compounds are recorded in all the four land use types (Fig. 1, 2, 3, 4). The band C (1660-1630cm^{-1}) suggest to the presence of the amide C=O stretching of the available humic acids. The peak intensity for this band was seen in all the four land use types. In addition, the band 1410-1380cm^{-1} associated with this group may confirm the presence of aromatic phenolic compounds C-O stretch and –OH deformation of humic acids and this band was commonly observed in all the studied four land use types4). The band E (1050-1000 cm^{-1}) corresponding to the stretching vibrations of saturated aliphatic compounds was found in all the four land use types. The band F (798-779 cm^{-1}) was usually associated with Si-O bond of quartz minerals and this band was prominently seen in the FTIR images of all the four land use types.

The occurrence of aliphatic compounds corresponds to labile carbon fractions and this peak is common in all the four land use types. The intensities at 2300-2925 cm^{-1} referred to aliphatic C-H stretch of humic acids. This condition may arise due to the availability of fresh litter content in all the land use types. Such a peak was observed in agricultural crop surface soil (0-10cm) samples in Brazil (Machado et al., 2020). The second peak registered at 1000-1050 cm^{-1} indicates the presence of saturated aliphatic compounds corresponding to polysaccharides and carbohydrates confirming the re-synthesised products from the litter in all the four land use types. Such a FTIR peak with stretching of -OH component of humic acid at the range of 3200-3700 cm^{-1} was also identified in the agroforestry soils of Brazil (Thabit et al., 2024). The conspicuous third peak in the range of 779-798 cm^{-1} referred to Si-O stretch in clay minerals is commonly present in all the four land use types suggesting that mineralized carbon is present in the passive carbon pool in all the land use types.

SOC comprises of not only litter products but also totally transformed litter components into humic molecules. The process of the breakdown of litter fragments usually increases the content of aromatic and aliphatic compounds. Based on this degree of transformation the change in the chemical structure of SOC occurs as well as the protection from microbial bio-decomposition occurs (Schnitzer and Monreal 2011). The aromatic compounds are mainly derived

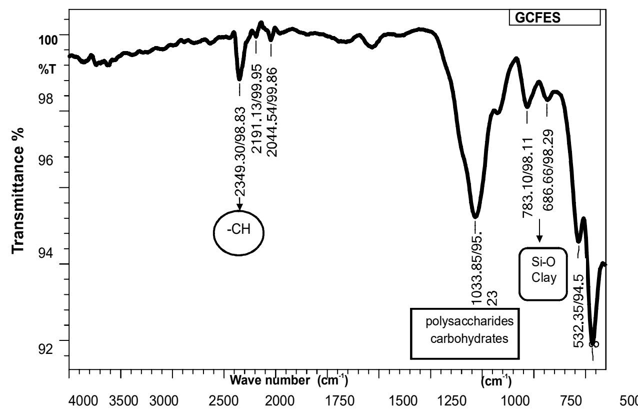

Fig. 1. FTIR spectra showing absorption bands of forest land use soil

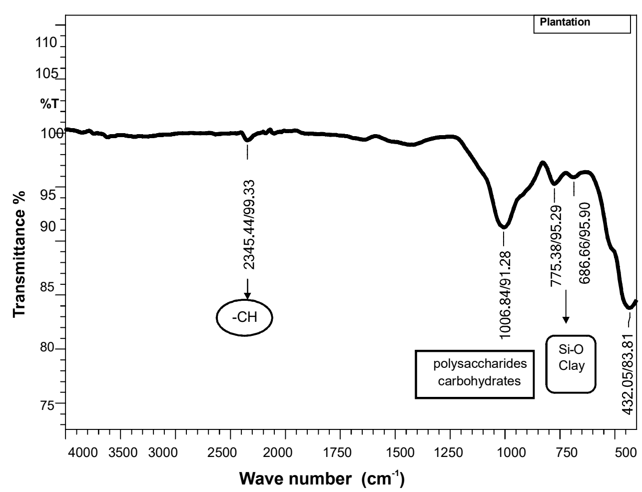

Fig. 2. FTIR spectra showing absorption bands of plantation land use soil

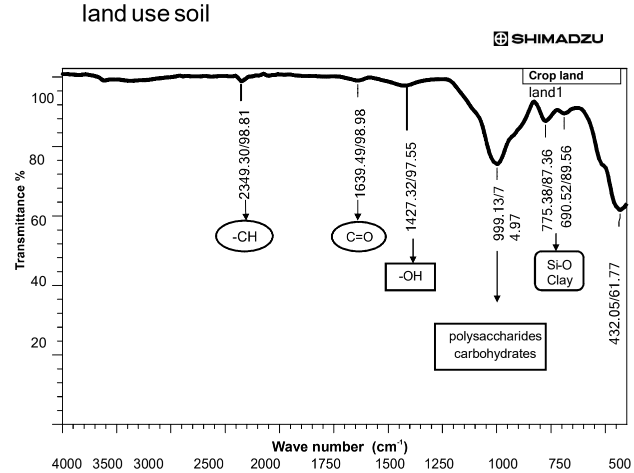

Fig. 3. FTIR spectra showing absorption bands of agricultural crop-land soil

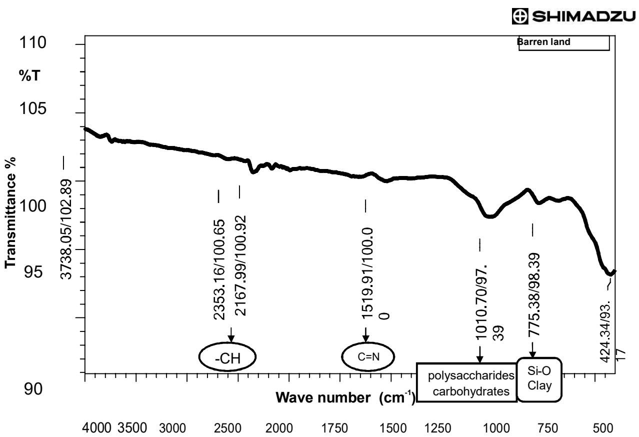

Fig. 4. FTIR spectra showing absorption bands of barren land soil

from lignin and tannin plant products, while alkyl components indicate the resistant products like waxes, cutin and suberins in the SOC (Thabit et al., 2024).The absorption spectra of SOC soil samples collected from four land use types produced similar structure indicating that the decomposition products of the litter comprise of re-synthesised compounds of similar nature although their fresh litter components are different.

CONCLUSIONS

The amount of SOC content is significantly different across the four land use types. But the FTIR data have produced similar functional groups across the land use types. The data indicates that the resulting decomposition products featured more or less same re-synthesised products. The decomposed products of SOC are due to complex interaction between the aboveground litter and its land use patterns. The study suggest that the processes regulating the composition and stability of SOC are related to the ecosystem properties rather than to specific individual plant properties.

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Soybean Productivity and Nutritional Quality as Influenced by Graded Phosphorus and Molybdenum Fertilization under *Typic Hapludalfs*

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Abstract: The present investigation was aimed to evaluate graded P (0, 30, 60 and 90 kg ha^{-1}) and Mo (0,1 and 2 kg ha^{-1}) levels through 12 treatment combinations on the yield, nutrient uptake and quality of soybean. The highest grain yield, NPK and Mo uptake was with highest application rates of both the tested nutrients. The protein content and protein yield also recorded remarkable improvements with the same treatments. The conjoint application of 90 and 2 kg ha^{-1} P and Mo exhibited a significant impact on number of pods per plant^{-1} and Mo uptake by grain. The study concluded that higher soybean yield with enhanced quality under prevailing acidic soil conditions, marked by P fixation and Mo deficiency can be achieved by applying these nutrients at their peak tested rates.

Keywords: Phosphorus, Molybdenum, Soybean yield, Nutrient uptake, Protein content

Heavy dependence solely on macronutrient fertilizers initially boosted the short-term crop yields but caused long-term soil degradation. Avoiding micronutrients' application disrupted the nutrient balance within the interconnected soil-plant-animal-human continuum. Amidst macronutrients, phosphorus (P), classified as the second most limiting after nitrogen in Indian soils, but plays crucial roles in energy production, signaling, homeostasis and nucleic acid synthesis (DNA/RNA) in humans (Shehzad et al., 2022). Despite regular/continuous application of phosphatic fertilizers to increase crop yields, P availability is often low, due to high affinity for the soil solid phase (Zhu et al., 2018). Concurrently, micronutrient deficiencies are widespread in India, particularly in lower-middle-income populations with calorie-dense, nutrient-poor diets. Among these, molybdenum (Mo), an essential element for humans, act as a cofactor for several enzymes essential for metabolism, cell signaling, differentiation and antioxidant defense has been reported deficient in about 13% of Indian soils impacting the soil- plant-human health (Behera et al., 2014).

In India, food and nutritional insecurity is on the rise particularly in the increasing demographic scenario and changing agro-climatic conditions. Under such situations, soybean (*Glycine max* L.), though a non-staple crop offers the inexpensive plant-based source of high-quality protein, rich in essential amino acids, micronutrients and vitamins, vital in combating malnutrition and ensuring food and nutritional security (Rashid et al., 2023). Above all, soybean's added advantage having remarkable ability to biologically fix nitrogen partially offsets the need for synthetic nitrogen fertilizers, emphasizing its importance in arresting the negative soil and environmental implications.

However, being nutrient intensive crop, often faces multi-nutrient deficiencies across major growing regions worldwide. Soybean specifically relies on adequate Mo fertilization to achieve maximum yield potential. However, non-replenishment of adequate Mo is causing its deficiency and significant yield and quality losses, severely impairing N-fixation, a cornerstone of soybean productivity (Zhang et al., 2023).

Deficiencies of both these nutrients exacerbates under humid subtropical climate with monsoonal influences matching the experimental site (Palampur region) where soils have been classified as *Typic Hapludalfs,* moderate to low in fertility and high susceptibility to nutrient leaching, exhibiting poor P availability locked away by sorption, precipitation (typically by reaction with Al^{3+} and Fe^{3+}) or microbial immobilization resulting in low P use efficiency and decline in soybean yield up to 29-45% (Vishwanath et al., 2022). Owing to acidic conditions, molybdenum is bound to be insufficient. It is involved in diversified functions and acts as a key to unlock nitrogen fixation in legumes like soybean. At low pH, the $HMoO_4$ is absorbed by silicate clays and oxides of Fe and Al through ligand exchange with hydroxide ions on the surface of the colloidal particles which hinders its availability. Oliveira et al. (2022) suggested that Mo concentrations below 0.1 ppm in mature leaf tissues in various crops indicate its deficiency, restricted flower development and nodule formation (Rana et al., 2020). However, limited research has been done with conjoint application of P and Mo on soybean under *Typic Hapludalfs*, hence this investigation was aimed with the hypothesis that graded P and Mo levels will enhance productivity, quality, and nutrient uptake by soybean.

MATERIAL AND METHODS

Study site: The present study was undertaken on soybean during *kharif* 2021, at CSK HP Agriculture University, Palampur (32° 6' N latitude and 76° 3' E longitude, 1290 m amsl). The site lies within the mid hill wet temperate zone (Zone II) of HP and is characterized as *Typic Hapludalfs*, with silty clay loam texture and pH of 5.32. During the experimental period, the crop received a total rainfall of 1882 mm, with mean maximum and minimum temperatures of 31.1 and 10.5°C, respectively and weekly relative humidity varying from 60 to 94.03%. The soil properties at the initiation of the experiment have been depicted in Table 1.

Experimental details: The experiment was arranged in randomized block design using 4 levels of P *viz.,* P_0: 0, P_1: 30 P_2: 60 and P_3: 90 kg P_2O_5 ha^{-1} and 3 levels of Mo *viz.,* Mo_0:0, Mo_1: 1 and Mo_2: 2 kg Mo ha^{-1}, thus, having 12 treatment combinations (T_1: P_0Mo_0, T_2: P_0Mo_1, T_3: P_0Mo_2, T_4: P_1Mo_0, T_5: P_1Mo_1, T_6: P_1Mo_2, T_7: P_2Mo_0, T_8: P_2Mo_1, T_9: P_2Mo_2, T_{10}: P_3Mo_0, T_{11}: P_3Mo_1 and T_{12}: P_3Mo_2) replicated thrice. Basal applications of 20 kg N and 40 kg K_2O ha^{-1} through urea and MOP while, P and Mo as per treatment structure were done through SSP and sodium molybdate, respectively. The standard agronomic measures were followed during the entire growth period of the crop.

Sampling and analysis: After harvest, the plant samples (grain and straw) were collected, dried in an oven at 60 °C, grounded in a Wiley mill and stored in moisture free paper bags for further analysis. The protein content and protein yield of the digested samples was worked out.

Protein content (%)= N content (%) x 6.25 (Walinga et al., 1989)

Protein yield (kg ha^{-1}) = Grain yield (kg ha^{-1}) x Protein content (%) / 100

Whereas, the nutrient uptakes were calculated using the following formulae:

Nutrient uptake by grain (kg ha^{-1}) = Nutrient content in grain (%) x Grain yield (q ha^{-1})

Nutrient uptake by straw (kg ha^{-1}) = Nutrient content in straw (%) x Straw yield (q ha^{-1})

Statistical analysis: The data was analyzed using the technique of analysis of variance for randomized block design (Gomez and Gomez 1984) using Microsoft Excel.

RESULTS AND DISCUSSION

Yield Attributes

Number of pods $plant^{-1}$: The individual application of P and Mo along with their interaction effect, significantly increased the pod count. The exclusive application of P and Mo at respective highest rates exhibited a significant edge over the lower levels (Table 2). With the graded P application, the pod count followed the trend 90 > 60 > 30 > 0 kg P ha^{-1}. However, the control treatment showed diminution of about 27 and 20 % over 90 and 60 kg P ha^{-1}, respectively. In contrast, Manoj et al. (2023) recorded maximum pod count in soybean with 40 kg P ha^{-1}. The adequate P fertilization in the regions prone to its high fixation, perfectly matching our situations (acid soils), might have improved its availability during the early crop growth stages (Johan et al., 2021). In a comparable manner, the progressive reductions in Mo doses at each level significantly reduced the pod number. But, with raised dose from 0 to 2 kg Mo ha^{-1}, increase from 73.5 to 80.4, respectively was observed, emphasizing the significance of Mo application addressing its deficiency at the experimental site. The application of 1 and 2 kg Mo ha^{-1} demonstrated noteworthy increases of about 5 and 10 %, respectively over the control. The probable reason could be the efficient use of Mo applied at the time of sowing, regulated effective nodulation, nitrogen fixation and contributed to better performance. Padhi and Pattanayak (2018) also observed maximum enhancement in yield attributes of mungbean with the use of Mo. Additionally, omitting phosphorus resulted in a more significant reduction in the pod number (63.0) compared to the exclusion of molybdenum (73.4) highlighting more reliance on P.

Interaction of P and Mo on number of pods $plant^{-1}$: The conjoint use of P and Mo exerted a synergistic effect on the pod count and the highest was recorded with 90 kg P ha^{-1} and 2 kg Mo ha^{-1} (88.9) trailed by 90 kg P ha^{-1} and 1 kg Mo ha^{-1}

Table 1. Initial characteristics of the soil

Soil property	Value
Mechanical separates (%)	
Sand	19.30
Silt	44.60
Clay	34.40
Textural class	Silty clay loam
Chemical analysis	
Soil pH (1:2.5)	5.32
Organic carbon (g kg^{-1})	7.31
Available nutrients (kg ha^{-1})	
N	307
P	16.3
K	119
Mo (ppm)	0.160

(85.4) and the least under control (61.8) (Table 3). The response of Mo to graded P application highlighted that in the absence of P, a consistent increase was up to 2 kg ha^{-1}. Conversely, at 30 and 60 kg P ha^{-1}, significant variations were observed only at 2 kg Mo ha^{-1} but, the use of 90 kg P ha^{-1} recorded a consistent increase up to 2 kg Mo ha^{-1}. Likewise, the performance of P under varied Mo doses revealed that with the omission of Mo, a marked increase was documented only up to 60 kg P ha^{-1}. But, at 1.0 and 2.0 kg Mo ha^{-1}, a steady increase up to 90 kg P ha^{-1} was observed. Ahmad et al. (2022) also reported significantly higher pods $plant^{-1}$ in mung bean with the conjoint use of 60 kg P ha^{-1} and 1.5 kg Mo ha^{-1}.

Number of seeds pod^{-1}: Significant variations in number of seeds pod^{-1} were observed in response to the graded applications of P and Mo (Table 2). The use of 30, 60 and 90 kg P ha^{-1} demonstrated improvements of about 8, 10 and 12 %, respectively over control. This enhancement might be attributed to the vital role of P in improving photosynthesis, root expansion, carbohydrate transfer, increasing sink capacity through improved flowering and metabolite transfer to pods, thereby aiding the seed establishment (Nikfarjam and Aminpanah 2015). Whereas, scarcity can lead to reduced seed number, as evidenced from the significant drop in the control. Shenoy and Kalagudi (2005) also highlighted that the deficiency of P can reduce the soybean yield by about 10-15%. Khan et al. (2017) observed higher seed count per pod compared to lower P rates. The 2 kg Mo ha^{-1} resulted in highest count (2.66) owing to the better supply of this deficient nutrient. In contrast, the lowest number was observed under Mo-devoid treatment (2.55), registering a diminution of about 3 and 4% over 1 and 2 kg Mo ha^{-1}, respectively. The significant reduction in the seed count pod^{-1} (2.42) was with the exclusion of P compared to the omission of Mo (2.55). These results corroborate the findings of Oliveria et al. (2022). However, the conjoint use of both the nutrients showed statistical equivalence.

Grain yield: The significant increase in the grain yield was attained with the graded application of both the nutrients (P and Mo) (Table 2), though their interaction exhibited no significant impact. Among the P levels, the yield response followed the trend 90 > 60 > 30 kg P ha^{-1} and registered an increase of about 61, 45, and 26 % over the control, respectively, underscoring the positive impact of P fertilization in acid soils exhibiting high fixation of phosphate radicals by acidic cations (Chen et al., 2022) or their complexation with soil organic matter and microorganisms (Huang et al., 2017). Adequate P improves the rhizosphere nutritional environment, accelerates root growth, flowering, seed formation, facilitates translocation of stored assimilates to seeds, ultimately aids in boosting the crop productivity (Khan et al., 2020). Similarly, the reductions in Mo doses at each level documented a declining trend in the yield and the control registered a drop of about 10 and 19 % over 1 and 2 kg Mo ha^{-1}, respectively. The proportionate increase with the graded doses might be owing to its predominant role in the biological nitrogen fixation, enhancing nitrogen absorption

Table 3. Interaction effect of graded P and Mo application on number of pods $plant^{-1}$

Treatment	Phosphorus levels			
Molybdenum levels	P_0	P_1	P_2	P_3
Mo_0	61.8	72.7	78.2	81.4
Mo_1	65.7	74.9	80.9	85.4
Mo_2	73.4	77.9	81.5	88.9
CD ($p \le 0.05$)	3.4			

Table 2. Impact of graded P and Mo application on yield attributes, yield and quality parameters of soybean

Treatment	No. of pods $plant^{-1}$	No. of seeds pod^{-1}	Grain yield (tonnes ha^{-1})	Protein content (%)	Protein yield (q ha^{-1})
Phosphorus levels					
P_0	67.0	2.42	1.54	34.4	4.81
P_1	75.2	2.61	1.94	35.7	6.04
P_2	80.2	2.65	2.23	37.8	8.52
P_3	85.2	2.72	2.48	38.4	9.96
CD ($p \le 0.05$)	2.0	0.03	0.06	1.6	0.29
Molybdenum levels					
Mo_0	73.5	2.55	1.87	35.4	6.83
Mo_1	76.7	2.58	2.05	36.8	7.33
Mo_2	80.4	2.66	2.21	37.5	7.84
CD ($p \le 0.05$)	1.7	0.03	0.05	1.3	0.25

thereby the yield (Guo et al., 2023). The increased Mo application under the deficient conditions might also have elevated the concentration in the soil solution for the uptake. The omission of P resulted in a greater yield reduction over Mo. Oliveira et al. (2017) also highlighted a significant impact of P and Mo fertilization on the soybean yield.

Quality Attributes

Protein content and protein yield: The individual factors (P and Mo) exerted a significant influence on the protein content and protein yield (Table 2), whereas their conjoint impact failed to register any significant improvement. The maximum protein content was with 90 kg P ha^{-1} (38.4 %) which showed statistical parity with 60 kg P ha^{-1} (37.8%) and the lowest under control (34.4%). The applications at 30, 60 and 90 kg P ha^{-1} increased the content by 4, 10 and 12%, respectively over control owing to the complementary relationship between nitrogen and phosphorus; which aids in the formation of stable phospho-protein compounds as nitrogen facilitates amino acid synthesis while phosphorus supports energy intensive processes such as nitrogen fixation, uptake and assimilation (Zhao et al., 2023). The addition of sulphur through SSP might have contributed significantly in protein synthesis as S helps in the formation of disulfide bonds which have paramount role in formulating and regulating the stability and configuration of the protein structures (Patel et al., 2019). The reductions in Mo doses registered a decline in the protein content and the lowest was documented under control (35.4 %) followed by 1 kg Mo ha^{-1} (36.8%) which behaved statistically alike with 2 kg Mo ha^{-1} (37.5%). The treatment devoid of Mo, reduced the content by about 6% over 2 kg Mo ha^{-1}. As Mo is a vital component of dual enzymes, nitrate reductase and nitrogenase; helps in producing more nitrogen through nitrate reduction and fixation respectively, leading to enhanced protein content in seeds (Ahmad et al., 2022). In case of protein yield, skipping both P and Mo led to substantial reductions in protein yields over their peak levels, but was more pronounced with P omission (about 107%) compared to Mo (about 15 %). The use of 30, 60 and 90 kg P ha^{-1} yielded about 6.04, 8.52 and 9.96 q ha^{-1} protein, respectively. The application of 1 and 2 kg Mo ha^{-1} produced 7.33 and 7.84 q ha^{-1} protein yields, respectively. The quantum jump with the highest levels of both P and Mo could be ascribed to the higher protein content and grain yields. The findings are in total agreement with Singh et al. (2017) in lentil advocating the same dose, however, Khaswa et al. (2014) recorded higher values with 40 kg P ha^{-1} in soybean.

Nutrient Uptake

Nitrogen uptake: Increased P and Mo rates exhibited significant improvements in nitrogen uptake by both grain and straw over rest of the treatments (Table 4). However, their conjoint application did not exhibit any significant interaction. Application of 90 kg P ha^{-1} demonstrated peak uptakes by both grain (159.4 kg ha^{-1}) and straw (26.9 kg ha^{-1}) followed by 60 (136.4 and 23.6 kg ha^{-1}) and 30 (96.7 and 19.7 kg ha^{-1}) kg P ha^{-1} registering improvements of about 107 and 65 % by grain and straw, respectively over respective controls as the uptake is a function of concentration within the plant and the overall crop yield (Gourav et al., 2019). P, being energy currency plays essential role in improving photosynthesis and metabolic processes, collectively boosting the grain nitrogen content. The improved grain yield with elevated P application could be ascribed to the better nutrient absorption, greater dry matter accumulation and

Table 4. Influence of varied P and Mo applications on the nutrient uptake by grain and straw

Treatment	Nitrogen uptake (kg ha^{-1})		Phosphorus uptake (kg ha^{-1})		Potassium uptake (kg ha^{-1})		Molybdenum uptake (g ha^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Phosphorus levels								
P_0	77.0	16.3	5.77	0.92	11.0	32.5	2.93	2.21
P_1	96.7	19.7	7.57	1.76	14.1	40.8	4.16	3.15
P_2	136.4	23.6	9.29	2.41	16.9	46.7	4.68	3.77
P_3	159.4	26.9	11.09	3.41	19.2	52.4	5.70	4.40
CD ($p\leq0.05$)	4.7	1.4	1.3	0.6	1.7	5.2	0.5	0.4
Molybdenum levels								
Mo_0	109.3	19.3	7.12	1.56	12.9	40.3	3.66	2.99
Mo_1	117.4	22.1	8.46	2.14	15.3	42.3	4.36	3.39
Mo_2	125.4	23.5	9.72	2.67	17.7	46.7	5.07	3.80
CD ($p\leq0.05$)	4.1	1.2	1.1	0.5	1.5	4.5	0.4	0.4

effective translocation of photosynthates to the grain (Ahmad et al., 2018, Anwar et al., 2018). The graded Mo levels also behaved alike to the graded P doses and use of 2 kg Mo ha^{-1} accelerated the uptake by both grain and straw (125.4 and 23.5 kg ha^{-1}, respectively) trailed by 1 kg Mo ha^{-1} (117.4 and 22.1 kg ha^{-1}, respectively). The uptake at 2 kg Mo ha^{-1} increased uptakes by about 15 and 22% by grain and straw, respectively over their controls owing to its roles in improving the N metabolism and use efficiency (Probst et al., 2021). As Mo is a key component of enzymes nitrogenase and nitrate reductase, plays pivotal role in nitrogen assimilation and transport which further contribute to its enhanced uptake (Oliveria et al., 2022). Omission of P (77 and 16.3 kg ha^{-1}) caused a greater decline in uptake compared to Mo (109.3 and 19.3 kg ha^{-1}) by grain and straw, respectively.

Phosphorus uptake: Significant variations in P uptake by soybean grain and straw were recorded in response to varied rates of P and Mo (Table 4), but their interaction effect showed statistical equivalence. The uptakes by grain and straw followed the trend 90 > 60 > 30 >0 kg P ha^{-1} and 2 > 1 > 0 kg Mo ha^{-1}. The use of 90 kg P ha^{-1} registered improvements of about 92 and 270 % in grain and straw, respectively, over respective controls. The increased utilization of P by the crop might be due to higher soil P concentration owing to higher addition which likely satisfied the crop's initial nutrient needs thus enhancing nutrients uptake. The low P use efficiency (approximately 15-20%) by most crops in acid soils owing to high fixing capacity leads to accumulation of large amounts of legacy P which prevents plant absorption and necessitates substantial phosphate fertilization (Zhu et al., 2018). The graded Mo application exhibited analogous results to varied P levels. Omitting Mo resulted in decline of about 37 and 71 % by grain and straw respectively, over 2 kg Mo ha^{-1}. Mo stimulates the secretion of root exudates *viz.,* succinic acid, malic acid and acid phosphatase in leguminous crops, thus promoting the conversion of soil-insoluble phosphorus into plant -available forms (Qin et al., 2023). Rana et al. (2020) also emphasized the role of Mo application in enhancing P availability within the rhizosphere soil of leguminous crops and ultimately the crop yields.

Potassium uptake: The potassium uptake was significantly influenced by graded P and Mo doses (Table 4). The peak uptakes by grain and straw were with 90 kg P ha^{-1} (19.2 and 52.4 kg ha^{-1}, respectively) and the minimum under control (11 and 32.5 kg ha^{-1}, respectively). The enhancement might be owing to the increased dry matter production and higher nutrient content in the P- treated plots. P plays a crucial role in stimulating root growth, increasing root surface area for nutrient absorption, providing energy in the form of ATP to support active potassium transport, thereby helped in improving it's uptake. Mo application enhanced grain uptake from 12.9 kg ha^{-1} under control to 17.7 kg ha^{-1} at 2 kg Mo ha^{-1}; accounting for an increase of about 37 %. Conversely, all the Mo doses behaved statistically alike with respect to K uptake by straw, and ranged from 40.3 kg ha^{-1} under control to 46.7 kg ha^{-1} at 2 kg Mo ha^{-1}. The numerical increase might be due to the favorable environmental conditions for growth and biomass production of soybean (Lateef et al., 2021). The interaction effect of both the nutrients exhibited non-significant impact on the uptakes.

Molybdenum uptake: The sole applications of P and Mo exhibited a marked impact on the Mo uptake by grain and straw and was maximum at highest levels (Table 4), though the interaction between the two was significant only in grain (Table 5). An exponential increase in uptake was observed with the increasing P doses. The uptakes at 90 kg P ha^{-1} recorded an increase of about 94 and 99 % in grain and straw, respectively over their controls. Huang et al. (2023) also highlighted the synergistic relationship between P and Mo. The graded application of both the nutrients enhanced their absorption, improved photosynthesis and utilization, higher use efficiency and ultimately yield. Similarly, Mo doses exhibited linear increase in uptake and the use of 2 kg ha^{-1} increased the uptakes in grain and straw by about 38 and 27 %, respectively, over their controls. The reduced uptake in control can likely be attributed to the absence of Mo supplementation and its inherent deficiency in the prevailing acidic soil conditions. The application of graded doses probably elevated its concentration in the soil solution, enhancing availability and subsequent utilization.

Interaction of P and Mo on Mo uptake by grain: The response of Mo to graded P application revealed that in absence of P, a consistent increase in uptake was documented upto 2.0 kg Mo ha^{-1} (Table 5). Whereas, at 30 and 60 kg P ha^{-1}, graded Mo application behaved statistically alike. But, at 90 kg P ha^{-1}, again consistent increase upto 2.0 kg Mo ha^{-1} was observed. Likewise, the response of P to varied Mo levels revealed that when 0 and 1 kg Mo ha^{-1} was applied, significant variations in uptake were observed only upto 30 kg P ha^{-1} which behaved statistically alike with 60 kg P

Table 5. Interaction effect of graded P and Mo applications on the Mo uptake (g ha^{-1}) by grain

Treatment	Phosphorus levels			
Molybdenum levels	P_0	P_1	P_2	P_3
Mo_0	1.74	3.72	4.16	5.01
Mo_1	2.65	4.15	4.83	5.83
Mo_2	4.39	4.62	5.03	6.26
CD (p=0.05)		0.8		

ha^{-1}, but thereafter a significant increase upto 90 kg P ha^{-1} was observed. When 2.0 kg Mo ha^{-1} was applied, a significant response was found only when combined with 90 kg P ha^{-1}. Huang et al. (2023) also highlighted that the co-application of Mo and P strengthens the root system of soybean, leading to enhanced nutrient absorption. Rana et al. (2020) has reported that phosphomolybdate complexes, formed by the synergistic interaction between phosphate and molybdate ions create a readily available form of phosphorus for plants, highlighting the role of Mo in enhancing P uptake in crops.

CONCLUSION

In acid soils, P in spite of having sufficient content experiences fixation, whereas, Mo is inherently deficient. Coupling P and Mo at their highest rates (90 and 2 kg ha^{-1}) enhanced soybean yield, quality attributes and uptakes. The increased quality attributes will certainly be a step to address the nutritional security issues. However, further higher doses may be tested in such soils involving different crops and cropping systems.

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Biological Activity as Influenced by Cropping Systems in Various Farming System under Southern Transition Zone of Karnataka

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Abstract: Soils are the basic unit of the agricultural production, maintaining the soil health with respect to nutrient availability and organic carbon content infers the soil suitability to grow the crops. To protect the soil native population of microorganisms and their efficiency, selection of suitable farming types and cropping systems are very much important in order to harvest much benefits without compromising degradation, in that view an experiment was executed at Zonal Agricultural and Horticultural Research Station, Navile, Shivamogga during *Kharif* 2021 and 2022 in sandy loam soils under split plot design, where main plots comprises conventional, organic and natural farming along with subplots consists of sole crops of groundnut, maize, finger millet, intercropping of maize and finger millet with groundnut. Organic farming recorded higher population of beneficial microorganisms (N fixers, P and K solubalizers) and higher enzymatic activity (dehydrogenase, alkaline and acid phosphatase) which was significantly higher than conventional farming. Among cropping systems, intercropping method beneficial microorganisms and enzymatic activity were significantly higher than sole crops, particularly in maize as an intercrop. Irrespective of the farming types and cropping systems biological activities were higher at 67 days.

Keywords: Farming types, Cropping systems, Enzymatic activity, N fixers, Dehydrogenase

Soils are the lifeline of crop production, which not only supports the plant growth but acts as hot spot of biodiversity by encouraging growth and development of various living organisms in it. Soil ecology infers the interaction of different species with their surrounding soil environment for nutrient recycling, organic matter decomposition and soil structure (Pavao-Zuckerman 2008). Interaction of soil physical, chemical and biological entities will made complicated reactions into resolvable matter. Proper depiction of these three entities are required to answer any of the reactions, in that view biological properties plays significant role along with physical and chemical properties (Vellend et al., 2017). Biological properties includes the population of living organisms which majorly constitutes bacteria, fungi, protozoa, insects, earthworms and enzymatic activity in the soil (Deng et al., 2015). Biological activities answers to the activities of soil respiration, mineralization and fertility status.

Population of various microorganisms and their activity depends on different factors *viz.,* status of organic carbon content in the soil, water holding capacity, C N ratio of the organic source to maintain remarkable soil health status (Cheng et al., 2012). In that view, cropping pattern practiced in particular land followed by method of cultivation of crops also decides the efficiency of the biological entities over long period, to understand the efficiency of farming methods and cropping systems. The current study was undertaken to understand the effect of different cropping systems and farming types on population of soil beneficial microbes.

MATERIAL AND METHODS

Field experiment was conducted at Zonal Agricultural and Horticultural Research Station, Navile, Shivamogga during *Kharif* 2021 and 2022 to evaluate the performance of groundnut following different farming types and groundnut based cropping system. The soil of the experimental site was sandy loam in texture with slightly acidic in nature (6.22) and lower in salt load, further soil was medium in organic carbon content (0.51 %), available nitrogen (262 kg ha^{-1}), available potassium (202 kg ha^{-1}) and higher in available phosphorus content (86.9 kg ha^{-1}). The field experiment was laid out in split plot design with three farming types *viz*., conventional farming, organic farming and natural farming as main plots and five cropping systems *viz*., groundnut + maize (4:1) intercropping, groundnut + finger millet (4:1) intercropping, sole groundnut, sole maize and sole finger millet as subplots (Table 1).

Conventional farming includes management of crops as per standard package of practice which includes seed treatment with biofertilizers, fungicides and insecticides followed by nutrients through both organic and chemical means, chemicals was used for insect, diseases and weeds (Ragini 2022). Natural farming treatment followed seed treatment with beejamrutha, nutrient management with ghanajeevamrutha and jeevamrutha and insect pest management was done through botanical extracts (agniastra, neemastra and dashaparni kashayam) and diseases by sour butter milk and weeds through hand weeding and mulching.

The population of beneficial micro flora was determined by serial dilution plate count technique. The population of N-fixers, P-solubilizing (PSM) microorganisms and Potash-solubilizing microorganisms was estimated by transferring 1 ml of 10^4 and 10^3 dilutions, respectively to sterile petri dishes. Approximately 20 ml of Waksman medium for N fixers, Sperber's medium for PSM and Alexandros for Potash solubilizing microorganisms were poured into the sterilized plates and kept for incubation in an inverted position at 30 ± 1 °C for a week time and emerged colonies were counted (Tate 1995). Activity of dehydrogenase, phosphatase and urease were estimated according procedure encasted by Casida et al. (1964), Tabatabai and Bremner (1972) and Kandeler and Gerber (1988) respectively. Enzymatic activity was compared between 67 DAS and at harvest. The data was analyzed in the M S office excel spread sheets.

RESULTS AND DISCUSSION

Organic farming recorded significantly higher population of nitrogen fixers, phosphorus solubalizers and potassium solubalizers than conventional farming. The higher beneficial microorganisms were due to application of greater amount of organic manures in the particular treatment enhanced the substrate availability for their multiplication, along with organic manures improves the soil water holding capacity and increases the porosity which further helps to microorganisms for their active division. Natural farming recorded similar observation for beneficial microorganisms next to organic farming may be due to application of ghanajeevamrutha at the time sowing followed by application of liquid jeevamrutha at the interval of 20 days and mulching

Table 1. Details of the experiment

Design		Split plot
Treatment combinations		15
Replications		03
Gross plot size		7.8 m × 4.2 m
Net plot size		3.6 m × 3.0 m
Season		*Kharif* 2021 and 2022
Location		ZAHRS, Shivamogga Latitude: 13.9711 N Longitude: 75.5776 E
Crops		Groundnut as main crop, Maize and Finger millet as intercrops and sole crops
Variety	Groundnut	TMV-2
	Maize	P-3304
	Finger millet	GPU 28
Spacing	Groundnut	30 cm × 10 cm
	Maize	60 cm × 30 cm
	Finger millet	30 cm × 10 cm
Recommended dose of fertilizers	Groundnut	25: 50: 25 kg N, P_2O_5 and K_2O ha^{-1}
	Maize	100: 50: 25 kg N, P_2O_5 and K_2O ha^{-1}
	Finger millet	50: 37.5: 37.5 kg N, P_2O_5 and K_2O ha^{-1}
Organic manures	Groundnut	Based on P equivalent basis
	Maize	Based on N equivalent basis
	Finger millet	Based on N equivalent basis
Natural farming	Groundnut	1000 kg of ghanjeevamrutha at the time of sowing 500 l of jeevamrutha at 20 days interval
	Maize	
	Finger millet	
Date of sowing	2021	27-07-2021
	2022	24-07-2022

which created the favourable condition *viz*., organic source, moisture availability, soil temperature and micro climate for their multiplication. Similar observations were observed in earlier studies (Gore and Sreenivasa 2011, Chandrashekara et al., 2020, Choudhary et al., 2022).

Among cropping systems, the statistically higher nitrogen fixers, phosphorus solubalisers and potassium solubalizers were obtained in intercropping system as compared to sole crops, further under intercropping higher values were obtained in the groundnut + maize intercropping. However, groundnut + finger millet intercropping was on par with sole groundnut and significantly higher values over sole maize and sole finger millet. Higher values in the intercropping was due to inter specific interaction of cereal legume root structures enhances the release of various secondary metabolites from the roots which were rich in polysaccharides helps in enhancing the microbial multiplication and acts as extra dose for their effective activity. Concurrent findings were postulated by Devkumar et al. (2018), Thakur (2021), Aravind et al. (2022). Interactions were non-significant among of various farming types and cropping systems for nitrogen fixers, phosphorus and potassium solubalizers.

Among different farming types organic farming recorded significantly higher dehydrogenase and phosphatase activity (acid and alkaline) than conventional farming this may be due to greater amount of substrate availability in organic farming followed by avoiding chemical inputs enhanced the effectiveness. In converse, conventional farming observed significantly higher values than organic and natural farming for urease activity at 67 DAS due to the supply of synthetic source of nitrogen in conventional farming enhanced the activity as it obtained the substrate for effective functioning ability as observed by Gowtham Chand et al. (2020) and Ragini (2022).

Among cropping systems, groundnut intercropped with maize was on par with groundnut intercropped with finger millet and significantly higher than sole crops of groundnut, maize and finger millet for dehydrogenase, alkaline phosphatase and acid phosphatase activity because inter specific interaction of roots of different crops under intercropping helps in production of flavanoids and polysaccaride compounds which helps in enhancing the soil dwell microorganisms, further improves their catalytic activity (Li et al., 2016). However, sole maize and sole finger millet recorded higher urease activity than sole groundnut and intercropping system at 67 days after sowing due to application of recommended dose of nitrogen increases the substrate for the enzyme activity as reported by Chen et al. (2018) and Babu et al. (2020).

Interaction of various farming types and cropping systems was non-significant differences for dehydrogenase, urease, acid phosphatase and alkaline phosphatase activity (Table 3).

Activity of nitrogen fixers and phosphorus solubalizers at 47 DAS was on par with their activity at 67 DAS, however, 20 per cent reduction in nitrogen fixers and phosphorus solubalizers each at 27 DAS. Population of microorganisms

Table 2. Effect of various farming types and cropping systems on beneficial microorganisms

Treatments	Nitrogen fixers (cfu × 10^4 g^{-1})	Phosphorus solubalizers (cfu × 10^4 g^{-1})	Potassium solubalizers (cfu × 10^4 g^{-1})
Main plot (Farming types)			
M_1- Conventional farming	5.01	4.29	2.12
M_2-Organic farming	7.80	6.49	3.41
M_3- Natural farming	7.02	6.05	3.15
CD (p=0.05)	0.19	0.22	0.19
Sub plot (Groundnut based cropping systems)			
S_1-Groundnut + Maize intercropping	7.10	6.28	2.84
S_2- Groundnut + Finger millet intercropping	6.80	5.82	2.98
S_3- Sole Groundnut	6.67	5.51	2.96
S_4- Sole Maize	6.18	5.31	2.83
S_5- Sole Finger millet	6.31	5.14	2.85
CD (p=0.05)	0.30	0.32	NS
Interaction			
CD (p=0.05) (SP at same level of MP)	NS	NS	NS
CD (p=0.05) at same or different level of SP)	NS	NS	NS

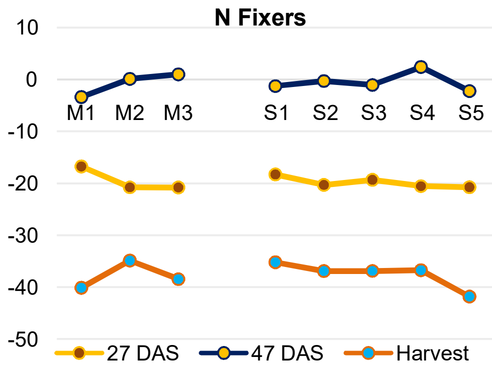

Fig.1a

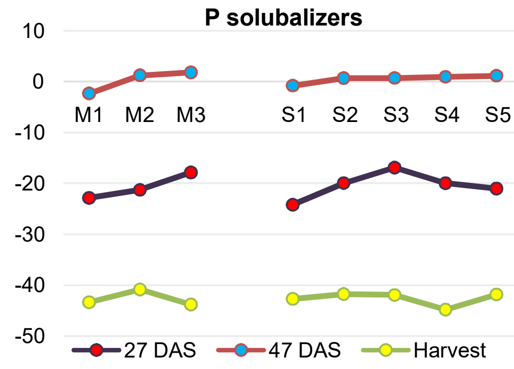

Fig. 1b

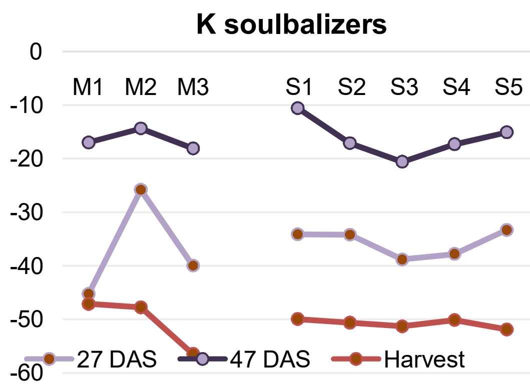

Fig.1c

Fig. 1. Percentage variation in the activity of beneficial microorganisms at 27 DAS, 47 DAS and at harvest compared at 67 DAS

Table 3. Effect of various farming types and cropping systems on enzymatic activity

Treatments	Dehydrogenase (µg TPF g^{-1} day^{-1})	Acid phosphatase (µg pNP g^{-1} hr^{-1})	Alkaline phosphatase (µg pNP g^{-1} hr^{-1})	Urease (µg urea hydrolysed g^{-1} hr^{-1})
Main plot (Farming types)				
M_1- Conventional farming	13.19	13.60	2.38	4.92
M_2-Organic farming	21.12	19.54	3.25	4.27
M_3- Natural farming	17.89	17.64	3.35	3.61
CD (p=0.05)	0.91	0.54	0.14	0.25
Sub plot (Groundnut based cropping systems)				
S_1-Groundnut + Maize intercropping	19.36	18.21	2.86	4.23
S_2- Groundnut + Finger millet intercropping	18.08	17.14	3.12	4.18
S_3- Sole Groundnut	17.80	17.45	2.97	4.00
S_4- Sole Maize	16.46	16.23	2.93	4.56
S_5- Sole Finger millet	15.30	15.60	3.07	4.37
CD (p=0.05)	1.20	0.67	0.12	0.20
Interaction				
CD (p=0.05) (SP at same level of MP)	NS	NS	NS	NS
CD (p=0.05) (MP at same or different level of SP)	NS	NS	NS	NS

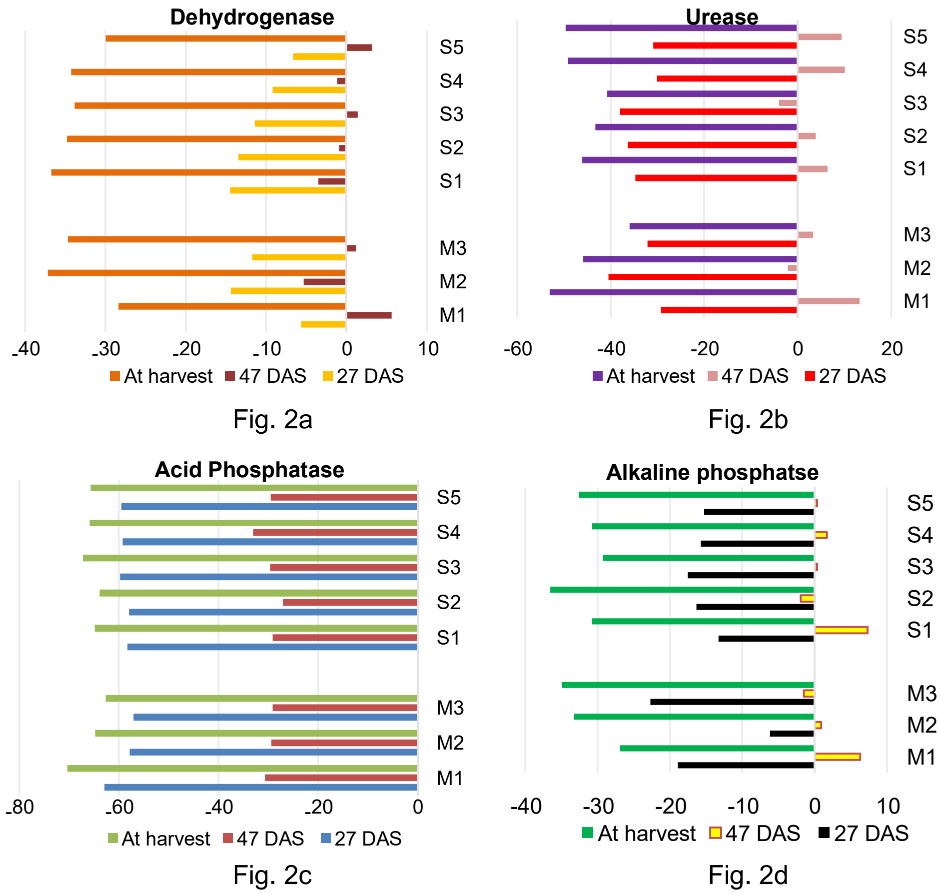

Fig 2. Percentage variation in the activity of enzymes at 27 DAS, 47 DAS and at harvest compared at 67 DAS

reduced drastically at the time of harvest to the extent of 30 per cent for nitrogen fixers and beyond 40 per cent for phosphorus solubalizers. Further, potassium solubalizers was recorded up to 20, 40 and 50 per cent reduction at 47 DAS, 27 DAS and at harvest, respectively as compared to 67 DAS. Population of microorganisms was directly related to the amount of organic substrate availability, moisture content of the soil followed by activeness of root system of the crops and its interaction with rhizosphere, in that view, activity of roots and release of nutrients to the soil nutrient pool by the microorganisms was maximum at flowering which was coincides between 40-70 DAS resulting in greater amount microbial activity during peak stage of crop development (Fig. 1). These results were in line with earlier findings (Chakraborty and Sarkar 2019, Amalraj et al., 2013, Aulakh et al., 2013).

Activeness of all the enzymes was higher at 67 DAS as compared to their vigour at 27 DAS and at harvest, however, on par at 47 DAS except for acid phosphatase activity which infers that there was a drastic reduction in the performance of all the major enzymes. Performance of enzymes at harvest was reduced beyond 30, 40, 60 and 20 per cent for dehydrogenase, urease, acid and alkaline phosphatase activity, respectively due to reduction in root exudates production along with lower moisture availability. Activity at 27 DAS was also lower because of delayed release of nutrients from the organic sources as observed by Li et al. (2019) and Sharma et al. (2022) (Fig. 2).

CONCLUSIONS

Organic carbon in the soil plays major role in the effective performance of the biological activities, amount of the organic matter in the soil was also influences the soil physical and chemical properties. Adding greater amount of organic matter in organic farming treatment noticed higher values of nitrogen fixers, phosphorus and potassium solubalisers population similarly, soil enzymatic performance was also follows same trend except for urease activity. Intercropping system performed better with respect to soil beneficial microorganisms and enzymatic performance as compared to sole cropping system, particularly maize intercropped with groundnut yielded more vivacity among the cropping systems.

CONTRIBUTION

SV Akarsh developed, performed the experiments, analyzed the data and wrote the manuscript. HK Veeranna contributed to the final version of the manuscript and supervised the project, further M Dinesh Kumar, GK Girijesh, BC Dhanajaya and MS Nandish provided critical feedback and helped in to shape the research, analysis and manuscript.

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Performance of Different Jute (*Corchorus olitorius* L.) Varieties Based on Growth and Yield in Coastal Soils of West Bengal

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***Abstract*:** Jute (*Corchorus olitorious* L.) is an environment friendly natural fibre crop responsible for out doing synthetic fibres. However, fibre yields have been decreasing daily in recent decades due to lack of long-term promising varieties, climate change issues and complexity of crop yields. The field experiment was carried out to study the performance of different *olitorius* jute varieties under coastal soils of West Bengal during *pre-kharif* season of 2023 comprising seven *olitorius* jute varieties - JRO 524, JRO 2407, JRO 204, CO 58, JBO 1, JROM 1 and NJ 7005. Variety JBO 1 showed superiority on growth and yield contributing characters and resulted in maximum plant height, leaf per plant and aerial dry biomass at harvest among the tested varieties. JBO 1 was superior with respect to fibre yield (28.2 q/ha), stick yield (78.3 q/ha), and harvest index (26.04) among all the tested jute varieties. Variety JBO 1 could be more successful productive variety under coastal soils of West Bengal.

***Keywords*:** Varietal performance, Natural fibre, Growth parameters, Fibre yield, Economics

Jute (*Corchorus spp*.) is an important natural fibre crop in India next to cotton. It is versatile, biodegradable, recyclable, eco-friendly lignocellulose and considered as the second most common natural cellulosic fibre in the world. *Corchorus olitorius* is the most cultivated species in the country as well as other parts of world. It is one of the important bast fibre crop species being cultivated in India, Bangladesh, China, Nepal, Thailand and some South-East Asian countries (Miah et al., 2020). In India, West Bengal is the major producing state contributing about 71% of national production and is mainly restricted to the Terai regions of the state. Jute production is essential for the agrarian economy since it not only generates revenue but also creates rural employment. The economic importance is further enhanced by its quick growth and capacity to flourish in low-input agricultural systems (FAO 2021). In an era marked by concerns about environmental sustainability, jute has emerged as an eco-friendly alternative to synthetic materials. Its biodegradability and low carbon footprint make it a favoured choice in various industries seeking to reduce their environmental impact (GCI and UNCTAD 2018). Despite technological advancements in crop production, jute yields have been decreasing daily in recent decades for a number of reasons (Islam and Ali, 2017, Karim et al., 2020, Karki et al., 2021). One of them is the lack of long-term promising varieties and the complexity of crop yields, which are greatly influenced by a variety of genetic factors as well as a series of environmental changes. Furthermore, with continuous variation in the present weather conditions, jute crop production is greatly affected by such climate change issues (Kalita and Bhuyan 2018). The jute industry is plagued by a number of issues, such as small and marginal growers with little negotiating power, a lack of institutional credit, a flawed marketing structure, declined productivity, competition from synthetics, high labour costs, frequent industrial disruptions, outdated machinery, and stagnating export facilities. These issues have all contributed to the widespread sickness in the jute industry. Varietal performance is the best tool to combat this situation by producing higher yield and good quality fibres of jute (Karki et al., 2021). The present experiment was undertaken to assess the performance of different *olitorius* jute varieties for their adaptability depending on crop growth and yield in coastal soils of West Bengal.

MATERIAL AND METHODS

Experimental site: Field trial was conducted at The Neotia University, Diamond Harbour, South 24 Parganas, West Bengal to evaluate the performance of few *olitorius* jute varieties during the *pre-kharif* season of 2023. The experimental field is situated at 22°26' N latitude and 88°19' E longitude with an average altitude of 8 m above MSL. The soil type of experimental field was clayey in nature, having 213.6 kg/ha available N, 22.08 kg/ha available P_2O_5, 312.26 kg/ha available K_2O and 0.46 % organic carbon. The experimental period was hot and received moderate rainfall partly effective for the crop grown (Table 1).

Field layout and treatment details: The experiment was carried out in a randomized complete block design comprising seven *olitorius* jute varieties (treatments) with three replications (Table 2). The size of each plot was 4 m × 3 m and gross cropped area was 336 m^2. Irrigation channels of 1 m width were provided along the length of the experimental field whereas the bund width of each plot was 0.5 m. Jute seeds were collected from ICAR-Central Research Institute for Jute and Allied Fibres, Nilganj, Barrackpore, West Bengal.

Crop management: Jute seeds were sown on 3rd week of March, 2023. All the standard agronomic practices were followed for raising the crop. Recommended dose of N-P_2O_5-K_2O @ 60-30-30 kg/ha were applied for the crop with seed rate of 5 kg/ha. Crop was harvested on 4th week of July, 2023 and thereafter, it was continuously kept below 10 cm of water depth for retting by making bundles after 3 days of harvesting. Considering the complete retting (after 14 days of complete submergence) of jute fibre it was then separated from the sticks and kept under the sun for drying and finally stored by making bundles.

Data recorded: Agronomic observations were taken by selecting ten plants randomly from each plot. Observations on crop growth parameters *viz*. plant population, plant height, leaf/plant, basal girth, aerial dry biomass, LAI, NAR, RGR were taken on regular interval and yield components *viz*. fibre yield, stick yield, biological yield and harvest index were recorded finally at harvest. Occurrence and severity of different pests were also monitored to understand the resistance of the tested varieties against a particular pest.

Statistical analysis: Statistical data analysis was carried out using OPSTAT software and Fisher's least significant test was used to compare the mean values at a probability level of 0.05.

RESULTS AND DISCUSSION

Crop growth parameters: Various crop growth parameters *viz.* plant population, plant height, leaf/plant, basal girth and aerial dry biomass of *olitorius* jute were significantly varied with different cultivars during the period of experimentation (Table 2). The highest plant population was observed under variety JBO 1 (49.7) while the lowest (44.7) in JRO 2407. Survival of more plants per unit area might be attributed to higher adaptability of JBO 1 variety in local environment as compared to all other jute varieties. Greater plant height (388.6 cm) and leaf/plant (54.26) were in JBO 1 which was closely followed by CO 58, NJ 7005, JROM 1 and JRO 524. Variation in plant height might have been seen due to genetic makeup of all the cultivars and their suitable adaptation in these soils of coastal Bengal. More thicker plants (7.21 cm) with maximum aerial dry biomass (2732.20 g/m^2) were in JBO 1 which was statistically at par with JRO 524 and CO 58. This increment in aerial dry biomass by JBO 1 might be attributed to a greater number of taller plants and more leaf/plant as achieved by the variety. Similarly, Karim *et al.,* (2020) reported that total aerial dry biomass of jute differed significantly among the cultivars depending upon the height

Table 1. Meteorological data pertaining to the year of experimentation

Months	Temperature (^{0}C)		Rainfall (mm)	Relative humidity (%)		Bright sunshine (hrs)
	Maximum	Minimum		Maximum	Minimum	
March	32.4	20.5	36.2	95.7	64.5	7.1
April	35.9	24.4	12.2	95.7	66.4	6.3
May	38.8	22.5	21.4	93.7	57.0	6.2
June	35.7	25.4	41.5	94.8	57.1	6.0
July	33.9	27.5	61.5	83.9	61.5	5.9

Source: Department of Soil Science & Agricultural Chemistry, SAAS, TNU

Table 2. Performance of different *olitorius* jute varieties based on morpho-physiological characters

Variety		Plant population	Plant height (cm)	Leaf/plant	Basal girth (cm)	Aerial dry biomass (g/m^2)
T_1	JRO 524	47.7	350.9	53.73	6.57	2595.40
T_2	JRO 2407	44.7	322.8	45.51	5.76	2192.86
T_3	JRO 204	45.7	350.0	47.26	5.90	2393.96
T_4	CO 58	48.3	374.8	52.73	6.75	2591.70
T_5	JBO 1	49.7	388.6	54.26	7.21	2732.20
T_6	JROM 1	46.7	352.0	47.33	6.20	2461.96
T_7	NJ 7005	46.3	372.6	51.03	6.41	2522.73
CD (p=0.05)		3.43	38.51	5.52	0.76	184.25

of plants. In contrast, JRO 2407 recorded the shortest plant (322.8 cm), lowest number of leaf/plant (45.51), basal girth (5.76 cm) and aerial dry biomass (2192.86 g/m^2) among the tested jute varieties during the study period.

The leaf area index (LAI) was significantly influenced while there was no significant influence on net assimilation rate (NAR) and relative growth rate (RGR) of all the tested *olitorius* jute varieties (Table 3). The increasing LAI and NAR was observed with the progress of crop growth while RGR recorded the opposite trends with time advancement. The highest LAI of 6.91, 7.28 and 7.77 was in JBO 1 during 31 - 60 DAS, 61 - 90 DAS and 91 - harvest, respectively. NAR followed a similar trend during 31 to 60 DAS and 61 to 90 DAS. The highest value of NAR (1.0504 g/dm^2/day) was in JBO 1 which was closely followed by JRO 204 and CO 58 at harvest. This increased NAR values of variety JBO 1 might be attributed to its positive correlation with LAI as the optimum leaf area, in general, should give highest rate of net assimilation in any crop. Initially all the tested jute varieties recorded higher values of RGR thereafter resulted in decreased value with time progress. Among all the jute varieties the highest RGR (0.0079 g/g/day) was observed in JBO 1 which was followed by the variety CO 58, JROM 1, NJ 7005 and JRO 204 from 91 DAS to harvest. In contrast, variety JRO 2407 recorded the lowest LAI, NAR and RGR.

Yield performance: Different *olitorius* jute varieties revealed significant variations in terms of dry fibre yield, stick yield, biological yield and harvest index under coastal soil conditions (Table 4). The highest fibre yield (28.2 q/ha) was in JBO 1 which was significantly differed with all other varieties while JRO 2407 recorded the least yield (21.2 q/ha). JBO 1 recorded an increase in fibre yield to the tune of 11.46% to 33.02% in respect of all other varieties. This increased fibre yield is directly linked with taller and thicker plants with higher dry matter partitioning that obtained in JBO 1. Plant height is the most efficient morphological trait which is directly related to increased fiber yield of jute (Hassan et al., 2018). It was also reported that maximum plant height returned the highest fibre yield of jute in Bangladesh (Karim et al., 2020, Rafiq et al., 2020). The highest stick and biological yield (78.3 q/ha and 108.2 q/ha respectively) were in JBO 1 followed by CO 58, JRO 524, NJ 7005 and JROM 1. Taller and thicker plants

Table 3. Leaf area index, net assimilation rate and relative growth rate of different *olitorius* jute verities in coastal soils of West Bengal

Variety	Leaf area index			Net assimilation rate (g/dm^2/day)			Relative growth rate (g/g/day)		
	31 - 60 DAS	61 - 90 DAS	91 DAS - Harvest	31 - 60 DAS	61 - 90 DAS	91 DAS - Harvest	31 - 60 DAS	61 - 90 DAS	91 DAS - Harvest
T1	6.57	7.20	7.39	0.7502	0.7764	1.0013	0.0143	0.0097	0.0075
T2	6.07	6.30	6.41	0.6788	0. 7390	0.9681	0.0148	0.0091	0.0075
T3	6.32	6.55	7.06	0.6914	0. 7619	1.0424	0.0151	0.0094	0.0076
T4	6.60	6.94	7.44	0.7487	0.7509	1.0323	0.0143	0.0097	0.0077
T5	6.91	7.28	7.77	0. 7297	0.7817	1.0504	0.0146	0.0093	0.0079
T6	6.31	6.87	7.19	0.7223	0.7263	0.9992	0.0139	0.0095	0.0077
T7	6.59	7.01	7.23	0.7280	0.7473	1.0027	0.0140	0.0095	0.0076
CD (p=0.05)	0.537	0.567	0.515	NS	NS	NS	NS	NS	NS

See Table 2 for treatment details

Table 4. Yield performance of different *olitorius* jute varieties in coastal soils of West Bengal

Variety	Fibre yield (q/ha)	Stick yield (q/ha)	Biological yield (q/ha)	Harvest index (%)
T1	24.7	71.9	96.9	25.48
T2	21.2	61.8	85.3	24.84
T3	22.8	66.2	89.6	25.41
T4	25.3	73.8	99.2	25.72
T5	28.2	78.3	108.2	26.04
T6	23.5	68.3	92.8	25.30
T7	24.4	70.9	95.9	25.45
CD (p=0.05)	2.32	6.07	9.01	0.26

See Table 2 for treatment details

resulted from the varieties with higher dry matter partitioning increases stick as well as biological yield of jute (Maji et al., 2024). Similar findings were also observed by Khan and Tareq (2018), Karim et al. (2020) and Miah et al. (2020). In contrast, JRO 2407 resulted in the least stick and biological yield. The highest harvest index (26.04) was observed in JBO 1 because of its increased economic yield in terms of dry fibre production while the lowest (24.48) was recorded in JRO 2407.

Pest infestation and its severity: Significant infestation of anthracnose and stem rot disease in terms of percent plant infestation and severity had been observed in all the jute varieties (Table 5). Maximum percent plant and leaf infestation of anthracnose was in NJ 7005 (23.07 and 11.22, respectively) which was statistically at par with JROM 1, JRO 204 and CO 58. JBO 1 showed the least percent plant and leaf infestation (13.43 and 6.77). Disease severity of anthracnose was maximum in JROM 1 (1.37) while JBO 1 recorded the minimum severity (0.70). The maximum percent plant infestation (34.70) and disease severity (4.20) of stem rot was noted in NJ 7005 followed by JROM 1, JRO 204 and JBO 1 while variety JRO 2407 recorded the minimum percent plant infestation and severity. JRO 2407 variety showed less infestation from anthracnose and stem rot disease as it is less susceptible to both the diseases while NJ 7005 showed highest susceptibility during the study period.

Infestation of insect pests like yellow mite and Bihar hairy caterpillar varied significantly in terms of percent plant and leaf infestation (Table 6). The maximum percent plant and leaf infestation of yellow mite was in JRO 204 (46.59 and 15.96 respectively) which was followed by CO 58 and JRO 2407. Variety JRO 524 attained minimum percentage of plant infestation (25.56) while JBO 1 recorded the least percent infestation in leaf (8.56). The maximum percent plant and leaf infestation of Bihar hairy caterpillar was observed in NJ 7005 (32.41 and 16.04 respectively). JRO 524 recorded the minimum percent plant and leaf infestation because of its least susceptibility to yellow mite and Bihar hairy caterpillar.

Production economics

The cost of production for all the jute varieties under cultivation was equal *i.e.* Rs. 55997 per ha as because similar types of management practices and inputs were applied to all the treatments (Table 7). Overall, JBO 1 recorded highest

Table 5. Disease infestation in different *olitorius* jute varieties in coastal soils of West Bengal

Treatment	Stem rot (percent)		Anthracnose		
	Plant infestation (%)	Severity	Plant infestation (%)	Leaf infestation (%)	Severity
T_1	18.09	1.20	13.99	7.43	0.73
T_2	15.90	1.16	14.31	7.35	0.73
T_3	25.62	2.80	19.15	9.94	1.03
T_4	19.30	1.20	16.55	10.07	1.03
T_5	21.61	1.73	13.43	6.77	0.70
T_6	25.87	3.06	20.03	11.16	1.37
T_7	34.70	4.20	23.07	11.22	1.33
CD (p=0.05)	12.803	2.278	6.046	3.466	0.324

See Table 2 for treatment details

Table 6. Insect infestation in different *olitorius* jute varieties in coastal soils of West Bengal

Treatment	Yellow mite (Per cent)		Bihar hairy caterpillar (Per cent)	
	Plant infestation	Leaf infestation	Plant infestation	Leaf infestation
T_1	25.56	9.37	18.94	11.24
T_2	37.58	12.64	25.61	12.64
T_3	46.59	15.96	29.01	15.98
T_4	42.05	14.31	26.79	15.22
T_5	30.96	8.56	19.50	12.17
T_6	29.98	10.44	28.61	14.22
T_7	31.72	9.56	32.41	16.04
CD (p=0.05)	9.147	3.828	5.583	4.499

See Table 2 for treatment details

Table 7. Production economics of *olitorius* jute as influenced by different cultivars

Variety	Cost of production (Rs./ha)	Gross return (Rs./ha)	Net return (Rs./ha)	B:C ratio
T1	55997	161662	105665	1.88
T2	55997	138814	82817	1.48
T3	55997	149044	93047	1.67
T4	55997	165882	109885	1.97
T5	55997	182196	126199	2.25
T6	55997	153654	97657	1.74
T7	55997	159714	103717	1.85

See Table 2 for treatment details

gross return (Rs. 182196 per ha), net return (Rs. 126199 per ha) and B:C ratio (2.25) followed by CO 58, JRO 524, NJ 7005, JROM-1 and JRO 204 while JRO 2407 recorded the lowest value of each parameter. Much variation in gross return, net return and B:C ratio was seen due to significant variation in fibre and stick yield of different jute varieties during the study period.

CONCLUSIONS

JBO 1 recorded a greater number of taller and thicker plants with maximum aerial dry biomass among all the tested jute varieties. Highest fibre, stick yield and harvest index was also observed in this variety along with maximum monetary return and B:C ratio. Therefore, it can be concluded that the variety JBO 1 could be more successful, efficient and productive compared to the other tested *olitorius* jute varieties under coastal soils of West Bengal.

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Study on Effect of Replacing Urea Top Dressing by Nano Urea on Yield and Quality of Direct Seeded Rice (*Oryza sativa* L.) under Rainfed Condition

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Abstract: Field experiment on effect of replacing urea top dressing by nano urea on yield and quality of direct seeded rice (*Oryza sativa* L.) under rainfed condition was conducted on clay soils during *kharif* season, 2023 at Central Agricultural University, Iroisemba, Imphal to evaluate the possibility of replacing urea top dressing by nano urea and its efficacy. Initial soil of the experimental field was acidic (pH 5.25). Replacing urea top dressing by nano urea had significant effect on the yield attributing characters. Highest yield attributes were observed under top dress (urea) at active tillering + nano urea (foliar spray) at panicle initiation + nano urea (foliar spray) at heading (T_6). The nutrient uptake (NPK), in T_6 was the highest. On the basis of above findings, it can be inferred that foliar application of nano urea twice at panicle initiation and heading stages can effectively replace urea top dressing at panicle initiation stage of crop growth.

Keywords: Rice, Nitrogen, Urea, Nano urea, Foliar spray

Rice (*Oryza sativa* L.) is the main staple food for over half the population of the world producing highest next to wheat with a record of 520.5 mt produced in 2022 (Anonymous, 2022). In India rice is grown in an area of 43.79 million ha^{-1} producing 112.91 million tonnes with an average productivity of 2578 kg ha^{-1} (Singh et al., 2020). Direct seeded rice is a resource conservation technology and reduces water and labor use by 50%. Productivity of DSR is 5-10% more than the yield of transplanted rice. It is considered as prosperous provided that there is good crop establishment and adequate weed control methods by maintaining the crop free from weeds (Rao et al., 2007, Rao and Nagamani 2007). Methane gas emission is also lower in DSR than with conventionally tilled transplanted puddle rice giving better option in the era of climate change(Marasini et al., 2016).

Nitrogen continues to be the "kingpin" of the nutrient kingdom and its management is a critical issue to be addressed across the globe including in India. In recent years, the input of chemical fertilizer is rising rapidly and N and P have been overused in rice production, leading to not only environmental pollution but also an increase in production cost (Wang et al., 2020). Nevertheless, soil N supply is often limited (Kumar et al., 2017), which forces farmers to increase the amount of N fertilizers in order to accomplish better crop yield. However, farmers may provoke nitrogen over fertilization, which effect optimum plant productivity as plants are not able to absorb the excess of N-fertilizer(Shrestha et al., 2020). Since a major portion of added N got lost through leaching, volatilization, and denitrification, the N use efficiency of crops hardly exceeds 30-35% (Ladha et al., 2005). The primary target of nano fertilizers in field of agronomy is to increase the plant nutrient use efficiency and diminish losses of nutrients (Ingale et al., 2013) and slowly and steadily release nutrients to the crop as needed to boost crop output, enhance crop quality, and raise the overall sustainability of agricultural systems, (Tarafdar et al., 2014). Nano fertilizers also contribute to the health of the soil by increasing soil organic carbon, enhancing soil aggregation, and enhancing soil's capacity to store water, (Rai et al., 2012). Nano fertilizers are ecologically sound and were created with the same nutrient content and application rates as inorganic fertilizers (Liu and Lal 2015). Foliar application of nutrients can improve nutrient utilization and minimizes environment pollution by reducing the amount of fertilizers added to soil (Miah *et al*., 2017). It would be exceptionally useful if we use nano fertilizer in rice crops to limit the potential negative impacts realized by the broad utilization of synthetic fertilizers without bargaining production and nourishing advantages (Benzon et al., 2015). With this background in view the present research was conducted to study the effect of replacing urea top dressing by nano urea on yield and quality of direct seeded rice (*Oryza sativa* L.).

MATERIAL AND METHODS

Experiment site: The present research was conducted at the Research field of College of Agriculture, CAU, Imphal during *kharif* 2023 located in 24º45' N latitude and longitude of 93º54' E with an altitude of 774.5 m above mean sea level. The experimental area comes under the Eastern Himalayan

Region (II) and the agro climatic zone is Sub Tropical Zone (NEH-4) of Manipur (Experimental Agromet Advisory Service ICAR Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal).

Soil status: The soil of the experimental field was clayey in texture with pH of 5.25, containing organic carbon 1.43 %, available N 275.34 kg ha^{-1}, P_2O_5 16.89 kg ha^{-1} and K_2O 276.32 kg ha^{-1} (Table 1).

Treatment details: The study was replicated three times in randomized block design with seven treatments viz., T_1: Top dress with urea at active tillering + top dress with urea at panicle initiation, T_2: Foliar spray with nano urea at active tillering + foliar spray with nano urea at panicle initiation, T_3: Top dress with urea at active tillering + foliar spray with nano urea at panicle initiation, T_4: Foliar spray with nano urea at active tillering + top dress with urea at panicle initiation, T_5: Foliar spray with nano urea at active tillering + foliar spray with nano urea at panicle initiation + foliar spray with nano urea at heading, T_6: Top dress with urea at active tillering + foliar spray with nano urea at panicle initiation + foliar spray with nano urea at heading, T_7: Foliar spray with nano urea at active tillering + top dress with urea at panicle initiation + foliar spray with nano urea at heading. All treatments are given equal basal dose of NPK (20:40:30 kg ha^{-1}), urea top dressing is given in two equal split doses at active tillering stage (35 DAS) and/or panicle initiation (50 DAS) @ 20kg N ha^{-1} each as per treatment and foliar spray of nano urea is given at the concentration of 0.4% as per treatment.

Yield attributes and parameters analysis

i. Number of effective tillers hill^{-1} was determined by counting panicle bearing tillers taken as effective tillers from each plot. The tillers of the representative samples were recorded in number and the values averaged for each treatment.
ii. Number of filled grains per panicle was determined after harvest of the crop by counting the number of filled grains from every ten panicles from five selected sample plants and recording the mean values as total filled grains for each treatment.
iii. Number of unfilled spikelets per panicle was determined after the harvest of the crop by counting the number of unfilled spikelets from every ten panicles from five selected sample plants and mean values were recorded as total filled grains from each treatment.
iv. Spikelet sterility percentage was calculated as a ratio between difference of total spikelets to filled grains and total spikelets multiplied by 100 to get the value in percentage.

$$\text{Harvest index (\%)} = \frac{\text{Economical Yield (q/ha)}}{\text{Biological Yield (q/ha)}} \times 100$$

v. Test weight was determined by taking the weight of 1000 randomly selected filled grains in gram after proper sun dry and recording for each plot.
vi. Grain yield (q/ha) was determined by weighing the cleaned seeds threshed dried at 14% moisture content after leaving the border rows in each plot. Grain yield for each net plot was recorded in kilogram and converted to quintal per hectare for statistical analysis.
vii. Straw yield (q/ha) in kilogram was recorded after proper sun drying of the straw separately for each plot, and converted to quintal per hectare.
viii. Harvest index (%) was calculated as a ratio between economic (grain) yield and biological yield (grain + straw) of the harvested plants which was introduced by Donald (1962).

$$\text{Spikelet sterility (\%)} = \frac{\text{Total number of spikelets- Number of filled grains}}{\text{Total number of spikelets}} \times 100$$

Table 1. Chemical properties of the experimental soil (initial)

Parameter	Value	Interpretation
pH	5.25	Acidic
Organic carbon (%)	1.43	High
Sand (%)	9.65	Clay soil
Silt (%)	15.68	
Clay (%)	74.67	
Available nitrogen (kg ha^{-1})	275.34	Low
Available phosphorus (P_2O_5) (kg ha^{-1})	16.89	Low
Available potassium (K_2O) (kg ha^{-1})	276.32	Medium

Quality parameters analysis: Plant samples were collected for nutrient content (NPK) at the harvest stage. Crop samples at harvest were used for nutrient analysis. These samples were dried and ground to a fine powder using a Willey mill and used for the analysis of the uptake of nutrients by crop.

I. Nitrogen content (%) in the grain and stover were assessed by the micro Kjeldhal method (Jackson, 1967) using a Kelplus N analyzer after digesting the samples with H_2SO_4 and H_2O_2.
ii. To analyse phosphorus content (%), one gram of plant sample and 10 ml of di-acid mixture was digested over a sand bath till a clear colourless solution was obtained. Then a known volume of the digested samples was taken for total phosphorus content determination by adopting the Vanado molybdophosphoric yellow color method as

described by Jackson (1973).

iii. For analysis of potassium content (%) one gram of plant sample was digested with di-acid to determine the amount of potassium in plant samples. Following this, 10 ml of the digested material was taken in a volumetric flask and make up volume up to 50 ml od distilled water. The total potassium content was estimated by atomizing the diluted digest to a calibrated flame photometer under suitable measuring conditions as described by Jackson (1973).

iv. For calculation of nutrient uptake (kg/ha) in grain and stover, the respective percent content of nitrogen in grain and stover were analyzed separately and the uptake of nutrient by rice was worked out for different treatments by multiplying the nutrient content and dry matter yield of the rice as given in the following formula.

Nutrient uptake (kg ha^{-1}) = Nutrient concentration (%) X Dry matter Yield (kg/ha)

RESULTS AND DISCUSSION

Effects of top dressing of nano urea on the yield attributes and parameters: The replacing urea top dressing by nano urea at different stages of crop growth had a significant effect on the yield attributes and parameters (Table 2). However, there was no significant effect of the treatments on test weight (g) and harvest index (%). T_6 produced highest number of effective tillers $hill^{-1}$ (16.83), no. of filled grains $panicle^{-1}$ (168.78), grain yield (46.80 q ha^{-1}) and stover yield (66.88 q ha^{-1}). Consequently, T_6 recorded the minimum number of unfilled spikelets $panicle^{-1}$ (17.11) and spikelet sterility (9.22 %) over other treatments. This might be due to combined application of conventional fertilizer as basal dose and split dosage application of Nano urea at panicle initiation and heading stage of rice had synergistic effects which leads to slow release of nitrogen resulting in higher nitrogen use efficiency, minimized loss and storage of remaining nitrogen in plant cells that can prevent the plant biotic and abiotic stress produces number of effective tillers per hill. Foliar application of nano urea resulted in more photosynthates accumulation and translocation of nitrogen and abundant availability and timely supply of nitrogen stimulated initiation and development of grain formation resulting in more number of filled grains per panicle, lesser number of unfilled spikelet per panicle and consequently lower spikelet sterility (%) in rice. Synergetic effect of conventional fertilizers as basal dose and split dose application of foliar nano urea improving nitrogen uptake by the plant through slow release mechanism that can prevent the plant from biotic and abiotic stress and increasing grain and stover yield. Similar findings were reported in earlier studies(Benzon et al., 2015, Yadav et al., 2021, Attri et al., 2022), Kothari et al. (2023).

Effects of top dressing of nano urea on quality parameters: P content in stover (%) and K content in grain (%), K content in stover (%) were not significantly influenced by replacing urea top dressing by nano urea in direct seeded rice under rainfed condition (Table 3). However, N content in grain (%), N content in stover (%) was significantly influenced by replacing urea top dressing by nano urea in direct seeded rice under rainfed condition . The T_6 recorded the maximum N content (1.49% in grain; 0.97% in stover) and T_2 recorded minimum N content (1.41% in grain; 0.91% in stover). Nutrient uptake (kg ha^{-1}) by grain and stover was significantly influenced by replacing urea top dressing by nano urea in rice (Table 2). Maximum N uptake by grain (kg ha^{-1}) and stover was recorded under T_6 (59.71 kg ha^{-1} for grain; 35.71 kg ha^{-1} for stover). This was followed by T_7 , , T_3 , , T_4, and T_5 .Minimum nitrogen uptake was observed under T_2 (41.59 kg ha^{-1} for

Table 2. Yield attributes and parameters as influenced by replacing urea top dressing by nano urea in direct seeded rice (*Oryza sativa* L.) under rainfed condition

Treatments	No. of effective tillers per panicle	No. of filled grains per panicle	No. of unfilled spikelets per panicle	Spikelet sterility (%)	Test weight (g)	Grain yield (q/ha)	Stover yield (q/ha)	Harvest index (%)
T_1	14.99	150.47	20.05	11.77	27.13	44.19	64.14	0.79
T_2	10.37	106.36	26.39	19.89	26.27	38.69	58.32	39.88
T_3	13.48	135.96	22.38	14.13	26.03	42.46	62.75	40.36
T_4	11.94	121.14	24.04	16.86	25.33	40.72	61.30	39.92
T_5	11.87	120.68	24.47	16.58	28.18	40.18	60.44	39.93
T_6	16.83	168.78	17.11	9.22	28.33	46.80	66.88	41.17
T_7	15.18	153.72	19.62	11.32	29.00	44.84	64.90	40.86
CD (p=0.05)	1.40	14.07	1.49	0.96	NS	1.51	1.21	NS

Table 3. Quality parameters (both grain and stover) as influenced by replacing urea top dressing by nano urea in direct seeded rice (*Oryza sativa* L.) under rainfed condition

Treatments	N Content (%)		P Content (%)		K Content (%)		N Uptake (kg ha^{-1})		P Uptake (kg ha^{-1})		K Uptake (kg ha^{-1})	
	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover
T_1	1.45	0.94	0.26	0.21	1.05	2.06	54.37	32.95	9.65	7.25	39.46	72.10
T_2	1.41	0.91	0.24	0.19	1.03	2.04	41.59	27.45	7.06	5.63	30.21	61.43
T_3	1.44	0.93	0.25	0.20	1.04	2.05	49.77	31.07	8.54	6.59	36.03	68.72
T_4	1.43	0.92	0.24	0.18	1.04	2.06	46.61	29.05	8.21	5.98	33.56	65.05
T_5	1.42	0.91	0.25	0.19	1.02	2.03	45.75	29.05	7.55	5.66	33.09	64.82
T_6	1.49	0.97	0.28	0.22	1.06	2.09	59.71	35.71	11.06	8.10	42.38	76.95
T_7	1.47	0.94	0.27	0.20	1.06	2.07	56.20	33.66	10.45	7.25	40.65	73.73
CD (p=0.05)	0.06	0.02	NS	NS	NS	NS	0.56	0.87	1.10	0.87	1.24	2.78

grain; 27.45 kg ha^{-1} for stover). Maximum P uptake by grain (kg ha^{-1}) and stover was in T_6 (11.06 kg ha^{-1} for grain; 8.10 kg ha^{-1} for stover) followed by T_7, T_1, T_3, T_4 and T_5 Minimum P uptake was observed under T_2 (7.06 kg ha^{-1} for grain; 5.63 kg ha^{-1} for stover. Maximum K uptake by grain (kg ha^{-1}) and stover was in T_6 (42.38 kg ha^{-1} for grain; 76.95 kg ha^{-1} for stover) followed by T_7, T_1, T_3, T_4, T_5. Minimum nitrogen uptake was observed under T_2 (30.21 kg ha^{-1} for grain; 61.43 kg ha^{-1} for stover). The positive influence of foliar nano urea application in panicle initiation and heading stages in rice might be due to improved nitrogen availability and absorption in both the root zone and plant system. The increased availability and accumulation of nutrients again leads to more metabolic activity at cellular level which in turn increased nutrient uptake resulting in greater translocation of nutrients to reproductive organs of the crop and ultimately increased the nitrogen contents in both grain and stover. Moreover, the nano particles have more specific surface area and number of particles per unit area of a fertilizer and can easily penetrate into the plants from applied surface and improve uptake and nutrient use efficiency of rice. The findings were in close agreement with the findings of Bora and Pandey (2018), Burhan and AL-Hassan (2019) and Mehta and Bharat (2019), Yadav et al. (2021), Sahu et al. (2022) and Sharma et al. (2022).

CONCLUSION

The treatment Foliar spray with nano urea at active tillering + top dress with urea at panicle initiation + foliar spray with nano urea at heading was the best treatment on replacing urea top dressing by nano urea in direct seeded rice (*Oryza sativa* L.) under rainfed condition with highest yield attributes and quality parameters of both grain and stover. Therefore, foliar application of nano urea twice at the panicle initiation and heading stages of rice growth can effectively replace urea top dressing at panicle initiation stage.

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Modulation of Antioxidant System by Glutathione in Maize Seedlings under Salt Stress Conditions

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Abstract: Effect of glutathione on seed vigour parameters and antioxidant system at different salt stress levels was observed in maize . Seeds of maize (*Zea mays* L.) cv J 1006 were soaked for 4hrs in water (hydration), glutathione 100 and 500 ppm solutions and surface dried. The treated seeds were subjected to salt stress levels of 50 , 75 and 100 mM NaCl. Vigour and biochemical parameters were recorded from 10 days old seedlings. Seed treatments increased percent germination, seedling length and biomasss, proline content and activities of antioxidant enzymes *viz.* catalase, peroxidase and superoxide dismutase of seedlings whereas decreased the malondialdehyde content over untreated seeds. The glutathione 500 ppm was more effective in alleviating salt stress compared to 100 ppm. The findings conclude that glutathione 500 ppm will be useful in ameliorating the adverse effects of salt stress by using seed treatments of glutathione for growing maize in salt affected areas.

Keywords: Catalase, Glutathione, Maize, Peroxidase, salt stress, Superoxide dismutase

Maize (*Zea mays* L.) is third important cereal crop after wheat and rice. Besides being important as staple food crop in developing countries and is in demand due to use for animal feed and ethanol production. Being a moderately salt sensitive crop, it shows visible signs of salt stress including reduced root and shoot length. Restricted water absorption is the main reason for decreased seedling growth. Salinity is one of the major abiotic stresses that hinders the development of plant resulting in a variable yield loss. It is mainly because of osmotic stress, imbalance of ions, oxidative damage, negative impact of sodium (Na+) and chloride (Cl^-) ions accumulation (Hussain et al., 2018). Inhibition of germination index, seedling growth by salt stress is reported in sorghum (Chen et al., 2021). Salt stress is the major factor affecting the seedling growth and yield of the crop plants (Billah et al., 2017). Reduction in yield by salt stress is caused due to adverse effects on the physiology of the plants (Hemalatha et al., 2017). Besides salt stress reduces the early seedling growth of crop plants (Chuchra and Gupta 2020). The plant responds to these stresses by activating its defense system. There is a boost in the activity of antioxidant enzyme like catalase, peroxidase and superoxidedismutase (Bhattarai et al., 2020). Under salt stress, the membrane lipids are damaged due to lipid peroxidation by reactive oxygen species (Yu et al., 2020).

For adapting seeds to adverse environments, seed treatment is considered as an important technique as it helps in enhancing salt stress tolerance. Seed treatment is done by pretreating seeds with stimulants which increase the resistance to negative effect (Tanou et al., 2012). Amongst the non- protein thiol in plants, glutathione (GSH) is the is most prevalent and widely distributed in cellular components (Frrat et al., 2003) . Reduced glutathione detoxifies reactive oxygen species and controls the GSH dependent ROS detoxifying enzymes activity (Nahar et al., 2015). It regulates both enzymatic and non-enzymatic antioxidants and osmporotectants. Glutathione treatments improve SOD activity, thus stimulates the conversion of superoxide radical to H_2O_2, an important step in imparting protection to the cellular contents. Treatment with GSH recorded increased biomass of plants and yield (Pei et al., 2019). Taking into consideration the above findings, the present investigation was planned to mitigate the adverse effects of salinity in maize by seed treatments with glutathione.

MATERIAL AND METHODS

Plant material: The present investigation was done at, Punjab Agricultural University, Ludhiana. Seeds of maize (*Zea mays* L.) cv were used to study the effect of seed treatments on early seedling growth and activity of antioxidant enzymes under salt stress conditions. The seeds were sterilized with 0.1 % mercuric chloride and washed with distilled water to prevent any possibility of seed borne infection. The seeds were later soaked for 4 hrs in different seed treatment solutions and were exposed to salt stress. For salt stress stimulation, the germination paper in Petri plates(150mm) was moistened with solutions of different concentrations of NaCl (50, 75 and 100mM). The Petri plates were kept at 25°C and relative humidity (RH) 80 ± 5 % in the seed germinator. Each treatment was replicated thrice and 20 seeds were used per replication.

Percent germination (%): Final count of normal seedlings was recorded after 10 days and was expressed as germination percentage.

Seedling length (cm), seedling fresh and dry weight (mg): Average of five normal seedlings were taken. Root and shoot length was expressed in centimeter and fresh and dry weight in milligrams. For dry weight seedlings taken for fresh weight were oven dried at 65˚C for 24 hours.

Measurement of antioxidant enzymes (CAT, POX, SOD) (ΔA/min/g FW): The known amount of seedlings was homogenized in 50 mM Na-phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone. The homogenate was filtered and then centrifuged at 4^0C for 20 min at 10,000g. Catalase activity was measured by following the decomposition of H_2O_2 at 240 in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 as described by Chance and Maehly (1955). Peroxidase activity was assayed by following the increase in absorbance at 470 nm due to guaiacol oxidation for 3 min (Chance and Maehly 1955). The assay mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol. Super oxide dismutase (SOD) was assayed (Marklund and Marklund 1974)

Measurement of malondialdehyde and proline contents: For MDA extraction, shoot samples were homogenized in 10 ml of 0.1% trichloroacetic acid (TCA) followed by centrifugation at 10000g and was estimated by method of Heath and Packer (1968). For proline extraction, 0.1 gm of seedlings were homogenised in sulphosalicylic acid (3%) followed by centrifugation at 3000 g and supernatant was used for proline estimation by the method of Bates et al. (1973)

Statistical analysis: Analysis of variance was carried out with software SAS 9.3 .

RESULTS AND DISCUSSION

Percent germination: Germination percentage in maize reduced as the salt concentration increased from 50 to 100 mM (Table 1). Increase in salt stress caused a reduction in germination percentage. Salt stress decreases the water absorption by decreasing the osmotic potential exterior to the seed thus cell expansion (Migahid et al., 2019). The findings are in agreement with the earlier study on sorghum genotypes, wheat and tomato where a decrease in percent germination was reported with increasing NaCl stress (Chuchra et al., 2020, Kaur et al., 2023, Singh et al., 2025a). In maize at all salt stress levels, GSH 100 and 500 ppm enhanced the germination percentage over control under salt stress conditions. GSH 500 ppm increased the germination

Table 1. Effect of seed treatments on percent germination, root and shoot length, fresh weight and dry weight in seedlings subjected to salt stress in maize

Salt stress (mM NaCl)	Treatments	Percent germination	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
0	Control	90.0^c	10.24^c	6.42^c	401.23^c	42.67^b
	Hydropriming	91.67^c	10.37^c	6.40^c	415.34^b	45.67^b
	GSH 100 ppm	95.00^b	11.45^b	7.23^b	485.67^a	60.43^a
	GSH 500 ppm	98.33^a	12.85^a	9.10^a	483.66^a	62.02^a
50	Control	86.67^c	9.29^c	5.83^c	386.33^d	39.33^b
	Hydropriming	88.33^c	9.75^c	5.44^c	400.00^c	40.33^b
	GSH 100 ppm	93.33^b	10.94^{bc}	6.74^b	458.00^a	58.00^a
	GSH 500 ppm	95.00^a	12.10^a	8.56^a	419.33^b	61.01^a
75	Control	81.67^c	7.39^c	4.93^d	302.33^d	36.34^c
	Hydropriming	81.67^c	8.65^b	5.45^{cd}	341.33^c	33.00^c
	GSH 100 ppm	91.67^b	11.02^a	7.61b	378.67^b	46.67^b
	GSH 500 ppm	98.33^a	11.90^a	10.19^a	384.00^b	55.75^a
100	Control	61.33^c	6.73^c	3.41^c	138.33^c	30.67^c
	Hydropriming	55.00^b	6.66^c	4.57^b	172.67^b	33.68^c
	GSH 100 ppm	85.00^a	9.42^b	7.13^a	352.67^a	41.31^b
	GSH 500 ppm	85.67^a	11.24^a	7.22^a	350.33^a	49.14^a

GSH= Glutathione. Different small letters indicated that means are significantly different ($p \leq 0.05$).

percentage maximally at all salt stress levels. It enhanced the percentage of germination significantly from 86.67% control to 98.33% at 75 mM NaCl. However, hydration of seeds was either at par with the control at 50 and 75 mM or decreased the germination percent at 100mM.The effects of salt stress on maize seed germination were reduced by treating the seeds with glutathione which in turn increased the germination over untreated seeds.

GSH is a redox molecule that could help in seedling establishment by enhancing the reductive status of seed. This molecule helped in combating oxidative stress that might increase seedling growth by stimulation of cell repair mechanisms and activation of germination and antioxidant enzymes (Martínez-Ballesta et al., 2020). There was a marked decrease in seedling length and weight under salt stress. The seed treatments with GSH 100 and 500 ppm improved the root and shoot length of salt stressed seedlings. Under salt stress conditions GSH 500 ppm increased the root and shoot length maximally. It increased the root length significantly from 9.29 cm (control) to 11.24 cm (100 mM NaCl). Similarly increased the shoot length significantly from 5.83 cm (control) to 7.22 cm (100 mM NaCl). GSH 100 and 500 ppm improved the fresh and dry weight compared to the control under salt stress conditions. GSH 500 ppm increased the fresh weight maximally from 350.33 to 386.33 mg at 100 mM NaCl. GSH increased the dry weight from 39.33 mg (control) to 49.14 mg at 100 mM NaCl. For dry weight, amongst all treatments, GSH 500 ppm showed the most significant improvement, especially at 50 mM and 75 mM. Thus, the salt induced length inhibition could be reverted back by glutathione treatment. Roots and shoots act as an important marker of plant's response to salt stress as the roots tend to be in direct contact with soil. Salt stress induces negative effects in the germinating seeds, salinity stress impairs seedling growth and development (Liang et al., 2018). Similar results were reported in sorghum where increasing levels of salt stress reduced root and shoot growth (Dehnavi et al., 2020). In the present experiment, glutathione application significantly improved root and shoot length, thus considerably reversing the damaging effect of salt stress. Glutathione modulates the growth and development, cell proliferation and thus exogenous application of glutathione seems promising in enhancing plant's abiotic stress tolerance (Kumar et al., 2019). Salt stress severely affects the fresh weight and dry weight of plants. Fresh weight and dry weight decreased with the increase in salt stress. The reduction in biomass occurs because of the slowed down metabolic mechanisms and thus decrease in the growth of plants under increasing salt stress levels (Kamran et al., 2020). Similar inhibitory effects of salt stress have reported in maize (Pei et al., 2019) and oats (Iqbal et al., 2020). In the present study, GSH 100 and 500 ppm effectively increased seedling biomass of maize compared to the control. Ibrahim et al. (2017) reported similar findings in the salt sensitive cultivar of *Gossypium hirsutum*, which recorded improved biomass after treatment with glutathione under salt stress. External application of GSH, increased the endogenous GSH levels which could promote tolerance to many stresses in plants (Pei et al., 2019). Thus the damaging effect of salt stress may be reduced by decrease in the seedling growth.

Antioxidant enzyme activities: The increase in the activities of enzymatic antioxidants (CAT, POX, SOD) in maize seedlings under the effect of salt stress was observed (Tables 2). The activities were further enhanced significantly upon treatment with GSH 100 and 500 ppm. The activity of catalase increased from 50 to 100mM NaCl as GSH 500ppm maximally enhanced the catalase activity over the untreated control and GSH 100ppm at all the stress levels. Similarly peroxidase and superoxide dismutase were increased as the concentration of NaCl increased from 50 to 100mM NaCl when compared to the unstressed control. The treatment with GSH 100 and 500ppm significantly enhanced the activities of peroxidase and superoxide dismutase compared to the untreated control. The most injurious effect of salt stress in plants is the oxidative stress caused by the reactive oxygen species (ROS). In the present study, the levels of CAT, SOD and POX showed an increase with increasing salt stress. Similar results were observed in faba bean and wheat under salt stress where H_2O_2 increased which lead to an increase in the levels of CAT and SOD (Alzahrani et al., 2019, Singh et al., 2025b). This stress impairs the cellular membranes, damages proteins and organelles, especially of mitochondria, peroxisomes, chloroplast and affects cell's integrity (Mushtaq et al., 2020). In study, the activities of the antioxidant enzymes showed a significant increase with glutathione application compared to the control. GSH 500 ppm showed the maximum increase at all stress levels. Similar results were also reported in sunflower where seed treatment with GSH resulted in considerable improvement in SOD, CAT and POX enzyme activity under salt stress (Asmaa et al., 2020).

Malondialdehyde and proline content: Malondialdehyde content was upregulated with the increasing levels of salt stress (Table 3). Both the concentrations of glutathione decreased the MDA content as compared to the control under salt stress. GSH 500ppm was more effective. Increase in MDA levels with the increase in salt stress and is because the most harmful effect of salt stress is the destruction of lipids by chain reactions and generation of lipid radicals which then damage many other biomolecules (Mushtaq et

Table 2. Influence of seed treatments on Peroxidase (A/min/g FW) and Superoxide dismutase (A/min/g FW) in maize (*Zea mays* L.) seedlings under salt stress conditions

Salt stress level/Treatment	0 mM NaCl (No stress)	50 mM NaCl	75 mM NaCl	100 mM NaCl
Catalase (ΔA/min/g FW)				
Control	32.17±0.67	40.22±0.41	58.60±1.63	69.87±0.50
Hydration	38.34±0.42	43.82±1.91	65.03±0.66	76.31±1.57
GSH 100 ppm	48.76±1.06	60.93±1.75	84.42±1.13	84.96±1.46
GSH 500 ppm	66.79±0.23	77.10±0.50	96.42±0.67	107.66±1.92
CD (p=0.05)	2.24	2.34	2.70	2.71
Peroxidase (ΔA/min/g FW)				
Control	0.01±0.00	0.02±0.00	0.04±0.00	0.06±0.00
Hydration	0.01±0.00	0.03±0.00	0.03±0.00	0.06±0.00
GSH 100 ppm	0.01±0.00	0.03±0.00	0.04±0.00	0.06±0.00
GSH 500 ppm	0.03±0.00	0.04±0.00	0.05±0.00	0.07±0.00
CD (p=0.05)	0.0039	0.0041	0.0040	0.0044
Superoxide dismutase (ΔA/min/g FW)				
Control	81.56±3.11	88.36±2.69	96.57±2.12	108.67±1.10
Hydration	88.32±3.55	93.95±1.55	104.18±0.68	114.99±3.15
GSH 100 ppm	121.65±1.22	131.42±2.25	130.30±1.98	136.49±3.17
GSH 500 ppm	134.23±1.45	147.67±3.75	156.06±5.45	150.57±1.52
CD (p=0.05)	1.03	4.33	5.16	3.80

al., 2020). Similar results were also reported in sweet pepper in which MDA levels increased by 2-fold when exposed to salt stress (Abdelaal et al., 2020). Glutathione plays a crucial role in maintaining the equilibrium between oxidation and antioxidation. Salt stressed mung bean seedlings also showed a decrease in MDA levels due to improved antioxidant enzymes by GSH application (Nahar et al., 2015).

Proline concentration showed a marked increase in the maize seedlings when seeds were grown in an exposure to salt stress (Table 3). Both concentrations of glutathione improved the proline content. However GSH 500ppm recorded the maximum increase in proline content when compared to untreated but stressed control. Proline content increased in salt stress exposed plants of both the cultivars. Proline protects the protein integrity by functioning as a molecular chaperone. To maintain the redox balance, it also acts as quencher of singlet oxygen species (Mansour et al., 2017). Similar results were also observed in mustard genotypes in which salt stress significantly elevated the proline content (Hossain et al., 2020) and in *Cicer arietinum* in which exogenous GSH treatment increased the accumulation of proline under salt stress (Sadak et al., 2017).

AUTHORS' CONTRIBUTION

N. Gupta: Conceptualization of research, designing of the experiments; H. Chhatwal: Execution of experiments, data collection, analysis of data and interpretation, preparation of the manuscript; G Kaur Conceptualization of research, designing of the experiments and supervised the manuscript; M. Goyal: Supervised the manuscript; P. Goyal: Supervised the manuscript; N. Garg Helped in editing the manuscript.

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Biodegradation of Phenoxyacetic Acid Herbicides (MCPA AND 2,4-D) by *Escherichia Coli*: Insights from HPLC Detection

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Abstract: The present study investigates the herbicide degradation potential of *Escherichia coli* strain LKDA3 under varying concentrations of two commonly used phenoxyacetic herbicides: 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA). Strain LKDA3, previously isolated using 500 mg/L herbicide, was evaluated for its degradation efficiency at 300, 500, and 700 mg/L concentrations over a 5-day incubation period in minimal salt medium supplemented with 0.2% glucose at 30 °C. Residual herbicide concentrations were quantified using high-performance liquid chromatography (HPLC), and degradation efficiency was determined by comparing chromatographic peak areas to those of untreated controls. The strain achieved complete (100%) degradation of 2,4-D at 300 mg/L, 99% at 500 mg/L, and 38.2% at 700 mg/L. A similar pattern was observed with MCPA, with ~99% degradation recorded across all concentrations. However, growth kinetics, measured via OD_{600}, showed concentration-dependent inhibition, particularly at 700 mg/L. Notably, MCPA induced a stronger inhibitory effect on bacterial growth compared to 2,4-D, despite high degradation efficiency. These results suggest that *E. coli* LKDA3 is capable of metabolizing both herbicides effectively, although elevated concentrations exert growth-limiting effects. The findings highlight the potential application of LKDA3 in bioremediation strategies targeting phenoxy herbicide-contaminated environments.

Keywords: Biodegradation, *Escherichia coli,* Phenoxy herbicides, 2,4-D, MCPA, HPLC

Human activities have played a major role in accelerating climate change, primarily through the release of greenhouse gases into the atmosphere. This has contributed to a rise in extreme weather conditions, including prolonged droughts, reduced rainfall, and escalating global temperatures (Tejada et al., 2023). Since the 20th century, the use of agrochemicals-particularly pesticides has expanded considerably in agricultural practices. This overreliance has led to the contamination of both surface and groundwater resources (Barba et al., 2020, Zhang et al., 2021). In countries such as those in the European Union, the United States, and various developing nations, the herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA) is commonly applied in agriculture to manage a broad spectrum of weeds. MCPA is a phenoxy-acid herbicide categorized under the auxins group, functioning by mimicking natural plant growth hormones. This imitation disrupts normal growth processes, resulting in tissue breakdown and ultimately plant death (Gorodylova et al., 2022). MCPA is effective in controlling weed populations, its removal from the soil is crucial before planting the next crop season. Residual MCPA in the soil can adversely affect subsequent crops, particularly sensitive ones such as cotton, soybeans, and corn (Zhang et al., 2021). Due to its high water solubility and low soil adsorption, MCPA easily leaches into surface and groundwater, posing a risk to water quality. This herbicide has been linked to adverse health effects in humans, particularly gastrointestinal and endocrine system disturbances (Wang et al., 2021). Similarly, 2,4-Dichlorophenoxyacetic acid (2,4-D), another member of the phenoxy herbicide group, is widely used to manage broadleaf weeds in agricultural lands and grasslands. As a hormonal herbicide, it disrupts plant growth by interfering with the hormonal balance within plants (Mustafa et al., 2015; Barba et al., 2020). Despite their effectiveness in weed control, both MCPA and 2,4-D are harmful to human health and the environment due to their persistence and toxicity. Therefore, there is an urgent need to develop efficient microbial solutions for their degradation. Studies have shown that 2,4-D residues can induce cytotoxic and genotoxic effects, trigger mutations in plants, and cause histological., physiological., and behavioral changes in animals (Aguiar et al., 2020). Researchers worldwide commonly rely on degradation technologies to eliminate environmental pollutants and their toxic byproducts. Among these methods, biodegradation stands out due to its cost-effectiveness and environmentally friendly nature (Singh et al., 2022). The effectiveness of a microbe in breaking down contaminants depends not only on its inherent biodegradation capacity but also on how accessible the pollutant is, which can be influenced by its interaction and binding with soil particles (Gorodylova et al., 2022). Microorganisms exhibit a wide range of capabilities in degrading various classes of pesticides. The natural breakdown of these substances can be enhanced through bioremediation techniques, which involve the addition of nutrients, carbon sources, or electron donors to support microbial activity (Mustafa et al., 2015). Numerous studies have reported the occurrence of microorganisms capable of degrading pesticides and herbicides in environments such as soil, sewage sludge, and water. These microbes were often isolated from sites contaminated with such pollutants (Nguyen et al., 2021; Gorodylova et al., 2022).

An essential aspect of isolating herbicide-tolerant microbial strains involves carefully selecting suitable strains and creating favorable conditions that support their survival and activity. Typically, these microorganisms are cultured in environments containing pesticides and herbicides often at concentrations exceeding typical toxicity levels, to assess their resistance and degradation capabilities (Gorodylova et al., 2022). Although many studies have explored microbial degradation of herbicides, only a limited number of bacterial and fungal species have been successfully isolated when exposed to lower pesticide concentrations. The objective of the present study was to isolate microbial strains capable of tolerating the highest possible concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA), both individually, within the shortest incubation period.

MATERIAL AND METHODS

Chemicals and solutions: HPLC grade 4-chloro-2-methylphenoxyacetic acid (MCPA) was procured from Merck, while 2,4-dichlorophenoxyacetic acid (2,4-D) of 99% purity was obtained from SRL. All other analytical-grade reagents were supplied by HiMedia. The mineral salt medium (MSM) used for microbial cultivation consisted of the following components (g/L): dipotassium phosphate (K_2HPO_4) - 1.5, potassium dihydrogen phosphate (KH_2PO_4) - 0.5, magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) - 0.5, sodium chloride (NaCl) - 0.5, and ammonium nitrate (NH_4NO_3) - 1.5, as described by Li et al. (2008).

Enrichment and Identification of herbicide-degrading bacteria: Soil samples were obtained from sugarcane and wheat cultivation fields in the vicinity of Bakshi Ka Talab (26.9897° N, 80.9205° E), Lucknow, Uttar Pradesh, India, at a depth of approximately 10 cm. Prior to bacterial isolation, the samples were air-dried, passed through a sieve to remove debris, and stored at 4 °C for later use. Bacterial isolation was conducted using a minimal salt medium (MSM), in which herbicides served as the sole carbon source to promote the selective growth of herbicide-tolerant strains. For the enrichment process, 10 g of each soil sample was introduced into 250 mL Erlenmeyer flasks containing 100 mL of MSM supplemented with either 2,4-D or MCPA at a concentration of 500 mg/L. Enrichment was continued by transferring 1% (v/v) of the culture into fresh herbicide-containing MSM three times per week. After several enrichment cycles, cultures were serially diluted and spread onto MSM agar plates. Following a 4-day incubation period, morphologically distinct colonies were isolated and subjected to further screening for their ability to degrade the target herbicides.

Molecular identification of herbicide-degrading bacterial strains: The bacterial isolate designated as strain A3 was identified through amplification and sequencing of the 16S rRNA gene. Universal primers 16-GT-F (5'-AGAGTTTGATCATGGCTCA-3') and 16-GT-R (5'-TCGGATGGGAACTTTAAGT-3') were employed for PCR amplification and characterization of the bacterial strains. Each PCR reaction was prepared in a total volume of 25 µL, containing 1X Premix Taq, 0.4 µM of each primer, and 50 ng of genomic DNA extracted from the bacterial culture. Thermal cycling was performed on an Eppendorf thermal cycler (Eppendorf AG, Hamburg, Germany) with the following program: initial denaturation at 95 °C for 5 minutes; 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 55 °C for 45 seconds, and extension at 72 °C for 90 seconds; followed by a final extension at 72 °C for 7 minutes. The PCR amplicons were confirmed by agarose gel electrophoresis and purified for subsequent Sanger sequencing. Sequence data were analyzed via the NCBI BLASTN tool to compare with sequences in the GenBank database, facilitating the identification or potential discovery of novel strains. In total, five bacterial strains exhibiting the ability to degrade the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) were isolated and classified based on the sequence similarity of their 16S rRNA genes to known taxa in GenBank.

Biodegradation kinetics of 2,4-D and MCPA by soil bacteria in MSM: Strain A3 was isolated using minimal salt medium (MSM) supplemented with 500 mg/L of the target herbicide. Post-identification, the strain was preserved by preparing a 70% glycerol stock, which was stored at –20°C for future use. Freshly cultured cells were employed for all experimental assays to ensure consistent and active microbial growth. To evaluate the effects of environmental parameters on strain A3 growth, experiments were performed by varying pH and temperature while maintaining a constant herbicide concentration in MSM. The growth kinetics of the isolated strain LKDA3 were investigated in Luria-Bertani (LB) broth under both herbicide-free and herbicide-supplemented conditions to evaluate the impact of herbicide exposure on bacterial proliferation. A control culture (without herbicide) was maintained alongside the treatment group to enable comparative analysis. Bacterial growth was assessed over a 26-hour period, with optical density measurements at 600 nm (OD_{600}) recorded hourly using a UV/VIS spectrophotometer. Growth kinetics were monitored in MSM containing 500 mg/L herbicide and supplemented with 0.2% (w/v) glucose as an additional carbon source. Cultures were incubated at 30 °C with agitation at 150 rpm for five days. Daily optical density measurements at 600 nm (OD600) were recorded using a UV/VIS spectrophotometer to track microbial proliferation. For pH-dependent growth analysis, strain A3 was inoculated into MSM adjusted to pH values of 4.0, 5.0, 6.0, and 7.0, while maintaining the herbicide concentration at 500 mg/L and incubation temperature at 30 °C. Glucose supplementation remained constant at 0.2%. Cultures were incubated under shaking conditions for five days, with daily OD600 measurements to assess growth across different pH levels. To investigate the impact of temperature, strain A3 was cultivated in MSM (pH 7.0) at 20 °C, 30 °C, 40 °C, and 50 °C, with a fixed herbicide concentration and 0.2% glucose. Growth was monitored daily over a five-day incubation period by measuring OD600. All experiments, including those evaluating pH and temperature effects, were conducted in triplicate to ensure reproducibility and statistical reliability. Media blanks without bacterial inoculation served as controls in all spectrophotometric analyses. Additionally, the biodegradation potential of strain A3 was examined at varying herbicide concentrations (300 mg/L, 500 mg/L, and 700 mg/L) in MSM supplemented with 0.2% glucose. Cultures were initiated at an OD600 of 1.0 and incubated at 30 °C with shaking at 150 rpm for five days. Both microbial growth and herbicide degradation capacity were assessed at the end of the incubation period via OD600 measurements. This comprehensive experimental design facilitated an in-depth understanding of strain A3 growth dynamics and its capacity to degrade herbicides under diverse environmental conditions.

Analytical determination of 2,4-D and MCPA degradation products: During the biodegradation assays conducted in minimal salt medium (MSM) under optimized conditions, culture samples were periodically collected to monitor and analyze the degradation products. The culture broth was initially centrifuged at 12,000 × g for 15 minutes to separate bacterial cells from the supernatant. Both fractions were processed independently to extract metabolites and degradation compounds. The supernatant was concentrated by lyophilization and subsequently reconstituted in 3 mL of methanol. This methanolic solution was centrifuged at 6,000 × g for 15 minutes to remove particulate impurities, followed by filtration through 0.22 µm sterile syringe filters (Xia et al., 2017) to obtain a clear filtrate suitable for analysis. Concurrently, the bacterial cell pellet was resuspended in 3 mL of methanol and incubated overnight at room temperature to facilitate extraction of intracellular metabolites. Post-incubation, the suspension underwent sonication for 1 minute at ambient temperature to lyse the cells and enhance metabolite release. The mixture was then allowed to settle overnight to enable sedimentation of cell debris. Subsequently, the suspension was centrifuged at 6,000 × g for 15 minutes, and the supernatant was collected and filtered through sterile 0.22 µm PVDF syringe filters (AXIVA) to remove any residual particulates. Both extracellular and intracellular extracts were thus prepared for high-performance liquid chromatography (HPLC) analysis to characterize herbicide degradation products.

RESULTS AND DISCUSSION

Enrichment and identification of herbicide-degrading bacteria: A total of five bacterial isolates demonstrating herbicide-degrading potential were successfully obtained from agricultural soil samples using an enrichment culture technique, wherein herbicides served as the sole carbon and energy source to selectively enrich the degrading microbial population. Identification of all isolates was conducted through amplification and sequencing of the 16S rRNA gene. The resulting nucleotide sequences were analyzed using the NCBI BLASTN tool to determine taxonomic affiliations, and all validated sequences were submitted to the GenBank database. Among the five isolates, strain A3 was selected for detailed investigation due to its superior degradation capacity. Sequence alignment using BLASTN revealed that strain A3 shared 99% similarity with *Escherichia coli*, based on a 1438 bp region of the 16S rRNA gene. Consequently, the strain was identified as *Escherichia coli* and designated as *E. coli* LKDA3. The corresponding sequence was deposited in the NCBI GenBank under accession number OQ881067. To confirm the taxonomic position of strain A3, a phylogenetic analysis was performed by comparing its 16S rRNA gene sequence with those of closely related species retrieved from GenBank. The phylogenetic relationships are depicted in Figure 1.

Biodegradation kinetics of 2,4-D and MCPA by soil bacteria in MSM: The resulting growth curves (Fig. 2a) demonstrated that herbicide exposure did not significantly inhibit the growth of *E. coli* LKDA3 under the enriched conditions provided by LB medium. Both treated and control cultures exhibited similar growth trends, indicating a high level of tolerance to the tested herbicides. An initial lag phase of approximately 4 hours was observed, during which cellular activity was primarily focused on physiological adaptation rather than active division. This was followed by a pronounced exponential growth phase starting around the

6th hour, characterized by rapid cell proliferation and a steady increase in OD_{600}. The exponential phase persisted until approximately the 18th hour, with the OD_{600} reaching ~1.2 units. Maximum cell density was recorded at approximately OD_{600} = 2.5 after 20 hours of incubation. Subsequent measurements showed a plateau in optical density, marking the onset of the stationary phase, likely resulting from nutrient depletion and/or accumulation of inhibitory metabolic by products.

Following the growth kinetics assessment in Luria-Bertani (LB) medium, the growth behavior of *E. coli* strain LKDA3 was further evaluated in minimal salt medium (MSM) to investigate its ability to utilize herbicides under nutrient-limited conditions. Initially, the strain was cultured in MSM without any additional carbon source, both in the presence and absence of herbicides. No detectable growth was observed over a 5-day incubation period at 30 °C, suggesting that the strain was unable to metabolize either the herbicide compounds or the MSM constituents as sole sources of carbon and energy under these conditions. To enhance the growth potential, MSM was supplemented with 0.2% (w/v) glucose and 500 mg/L of either 2,4-dichlorophenoxyacetic acid (2,4-D) or 2-methyl-4-chlorophenoxyacetic acid (MCPA). In the control group (MSM + 0.2% glucose without herbicide), minimal growth was observed after 5 days, indicating that the limited glucose alone was insufficient for robust proliferation. When 2,4-D was present, a slight increase in OD_{600} was recorded on day 1 (~0.04), which subsequently declined by day 5, suggesting an initial adaptive response followed by inhibition due to herbicide toxicity. A comparable trend was observed in MCPA-supplemented cultures, where OD_{600} similarly peaked at ~0.04 on day 1 but exhibited a modest increase to ~0.08 by day 5 (Fig. 2b), indicating a delayed but partial adaptation to

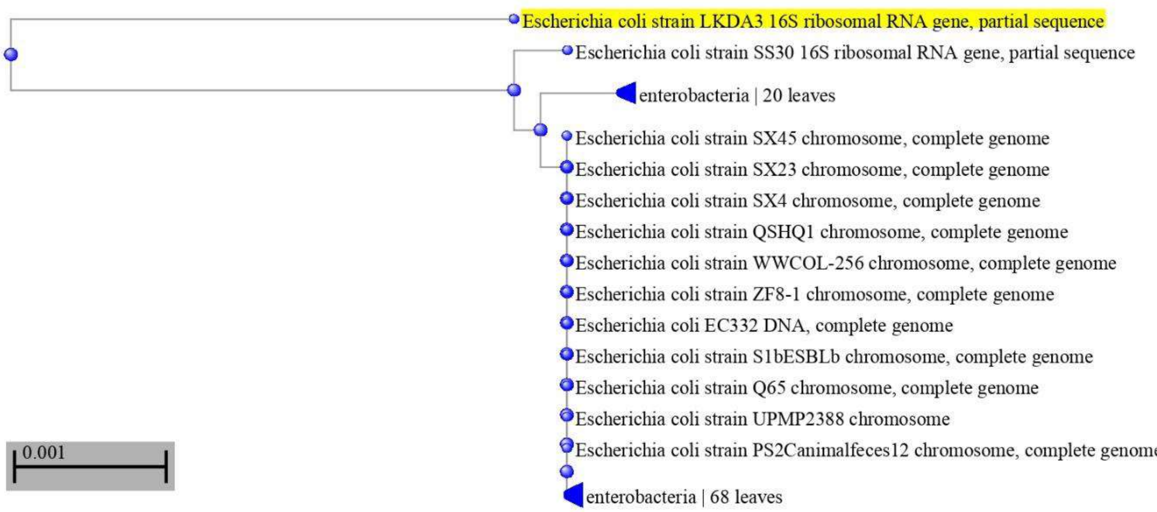

Fig. 1. Phylogenetic tree of strain LKDA3 with other microorganisms

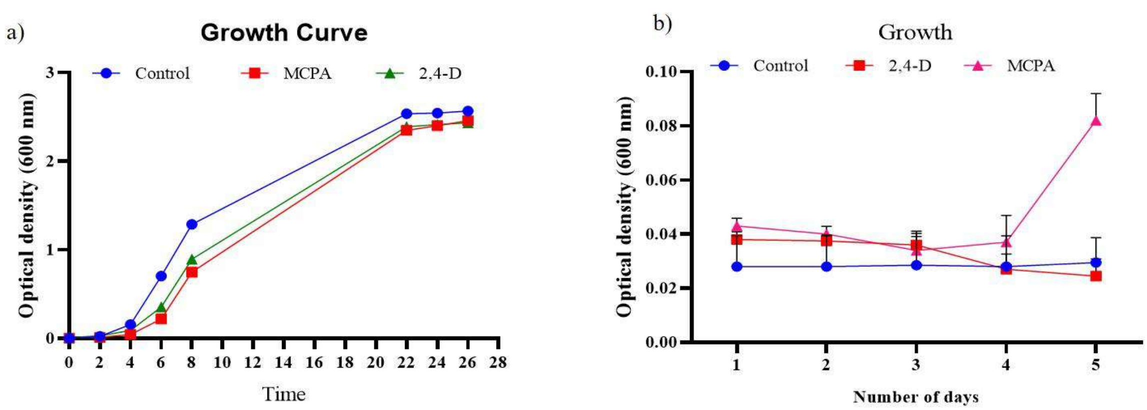

Fig. 2. Growth Curve in (a) enriched medium (LB broth) (b) Minimal salt medium with 0.2% glucose in the presence of 2,4-D and MCPA at 500 mg/L concentration

the herbicide-induced stress. These results suggest that both herbicides exert inhibitory effects on *E. coli* LKDA3 under minimal nutritional conditions. While the strain exhibited limited initial survival., it showed some capacity for physiological adaptation, particularly in the presence of MCPA. To further investigate environmental influences on growth, the effect of pH was assessed. Cultures were grown in MSM supplemented with 0.2% glucose and 500 mg/L of herbicide, across a pH gradient (5.0, 6.0, 7.0, and 8.0), at 30 °C under continuous shaking (150 rpm) for 3 days. In the presence of 2,4-D, the highest OD_{600} (~0.12) was observed at pH 6.0, indicating that mildly acidic conditions favor growth under herbicide stress. Reduced growth was observed at pH 7.0 and 8.0, while no growth occurred at pH 5.0 (Fig. 3a), indicating that strongly acidic conditions impair strain viability. In contrast, cultures exposed to MCPA demonstrated limited survival across all pH levels, with OD_{600} values remaining around ~0.05, and no detectable growth at pH 5.0 (Fig. 3b). These findings highlight the pronounced inhibitory effect of MCPA, particularly under acidic conditions, and suggest a narrow window of environmental tolerance for strain LKDA3 in herbicide-amended minimal media.

Following growth kinetics analysis in LB medium, the growth response of *Escherichia coli* strain LKDA3 was further examined in minimal salt medium (MSM) to assess its ability to utilize herbicides under nutrient-limited conditions. No visible growth was observed in MSM alone, with or without herbicides, after 5 days of incubation at 30 °C, indicating that the strain could not metabolize the herbicides or MSM constituents as sole carbon sources. When MSM was supplemented with 0.2% glucose and 500 mg/L of 2,4-D or MCPA, a slight increase in OD_{600} (~0.04) was recorded on day 1, followed by either stagnation or a marginal rise by day 5 (~0.08 in MCPA), indicating initial stress adaptation. Minimal growth was also noted in the glucose-only control, suggesting limited nutrient availability. To investigate environmental tolerance, growth was evaluated at different pH levels (5.0–8.0) in MSM with glucose and 500 mg/L herbicide. Optimal growth with 2,4-D was observed at pH 6.0 (OD_{600} ~0.12), while growth declined at neutral and alkaline pH and was absent at pH 5.0. In MCPA treatments, OD_{600} remained low (~0.05) across all pH levels, with no growth at pH 5.0. These findings suggest that strain LKDA3 shows limited growth under herbicide stress, with slightly better adaptation to 2,4-D than MCPA.

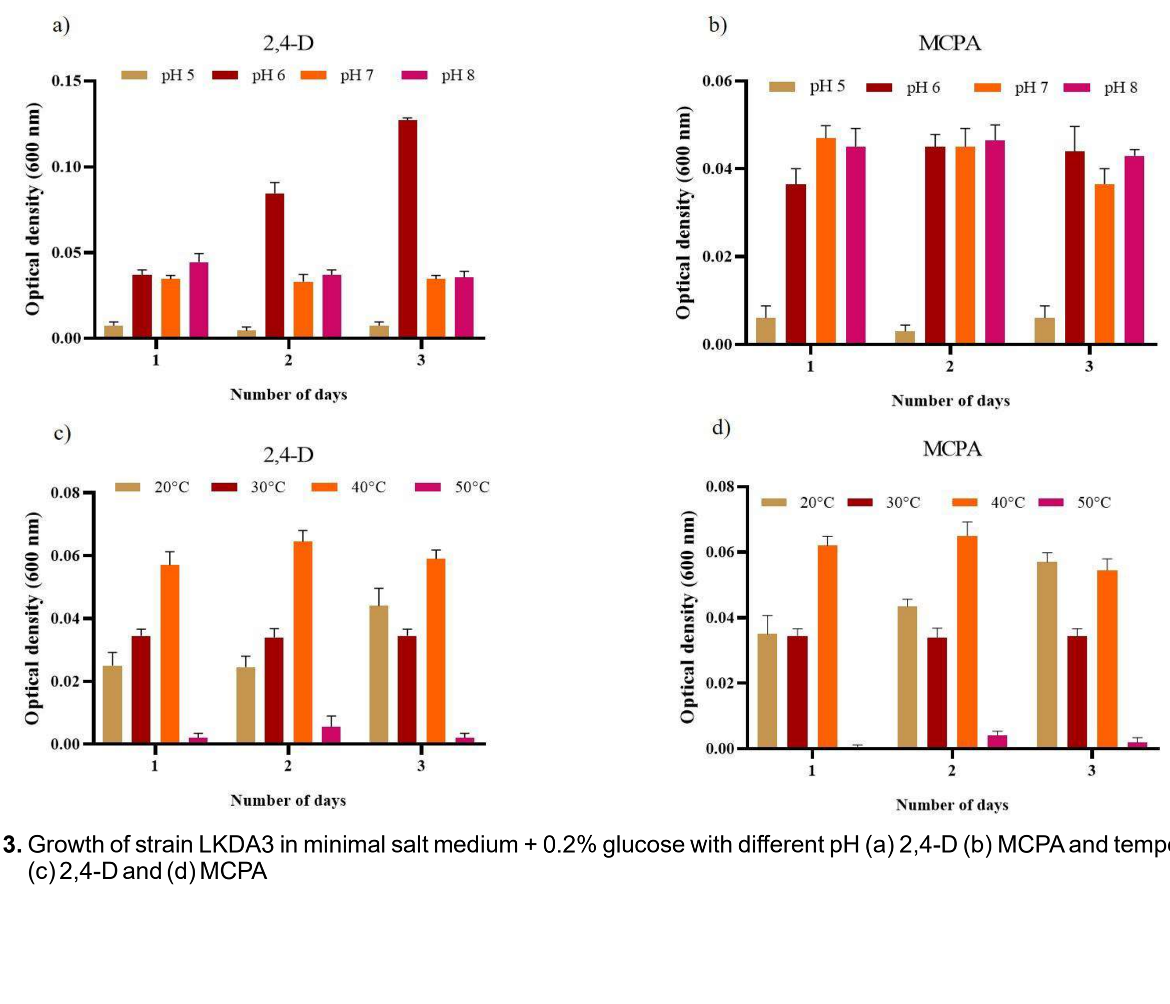

Fig. 3. Growth of strain LKDA3 in minimal salt medium + 0.2% glucose with different pH (a) 2,4-D (b) MCPA and temperatures (c) 2,4-D and (d) MCPA

Analytical determination of 2,4-D and MCPA degradation products: The herbicide degradation capability of *E. coli* strain LKDA3 was assessed at three initial concentrations (300, 500, and 700 mg/L) of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) following a 5-day incubation under optimized conditions (30 °C, 150 rpm). While agricultural usage typically involves herbicide concentrations between 200–300 mg/L, the strain was originally isolated using 500 mg/L, justifying the inclusion of higher concentrations to evaluate degradation efficiency under increased exposure. After incubation, culture supernatants were collected and analyzed via high-performance liquid chromatography (HPLC) to quantify residual herbicide levels. Degradation efficiency was calculated by comparing the sample peak areas to those of untreated standards (Fig. 5). Strain LKDA3 exhibited complete degradation of 2,4-D at 300 mg/L and 500 mg/L shows 99%, and 38.2% at 700 mg/L (Fig. 4b), indicating a concentration-dependent response. Concomitant growth measurements (OD_{600}) revealed more robust biomass accumulation at lower herbicide levels, with diminished growth (OD_{600} ≈ 0.12) observed at 700 mg/L (Fig. 4a), suggesting delayed adaptation or toxic effects at elevated concentrations. Comparable trends were noted for MCPA, where ~99% degradation occurred after 5 days at all concentrations. However, bacterial growth declined markedly with increasing MCPA levels. At 300 mg/L, OD_{600} reached ~0.5, while at 700 mg/L, it dropped to ~0.2, indicating stronger inhibitory effects of MCPA compared to 2,4-D.

Isolated *E. coli* LKDA3 shows 99% degradation rate at 300 mg/L and 500 mg/L concentration of 2,4-D herbicide, while at 700 mg/L it shows decline degradation rate of 38.2%, indicating potential toxicity at higher concentrations. Similarly, MCPA degradation was approximately 99% at 300 mg/L, with reduced bacterial growth observed at higher concentrations. These findings suggest that while strain LKDA3 can effectively degrade these herbicides at lower concentrations, higher levels may inhibit its activity.

Comparative studies have explored the degradation of 2,4-D by *E. coli* strains. Bhat et al. (2015) investigated the effects of sublethal levels of 2,4-D on *E. coli*, revealing that exposure induced oxidative stress, leading to filamentous cell morphology and alterations in metabolic pathways.

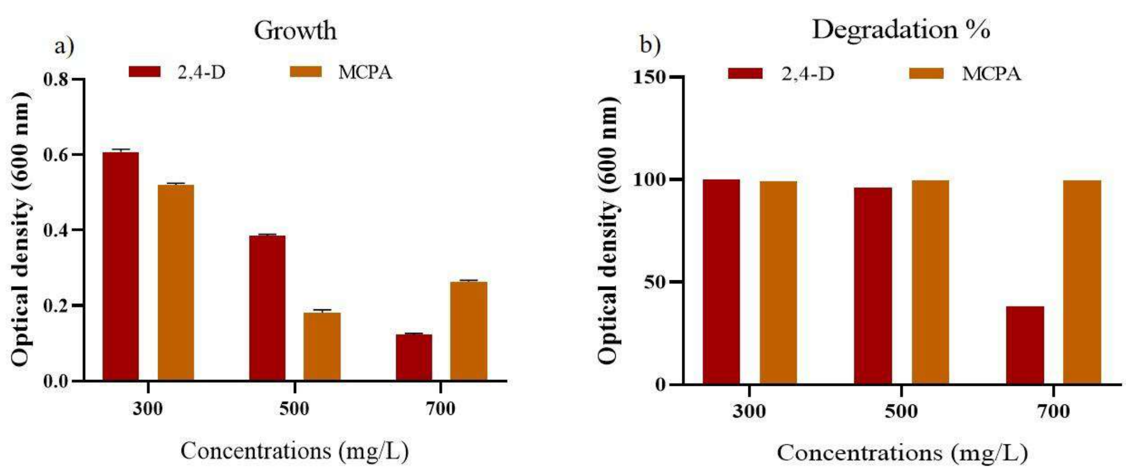

Fig. 4. Growth with different concentrations of herbicides (2,4-D and MCPA) after 5 days of incubation at 30˚C (a) degradation % of 2,4-D and MCPA by *E. coli* LKDA3 (b)

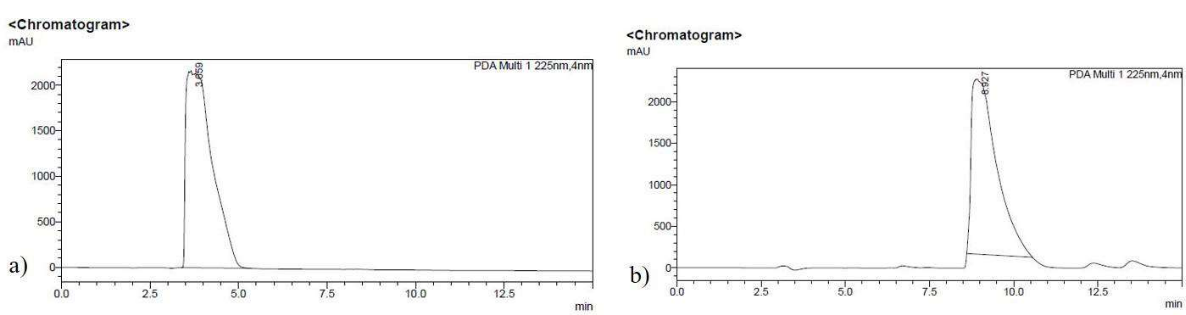

Fig. 5. Standard HPLC chromatograph of both herbicide (a) 2,4-D (b) MCPA

These stress responses could impact the bacterium's degradation capabilities. In contrast, a study by Wang et al. (2023) engineered *E. coli* strains with a complete 2,4-D degradation pathway, achieving rapid and complete degradation of 0.5 mM 2,4-D within 6 hours. This highlights the potential of genetic engineering to enhance degradation efficiency. Further, Bhat et al. (2018) demonstrated that sublethal concentrations of 2,4-D arrested cell division in *E. coli* by disrupting the divisome complex, leading to DNA damage and activation of the SOS response. These cellular disruptions could compromise the bacterium's viability and degradation capacity under herbicide stress. Additionally, a study by Singh et al. (2022) using Raman spectroscopy found that exposure to 2,4-D and glyphosate induced distinct biochemical changes in *E. coli*, potentially leading to phenotypic antibiotic resistance. Such stress-induced adaptations may influence the bacterium's metabolic functions, including herbicide degradation. Collectively, these studies underscore the complex interplay between herbicide concentration, bacterial stress responses, and degradation efficiency. While strain LKDA3 exhibits promising degradation capabilities at lower herbicide concentrations, its performance diminishes at higher levels, likely due to induced stress responses similar to those observed in other *E. coli* studies. Future research could focus on enhancing the resilience and degradation efficiency of such strains, potentially through genetic modifications or adaptive evolution strategies, to improve bioremediation outcomes in environments with varying herbicide concentrations.

CONCLUSION

The present study demonstrates the efficacy of selected strain *E. coli* LKDA3 in degrading phenoxyacetic herbicides 2,4-D and MCPA under varying environmental conditions. The results reveal that bacterial growth and degradation efficiency are significantly influenced by pH, temperature, and herbicide concentration. Optimal bacterial proliferation and maximum degradation were observed at pH 6 and temperatures between 30°C and 40°C, 40˚C being the best suggesting that these conditions enhance enzymatic activity and microbial adaptation. Although both herbicides exhibited some inhibitory effects on early bacterial growth, particularly at higher concentrations, the strains retained exceptional degradation capacities across all tested levels, consistently achieving degradation efficiencies to 99%. MCPA was found to be slightly less inhibitory than 2,4-D, as indicated by higher growth rates in its presence. This may reflect structural differences between the compounds affecting microbial uptake and enzymatic breakdown. Further studies involving molecular characterization of the degradation pathways and field-scale validation are recommended to enhance the application potential of strains. Overall, this research reinforces the viability of using microbial agents for sustainable and cost-effective remediation of phenoxy herbicide pollution. The ability of the bacteria to maintain high degradation performance under suboptimal conditions highlights their potential application in bioremediation of herbicide-contaminated soils and aquatic environments.

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Effect of Mulching on Growth of Dragon Fruit [*Hylocereus costaricencis* (Web.) Britton and Rose] in Sub Tropical Climate

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***Abstract*:** The experiment on mulching practices for the better use of soil moisture for the growth of dragon fruit which is a hardy Cactaceae family was conducted at sub-tropical climate of Lucknow region. There were 8 treatments (mulching with sugarcane trash, paddy straw, wheat straw, dry leaves, saw dust, black polyethylene and transparent polyethylene). Mulching was done round the base of plants on 60 cm radius. The mulching with transparent polyethylene showed better growth in terms of plant length, number of segment, distance between areole, number of new branches as well as chlorophyll content. However, among the organic mulches saw dust, dry leaves and wheat straw beneficial for improving vegetative growth of dragon fruit.

Keywords: Dragon fruit, *Hylocereus costaricencis* (Web.), Organic mulching

In India, dragon (*Hylocereus spp.)* fruit was introduced during the late 1990s (Arivalgan et al., 2019). Between 2005 and 2024, the cultivation of dragon fruit saw a gradual expansion, with the cultivation area increasing from 4 to 4000 hectares. Although, India has not a significant mark in global export market, the ongoing expansion of dragon fruit cultivation in the country presents promising opportunities for the future. *Hylocereus costaricensis* Syn. *selenicereus costaricensis* has a purple red flesh having more phyto-nutrients and high market demand (Mori et al., 2023, Maji 2019). In India around 95% of market demand is fulfilled by importing in India and mostly are white fleshed.

Mulching is well-known and recommended practice for conserving soil and water as well as weed control (Pabin et al., 2003, Becher 2005, Prasad et al., 2017, Maji et al., 2021). Mulched crops approach also increases soil organic matter and microbial biomass in long term (Sun et al., 2021). It further improves nutrient retention and N-uptake efficiency. The other profits of mulching are favourable changes in microclimate within fields and reduction soil temperature variations (Sharratt 2002). The use of surface mulch can result in storing more precipitation water in soil by reducing runoff, increasing infiltration and decreasing evaporation (Ji and Unger 2001). Organic mulching like wheat straw mulching could increase soil C sequestration and microbial biomass (Wang et al., 2018). Comparative study on use of organic and inorganic mulching materials specially on dragon fruit is lacking. Thus, there is a scope to explore the present study to effective utilization of available resources like wheat straw, rice straw, saw dust, grass clippings, leaf debris, sugarcane trashes etc. which can also address the issue of stubble burning. It is not only cost-effective but also helps conserve water, soil temperature, reduce waste and improve soil health. Mulch around the base of dragon fruit plants can create a more favourable environment for dragon fruit growth.

MATERIAL AND METHODS

The present investigation was carried out at Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India (26°55′N latitudes and 80°54′E, 123 m above MSL) situated under the subtropical climate of central Uttar Pradesh during 2023-24. Lucknow exhibits distinct seasonal variations, including hot, dry summers and cold winters. The soil of experimental site was sandy loam with uniform topography having slightly alkaline soil with pH 8.2. The experiment consisted of 8 treatments having mulching with sugarcane trash, paddy straw, wheat straw, dry leaves, saw dust, black polythene and transparent polythene in randomised block design with 3 replication having 96 plants (4 years old) .

Sugarcane trash was obtained from the nearest sugarcane juice corner, underwent treatment with insecticide and was subsequently dried in the sun before being applied. Rice straw and wheat straw were acquired from a farmer field. Dry leaves were collected from the campus area. Additionally, black and transparent (white) polythene mulch, each with a thickness of 15 microns, was purchased from market. Mulches were applied at root zone around the pole each having 4 plants was completely covered by these mulching materials along 60 cm radius leaving sufficient space at the crown area for excess heat to dissipate into the environment.

Observations were recorded for its vegetative growth parameters like plant height (cm), stem circumference (cm), stem thickness (cm), number of segments per plant, number of primary branches, distance between areoles (cm), number of new primary branches per plant, dry weight of stem (g), moisture content (g), moisture percentage of the stem (%) and chlorophyll content (mg/100g) using standard methods of biochemical analysis (Thimmaiah 2009). The observed data were analyzed by OPSTAT software (Sheoran et al., 1998).

RESULTS AND DISCUSSION

Plant height: The maximum plant height (12.99 cm) was in T_8 (transparent polythene) with 6.75% increase from 0 to 90 DAT followed by T_3 (paddy straw), T_5 (dry leaves) and T_6 (saw dust) with non-significant difference in T_3, T_5, T_6 and T_7. Deb et al. (2014) also reported that transparent polythene mulch comparable to black polythene mulch, indicating its effectiveness in promoting plant growth.

Stem circumference: The maximum increase (1.60 cm) in stem circumference was in T_6 (saw dust) followed by T_2 (sugarcane trash) while, minimum increase of 4.18% was recorded (0.63 cm) in T_5 mulching.

Stem thickness: T_5 caused maximum increase in stem thickness (0.96 cm, 22.59 %) followed by T_2 and T_7 (black plastic mulch) but were statistically at par. Minimum stem thickness (0.40cm) was in T_8. Sharma et al. (2023) rice husk was more effective in increasing stem diameter, even better than black plastic followed by straw and sawdust treatments in tomato.

Number of stem segment per plant: The highest increase (23.13%) in number of stem segment per plant from 0 to 90 DAT was in treatment T_8 which was significantly higher from T_5 (12.49%). Control (T_1) was at p*ar* with T_2 and T_3. Puri et al. (2022) concluded that plastic mulch gave best result in increasing number of leaves in okra. Besides plastic mulch, rice straw and rice husk mulch were effective.

Distance between areoles: Maximum increase (0.64 cm, 25.71 %) of distance between areole was in T_8 mulch followed T_5 and T_3 while, minimum increase (0.10 cm, 3.30 %.) was in T_2. Increase in areole distance was most probably due to effect of mulch as it also resulted in the increase of plant height.

Number of primary branches: The highest increase in number of primary branches (14.00) was in T_8 and was significantly different from minimum increase (4.67) in T_1. Rajbir et al. (2006) suggested that thick, matted surface covering capacity of polythene mulches helps to better water retention and nutrient availability to the plant leading to increased vegetative growth.

Stem dry weight and moisture content: The dry weight of the stem was significantly influenced by the application of mulches. The maximum stem dry weight (6.83 g) was in T_4. However, highest moisture content (45.50 g) was observed in T_6 followed by T_7 which was statistically *at par* with T_1. Kazemi and Jozay (2020) also reported that the fresh and dry weights of the plants under mulch treatments had significant difference might be due to less evaporation from soil. The water vapours that evaporate from the soil surface further trapped in the plastic and dropped again into the upper soil surface which increases soil moisture content in the root zone. Khan and Singh (2005) reported similar observation in tomato crop.

Chlorophyll content: Highest Chlorophyll-A was 10.51 mg/100g) in T_8 followed by T_6 (saw dust) and least was in T_1 (1.50 mg/100g. Similarly, Chlorophyll-B as well as total chlorophyll was also maximum (9.64 and 12.07 mg/100g, respectively) in T_8 followed by T_6 (8.64 and 10.63 mg/100g). Kazemi and Jozay (2020) reported that mulch type significantly affect chlorophyll levels in *Gaillardia* sp.

Table 1. Influence of mulching on plant height, stem circumference and stem thickness of dragon fruit

Treatments	Plant height (cm)				Stem circumference (cm)				Stem thickness (cm)			
	At 0 DAT	At 90 DAT	Total increase	Per cent increase	At 0 DAT	At 90 DAT	Total increase	Per cent increase	At 0 DAT	At 90 DAT	Total increase	Per cent increase
T_1	222.50	228.19	5.66	2.56	15.60	16.76	1.16	7.59	4.92	5.51	0.59	12.03
T_2	268.22	274.25	6.02	2.24	15.66	17.05	1.39	8.93	4.58	5.43	0.84	18.78
T_3	231.64	240.44	8.80	3.80	13.97	14.65	0.68	4.91	3.92	4.61	0.70	17.91
T_4	237.74	243.40	5.69	2.39	13.38	14.56	1.19	8.92	4.07	4.55	0.47	11.60
T_5	212.71	221.20	8.49	4.00	15.24	15.87	0.63	4.18	4.27	5.23	0.96	22.59
T_6	228.60	236.63	8.04	3.52	16.93	18.53	1.60	9.44	5.30	5.88	0.58	10.95
T_7	234.85	242.81	7.96	3.39	12.52	13.61	1.09	8.76	3.36	4.08	0.73	22.59
T_8	193.04	206.03	12.99	6.75	12.36	13.70	1.34	10.88	3.80	4.20	0.40	10.52
CD (p=0.5)			1.55	0.86			0.39	3.33			0.16	6.92

T_1-Control (no mulch), T_2-Sugarcane trash, T_3-Paddy straw, T_4-Wheat straw, T_5-Dry leaves, T_6-Saw dust, T_7-Black polythene, T_8-White polythene

Table 2. Influence of mulching on change of number of segments, distance between areoles and number of primary branches of dragon fruit

Treatments	Number of stem segment/plant				Distance between areoles				Number of primary branches			
	At 0 DAT	At 90 DAT	Total increase	Per cent increase	At 0 DAT	At 90 DAT	Total increase	Per cent increase	At 0 DAT	At 90 DAT	Total increase	Per cent increase
T_1	6.00	6.37	0.37	6.22	2.71	2.92	0.21	7.83	0.00	4.67	4.67	
T_2	5.67	5.95	0.28	6.81	3.04	3.14	0.10	3.30	0.00	6.00	6.00	
T_3	5.67	6.00	0.34	6.79	2.47	2.86	0.39	15.71	0.00	12.00	12.00	
T_4	5.67	6.49	0.82	20.89	2.87	3.06	0.19	6.50	0.00	7.00	7.00	
T_5	5.00	5.06	0.06	1.13	2.89	3.36	0.47	16.49	0.00	9.33	9.33	
T_6	4.00	4.64	0.64	22.81	3.00	3.29	0.29	9.78	0.00	9.00	9.00	
T_7	5.67	6.33	0.66	12.49	3.04	3.28	0.24	8.01	0.00	6.67	6.67	
T_8	5.00	6.03	1.03	23.13	2.48	3.12	0.64	25.71	0.00	14.00	14.00	
CD (p=0.05)			0.20	0.03			0.05	2.96			3.34	

Table 3. Effect of different mulches on stem dry weight, moisture content and chlorophyll content

Treatments	Dry weight of stem (g)*	Moisture content (g)	Moisture percentage (%)	Chlorophyll A	Chlorophyll B	Total chlorophyll
T_1	5.54	44.46	88.91	1.50	2.49	2.81
T_2	6.04	43.46	87.91	4.99	5.03	5.47
T_3	6.11	43.89	87.79	6.85	6.17	8.18
T_4	6.83	43.17	86.33	5.98	6.94	9.18
T_5	5.62	44.38	88.76	4.75	5.15	6.53
T_6	4.50	45.50	91.00	10.06	8.64	10.63
T_7	5.23	44.77	89.54	8.49	7.96	10.42
T_8	6.06	43.94	87.88	10.51	9.64	12.07
CD (p=0.05)	1.05	1.05	2.10	0.40	0.56	0.20

* Dry weight was estimated on the basis of 50 g fresh weight

CONCLUSION

Application of organic and inorganic mulches both are beneficial for dragon fruit cultivation. The transparent polythene mulch under inorganic category while dry leaves and saw dust under organic mulching category may be recommended for better growth of dragon fruit in Lucknow region having maximum vegetative growth in terms of stem length, stem thickness, higher plant branches, dry weight, moisture content and chlorophyll content.

AUTHOR'S CONTRIBUTION

This is a part of the M.Sc. Thesis of first author Kanak Lata. She executed most part of the field experiment. Sutanu Maji planned this investigation. Manya Kumari, Tannu Kumari, Tanay Anand and Ashish Kumar Murmur are the coworkers who have assisted her in field as well as for laboratory works.

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Effect of Girdling on Bio-chemicals Parameters in Plum cv. Kala Amritsari

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***Abstract*: E**xperiment was with the objective to improve the fruit quality through girdling in sub-tropical (low chilling requiring) plum cv. Kala Amritsari during 2022 and 2023 at CCS HAU, Hisar. The experiment encompasses of five treatments *viz*. girdling at full bloom stage (more than 70 % flower anthesis), girdling after fruit set (14 days after petal fall), girdling at pit hardening stage, girdling at 15 days before harvesting and control (un girdled). Among various girdling treatments, girdling at pit hardening stage significantly increased fruits bio-chemical quality (total soluble solids, sugars, anthocyanin content) and decreased acidity, carotenoid content followed by girdling after fruit set over the other remaining girdling treatments. The girdling at full bloom stage was inferior to control (un girdled) in all parameters studied except carotenoid content parameter. Ascorbic acid parameter was non-significant with girdling treatments.

Keywords: Plum, Anthocyanin, Carotenoids, Quality, Bio-chemicals.

Plum is a delicious stone fruit of temperate regions. In Europe, its importance is next to apple. In India, it is grown in hilly tracts of north- eastern states, Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand and parts of Uttar Pradesh. Some low chilling and early ripening cultivars can also be grown in sub-tropical regions. At national level it has covered an area of 17930 ha with production of 69530 MT (Anonymous 2023a) but, in Haryana it was grown on 66.2 ha with production of 1314.71 MT (Anonymous 2023b). The growth and fruitfulness of a plant is greatly influenced by the relative proportions of carbohydrates and nitrogen. The C:N ratio of crop plants can be altered through simple special horticultural practices like girdling. It is basically an intervention in the phloem transport between canopy and roots, an attempt to manipulate the distribution of photosynthate, mineral nutrients and plant bioregulators. Wide variety of fruit species are girdled to induce flowering, improve fruit set, increase in yield, enlarge fruit size, advance maturity and improve quality. This methodology is extremely effective controlling vegetative growth, enhancement of fruit yield and quality attributes in horticultural crops. Hence, considering the above importance and keeping in view the potential of girdling for quality improvement the present investigation effect of girdling on bio-chemicals parameters in plum cv. Kala Amritsari was planned with objectives to systematize the time of girdling for quality enhancement in plum.

MATERIAL AND METHODS

Experimental site and climate: The present study was carried out at experimental orchard, Department of Horticulture, CCS HAU, Hisar, Haryana, situated at an altitude of 215.2 m above mean sea level with coordinates of 29°15' North and 75°68' East of Haryana. Hisar has typically semi-arid with very hot dry summers and excessively winter weather condition. The climate is characterized by dryness, high temperature and light rainfall. Temperature reaches around 45^0C accompanied by hot and dry winds in May-June, however, sometimes the temperature drops to freezing point followed by occasional frost in December-January. Hisar receives 80-85 per cent of total rainfall *i.e.* 450 mm during July to September and 10-15 per cent during winter month *i.e.*, December to February which is due to western disturbances.

Treatment details: The field experiment was conducted on 15 years old plum cv. Kala Amritsari with spacing 6m×6m. Twenty uniformly grown plants having similar growth were selected which were under uniform agronomic practices as per recommended (Anonymous 2021). All plants were maintained under uniform practices of orchard management during the study period. The experiment was laid out in randomized block design with three replications by taking one tree per replication and girdling was done with girdling knife of 2 mm width four stages at full bloom stage (more than 70 % flower anthesis), after fruit set (14 days after petal fall); at pit hardening stage; at 15 days before harvesting and control (no girdling).

RESULTS AND DISCUSSION

Quality attributes: TSS (^{0}Brix): The maximum total soluble solids 15.22 ^{0}Brix and total sugars 12.13 per cent (Table 1); reducing sugars 6.79 per cent and non-reducing sugars 5.35 per cent was observed in girdling at pit hardening stage (T_4) followed by treatment girdling after fruit set (T_3) and minimum were in girdling at full bloom (T_2) (Table 2). The improvement in fruit quality in terms of TSS and sugars might be due to the accumulation of more assimilates above the girdle at pit hardening stage and their translocation to the parts above it with a reduction in supply of basipetal mobile factors to the root system. Kaur et al. (2019) observed in peach that treatment of urea @ 0.2 per cent with branch girdling had maximum TSS, sugars. Similarly, El-Kenawy et al. (2018) in grape vines noticed that jasmonic acid 40 ppm+ girdling gave the highest significantly values in SSC and total sugars.

Acidity (%): Different girdling treatments such as girdling at full bloom and control showed higher value of acidity than treatments girdling after fruit set, girdling at pit hardening stage and girdling 15 days before harvesting. The maximum acidity of 1.64 per cent was in girdling at full bloom (T_2) and minimum in girdling at pit hardening stage (T_4) followed by girdling after fruit set (T_3). Reduction in acidity in T_4 and T_5 might be due to conversion of organic acids into sugar and dilution effect as a result of increased fruit size or might be due to increase in total soluble solids at the expense of acid content. The acids under the influence of nutrients might get converted into sugars and their derivative by the reaction involving the reversal of glycolytic pathway. These results was supported by Azizi et al. (2022) in Kiwi vines where highest TSS, total sugar, reducing sugar, non-reducing sugar and lowest in acidity in f girdling in grafted vines. Similarly, Kaur et al. (2019) observed in peach that urea @ 0.2 per cent with branch girdling had maximum TSS, sugars and lowest in acidity. El-Kenawy et al. (2018) in grape vines observed that jasmonic acid 40 ppm+ girdling recorded significantly higher SSC, total sugar and lowest acidity.

Ascorbic acid (mg/100 g pulp): There was non-significant effect of girdling on ascorbic acid. But, the girdling treatments such as girdling after fruit set, girdling at pit hardening stage and girdling 15 days before harvesting showed higher ascorbic acid than control. However, the maximum numeric

Observations

Parameters	Method suggested by
TSS, Acidity, Ascorbic Acid	A O A C (Association of Official Analytical Chemists - 1990)
Total, Reducing and Non-reducing sugars	Hulme and Narain (1993)
Anthocyanin content	Harborne (1973)
Carotenoid content	Hiscox and Isrealstam (1979); calculated as per the formulae given by Venkatarayappa et al. (1984)

Table 1. Effect of girdling on TSS (ºBrix) and total sugars (%) in plum cv. Kala Amritsari

Treatments	TSS (ºBrix)			Total sugars (%)		
	2022	2023	Pooled	2022	2023	Pooled
Control-T_1	14.67	14.3	14.49	11.74	11.36	11.55
Girdling at full bloom- T_2	13.97	13.5	13.74	11.18	10.8	10.99
Girdling after fruit set- T_3	15	14.9	14.95	12.11	11.92	12.02
Girdling at pit hardening stage- T_4	15.3	15.13	15.22	12.16	12.1	12.13
Girdling 15 days before harvesting- T_5	14.92	14.63	14.78	11.93	11.7	11.82
CD (p=0.05)	0.32	0.35	0.34	0.35	0.41	0.38

Table 2. Effect of girdling on reducing sugars (%) and non-reducing sugars (%) in plum cv. Kala Amritsari

Treatments	Reducing sugars (%)			Non-reducing sugars (%)		
	2022	2023	Pooled	2022	2023	Pooled
Control-T_1	6.57	6.36	6.47	5.17	5	5.09
Girdling at full bloom- T_2	6.26	6.04	6.15	4.92	4.76	4.84
Girdling after fruit set- T_3	6.77	6.67	6.72	5.34	5.25	5.30
Girdling at pit hardening stage- T_4	6.8	6.77	6.79	5.36	5.33	5.35
Girdling 15 days before harvesting- T_5	6.68	6.55	6.62	5.25	5.15	5.20
CD (p=0.05)	0.18	0.24	0.21	0.15	0.13	0.14

values of ascorbic acid 4.38 mg/100 g pulp (Table 3) was observed in treatment girdling at pit hardening stage (T_4) and minimum in girdling at full bloom (T_2). The improvement in fruit quality in terms of ascorbic acid might not be due to the lower rate of conversion of dehydro- ascorbic acid to ascorbic acid. The data presented was contrast to the findings of Azizi et al. (2022) in Kiwi vines where highest ascorbic acid was girdling at 1/4 girdled grafted vines whereas, Kaur *et al*. (2019) observed maximum ascorbic acid with branch girdling with combination of 0.2 per cent urea in peach cv. Shan-i - Punjab.

Anthocyanin content (mg/100 g pulp wt.): The girdling after fruit set, girdling at pit hardening stage and girdling 15 days before harvesting showed higher anthocyanin content than control. The maximum pulp anthocyanin content of 6.04 mg/100 g pulp wt. was observed in girdling at pit hardening stage (T_4) followed by girdling after fruit set (T_3) and minimum was observed in t girdling at full bloom (T_2). The improvement in fruit quality in terms of anthocyanin content might be due to girdling treatment influenced the pigment composition in both fruit pulp and skin, mainly promoting the biosynthesis of anthocyanin content that were increased linearly with the girdling duration in both fruit pulp and skin. In particular, the red colouration increased in the pulp. In plum fruit, the increase in reddish colouration can be ascribed to the increased biosynthesis of red/purple pigments (anthocyanin) and/or the degradation of other coloured molecules such as chlorophylls and carotenoids (Olivares et al., 2017) . Solfanelli et al. (2006) in Arabidopsis found that at the molecular level, anthocyanins can be induced by sugar accumulation in fruit pulp under girdling conditions likely induced the anthocyanin content biosynthesis making the pulp more reddish than that of control. Further, the anthocyanin increase induced by girdling was reported in some fruits, e.g. in grapes (Basile et al. 2018), red kiwifruit (Nardozza et al. 2018), cherries (Michailidis et al. 2020) and red plum (Piccolo et al. 2021).

Carotenoid content (mg/100g pulp wt.): The girdling treatments such as girdling after fruit set, girdling at pit hardening stage, girdling 15 days before harvesting showed lower pulp carotenoid content than control except treatment girdling at full bloom. The maximum carotenoid content 10.46 mg/100 g pulp wt. was observed in treatment of girdling at full bloom stage (T_2) which was significantly higher to control and other remaining treatments (Table 4) .The minimum carotenoid content was observed in girdling at pit hardening (T_4). The improvement in fruit quality in terms of carotenoid at pit hardening might be due to interaction between anthocyanin and carotenoid. Rivas et al. (2011) also showed that girdling significantly increased leaf carotenoids, carotenoids: chlorophylls ratio and xanthophylls content in citrus.

Table 3. Effect of girdling on acidity (%) and ascorbic acid (mg/100 g pulp) in plum cv. Kala Amritsari

Treatments	Acidity (%)			Ascorbic acid (mg/100 g pulp)		
	2022	2023	Pooled	2022	2023	Pooled
Control-T_1	1.45	1.52	1.49	4.59	3.66	4.13
Girdling at full bloom- T_2	1.65	1.62	1.64	4.32	3.1	3.71
Girdling after fruit set- T_3	1.36	1.4	1.38	4.81	3.86	4.34
Girdling at pit hardening stage- T_4	1.29	1.32	1.31	4.86	3.9	4.38
Girdling 15 days before harvesting- T_5	1.39	1.45	1.42	4.61	3.68	4.15
CD (p=0.05)	0.06	0.07	0.07	NS	NS	NS

Table 4. Effect of girdling on anthocyanin content (mg/100 g pulp wt.) and carotenoid content (mg/100 g pulp wt.) in plum cv. Kala Amritsari

Treatments	Anthocyanin content (mg/100 g pulp wt.)			Carotenoid content (mg/100 g pulp wt.)		
	2022	2023	Pooled	2022	2023	Pooled
Control-T_1	5.62	5.85	5.74	9.5	10.18	9.84
Girdling at full bloom- T_2	5.3	5.44	5.37	10	10.92	10.46
Girdling after fruit set- T_3	5.79	6.08	5.94	9.12	9.84	9.48
Girdling at pit hardening stage- T_4	5.89	6.18	6.04	9.02	9.75	9.39
Girdling 15 days before harvesting- T_5	5.68	5.96	5.82	9.15	10.05	9.6
CD (p=0.05)	0.16	0.13	0.15	0.26	0.32	0.29

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Effect of Weather Condition on Vegetative Propagation of Black Plum (*Syzygium cumini*) and Glue Berry (*Cordia myxa*)

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Abstract: The experiment on effect of growing season on propagation of glue berry (*Cordia myxa*) and black plum (*Syzygium cumini*) was conducted at the regional research station, CCS Haryana Agricultural University, Hisar during 2017-2023. There were two treatments i.e., time of planting (eight months i.e., February to September) and method of propagation. The maximum success rate of budding 63.3 % was during August and July in black plum and glue berry, respectively over the years. The per cent success rate of soft wood grafting was maximum 53.3 and 56.3 % in August and July in black plum and glue berry respectively. However, in black plum lowest budding and soft wood grafting success was as 10.0 % in February, 2017-23. Similarly, in glue berry minimum budding and soft wood grafting success was 13.3 % in February. Positive and significant correlation existed between percent success budding with minimum temperature (r=0.826) while a negative and significant correlation existed between percent success budding with sun shine hours (r= -0.808) in black plum and glue berry. There was non-significant correlation existed between percent success soft wood grafting with maximum temperature, humidity, rainfall and evaporation weather parameters.

Keywords: *Syzygium cumini*, *Cordia myxa*, Propagation, Temperature, Sunshine

There is a plenty of scope for quantum jump in fruit production in semi-arid areas. These fruits are source of income and nutritional security to inhabitants in arid and semi-arid regions. The importance of underutilized fruits is increasing because people are realizing the potential of these fruits. Looking into the importance of these fruits, the demand of their genuine planting material is increasing day by-day. To meet the demand of planting material, vegetative propagation techniques have been standardized for commercial multiplication. The variability has been observed in plants raised through seeds. Except few plant species, vegetative methods of propagation are used for their multiplication. Propagation through vegetative methods, viz. stem cutting, layering, stooling and grafting have been described for many semi-arid fruits. Under dryland condition, in-situ establishment of black plum orchard has been found successful with better survival (Singh et al., 2020). It is established fact that to harness the maximum efficiency from a crop cultivation, use of genuine planting material is fore most requirement. However, information on propagation techniques for these species is scantily available. Further vegetative propagation is essential for due to heterozygous nature of fruit plants (Tripathi et al., 2022). In order to optimize the production of semi-arid fruit crops, propagation techniques of black plum, glue berry mimusops (*Mimusops hexandra*), elephant apple (*Limonia acidissima*), Madras thorn (*Pithecellobium dulce*), custard apple (*Annona squamosa*), mahua tree (*Madhuca longifolia*), stone apple (*Aegle marmelos*), cuddapah almond (*Buchanania lanzan*) have been standardized for large scale multiplication of plants. For better success and survival of semi-arid fruits, in-situ budding and grafting has been found better with vigorous growth of grafted plants under arid and semi-arid conditions (Singh et al., 2014). Since no attempt has been made earlier to standardized the propagation method of several wild fruits and very little information is available on multiplication of these fruits. The success and subsequent growth of grafted saplings depend on many factors including environmental conditions, variety, method of grafting and selection of scion and rootstock materials (Hartmann et al., 2002). The prevailing environmental conditions are primarily governed by the seasons under which budding and grafting is performed. The climatic factors like light, temperature, rainfall and humidity have a significant influence on the percentage of survival and establishment of grafts (Singh et al., 2020). In view of above present study was undertaken to find out the suitable method of propagation with respect to growing season in black plum and glue berry fruit crops.

MATERIAL AND METHODS

The experiment was conducted at CCS Haryana Agricultural University, Regional Research Station, Rewari during 2017-2023 on glue berry (*Cordia myxa*) and black plum (*Syzygium cumini*) This region is characterized by arid regions with hot and dry summer and cold winter. The experiment was completely randomized design with ten replications of each treatment. The two methods of propagation i.e. T-budding and soft wood grafting in both the crops and also to standardized time of vegetative propagation with eight treatments each *viz*. middle of February, March, April, May, June, July, August and September (eight dates of budding) were taken. The mature fruits were collected during their season. The freshly collected seeds were sown in poly bags. In grafting, One year-old rootstocks were selected was used with 3-4-month-old scion sticks. The data on success and survival of both the methods with respect to growing seasons were recorded. The statistical analysis was done using software OP STAT (Sheoran 2004).

RESULTS AND DISCUSSION

In black plum the maximum success rate of budding (63.3 %) and graft success (53.3 %) was in August over the years whereas the minimum bud intake and graft union success was during the February. The per cent success rate of soft wood grafting was maximum 56.3 % in July glue berry. The maximum bud intake success was in July in glue berry over the years. Similarly, in glue berry minimum budding and soft wood grafting success was 13.3 % in February, 2017-2023. It might be due the variation in graft and bud success in black plum and glue berry due to variation of temperature and relative humidity during different months and seasons. The maximum graft and bud success is directly related to prevailing optimum temperature and higher relative humidity. These congenial weather conditions facilitate early contact of the cambium layer of rootstock and scion resulting in early callus formation and initiation of subsequent growth. Mulla et al. (2011) on softwood grating in black plum observed the highest graft success (100%) in November and May and highest graft survival (93.33%). Uchoi et al. (2012) reported the highest graft survival (90.50%) during January in black plum.

There was positive and significant correlation between % success budding (T) with minimum temperature (r=0.826) in black plum and (r=0.858) glue berry while a negative and significant correlation existed between % success budding (T) with sun shine hours (r= -0.808) in black plum and (r=0.919) in glue berry . There was non-significant correlation existed between % success budding with other weather parameters (Table 1).

There was positive and highly significant correlation between percent success soft wood grafting with minimum temperature(r=0.872) in black plum and (r=0.769) in glue berry, while a negative and highly significant correlation existed between percent success soft wood grafting with sun

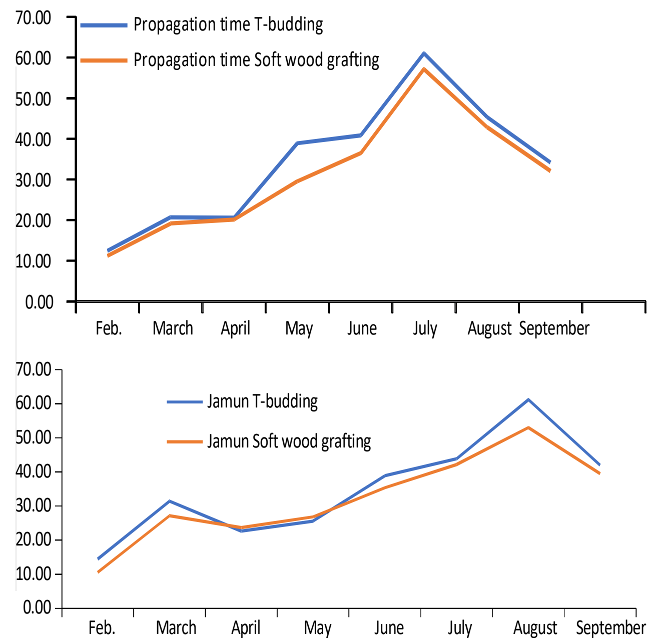

Fig. 1. Effect of seasonal variation on budding and grafting in glue berry and black plum

Table 1. Correlation of success budding (%T) in black plum (*Syzygium cumini*) and Glue berry (*Cordia myxa*) with different weather parameters

Percent budding success	Max T	Min T	Humidity (%) M	Humidity (%) E	Wind velocity	Sun shine	Evaportion	Rainfall
Black plum	0.215^{NS}	0.826*	0.205^{NS}	0.650^{NS}	0.599^{NS}	-0.808*	-0.028^{NS}	0.513^{NS}
Glue berry	0.316^{NS}	0.858**	0.102^{NS}	0.598^{NS}	0.597^{NS}	-0.919**	0.154^{NS}	0.750*

*,** Significant at p<0.05 and <0.01; NS: Non-significant

Table 2. Correlation of success soft wood grafting (%) Black plum (*Syzygium cumini*) & Glue berry (*Cordia myxa*) with different weather parameters

Percent success soft wood grafting	Max T	Min T	Humidity (%) M	Humidity (%) E	Wind velocity	Sun shine	Evaportion	Rainfall
Black plum	0.234^{NS}	0.872**	0.181^{NS}	0.678^{NS}	0.664^{NS}	-0.867**	0.014^{NS}	0.599^{NS}
Glue berry	0.163^{NS}	0.769*	0.237^{NS}	0.720*	0.548^{NS}	-0.946**	-0.016^{NS}	0.765*

*,** Significant at p<0.05 and <0.01; NS: Non-significant

Table 3. Regression equations for percent success T- budding (T) and soft wood grafting in black plum (*Syzygium cumini*) and glue berry (*Cordia myxa*)

$Y_1 = 3.018 X_1+1.82 X_2-1.063 X_3-0.126 X_4-0.008 X_5-8.928 X_6-14.148 X_7-0.053 X_8+121.27$
$Y_2= 1.997X1+1.338 X_2-0.901 X_3-0.311 X_4+4.394 X_5-6.459 X_6-12.197 X_7-0.008 X_8+105.485$
$Y_3 = 0.714 X_1-1.792 X_2+0.567 X_3+3.578X_4-22.435 X_5-2.415 X_6+24.103 X_7-0.216 X_8-153.691$
$Y_4 = 1.839 X_1-2.574 X_2-0.428 X_3+3.201X_4-15222 X_5-5.166 X_6+13.102 X_7-0.163 X_8-53.041$
where Y_1= % success budding (T) in Black plum , Y_2= % success soft wood grafting in Black plum , Y_3=% success budding (T) in Glue berry , Y_4=% success soft wood grafting in Glue berry , X_1= max temp., X_2= min temp., X_3= humidity (%) morning, X_4= humidity (%) evening, X_5= wind velocity, X_6= sun shine hours, X_7= evaporation and X_8= rainfall

shine hours (r= -0.867) in black plum and (r=0.946). There was non-significant correlation existed between % success soft wood grafting with other weather parameters (Table 2). The success/failure of grafting is highly correlated with the environmental conditions under which it is performed. The most important conditions for successful grafting are the selection of the appropriate season of grafting which is conducive to rapid graft healing and subsequently the formation of the graft union.

CONCLUSION

The July month is best for propagation in glue berry whereas August is best for black plum propagation by means of budding and grafting.

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Differential Responses of Planting Time and Auxin on Rooting and Growth Behaviour of Hardwood Cuttings of Wild Pomegranate (*Punica Granatum* L.)

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***Abstract*:** An investigation was carried out to elucidate the influence of planting time and auxin treatments on the rooting and growth of hardwood cuttings of wild pomegranate. The experiment was laid out in Randomized Block Design (Factorial) with 28 treatment combinations comprising 7 auxin levels (IBA 2000, 2500 & 3000 ppm, NAA 2000, 2500 & 3000 ppm and control without IBA/NAA) and 4 planting times (15^{th} & 30^{th} December and 15^{th} & 30^{th} January). 2500 ppm of IBA (auxin) was found to be the optimum concentration used and 30^{th} January was the most suitable time for planting hardwood cuttings of wild pomegranate under subtropical conditions to produce quality plants with a good root and shoot system.

***Keywords*:** Hardwood cuttings, Indole-Butyric Acid, N-Acetic Acid, Wild pomegranate

Wild pomegranate belongs to the family Punicaceae and is one of the important fruit scattered in wild form along the roadsides and upper extremities of sub-tropical forests in Western Himalayan regions of India including Himachal Pradesh, Jammu & Kashmir, Uttarakhand, and Punjab (Rana et al., 2022). *Punica granatum* L. originated from Iran and is found in almost all tropical and subtropical regions of the world and is an important fruit tree growing wild in hilly tracts and forests of Jammu and Kashmir state between 1000-2500 m above mean sea level (Ge et al., 2021). These fruits of wild pomegranate are collected by local people for self-use or petty scale to generate a part of their annual income.

The flowering season of wild pomegranate in the North western Himalayan region is observed to be from March to the last week of May and the collection of ripe fruits usually starts during August and continues upto October (Dhandar and Singh 2002). The Wild pomegranate being resistant to drought and diseases requires less after-care and therefore plays an important role in pushing up the economy of farmers of the areas lacking good cash crops. The rooting ability of cuttings is influenced by several factors, such as the genetic background of the rootstock, their horticultural management, and nutritional status, the age of the stock plant, the cutting collection season, the endogenous content of photoassimilates and hormones, the type of cutting, the environmental manipulation of cuttings, the rooting media, and the hormonal treatment of the tissues (Tewfic 2002). The propagation through cutting with the application of plant growth regulators (PGRs) is one of the most common practices. Auxin is one of the most important PGRs regulating the speed of rooting. Plants produce natural auxin in their branches and young leaves, but synthetic auxin should be applied for better rooting. Natural auxins are more sensitive to catabolism enzymes than synthetic auxins. In a study on the effect of different levels of IBA on the rooting of semi-hardwood cuttings like kiwifruit, the best results have been obtained with the treatment of 4000 ppm IBA. There are no systematic plantations of this crop and plants are available mostly in marginally and neglected areas along roadsides due to a lack of vegetative propagation means. Keeping in view these facts, the present study was conducted with objectives to standardize the doses for auxin and planting time for survival and growth of shoots of rooted hardwood cuttings of wild pomegranate.

MATERIAL AND METHODS

The study was carried out at Research Farm, Division of Fruit Science, SKUAST-Jammu, Chatha from 2020 to 2021, situated at an elevation of 300 m above mean sea level and lies at 32˚43" North latitude and 74˚54" East longitude. The climatic is sub-tropical with hot and dry summer, hot and humid, rainy season and cold winter months. The maximum temperature rises to 45˚C during summer and the minimum temperature falls to 10˚C during winter. The mean annual rainfall is about 1000-1200 mm. The meteorological data regarding the climatic conditions that prevailed during the period of experimentation. The soil of the experimental field is sandy loam with pH of 7.5. The available soil nitrogen, phosphorus and potassium were 237.21, 16.23 and 153.25 kg ha^{-1}, respectively.

Treatment details: The experiment was laid out according to randomized block design (factorial) with seven harmonal treatment combinations with dates of planting replicated thrice. Uniform, healthy, disease-free semi-hardwood cuttings of pencil-size thickness were selected from elite mother plants producing good quality anardana with the help of officials of the state horticulture and forest department in Chennai, Jammu. From the selected branches, 20 cm long cuttings having 4 to 5 nodes were taken from the hardwood portion of the branches. IBA and NAA solutions of different concentrations viz., 2000, 2500 and 3000 ppm were prepared by dissolving 2.0 g, 2.5.0 g and 3.0 g of IBA and NAA in distilled water and the volume in each concentration were made up to 1000 ml by adding distilled water**.** The basal 1.5-2.0 cm portion of the cuttings were dipped in IBA and NAA for 5-10 sec before planting. Cuttings were planted on the beds. The beds contained Soil: Sand: FYM. Weeding and watering were done at regular intervals when needed.

Survival percentage: The number of days taken for first sprouting is calculated observing the sprouting of cuttings daily and data was recorded for initiation of sprouting of each treatment**.** The number of shoots per cutting that emerged was calculated after 90 days of planting. The shoot diameter of the longest shoot was recorded using a vernier caliper and was expressed in millimeters. The length of the longest shoot was recorded from each cutting with the help of scale and measured in centimeters, length of the shoot was taken from point of its emergence to the tip of the fully opened leaf.

Leaf characteristics: The number of leaves per cutting that emerged was calculated after 90 days of planting. For measuring leaf area, five leaves were randomly sampled and their cumulative area was recorded with the help of a leaf area meter, Systronics -211 model at 90 days and expressed as average leaf area in square centimeters (cm^2).

Root characteristics: The mean root length of five roots per cutting was calculated by adding the length of each root of the same cutting and dividing it by the number of roots in that cutting. The diameter of the longest root was calculated using the vernier caliper and was expressed in millimeters. The roots detached from the plant and the average fresh weight of leaves and roots was also calculated. This sample taken for calculating the fresh weight was subjected to a temperature of 60˚C for 12 hours in an oven for drying. Electronic weighing balance was used to calculate the dry weight of leaves and roots only after complete drying of leaves and roots has taken place.

Chlorophyll content: Chlorophyll content in leaves of wild pomegranate was determined by the use of chlorophyll meter SPAD-502 manufactured by Konica Minalta Sensing, Inc. Japan. The data was expressed in percentage.

Partitioning coefficient: The partitioning coefficient of root and shoot of wild pomegranate cuttings was determined to know the distribution of photoassimilates in different parts of pomegranate cuttings (shoot and root) following equation was used to determine the partitioning coefficient of specific plant parts. The partitioning coefficient [(dry weight of plant part/total dry weight) x 100] was expressed in percentage.

Statistical analysis: The data analyses were performed using SPSS-21 software (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

The effect of plant growth regulators and planting dates on the survival rate of wild pomegranate indicated that there were no statistically significant differences between treatments (Table 1). Among different planting dates, 30th January planted cuttings showed maximum mean survival percentage followed by 30th December and minimum mean survival percentage was observed on 15th December. Similarly, wild pomegranate cuttings treated with IBA 2500 ppm resulted in the highest survival percentage and the lowest mean survival of cuttings was observed under control. The interaction effect of plant growth regulators and planting dates on survival percentage in wild pomegranate also exhibited positive effects. The planting dates and doses of plant growth regulator exhibited a positive impact on wild pomegranate. The delayed planting reduced root growth and also restricted soil volume exploited by roots and hence nutrient uptake, hormone synthesis and metabolism in the root system resulting in less survival of cuttings. The better development of the root system with good quality root and shoot parameters enables the rooted cuttings to make better use of nutrients resulting in luxuriant growth.

The minimum number of days for supporting when wild pomegranate cuttings were planted on 30th January and treated with IBA 2500 ppm. Cuttings took maximum number of days for sprouting in untreated cuttings planted on 15th December. Treating the cuttings with growth regulators such as IBA results in earliness in sprouting because of better utilization of stored carbohydrates, nitrogen and other factors. Earliness in sprouting, due to timely planting, the increase in the number of sprouts and sprout length may be due to greater utilization of stored carbohydrates, nitrogen and other factors with the help of growth regulators (Chandramouli 2001).

The maximum number of shoots per cutting is observed with IBA 2500 ppm planted on 30th January whereas, the minimum number of shoots per cutting (2.49) was in control planted on 15th December. Treatment of cuttings with IBA causes an increase in the number of roots which in turn results in an increased number of shoots because the increase in the number of roots enhances the nutrient uptake, which affects the cell division and cell expansion in the cambium resulting in an increased number of shoots (Kamboj et al., 2017). Maximum mean shoot diameter was in cuttings treated with IBA 2500 ppm planted on 30th January, while minimum mean shoot diameter was recorded in untreated cuttings on 15th December planting. This may be due to the maximum number of roots, which helped in nutrition and water absorption. The timely planting of cuttings might have resulted in a better-rooting establishment leading to a higher number of leaves, which in turn led to an increase in shoot diameter, gathering more biomass in the shoot.

The length of the longest sprout per cutting was markedly influenced by plant growth regulators and planting dates. The differences in shoot length may be due to better growth of cuttings during January. Poor growth and success of cuttings during December may be attributed to the reduced rate of division of cambial cells, their differentiation and consequent development in the healing of cuttings. This in turn may be due to decreased synthesis of endogenous auxin and mobilization of reserved food material caused can be correlated to higher cell activity and early sprouting which are responsible for a higher number of leaves and shoot length, thus synthesizing more food material and reduced activity of hydrolysis enzymes. The maximum mean the number of leaves per cutting was in wild pomegranate cuttings treated with IBA 2500 ppm planted on 30th January, which was closely followed by cuttings treated with IBA 2000 ppm planted on 15th January while the minimum mean number of leaves was recorded under control planted on 15th December (Table 2). The increased leaves per shoot might be the result of rapid cell division and cell elongation due to the treatment of cuttings with plant growth regulators. Kepinski and Leyser (2005) also observed that increase in the number of leaves was due to the auxin treatment, which increased the development of primary shoots and their number.

The influence on leaf area was observed during the present investigation with pre-treatment of pomegranate cuttings with rooting hormones and different times of planting. Maximum leaf area was in cuttings treated with IBA 2500 ppm planted on 30th January and minimum leaf area was under control when planted on 15th December. Since, the number of green leaves was significantly influenced by variation in dosages of plant growth regulators and consequently the leaf area also showed variation. The increase in leaf area can be attributed to the joint effect of timely planting and conducive soil and climatic conditions having a positive effect on water holding capacity, porosity, soil aeration and supplying a substantial amount of nutrients for good root and shoot growth over control. The maximum mean fresh and dry weight of stem was recorded when cuttings were treated with IBA 2500 ppm, whereas, untreated cuttings recorded minimum mean dry weight of stem (Table 2). The auxins activated shoot growth, which might have resulted in elongation of stems and leaves through cell division accounting for higher dry weight of shoot (Abraham

Table 1. Effect of hormones on survival and shoor parameters at different treatment dates

Treatment details	Survival percent (Number of days taken for first sprouting)					Number of shoots per cutting (Shoot diameter)				
	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean
T_1- IBA 2000 ppm	75.00 (15.60)	74.95 (15.35)	74.60 (15.20)	76.32 (15.08)	75.22 (15.31)	3.91 (2.48)	4.13 (2.51)	4.22 (2.97)	4.45 (3.01)	4.18 (2.74)
T_2- IBA 2500 ppm	75.82 (12.90)	76.09 (12.75)	76.68 (12.42)	76.73 (12.32)	76.33 (12.60)	4.31 (2.52)	4.18 (2.56)	4.56 (3.06)	4.85 (3.11)	4.48 (2.81)
T_3- IBA 3000 ppm	72.51 (17.00)	74.91 (16.85)	74.37 (16.70)	74.19 (16.45)	74.00 (16.75)	3.71 (2.41)	3.97 (2.45)	4.16 (2.74)	4.35 (2.88)	4.05 (2.62)
T_4- NAA 2000 ppm	73.22 (15.56)	75.16 (15.30)	74.31 (15.04)	73.78 (14.92)	74.12 (15.21)	3.31 (2.38)	3.53 (2.42)	4.13 (2.51)	3.92 (2.59)	3.72 (2.48)
T_5- NAA 2500 ppm	74.29 (13.75)	74.49 (13.55)	75.43 (13.30)	75.15 (13.16)	75.24 (13.44)	4.05 (2.49)	4.09 (2.52)	4.20 (2.61)	4.60 (2.65)	4.23 (2.57)
T_6– NAA 3000 ppm	73.06 (17.85)	73.77 (17.50)	72.58 (17.45)	73.71 (17.26)	73.28 (17.52)	3.27 (2.32)	3.75 (2.36)	3.95 (2.51)	3.76 (2.54)	3.68 (2.43)
T_7- Control	50.72 (19.91)	51.59 (19.75)	51.64 (19.43)	53.79 (19.28)	52.24 (19.59)	2.49 (1.08)	2.59 (1.10)	2.91 (1.14)	2.71 (1.19)	2.68 (1.13)
Mean	70.96 (16.08)	71.53 (15.86)	71.37 (15.65)	72.09 (15.50)		3.58 (2.24)	3.75 (2.27)	4.02 (2.51)	4.09 (2.57)	
	D	T	D x T				D	T	D x T	
CD (p=0.05)	1.27 (0.44)	1.67 (0.58)	3.35(1.17)				0.22 (0.22)	0.28 (0.28)	0.39 (0.39)	

Table 2. Effect of hormones on shoot, leaves and biomass at different treatment dates

Treatment	Length of longest shoots					Number of leaves (Leaf area, cm^2)					Fresh weight of shoots (dry weight of shoot, g)				
	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean
T_1-IBA 2000 ppm	22.69	23.72	24.11	24.56	23.77	206.00 (28.57)	208.67 (28.75)	209.00 (29.45)	210.67 (32.96)	208.59 (29.93)	17.89 (10.68)	17.77 (11.11)	17.90 (11.47)	18.58 (11.45)	18.04 (11.18)
T_2-IBA 2500 ppm	25.51	24.69	25.05	26.38	25.41	210.67 (28.71)	209.00 (30.12)	211.33 (31.56)	214.00 (33.45)	211.25 (30.96)	18.26 (11.26)	18.26 (11.30)	18.48 (11..44)	18.87 (12.09)	18.47 (11.52)
T_3- IBA 3000 ppm	22.45	22.93	23.88	23.72	23.25	208.00 (27.42)	207.00 (28.89)	210.33 (28.53)	210.33 (30.45)	208.92 (28.82)	17.82 (10.49)	17.97 (11.16)	18.05 (11.15)	18.54 (11.41)	18.18 (11.05)
T_4- NAA 2000 ppm	21.34	21.35	21.67	23.08	21.86	205.33 (27.98)	208.33 (27.42)	208.33 (29.77)	208.67 (31.52)	207.67 (39.17)	17.56 (10.79)	17.61 (10.88)	18.00 (11.12)	18.58 (11.42)	18.01 (11.05)
T_5-NAA 2500 ppm	23.08	22.97	24.85	24.99	23.97	208.00 (27.45)	208.67 (29.68)	209.00 (29.56)	211.00 (32.29)	209.17 (29.75)	17.03 (11.08)	17.54 (11.51)	18.00 (11.75)	18.64 (12.03)	17.78 (11.59)
T_6– NAA 3000 ppm	21.52	20.08	20.89	21.58	21.02	203.67 (26.98)	206.67 (27.26)	207.33 (27.88)	208.00 (29.85)	206.42 (27.99)	16.78 (10.38)	17.03 (10.64)	17.93 (11.13)	17.90 (11.15)	17.41 (10.82)
T_7- Control	12.65	12.72	13.75	13.55	13.17	169.00 (16.11)	174.33 (16.88)	173.33 (17.52)	172.00 (18.88)	172.17 (17.35)	13.26 (8.21)	13.50 (8.83)	13.90 (9.31)	14.50 (10.02)	15.65 (9.09)
Mean	21.32	21.21	22.03	22.55	21.78	201.52 (26.17)	203.24 (27.00)	204.09 (27.75)	204.95 (29.91)		16.94 (10.41)	17.10 (10.77)	18.04 (11.05)	18.51 (11.37)	
	D	T	D x T				D	T	D x T			D	T	D x T	
CD (p=0.05)	1.70	2.25	4.50			CD (p=0.05)	1.86 (0.08)	2.45 (0.11)	4.91 (0.22)		CD (p=0.05)	0.38 (0.26)	0.51 (0.35)	1.01 (0.70)	

1996). The total chlorophyll content was appreciably influenced by pre-treatment of rooting hormones and time of planting of wild pomegranate found in cuttings treated with IBA 2500 ppm and planted on 15th January after treatment with NAA. However, the lowest chlorophyll content was observed in untreated cutting planted on 15th December (Table 3). This was due to the the abundant supply of nutrients in balanced quantity and more particularly in addition the optimum time of planting cuttings. Wong et al. (1995) reported chlorophyll content increase in hormones treated plots in comparison to control.

A marked influence on the partitioning coefficient was observed during the present investigation with pre-treatment of pomegranate cuttings with rooting hormones and different times of planting. The maximum partitioning coefficient was recorded in cuttings treated with IBA 2500 ppm planted on 30th January whereas, the minimum partitioning coefficient was recorded in untreated cuttings planted on 15th January. Our result is supported by Law and Davis (1990), who reported that a close correlation was observed between the level of tree auxin and the rate of stem growth in a range of genetic lines of peas differing in height. For maintaining the height of the plant, the highest source-sink relationship shows a better partitioning coefficient of the photosynthates towards the stem. However, the present investigation revealed that wild pomegranate cuttings treated with IBA 2500 ppm and planted on 30th January resulted in maximum length of the longest root. However, the minimum length of the longest root was observed in cuttings planted on 15th December under control. Treatment of cuttings with auxins enhances the hydrolysis of carbohydrates, accumulation of metabolites at the site of application, synthesis of new protein, cell enlargement and cell division, which results in increased length of roots in cuttings. Treatment of cuttings with IBA promotes the translocation of metabolites and carbohydrate metabolism, which ultimately affects the beginning of rooting and increases the length of roots and number of roots. Auxins cause hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings, which results in accelerated cell elongation and cell division in a suitable environment.

The diameter of the longest root was significantly influenced by both rooting hormones and the time of planting of cuttings (Table 4). The maximum diameter of the longest root (1.59 mm) was observed in cutting treated with IBA 2500 ppm and planted on 30th January whereas, a minimum diameter of the longest root was found under control and cuttings were planted on 15th December. Gulifoyle and Hagen (2007) opinioned that auxins would bring about various physiological changes, but the mechanism by which these changes are brought about is not fully understood except for the effect on cell elongation and differentiation. This mechanism of cell differentiation was quite evident in terms of variability in root diameter. Similar results have also been recorded earlier by Bemkaireima et al. (2012) in passion fruit, Seiar (2017) in pomegranate and Rolaniya et al., (2018) in

Table 3. Effect of hormones on chlorophyll and root parameters at different treatment dates

Treatment details	Chlorophyll content (partitioning coefficient of dry shoots)					Average root length, cm (Diameter of longest root, mm)				
	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean
T_1-IBA 2000 ppm	45.79 (66.92)	46.54 (67.66)	48.89 (68.79)	51.35 (69.64)	48.14 (68.25)	22.61 (1.40)	23.59 (1.41)	22.85 (1.44)	24.42 (1.48)	23.37 (1.43)
T_2-IBA 2500 ppm	52.79 (70.51)	54.41 (71.45)	55.33 (72.03)	51.78 (75.14)	53.58 (72.8)	25.45 (1.43)	24.68 (1.47)	24.82 (1.52)	26.16 (1.59)	25.28 (1.50)
T_3- IBA 3000 ppm	46.45 (63.32)	48.05 (68.41)	49.31 (69.31)	50.30 (70.34)	48.53 (68.85)	21.44 (1.36)	22.83 (1.38)	23.81 (1.41)	23.66 (1.44)	22.94 (1.40)
T_4- NAA 2000 ppm	41.44 (67.90)	43.67 (66.38)	44.00 (67.60)	45.28 (68.33)	43.60 (66.55)	21.27 (1.28)	21.24 (1.31)	21.58 (1.36)	22.94 (1.39)	21.76 (1.34)
T_5-NAA 2500 ppm	45.93 (67.25)	47.47 (68.32)	51.38 (70.71)	55.22 (70.99)	50.00 (69.32)	22.72 (1.40)	22.89 (1.41)	24.14 (1.43)	24.87 (1.46)	23.66 (1.43)
T_6– NAA 3000 ppm	42.41 (63.96)	41.72 (64.46)	44.99 (67.54)	46.11 (70.80)	43.81 (66.69)	20.74 (1.29)	19.70 (1.31)	20.58 (1.35)	21.38 (1.38)	20.60 (1.33)
T_7- Control	26.45 (56.23)	29.64 (57.12)	29.53 (56.32)	30.59 (56.64)	29.05 (56.58)	12.67 (0.89)	12.72 (0.91)	13.21 (0.94)	13.14 (1.38)	12.93 (0.93)
Mean	43.04 (65.16)	44.50 (66.26)	46.20 (67.47)	47.23 (68.84)		20.99 (1.29)	21.09 (1.31)	21.57 (1.35)	22.37 (1.40)	
		D	T	D x T			D	T	D x T	
CD (p=0.05)		1.94 (1.08)	2.56 (1.43)	5.13 (2.87)		CD (p=0.05)	1.07	1.42	2.85	

Table 4. Effect of hormones on root biomass at different treatment dates

Treatment details	Fresh weight of roots (dry weight of roots. g)				
	15th December (D_1)	30th December (D_2)	15th January (D_3)	30th January (D_4)	Mean
T_1-IBA 2000 ppm	0.99 (0.83)	0.98 (0.90)	1.00 (1.00)	0.97 (0.99)	0.99 (0.91)
T_2-IBA 2500 ppm	1.04 (0.93)	1.12 (0.91)	1.02 (1.02)	1.11 (1.07)	1.07 (0.97)
T_3- IBA 3000 ppm	0.90 (0.78)	0.93 (0.89)	0.94 (0.94)	0.96 (0.93)	0.93 (0.86)
T_4- NAA 2000 ppm	0.92 (0.77)	0.97 (0.86)	0.93 (0.93)	0.91 (0.93)	0.93 (0.87)
T_5-NAA 2500 ppm	1.05 (0.89)	1.03 (0.94)	0.99 (0.99)	1.06 (1.03)	1.03 (0.95)
T_6– NAA 3000 ppm	0.89 (0.73)	0.93 (0.89)	0.93 (0.93)	0.95 (0.92)	0.92 (0.85)
T_7- Control	0.71 (0.60)	0.72 (0.63)	0.72 (0.72)	0.74 (0.72)	0.72 (0.65)
Mean	0.93 (0.79)	0.95 (0.86)	0.94 (0.87)	0.96 (0.89)	
		D	T	D x T	
CD (p=0.05)		0.05 (0.04)	0.06 (0.05)	0.12 (0.10)	

grapes. Both rooting hormones and the time of planting of cuttings significantly influenced the fresh weight and dry weight of roots. Maximum fresh and dry weight of root were recorded in cuttings treated with IBA 2500 ppm when planted on 30th January and minimum were observed in cuttings planted on 15th January under control. This may be due to the maximum number of primary and secondary roots, higher length, thickness and perhaps the ability to regenerate further new fibrous roots from main roots, which probably absorb more nutrients and water from the soil and resulted in an increment in weight of roots either fresh or dry (Kathrotia and Singh 1995).

CONCLUSION

The cuttings treated with IBA 2500 ppm and planted on 30th January was the most suitable concentration of IBA and time for planting hardwood cuttings, respectively of wild pomegranate under subtropical conditions to produce quality plants.

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Efficacy of Food Bait Attractants in Reducing *Sitophilus oryzae* Populations in Stored Paddy

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Abstract: The rice weevil (*Sitophilus oryzae* L.), a major pest of stored grains, relies heavily on olfactory cues for host selection and localization. This study evaluated the behavioral responses and attraction efficiency of *S. oryzae* to various food-based baits under laboratory conditions using olfactometer bioassays, trap-based assessments and bait ratio trials. Bait traps filled with different food attractants wheat flour, sorghum flour, pearl millet flour and combinations thereof were deployed in a simulated storage environment. Observations over 25 days revealed that wheat flour was the most attractive bait, achieving a total attraction rate of 64.70%, followed by sorghum flour (58.71%). Combination bait trials demonstrated that a 2:1:1 ratio (wheat flour: sorghum flour: pearl millet flour) yielded the highest insect recapture (181 out of 700 released), indicating optimal arresting efficacy. Four-arm olfactometer assays further confirmed the strong olfactory-driven orientation of *S. oryzae* towards wheat and sorghum volatiles. Behavioral observations showed a peak in insect settling at 25 minutes after release (MAR) and highest retention on wheat-based substrates. The results suggest that wheat and sorghum flours can serve as highly effective attractants for the monitoring and management of *S. oryzae* in stored grain systems. This study provides a foundation for the development of sustainable, bait-based pest control strategies in post-harvest storage environments.

Keywords: *Sitophilus oryzae,* Wheat flour, Sorghum flour, Pearl millet flour, Paddy storage godown

Rice is a widely consumed cereal grain and it is the staple food for many people in Asian countries (Devi et al., 2017). The impact of insect-related losses in storage significantly affects food availability. Most commonly the insects, mites, birds, rodents, fungi and moisture are the major problems in storage godowns. Adult beetles, especially stored product insects, exhibit a propensity for seeking shelter in the cracks and crevices of warehouses and storage godowns due to their harborage seeking behavior. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the most widespread and destructive primary pests of stored cereals such as rice, wheat, maize, barley, sorghum, buckwheat, pulses, dried beans, cashew nuts and products derived from them (Nwaubani et al., 2014; Mehta and Kumar 2020). Rice weevils damage rice by boring a hole into a grain or seed to lay their eggs, then sealing the opening. Once the eggs hatch, the larvae feed on the inside of the grain until they mature. The adult weevils then chew their way out, causing considerable loss in both the quantity and quality of the rice (Arafah et al., 2023). Control of this pest is difficult because the immature stages develop inside grain kernels (Mehta et al., 2021). *Sitophilus oryzae* shows host preferences for different stored products in terms of its feeding, development, oviposition and degree of damage (Jalaeian et al., 2021, Mehta and Kumar 2021). Worldwide, chemical control stands out as the most commonly utilized method for managing pests of stored products (Nayak et al., 2020). In addition, the behavior and performance of insects differ depending on the host's physicochemical characteristics, such as the occurrence of toxins, inhibitors, volatiles, macronutrients and micronutrients, as well as kernel hardness and texture. Infestations of *S. oryzae* have been observed in different types of stored commodities, in terms of both damage and the progeny production capacity (Jalaeian et al., 2021, Mehta and Kumar 2021). However, resistance among several species of stored product insects to conventional pesticides has increased over the last few decades (Isman 2006; Hagstrum and Phillips 2017, Nayak et al., 2020). These challenges and the growing awareness of environmental issues have prompted researchers to explore suitable alternatives to chemical pesticides. One such alternative is the volatiles from different grain commodities because they are environmentally friendly and safe for human health (Phillips and Throne 2010; Pavela and Benelli 2016; Hubert et al., 2018). In this study, the behavioral responses of *S. oryzae* to volatiles from different grain commodities, i.e. Crushed groundnut, wheat flour, cracked corn, sorghum flour, rice flour, pearl millet flour, rice bran, rice bran + rice flour, cracked sorghum and control (without bait) were assessed in storage godown and four arm olfactometer bioassays.

MATERIAL AND METHODS

Bait traps, filled with attractants, are strategically positioned between the stacked bags in the paddy storage godown. Designed with a 4mm entrance, they guide insects into the lower polyethylene receptacle (Fig. 1). Trap catches were recorded at 5, 10, 15, 20 and 25 days after bait installation (DAI). The number of insects captured in the control (without bait material) was compared with those caught in the various treatment containing bait (Sathiyaseelan et al., 2022). The attraction index was calculated (Smith et al., 1993).

$$\text{Attraction Index} = \frac{T\text{-}C}{N}$$

T- Number of insects drawn to the treatment, C- Number of insects drawn to the control

N - Total number of individuals

Rearing of test insects: Adults of the rice weevil, *Sitophilus oryzae*, were reared in plastic jars containing a mixture of wheat flour and whole grains as their diet. Each jar was provisioned with 250 g of grains and 20 to 30 insect pairs, and covered with a piece of kada cloth secured by rubber bands to allow ventilation while preventing escape. The rearing was conducted under controlled environmental conditions, with a 12:12 hour light: dark photoperiod, temperatures maintained between 26–28 °C, and relative humidity levels between 60–65%. All experiments were carried out under these standardized conditions to ensure consistency in culture maintenance.

Trapping efficiency of different food bait mixture: Based on initial bait attractiveness, three promising food bait sources were selected for further evaluation. An experimental setup was designed consisting of four 150 g capacity containers connected to a central 2-litre container using PVC pipes positioned at a 45° angle. The selected bait materials wheat flour, sorghum flour and pearl millet flour were combined in different ratios 1:1:1, 2:1:1, 1:2:1 and 1:1:2 with 20 g of each mixture placed in the respective chambers. A total of 700 *Sitophilus oryzae* adults were released into the central arena, and insect movement towards the bait mixtures was recorded 24 hours after release (HAR) (Fig. 2).

Behavioural / Orientation studies: The test insects, including the rice weevil, *S. oryzae*, underwent a 24h period of starvation in Petri plates prior to initiating the olfactory bioassay. Subsequently, 100 adult insects of mixed sex were introduced into the central chamber of the olfactometer, which featured a 7 mm aperture for movement. To minimize light-induced orientation, the entire setup was covered with cloth. The distribution of *S. oryzae* in response to different bait treatments was recorded at 5, 10, 15, 20 and 25 minutes after release (Vijay et al., 2020). Each treatment, involving wheat flour, sorghum flour and pearl millet flour as attractants, was replicated five times to ensure accuracy and reliability of the results (Fig. 3).

Statistical analysis: The data on attraction index and behavioural response/orientation of *Sitophilus oryzae* were statistically analyzed using a completely randomized design . The analysis was conducted using IBM SPSS Statistics version 22.0, differences considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The different attractants revealed significant variation in

Fig. 1. Structural design of the insect bait trap

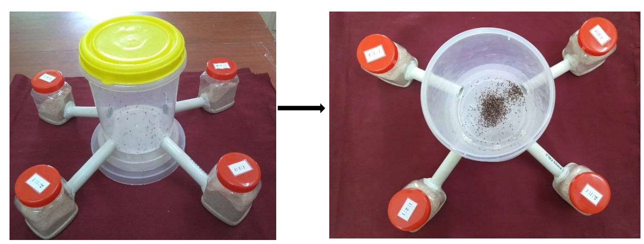

Fig. 2. Test chamber for evaluating insect attraction toward food bait mixtures

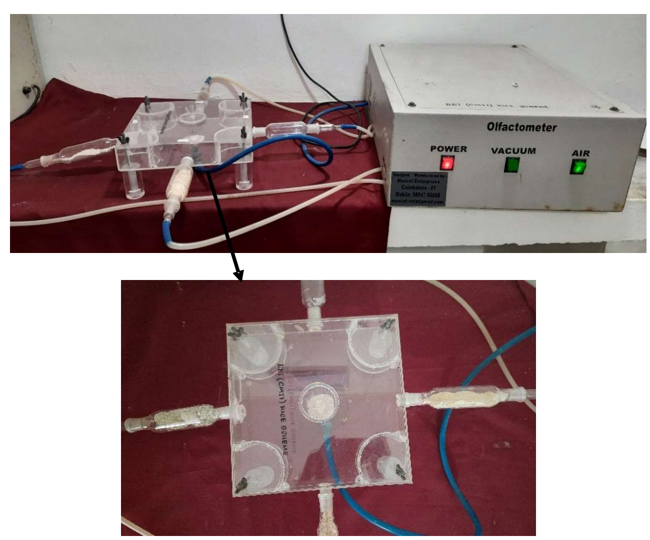

Fig. 3. Four-arm olfactometer arrangement for bioassay

their ability to attract the target species over a 25 days period. The wheat flour was the most attractive substrate, recording the highest total attraction of 64.70%. The weevils showed a consistent preference for wheat flour, with peak attraction observed on the 10th day after placement. Sorghum flour also elicited a strong response, with a total attraction of 58.71%, indicating its effectiveness as an attractant. Cracked sorghum attracted moderate weevil activity, with a total response of 44.90%. Other attractants such as pearl millet flour, rice flour and cracked corn showed intermediate levels of attraction, registering total values of 34.37, 28.46 and 27.60%, respectively. Rice bran and the combination of rice bran with rice flour recorded lower attractiveness, with total responses of 32.25 and 20.41%. Among all the tested substrates, crushed groundnut was the least attractive, with a total attraction of only 19.67%, suggesting limited interest from the weevils. The control treatment, showed no weevil activity throughout the observation period. These findings suggest that wheat flour and sorghum flour are highly attractive to *S. oryzae* and may be effectively utilized in monitoring or management strategies for this pest in stored grain systems (Table 1). Rice is recognized as a preferred host plant for *S. oryzae* (Subedi et al., 2009).

The response of *Sitophilus oryzae* to different bait source ratios composed of wheat flour, sorghum flour and pearl millet flour was assessed to determine the most effective combination for arresting insect movement. The 2:1:1 ratio (wheat flour: sorghum flour: pearl millet flour) recorded the highest recapture, with 181 insects, representing the most effective ratio for insect arrest. This was followed closely by the 1:2:1 ratio, which recorded 179 insects, and the 1:1:1 ratio with 162 insects. The 1:1:2 ratio was the least effective, with only 137 insects recaptured. Out of the total 94% were recaptured, while 6% insects did not respond to any of the bait treatments, possibly due to mortality or lack of attraction under the test conditions (Table 2).

Orientation of stored product insect in four-way olfactometer: Olfactometer bioassay revealed the significant variations on orientation/behavioural response of *S. oryzae* towards wheat flour, sorghum flour, pearl millet flour and control (without food bait) in a four-arm olfactometer. There was indicated significant variation in the percentage of insects settling on different food sources throughout the study duration, reflecting differences in the relative attractiveness and retention potential of each attractant over time. Wheat flour consistently attracted the highest percentage of settling insects. The settling increased steadily from 21% at 5 MAR to a peak of 38% at 25 MAR, indicating its sustained attractiveness over time. Sorghum flour also demonstrated an initially strong response, attracting 25% at 5 MAR, but the settling percentage declined

Table 2. Effect of bait source ratio on the arrest of insect movement (wheat flour: sorghum flour: pearl millet flour)

Bait ratio	Percent of insect captured	Number of insects recaptured
		S. oryzae
1:1:1	23.14	162
2:1:1	25.85	181
1:2:1	25.57	179
1:1:2	19.57	137
Total		660
Not responded		40

Table 1. Comparative response of *Sitophilus oryzae* to various food attractants

Attractants	**Relative attraction index (%)					Mean attraction	Total attraction (%)
	5 DAP *	10 DAP	15 DAP	20 DAP	25 DAP		
Crushed groundnut	5.23	4.69	3.35	3.77	2.63	3.93	19.67
Wheat flour	7.29	16.89	13.81	13.32	13.39	14.35	64.70
Cracked corn	6.18	5.61	3.97	6.28	5.56	5.52	27.60
Sorghum flour	5.84	13.19	14.40	13.52	11.76	11.74	58.71
Rice flour	5.18	5.67	5.37	5.06	7.18	5.69	28.46
Pearl millet flour	7.68	5.56	3.27	9.69	8.17	6.87	34.37
Rice bran	4.59	5.56	6.84	8.07	7.19	6.45	32.25
Rice bran + Rice flour	6.68	4.63	3.35	2.49	3.26	4.08	20.41
Cracked sorghum	10.90	9.43	8.84	7.56	8.17	8.98	44.90
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD Value	0.293	0.243	0.290	0.230	0.180	-	-

*DAP – Days after placement, **Mean of three replications

Table 3. Behavioural/Orientation response of *Sitophilus oryzae* to various food attractants

Food attractants	*S. oryzae* settled (%)					Mean attraction
	5 MAR*	10 MAR	15 MAR	20 MAR	25 MAR	
Wheat flour	21	26	33	31.33	38	29.87
Sorghum flour	25	20.33	16	24	20	21.07
Pearl millet flour	15	18	19	17	12	16.20
Control (without food)	4.67	4.67	5	5	4	4.67
Unsettled	34.33	31	27	22.67	26	28.20
CD Value	1.864	1.251	1.352	1.105	0.616	-

*MAR- Minutes after release

in the subsequent observations, dropping to 16% at 15 MAR before showing a slight increase by 20 MAR and a decline again at 25 MAR. This trend suggests a reduction in its long-term attractiveness compared to wheat flour. The relatively low and stable response indicates that pearl millet flour is less effective in encouraging insect settlement. The percentage of unsettled insects decreased over time from 34.33% at 5 MAR to 22.67% at 20 MAR, suggesting that more insects were gradually responding to the attractants, particularly wheat flour. However, a slight increase to 26% at 25 MAR indicates some fluctuation in insect behavior, possibly due to environmental or internal factors influencing activity (Table 3).

Responses to plant extracts and pheromones were researched by (Athanassiou et al., 2006), the traps with baits like oil and seeds were more attractive than traps without bait *to T. confusum* and *S. oryzae*. Vijay et al. (2020) observed that *Sitophilus oryzae* showed the strongest orientation towards sorghum.

CONCLUSIONS

The study demonstrates that *Sitophilus oryzae* exhibits distinct preferences for specific food-based attractants, with wheat flour emerging as the most attractive and consistently effective substrate across a 25-day period. Sorghum flour also proved to be a strong attractant, particularly in the initial days, while pearl millet flour showed moderate effectiveness. Combination baits, especially the 2:1:1 ratio of wheat flour, sorghum flour and pearl millet flour, resulted in the highest insect recapture rates, indicating their potential in enhancing bait efficacy. The four-way olfactometer assays confirmed that *S. oryzae* and related stored product pests are strongly oriented towards cereal-based volatiles, with minimal response to control setups lacking food cues. Based on these results, it is recommended that wheat flour and sorghum flour, either alone or in optimized ratios, be employed as key components in bait formulations for monitoring and managing *S. oryzae* populations in stored grain environments.

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Diversity of Orb-Weaving Spiders in Sree Kerala Varma College Campus, Kerala

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Abstract: The study aims to assess the diversity of orb-weaving spiders in Sree Kerala Varma College campus. A total of 19 species belonging to 14 included in genera of 4 families were identified. The family Araneidae was the dominant family with 8 species belonging to 6 genera *Anepsiora, Argiopa, Cyclosa, Cryptophora, Gastrocanella* and *Neoscana*. The family Tetragnathidae was the second dominant family comprising 6 species belonging to 3 genera *Leucauge, Tetragnatha* and *Tylorida*. The family Uloboridae comprises 3 species belonging to the 3 genera *Uloborus*, *Philoponella* and *Zosis* and the family Theridiidae comprises 2 species belonging to the genera *Argyrodes* and *Nihonhimea*.

Keywords: Araneidae, Checklist, Diversity, Orb-weavers, Structural complexity

Of the orb-web weaving spider families (e.g., Araneidae, Tetragnathidae and Uloboridae) are distinct or diverse and well known for their web pattern. As orb-wearers age, they tend to have less production of silk, many adult orb-weavers can then depend on their coloration to attract more of their prey (Galvez et. al., 2018). The capacity of orb-web species to catch the prey, led orbicularians to become the most dominant predators in many ecosystems (Blackledge et al., 2009). In orb-weavers, there are two major radiations of three-dimensional web spinning spiders; the cobweb spinning Theridiidae (Agnarsson 2004, Arnedo et al., 2004) and the aerial sheet-web spinning Linyphiidae (Arnedo et al., 2009, Miller 2007).

Araneoidea monophyly is well supported by both morphological and molecular data. Although Griswold et al. (1998) found homoplasious occurrences in non-orb-weaver species of some classic araneoid characters; several unambiguous morphological synapomorphies support Araneoidea, most notably the paracymbium and flagelliform and aggregate silk glands. Mimetidae is placed in Arachnoidea because of the presence of putative vestigial homology (Hormiga and Griswold 2014). Deinopoidea is also called the cribellate orb builder spider. Deinopoid web architecture is unique, web building behavior is homologous to other orb-weavers (Hormiga and Griswold 2014). The family Araneidae, one among the families of orb-weavers exhibits a worldwide distribution (Foelix 1982). It includes 3093 species under 184 genera (WSC 2022). The living fauna of Theridids are classified into 125 genera, constituting 2537 species around the world (WSC 2022). Theridiid webs are those structures of viscid silk lines that are apparently function in one way or another in prey capture (Eberhard et al., 2008).

Synotaxidae are slender and they resemble theridiid. It comprises 11 species under single genus (WSC 2022). Synotaxids occur mostly in the temperate parts of South America, Australia, and New Zealand. They usually build sheet or dome webs beneath which they hang, although some are tiny kleptoparasites that inhabit webs of other spiders (Hormiga and Griswold 2014). Cyatholipidae includes 23 extant genera and 58 extant species scattered across Africa, Madagascar and New Zealand. The family also has a rich European fossil record from Oligocene to Miocene amber deposits from Germany and Baltic (Hormiga and Griswold 2014). Synaphridae is a small (3 genera and 13 species), cryptic, and poorly known group of araneoids from southern Europe, the Middle East, and Africa (WSC 2022). Uloboridae, is a cribellate orb weaver. They are non-venomous spiders and also known as hackled orb weavers. This study, attempts to generate a preliminary checklist of orb-weaving spiders in Sree Kerala Varma College Campus, located in Kanattukara, Thrissur district, Kerala.

MATERIAL AND METHODS

Study area: The study was undertaken in Sree Kerala Varma College Campus. It is located in Kanattukara, west of Thrissur district, Kerala. It has a longitude of 76° 11 '48.84 " E and a latitude of 10° 31' 49.44” N. The campus covers about 30 acres of land and the annual temperature varies from 23° c to 32° c. The major plant communities are *Hibiscus indicus, Syzgium cumini, Psidium guajava, Mangifera indica, Delonix regia.*

Sampling: Observations of orb-weaving spiders were undertaken from February 2022 to July 2022. Spiders were collected early morning between 7.30 am - 9.30 am and late evening between 4.00 pm - 6.00 pm. The orb-weaving spiders were collected by different methods like aerial hand collection, ground hand collection, sweep netting, water spraying method and vegetative beating. The collected specimens were preserved in 70% ethyl alcohol. The identification of adult spiders was done by using taxonomic keys, available literature (Tikder and Malhotra 1980, Barrian and Listinger 1995, Sebastian and Peter 2009) and catalogues provided by NMBE (World Spider Catalogue 2022). Preserved specimens were examined under a stereo zoom microscope (Leica-M205C) in the laboratory for taxonomic identification. Photos of live specimens were done by using a digital camera and lens (Canon EOS 5D digital SLR and Canon 180 mm macro lens). Identification and classification were also done on the basis of morphometric characters of various body parts. The identification is also based on salient features like, presence of two or three claws, presence or absence of cribellum, number of cheliceral teeth, presence of one or two pairs of book lungs. Adult specimens were identified up to the species level**.**

RESULTS AND DISCUSSION

Nineteen species belonging to 14 genera of 4 families from four different families Araneidae, Tetragnathidae, Theridiidae and Uloboridae were recorded (Table 1). The family Araneidae was the most dominant orb-weaving family. They are typical orb-weavers and the orb-web has particular

Table 1. Checklist of orb-weaving spiders

Family: Araneidae (Clerck 1757)
Anepsion maritatum (O. Pickard-Cambridge 1877)
Argiope anasuja (Thorell 1887)
Argiope pulchella (Thorell 1881)
Cyclosa confraga (Thorell 1892)
Cyclosa hexatuberculata (Tikader 1982)
Cyrtophora cicatrosa (Stoliczka 1869)
Gasteracantha geminata (Fabricius 1798)
Neoscona nautical (L. Koch 1875)
Tetragnathidae (Menge 1866)
Leucauge decorate (Blackwell 1864)
Leucauge fastigata (Simon 1877)
Tetragnatha keyserlingi (Simon 1890)
Tetragnatha viridorufa (Gravely 1921)
Tylorida striata (Thorell 1877)
Tylorida ventralis (Thorell 1877)
Theridiidae (Sundevall 1833)
Argyrodes flavescents O. Pickard-Cambridge 1880
Nihonhimea mundula (L. Koch 1872)
Uloboridae (Thorell 1869)
Philoponella feroka (Bradoo 1979)
Uloborus danolius (Tikader 1969)
Zosis geniculata (Olivier 1789)

1. Araenidae Simon 1895 (Plates 1-5)


Plate 1. *Arjiope anasuja*


Plate 2. *Arjiope pulchella*


Plate 3. *Cryptophyta cicatrosa*


Plate 4. *Cyclosa confraga*

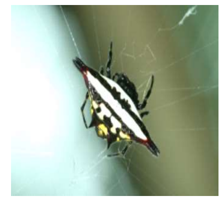
Plate 5. *Gasteracantha geminata*

2. Tetragnathidae Menge, 1866 (**Plates 6-10**)


Plate 6. *Leucauge decorata*


Plate 7. *Tetragnatha keyserlingi*

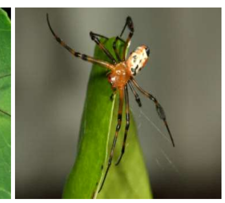
Plate 8. *Leucauge fastigata*


Plate 9. *Tylorida striata*


Plate 10. *Tylorida ventralis*

3. **Uloboridae** Thorell,1869 **(Plates 11-12**)


Plate 11. *Philoponella feroka*

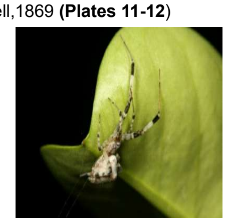
Plate 12. *Zosis geniculata*

4. Theridiidae Sundevall, 1833 (**Plates 13-14**)


Plate 13. *Argyrodes flave*


Plate 14. *Nihonhimea mundula*

Plates 1-14. Orb weaving spiders identified from Sree Kerala Varma College Campus, Thrissur, Kerala geometrical precision. Out of the four different families, Araneidae comprises eight species belonging to 6 genera, *Anepsion, Argiope, Cyclosa, Cyrtophora, Gastracantha and Neoscona*. The family Tetragnathidae was the second dominant family comprising 6 species belonging to 3 genera *Leucauge, Tetragnatha* and *Tylorida*. The family Uloboridae comprises 3 species belonging to the 3 genera *Uloborus*, *Philoponella* and *Zosis*. The family Theridiidae comprises 2 species belonging to the genera *Argyrodes* and *Nihonhimea*.

The study of specific diversity of orb-weavers are not common. Lowe et al. (2014) documented a total of 33 species belonging to 6 families in Caribbean Islands. The diversity was assessed by collecting samples from seven Islands in the Caribbean Island. In the present study also the family Araneidae is the most diverse family. The dominance of this family in the study area and the Caribbean Islands is directly proportional to the vegetational architecture (including gaps in the vegetation). Vegetation which is structurally more complex can sustain a higher abundance and diversity of spiders (Andrew and Hughes 2004). Vegetation provides an extensive option of microhabitat selection, web attachment and prey capture, especially among web builders (Haddad et al., 2009). Memah et al. (2014) suggested that the diversity and abundance of spider communities are generally determined by the complexity of the structure of the plant and their environmental conditions. Spiders usually exhibit humidity and temperature preferences that limit them to areas within the range of their physiological tolerances. Presence of large web near water bodies, construction of web in horizontal plane and construction of web over water are the important web characters of the collected genera of Tetragnathidae family. Presence of sticky silk, calamistra hairs are the major web characters of the collected genera of Uloboridae family. Family Theridiidae has web with araneophagy guy lines, viscous silk and suspended egg sac. These web characters of different genera contribute very much to the diversity by ensuring easy prey capture methods

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Assessing *Meloidogyne Incognita* Population Dynamics In Tomato: Comparative Study of Open Field and Polyhouse Conditions

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Abstract: Root knot nematode is an economically important pathogen affecting the crop in both the cultivation systems *i.e.* open and protected. The present research revealed that the reproduction of *M. incognita* population was comparatively higher in polyhouse as compared to open cultivation of tomato. Low inoculums levels reached to pathogenic levels due to higher multiplication of root knot nematode in polyhouses as compared to open cultivation system. There is substantial impact on buildup of nematode populations under prevailing environmental conditions. The infestation of *M. incognita* affected the growth parameters and health of the crop and the effects were more at higher inoculum levels in both the cultivation systems. Comparatively, due to higher multiplications, the infestation of root knot nematode may have more severe impact on the same crop grown in polyhouse.

Keywords: Agro-ecosystem, Inoculum levels, *Meloidogyne incognita*, Tomato

Plant-parasitic nematodes cause yield losses of $173 billion annually worldwide (Pires et al., 2022). Root-knot nematode, *Meloidogyne incognita* is one of the major limiting factors in production of tomato crop (Qiao et al., 2012, Onkendi et al., 2014, Abrar et al., 2020). Yield losses of 22-30% have been reported on tomato due to *M. incognita* (Ahmed et al., 2023). However, the losses caused by this nematode may vary according to the species. In India on an average, a national loss of tomato Rs 6035.2 million has been estimated due to plant parasitic nematodes (Kumar et al., 2018). Out of 100 species of *Meloidogyne* which were present throughout the world, four species *viz. Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* are commonly found in India (Khan et al., 2023). Among these four species, *M. incognita* is predominantly associated with tomato cultivation in India.

Tomato is grown worldwide in diverse agro-ecosystems in open fields as well as under protected structures /polyhouses. On the other hand, in protected cultivation, there are polyhouses which are framed structures covered with transparent or translucent material and are large enough to grow crops under partial or full controlled environmental conditions to obtain optimum growth and yield. This partial control of microclimatic conditions may have differential influence on prevalence, abundance or buildup of pathogens. The present studies have therefore been undertaken to know the buildup of root knot nematode at different inoculum levels in open and polyhouse cultivation of tomato.

MATERIAL AND METHODS

Experimental design: The experiment was conducted in two sets of environment from October to December (winter season crop) in open and protected cultivation for consecutive two years (2017 and 2018). The buildup of root knot nematode was estimated at different inoculum levels *i.e.* 100, 500, 1000, 2000, 4000 and 8000 juveniles/pot. The study showed no significant difference, so the two sets of data were combined for final analysis. In open field condition/natural conditions at Punjab Agricultural University, Ludhiana, 5x5 sq.m area was marked and covered with plastic sheet on which pots were kept. In second agro-ecosystem *i.e.* protected cultivation, in a polyhouse area was 250 sq.m. at Department of Plant Pathology, Ludhiana and area of 5x5 sq.m. was covered with plastic sheet on which pots were kept. In open cultivation, temperature from October to December ranged from 22.2°C to 32.7°C (2016-17) and 20.9°C to 33.3°C (2017-18) while the range of temperature was 27.1°C to 37.3°C (2016-17) and 25.1°C to 38.9°C (2017-18) in polyhouse. Pots were filled with sterilized soil and then ten seeds of tomato var. Punjab Ratta were sown in pots containing loamy sand soil (Sand-75.5%, Silt-19.3%, Clay-5.2%, ph-7.6, EC-0.24 dS m^{-1}, Organic matter-0.672%). At two leaf stage, the plants were inoculated with root knot nematode juveniles collected from pure culture obtained from single egg mass technique given by Zakaria (2013). In single egg mass technique, galled roots of tomato were carefully washed using gentle flow of water to remove the adhering soil particles. One egg mass was collected by the aid of a needle specially adapted for this technique. The obtained culture was reared on tomato seedlings planted in pots filled with sterilized soil. Pots were kept under glasshouse conditions for 45–60 days to maintain the nematode inoculum for further studies. The egg masses were collected from these plants and root knot nematode female was identified by morphological characteristics and perennial patterns of females. *M. incognita* females illustrated the presence of a high, squarish dorsal arch, which contains a distinct whorl in the tail terminal area. The striae are smooth to wavy. Distinct lateral lines are absent, but breaks and forks in striae are obvious.

Six inoculum levels were used *i.e.* 100, 500, 1000, 2000, 4000 and 8000 juveniles. These juveniles were concentrated in 5ml of water and added to soil by making 4-5 holes around the stem. The un-inoculated plants served as control. Each treatment was replicated four times in completely randomized factorial design ..

Data collection: Observations on soil nematode population and growth parameters were taken. The nematode infestation in roots was assessed on the basis of number of number of galls per root system and was referred to as root galling index (GI). The egg mass count was taken by manually counting the egg masses on 3g of root.

Plant growth parameters: The plants were uprooted after sixty days of inoculation. The roots were washed carefully with water to remove all the debris. The length and fresh weight of shoot and root were measured using centimeter scale.

Nematode population in soil as well as roots: Galling was scored on scale of 0-5 rating chart by Taylor and Sasser (1978) where 0 = no galls; 1 = 1–2; 2 = 3–10; 3 = 11–30; 4 = 31–100; and 5 = more than 100 galls. Washing of the individual soil sample was done by using Cobb's sieving and decanting technique (Cobb 1918, Schnidler 1961). This is the basic technique which involves the principle of gravity. The difference in the specific gravity and size between soil components and nematodes were used. Nematodes being lighter in weight can be disunited from the soil using this method. In this method, intermingling of the soil with voluminous water is done and then the admixture is poured through set of sieves of different mesh sizes (20, 200 and 325 mesh sieve) to retain nematodes. First of all, the individual sample was mixed thoroughly and then 250cc of the soil was taken using 250 ml beaker and poured into a plastic tray A of large capacity. Then, approximately 1litre of the water was added to the tray and the admixture was mixed properly by continuously stirring with hand and allowed to settle the debris or heavy particles for ten seconds. After that decantation was done by passing through a coarse sieve (20 mesh sieve) into another plastic tray B. During this procedure along with water suspension in tray B nematodes were also carried. The left over material in tray A and on the coarse mesh sieve were discarded. The mixture in the tray B was mixed, allowed to settle for 10 seconds and then poured into the tray A through 200 mesh sieve. The residue left in tray B was discarded. The content of 200 mesh sieve was backwashed and collected in the beaker. The mixture in tray A was again mixed, allowed to settle for few seconds and then poured into tray B through 325 mesh sieve and the residue in the tray A was discarded. The contents of 325 mesh sieve which includes the nematodes were backwashed using squeezed water bottle into a beaker. The suspension collected in the beaker was then passed through a tissue paper, which was placed on aluminium wire gauze. After that a Petri dish (10 cm in diameter) filled with distilled water was taken and the gauze along with tissue paper was placed over it in such a way that the gauze may touches the surface of water. Then, the sample was allowed to be undisturbed for 24 hours. After 24 hours, the suspension in the petiolate was collected and observed under stereo zoom binocular microscope for the nematode examination. Reproduction factor (Rf) which is the final population divided by the initial population was calculated. The roots of each plant were washed under tap water and spread on a white paper and on the basis of number of galls each plant was graded as given below: The root galling index was calculated.

$$RGI = \frac{\text{Sum total of grades of all the plants observed}}{\text{Total number of plants observed}}$$

Statistical analysis: The differences among means were compared by Tukey method ($P<0.05$). Nematode variables were regressed as the dependent variable with the initial inoculum level as the independent variable under two agro-ecosystems.

RESULTS AND DISCUSSION

Effect on soil nematode population, root galling index and growth parameters: The buildup of nematode population was greater in polyhouse as compared to open conditions (Table 2, 3) and even low inoculum levels crossed threshold levels in polyhouse indicating supportive build up for root knot nematode populations. The inoculum level of Pi=500 exhibited higher multiplication in polyhouse and thus reaching pathogenic levels 2025 J2/kg soil (>1J2/g soil) while at the same inoculum level the final population was 799.50 juveniles in open cultivation which was relatively less than the pathogenic levels marked for nematode species (1J2/g of soil) (Table 2, 3). The comparative account of root galling index (RGI) also indicated that number of galls were observed were higher in polyhouse cultivated tomato (Table 3). The number of egg masses were more on roots of tomato grown in polyhouse though it was not significant except at Pi= 500 (Table 3). In the two ecosystems, comparatively, the percent reduction in shoot length and shoot weight of tomato was higher in polyhouse as compared to open cultivation up to moderate levels of inoculum indicating more severity in polyhouses in comparison to control (Pi = 8000 J2) (Table 1).

Effect of different inoculum levels of *Meloidogyne incognita* on root galling and soil nematode population: The tomato plants inoculated with second stage juveniles of *Meloidogyne* spp. significantly affected both root galls and nematode's population after harvest. Significant increase in number of galls was also observed at all inoculum levels in polyhouse as well as open conditions. The increase in initial levels of *M. incognita* resulted in significant increase in soil nematode population, number of egg masses and root galling index (RGI) (Table 2, 3). Multiplication of nematode was observed at all inoculum levels. The highest number of egg masses were at Pi= 8000 and lowest at Pi= 100 (Table 4). Reproduction factor of nematode was reduced as initial population levels of nematode increased and minimum at Pi= 8000 (Table 3). Soil nematode population was observed to be highest at Pi= 8000 and minimum at Pi= 100. Soil nematode population was indicated direct relationship with inoculum levels (Table 2). The presence of root galls and number of root knot nematode juveniles inoculated has a strong negative correlation with the growth parameters. This indicate, with increase in the inoculums level, the root galling

Table 1. Effect of different inoculum levels of root-knot nematode on shoot length and shoot weight of tomato under polyhouse and open field conditions

Inoculum levels	Per cent reduction in shoot length (cm)		Per cent reduction in shoot weight (g)	
	Polyhouse	Open	Polyhouse	Open
100 J2s	0.79	2.24	4.68	9.27
500 J2s	11.03	4.59	20.97	14.5
1000 J2s	17.01	8.54	23.90	16.12
2000 J2s	20.83	13.97	33.70	19.75
4000 J2s	39.84	28.37	54.68	29.33
8000 J2s	81.74	71.89	66.85	50.30

Table 2. Effect of different inoculum levels of root-knot nematode on final soil nematode population in tomato under polyhouse and open field conditions

Agro-ecosystem	Soil population per 250 cc soil						
	0 J2s	100 J2s	500 J2s	1000 J2s	2000 J2s	4000 J2s	8000 J2s
Polyhouse	0.00[g]	249.50[f]	2025.00[e]	2483.00[d]	4325.00[c]	5799.50[b]	6033.16[a]
Open	0.00[g]	201.00[f]	799.50[e]	2166.50[d]	3153.00[c]	3841.50[b]	5316.66[a]

Means sharing common letters within columns do not differ significantly by Tukey's test at P < 0.05%.

Table 3. Effect of different inoculum levels of root-knot nematode on root galling index , egg mass index and reproduction factor of tomato under polyhouse and open field condition

Agro-ecosystem	Root gall index (RGI) 0-5 scale						
	0 J2s	100 J2s	500 J2s	1000 J2s	2000 J2s	4000 J2s	8000 J2s
Polyhouse	0.00[g]	1.16[f]	1.83[e]	2.50[d]	3.50[c]	4.16[b]	4.83[a]
Open	0.00[g]	1.00[e]	1.33[e]	2.16[d]	3.16[c]	3.83[b]	4.83[a]
Egg mass index (EMI)							
Polyhouse	0.00[g]	14.80[f]	36.60[e]	43.30[d]	55.60[c]	94.30[b]	106.20[a]
Open	0.00[f]	12.30[e]	15.90[e]	39.50[d]	51.90[c]	92.50[b]	102.45[a]
Reproduction factor (Rf)							
Polyhouse	0.00	2.49	4.05	2.48	2.16	1.44	0.75
Open	0.00	2.01	1.59	2.16	1.57	0.96	0.66

Means sharing common letters within columns do not differ significantly by Tukey's test at P < 0.05%.

of tomato plant increased which showed a significant negative impact on the plant height and weight. that increased number of nematode juveniles have a strong positive correlation with the presence of root galling. This implies that, as nematode population increases in the rhizosphere of the tomato plant, the presence of root galling of tomato plant roots also increases. The increase in initial inoculum levels of *M. incognita* was significant and increase in soil nematode population, number of egg masses and root galling index (GI). Multiplication of root knot nematode was observed at all inoculum levels. The highest number of egg masses were observed at Pi= 8000 and lowest at Pi= 100. However, reproduction factor of root knot nematode was reduced as initial population levels of nematode increased and was minimum at Pi= 8000 (Table 3).

Effect of inoculum levels of *Meloidogyne incognita* on growth parameters of tomato: The growth parameters of tomato were affected by infestation of *M. incognita* in both the cultivation systems. Shoot length as well as shoot weight decreased with increase in inoculum level of the nematode (Table 1). In tomato plants inoculated, minimum reduction was found in plants inoculated with Pi= 100, while maximum decrease in shoot length and weight was observed at Pi= 8000. The differential rates of buildup of nematodes in the two ecosystems. The infestation of *M. incognita* in the polyhouses was significantly higher as compared to open cultivation of tomato at all inoculum levels. This may be due to the difference of environmental conditions prevailing in the protected and open ecosystems. The multiplication of *M. incognita* was higher in polyhouse conditions and even low inoculum load in soil crossed the threshold levels (the minimum intensity or amount that must be reached for a specific event, or condition to occur or be noticeable) and had greater effects on the health of the crop as compared to plants which were grown in open field. Ndiffon (2024) reported that the overall multiplication rates are influenced by nematode species, the susceptibility of the host and the various environmental factors. Nematodes being invertebrates, have their life cycle dependent upon the environmental temperature. Their reproductive rates and metabolism are directly proportional and respond to fluctuations in temperature. Monitoring of temperatures during the conduct of experiment revealed that it was comparatively higher in polyhouses as compared to open. The, increased temperatures generally increase plant growth rates, which in turn gives more food source to nematode pests besides affecting plant phenology and increasing the whole ecosystem complexity. Both of these factors also lead to an earlier emergence of pests/diseases/vectors and crop attacks, longer life-cycles and reduced pest/disease generation times (Colagiero and Ciancio 2011). In earlier on population dynamics of root knot nematode, enhanced population build up from 1 to 30 J2/c.c. soil within a period of 6-12 months was observed in polyhouse which is comparatively higher in contrast to the open cultivation (Minuto et al., 2006, Engindeniz and Engindeniz 2006).

The different inoculum levels of *M. incognita* resulted in significant reductions in growth variables. In nematode infested plants there was reduction in shoot length and shoot weight, which could be due to the damage caused by increasing numbers of nematodes that invaded roots and hampered the nutrient and water uptake of plants (Karssen and Moens 2006). The effects of nematode on tomato increase as the inoculum levels increased. In the present studies, the multiplication of nematode was observed to be higher at low to moderate inoculum levels (Rf>1). High rate of multiplication of nematodes with low level of inoculum might be due to encouraging factors like plenty of food, reduced competition level and the ability of hosts to support these populations (, Bendezu and Starr 2003).

CONCLUSION

Meloidogyne incognita exhibits significantly higher reproduction and pathogenic impact in polyhouse conditions than in open fields, even at lower inoculum levels. This highlights the need for tailored nematode management strategies in protected cultivation systems.

AUTHOR'S CONTRIBUTIONS

Anupam Sekhon and Narpinderjeet Kaur Dhillon jointly planned and executed the research work. Anupam Sekhon and Harwinder Singh Buttar carried out data collection for root galling index and soil nematode population and statistical analysis. Narpinderjeet Kaur Dhillon and Sukhjeet Kaur supervised the entire study and critically reviewed the manuscript. All authors contributed to manuscript writing and approved the final version.

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Survey of Wild Edible Mushrooms in Darma Valley, Kumaun Himalaya, Uttarakhand

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Abstract: Wild edible mushrooms (WEM) form an important component of the dietary needs of the traditional people. A preliminary survey was conducted in the Darma valley, inhabited by the Rung sub-tribe, practicing a transhumant mode of sustenance. Exploitation of WEM is carried out in their summer homes, during the interim period from June-August. Altogether 19 species were identified as edible from the broad landscape, encompassing 6 villages. However, the inhabitants harvested only 9 species- for their consumption. The present study presents data about the quantity of the WEM harvested; the traditional knowledge base as to the medicinal uses of some of the species; and the mycorrhizal association of the species with the above-ground vegetation.Of significance are the 12 species, which exhibit mycorrhizal association with *Abies spectabilis,* while another 3 species exhibiting mycorrhizal association with *Betula utilis.* Apart from *Ganoderma* sp. and *Morchella esculenta*, none of the other species harvested is marketed. The paper highlights the conservation aspect of wild mushrooms, as well as probable methodologies for sustainable harvesting of the WEM.

Keywords*:* Anthropogenic pressure, Mycorrhizal association, Wild edible mushroom

Over the last few decades, the abundance and diversity of mushrooms marketed as food and medicine worldwide have steadily grown. The 2327 species of wild edible and medicinal fungi are collected, consumed, and traded worldwide (Boa 2004). As relates to their antiquity, the consumption of wild edible mushrooms (WEM) has been traced to around 13,000 years back, going by the archaeological records from Chile (Rojas and Mansur 1995), while the written records of their consumption go back to 2000 years in China (Boa 2004). The collection and scientific study of mushrooms in India began in the 19th century and has continued to the present day (Kaul 2002). The mushroom is the fruiting body, or better still, the reproductive structure of a macrofungus that generates spores. These spores can be observed without the aid of magnification, and the mushroom itself is visible and can be manually harvested. Mushrooms offer local people a source of seasonal food, medicine, and an alternative source of income, while maintaining forest health (Sysouphanthong et al., 2010, Mortimer et al., 2012). The abundance of wild mushrooms is also a bioindicator of the ecosystem health (Dai et al., 2009, Sysouphanthong et al., 2010, Egli 2011, Du et al., 2011a, b). In Yunnan Province, China, up to 700 species of wild mushrooms are known to be edible and are utilized by local indigenous people as both a source of food and income (Mortimer et al., 2012). Similarly, an estimated 300 species of fungi are used as food in Mexico (Garibay-Orijel et al., 2006). Presently, an estimated value worth more than two billion US$ is ascribed to only a few edible and marketed species of mushrooms (Wang and Hall 2004). In 2010, a total of 10572 tonnes of *Boletus edulis* alone was exported from China, worth US $71.83 million (Mortimer et al., 2012). This international trade is infact vital for the livelihoods of collectors (Hamayun et al., 2006), with more than half of the cash income in some rural areas of Tibet and China's Yunnan Province, derived exclusively from mushroom exports to Japan and Europe (Chen 2004).

Several principal bio-active compounds-polysaccharides, glycoproteins, ergosterols, triterpenes, and antibiotics are isolated from mushrooms, which are known for their myriad pharmacological activities, such as (i) anti-fungal, (ii) anti-inflammatory, (iii) antitumour, (iv) antiviral, (v) antibacterial, (vi) hepato-protective, (vii) anti-diabetic, (viii) hypolipidemic, (ix) anti-thrombotic/antifibrotic, (x) hypotensive activities, (xi) anti-fatiguing, (xii) anti-oxidative, and (xiii) chemoprotective (Stamets 2002, Paul and Snyder 2009). In addition to antibiotics used in medicine (Sur and Ghosh 2004), mushrooms also yield fermentation products used in the food industry (Koizumi 2001), enzymes used industrially for the biotechnology of wood (Mai et al., 2004), as well as find applications in eco-friendly bioremediation of contaminated sites (Stamets 2005, Brar et al., 2006). Such studies should be explored for other species of mushrooms, too, as a biocontrol agent for getting rid of insect pests.Traditionally, however, mushrooms form a most common Non-timber Forest Products (NTFPs), harvested, primarily as food. While some of the species are eaten raw, or roasted, many, however, are cooked as a most preferred culinary dish. In traditional societies, the mushrooms harvested from the wild undergo processing and are preserved to be consumed at times of food scarcity. From an ecological perspective, mushrooms and their mycorrhizal processes greatly impact nutrient cycling by playing an essential role in the transport, storage, and release of nutrients such as carbon (C), phosphorus (P), and nitrogen (N) (Varma et al., 2017, Bortier et al., 2018). Some of the mushroom species have very specific host plant species, while the others may be more general species (Pande et al., 2004). While commercial prospects of wild edible mushrooms, as well as their sustainable harvesting methods, have been dealt with (Boa 2004, Wang and Hall 2004, Mortimer et al., 2012), such studies are presently lacking altogether in this part of the country. Again, while one would subscribe to the exploitation of the WEM for the economic benefits of the inhabitants, more so, when it is known that picking large macrofungal species, such as *Lactarius* and *Russula,* for example, without disturbing the habitat has no negative impact on the future harvests (Egli et al., 2006). Similarly, unsustainable means, such as scouring the floor of the forest, or any perceptible change in the above-ground vegetation profile will severely impact upon the very viability of the WEM (Luoma et al., 2006). This is more so, in the case of the perennial species, e.g., *Fomitopsis spp.,* for the simple reason that they develop over many years, therefore would be much more susceptible to overharvesting (Berch et al., 2007).The present paper relates to the exploration of the diversity of the wild edible species (WEM), the principal species being harvested and the quantity being harvested there of, a preliminary listing of WEM exhibiting mycorrhizal associationship with the tree species, and lastly, the traditional knowledge base vis-à-vis wild edible mushrooms within the broad landscape, i.e., the Darma valley.

MATERIAL AND METHODS

The field survey was conducted within the Darma Valley, a constituent part of the greater landscape, referred to as Askote Conservation Landscape, located in Eastern Kumaun Himalaya, between 80°15' to 81°52' E longitude and 29°52' to 30°32' N latitude, with the Darma valley occupying just the central part of this landscape and lying across and along the River Dhauli, which is one of the major tributaries of the River Kali that again forms the international boundary between India and adjoining Nepal (Fig. 1). The villages studied lie within an altitudinal range between 3000 and 3400 meters amsl. The study sites were primarily dominated by *Abies spectabilis,* and in a few cases by *Betula utilis,* lying on an average between 3000-3400 meters amsl, i.e., between sub-alpine and alpine zone. The ambient temperature recorded during the conduction of the study period- June to August, ranged between 10 and 20°C, while the average humidity ranged between 72 and 86 percent. Within the broad landscape, the majority of the study sites are treated as sacred. These sites, however, do exhibit signs of tree lopping, primarily for fuelwood collection, and secondarily, tree felling for the erection of ritualistic Aalam Sammo (syn. Flag pole).

The study primarily comprised of identification of the wild edible mushroom species, their association with particular plant species, quantity being harvested per family, and ethnobotanical knowledge associated with any particular mushroom species. Toward quantifying the harvested lot, the individual households were visited, and the raw weight of the collected lot was measured. The field survey was conducted along with the harvesters. This apart, information was gathered through a semi-structured questionnaire, personal interviews, and consultation with local inhabitants, particularly with the elderly folks. The collected specimens of WEM were identified by seeking the help of the experts, as well as through an extensive literature survey.

RESULTS AND DISCUSSION

Altogether 19 species of mushrooms were identified as edible from the landscape. However, in the six villages surveyed for harvesting of WEM, this number dwindled to just 9 species- *Clavatia craniiformis, Ramaria sanguinea, R. fennica, R. flava, R. botrytis, Clavaria zollingeri, Gomphus clavatus, Pleurotus ostreatus,* and *Kuehneromyces mutabilis* (Fig. 2). The total yield of all mushroom species harvested across the six villages was 3051 kg (Table 1). However, the harvested lot, varied across the six villages, both in terms of

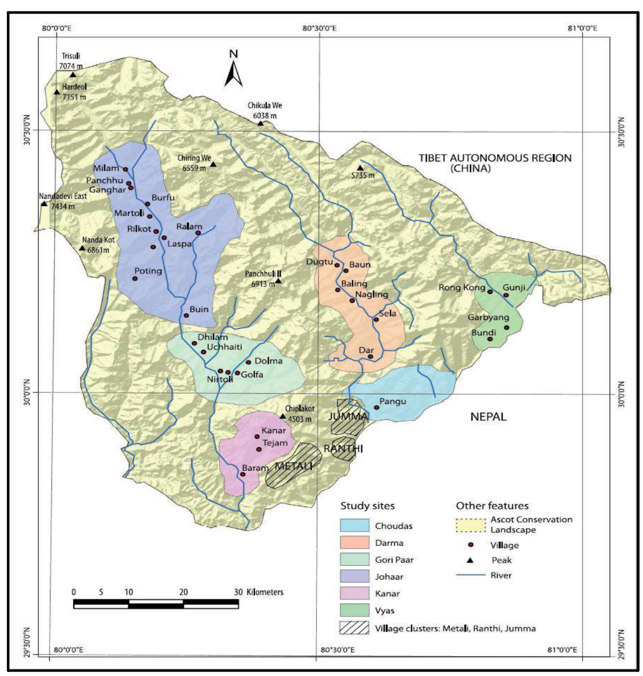

Fig. 1. Site location

the quantity of each species harvested and the number of WEMs being harvested. As concerns the volume, two species- *Gomphus clavatus* and *Ramaria spp.,* constituted the bulk of the harvested lot. These two species were abundant in *Abies*-dominated forests, lying adjacent to Baling, Philam and Bon villages (Fig. 3, Table 1).

There was significant variability in the seasonal harvesting of wild edible mushrooms across the Darma Valley. *Gomphus clavatus* is the most extensively harvested species, totaling 1449 kg, with a particularly high yield in Baling (1006 kg). *Ramaria spp.* also shows notable harvesting, with a total of 703 kg, highlighting its importance in the region. Other species, such as *Pleurotus ostreatus* and *Boletus edulis*, were collected in smaller quantities, indicating variable availability, or preference. Village-specific trends include a significant harvest of *Pleurotus ostreatus* in Nangling and *Boletus edulis* in Dugtu. The low total yield in Dantu (14 kg) suggests limited mushroom availability, or harvesting activity (Table 1, Fig. 2). Several mushrooms exhibited mycorrhizal associations with tree species (Table 2). The findings substantiate the results of Semwal (2003), Pande et al. (2004), Semwal et al. (2005, 2006) and Upadhyay et al. (2008). Significantly, the vernacular names given to one particular species either related to its invariable association with the particular plant/tree species or were derived according to its precise shape/morphology. Thus, *Bhuj mokshya* (*Kuehneromyces mutabilis*), and *Akhrot mokshya* (*Laetoporus sulphureus*) are associated with Bhojpatra (*Betula utilis*) and Akhrot (*Juglans regia*), respectively, while *Kuri mokshya* (*Boletus edulis*), *Puccham mokshya* (*Ramaria spp.*), *Damo mokshya* (*Clavatia craniiformis*), *Cherpiya* (*Auricularia auricula-judae*) are named as per their resemblance to bowl, rice, drum, and ear lobe, respectively.

Even though a substantial number of WEMs were identified from the landscape (Fig. 4), most were either unknown to the local inhabitants or were not harvested because of their relatively lower population size. Interestingly, even though the studied villages were located quite near to each other, some of the species being harvested in one village, remained unexploited in the other village. This could be traced to a look-alike of one edible species, which might have resulted in some unfortunate event. However, the precise cause remained unfounded. Such events, however, result in phenomena as exhibited in the landscape, where a

Fig. 2. (from top left to right): The edible mushrooms currently harvested within the broad landscape- 1. *Clavatia craniiformis* (Schwein.) Fr. ex De Toni 1888, 2. *Ramaria sanguinea* (Pers.) Quel. 1888, 3. *R. fennica* (P. Karst.) Ricken 1920, 4. *Clavaria zollingeri* Lev. 1846, 5. *Gomphus clavatus* (Pers.) Gray 1821, 6. *Ramaria flava* (Schaeff.) Quel. 1888, 7. *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. 1871, 8. *Kuehneromyces mutabilis* (Schaeff.) Singer & A.H. Sm. 1946, 9. *Ramaria botrytis* (Pers.) Ricken 1918

Table 1. Seasonal collection of wild edible mushrooms (raw weight in Kg) from different villages of Darma Valley (Based on the survey with harvesting households, n= 144)

Species	Quantity (raw, in Kg)						Total
	Baling	Nangling	Boun	Philam	Dugtu	Dantu	
Gomphus clavatus	1006	119	75	149	100	0	1449
*Ramaria spp.**	380	70	48	72	127	6	703
Clavatia craniiformis	0	0	81	33	0	8	122
Pleurotus ostreatus	0	363	0	0	0	0	363
Boletus edulis	2	0	39	6	289	0	336
Kuehneromyces mutabilis	4	0	0	0	74	0	78
Total	1392	552	243	260	590	14	3051

* Altogether 4 different species of *Ramaria* are exploited, which include *R. sanguinea, R. fennica, R. flava,* and *R. botrytis*

relatively higher age group is involved in the harvesting of WEM (Table 3). Whether the current harvesting of WEM is detrimental or not, could only be ascertained by periodic assessment of the yield of each WEM, over a period, say 5-6 years. However, presently, the lesser number of harvesters per household (between 1-2, Table 3), aided by a lesser number of individual forays made into the forest for collection, could well be judged as sustainable harvesting practice.

Within the forest located above the village Baling, there was greater abundance, as well as the diversity of WEMs in the relatively well-preserved forest segment, as compared to the forest segment experiencing more anthropogenic disturbance, primarily in terms of tree lopping and felling. It was found that the extent of forest canopy cover (own observation) affects the ambient temperature and humidity, as well as the moisture content in the underlying soil- all the factors that are necessary for the fruiting of the mushrooms. The canopy cover and the number of canopy branch layers, in fact significantly relate to the number/abundance of the fruiting bodies. Abrego et al. (2015) also highlighted that macrofungi are particularly sensitive to habitat loss and fragmentation. It is believed that increased opening resultant of tree felling and lopping could lead to higher variations in microclimate patterns, especially as concerns greater availability or percolation of light, resulting in turn in a substantial increase in the ambient temperature at the ground level (Dale et al., 2001, Greenberg and Forrest 2003). The above finding has two important applications- first, how the changes in the canopy cover relate to the yield of the particular WEM, and secondly, and more importantly, how best one could manage the forest cover to raise the productivity of the WEM of interest. This comprehensive data reveals considerable variation in mushroom species availability and harvesting levels, highlighting the impact of human activities on mushroom populations in the region. However, determining the sustainability of current harvesting practices remains challenging in the present landscape, necessitating more detailed studies are warranted to ascertain- (i) quantity harvested per household per visit, (ii) quality of the harvested lot- immature versus mature samples

Fig. 3. (from top left to right): The edible species reported to be edible in one village, but not reported as such from the adjoining village. 1. *Tremella fuciformis* Berk 1856, 2. *Paxillus involutus* (Batsch) Fr., 1838, 3. *Laetiporus sulphureus* (Bull.) Murrill 1920, 4. *Laccaria amethystine* (Huds.) Cooke, 5. *Collybia confluens* (Pers.) R. H. Petersen, 6. *Fomes fomentarius* (L.) Fr. 1849, 7. *Amanita rubescens* (Pers. ex Fr.) Gray, 8. *Auricularia auricula-judae* (Bull.) J. Schröt., 9. *Boletus edulis* Bull. 1782, and 10. *Cantharellus-cibarius* Fr. 11. *Morchella esculenta* Fr. and 12. *Ganoderma lucidum* Karst 1881. The last two species are exclusively harvested for the markets, and fetch good prices, nonetheless much below than what these two species command in the markets outside

Table 2. Mycorrhizal association of the mushroom species

Host species	The broad category of macrofungus
Abies spectabilis	*Lactarius sp., Gomphus clavatus, Ramaria spp., Auricularia auricula-judae, Fomes fomentarius, Hericium sp., Strobilomyces sp., Clitocybe sp., Inocybe sp., Galarina sp., Russula sp., Cortinarius sp.*
Betula utilis	*Kuehneromyces mutabilis, Boletus edulis, Pleurotus ostreatus*

Table 3. Characteristics of harvesting households surveyed in the different villages of Darma valley

Characteristics	Baling	Nangling	Boun	Philam	Dugtu/ Saun	Dantu	Total
Total households surveyed	30	26	23	20	30	15	144
Informants	95	41	70	35	111	28	380
Average age (years)	32	33	37	35	40	35	35
No. collectors per household	2	1	2	2	2	1	1.66

(mature samples though are not usually preferred by the locals), (iii) mode of traditional harvesting practices (whether few mature samples are left out for regeneration purposes, or not), and (iv) whether the harvester semi-process the harvested lot in the forest itself.

The sustainability or unsustainability of the harvesting practices could be ascertained through observations that relate to changes in the species diversity associated with the WEM of interest. Overharvesting is often a major concern, whether for economic gain or subsistence needs (Hens and Boon 2003a,b, Boa 2004). The high diversity of wild mushrooms is a vital requirement for a healthy forest ecosystem (Boa 2004), and a healthy forest is necessary to maintain a high diversity and productivity of wild mushrooms, Moore and Chiu 2001, Boa 2004). Studies that replicate the above findings, but conducted with WEMs of interest in the present landscape, will undoubtedly help in taking up the remedial measures. Till such studies are undertaken, it would be safe to institute a rotational harvesting method or policy, wherever, the locals cite a decline in yield (Lu 1998). Otherwise too, a regulatory or monitoring mechanism is instituted that stipulates a certain mature stage of each harvested WEM (Chen 2004).

CONCLUSION

Currently, the WEM harvested within the landscape could be categorized as sustainable, considering the quantity of the harvested lot, and the anthropogenic pressure exerted, i.e., 1-2 individuals per household engaged in the harvesting, since the market forces, as concerns the edible species of WEM being harvested, are relatively speaking, non-existent. However, the current lower anthropogenic pressure on the WEM, could also be because the harvesting season of WEM coincides with the harvesting of the most lucrative Yartsa Gunbu (*Ophiocordyceps sinensis*), when almost all the villagers occupy the alpine meadows, the habitat sites of Yartsa Gunbuis is another example of WEM. Even though, the present exploitation of WEM might seem sustainable, there is nonetheless, an urgent need to undertake studies related to the habitat ecology, phenology, and response/s of the WEMs to disturbances, such as over-harvesting, tree felling, as also the salient changes in the community dynamics, which could act as an ecological indicator, defining the change- both positive (in favour of the increased yield of WEM), or negative (signifying the negative impact of the increased abundance of the associated species) on yield of WEM of concern. Such studies would undoubtedly yield information, which then could be applied to the sustainable harvesting of the WEM, as well as in raising their productivity/yield. For the present landscape, with underdeveloped markets, this remains a sound option, which would not just result in stabilizing productivity, but would certainly open up opportunities for viable marketization of their harvested lot.

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Exploring Intricate Interplay between Water Quality Dynamics and Zooplankton Diversity in Chikkere Water Body, Karnataka, India

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Abstract: The study investigates the zooplankton diversity and water quality of Chikkere Waterbody in Sira town, located at 13°75'25'' N latitude and 76°90'70'' E longitude, covering an area of 10.54 hectares. Water samples were collected from February 2020 to January 2022 across four strategically selected stations. Physico-chemical analysis of the water indicated that all parameters fell within permissible limits, though the pH was slightly alkaline. Zooplankton diversity included groups such as protozoa, rotifers, cladocerans, and copepods, with rotifers being the most dominant, comprising 47.82% of the total zooplankton population, followed by cladocerans (26.1%) and copepods (17.39%). The diversity of fish species was notable, with 23 species belonging to five genera and five families, and the highest species dominance recorded during the summer season. The Shannon diversity index ranged from 2.918 to 3.098, peaking at 3.081 during the northeast monsoon season. The findings stress the importance of long-term conservation strategies to ensure the preservation and ecological health of Chikkere waterbody.

Keywords: Physico-chemical, Water quality, Zooplankton, Diversity, Chikkere water body

Freshwater ecosystems have a vital role in providing essential resources and services to human beings. These include the provision of drinking and irrigation water, food, as well as the creation and regulation of micro-climate (Vörösmarty et al., 2010, Green et al., 2015). However, it is alarming to note that freshwater ecosystems, such as rivers and inland lakes, are facing significant threats (Vörösmarty et al., 2010, Alahuhta et al., 2019). The rate of biodiversity loss in freshwater ecosystems surpasses that of terrestrial ecosystems (Geist 2011) and loss of biodiversity shows no signs of slowing down in recent years (Butchart et al., 2010). The severity of biodiversity loss in freshwater ecosystems may be much greater than particularly when comes to the response of hidden microscopic taxa like zooplankton to disturbances (Cazolla 2016).

Zooplankton have been recognized as organisms of ecological importance (Smitha et al., Jose et al., 2015), making them a vital and paramount role in the maintenance of ecosystems. Murulidhar and Yogananda Murthy (2014) utilized the Lange-Bertteart method to study the distribution and ecology of diatom communities in Tumkur's lakes, identifying a total of 46 species. Murulidhar and Yogananda Murthy (2015) investigated the seasonal variations in hydrography and phytoplankton diversity in the Teeta wetland of Tumkur district, discovering 66 species of phytoplankton across different groups. Shalini et al. (2018) focused on the limnological profile of the Gottla Gollahalli wetland in Tumkur district, reporting 45 species of phytoplankton. The water body is located near the city of Sira; nevertheless, remains unusable due to various pollutants. However, no reports were published on the Chikkere water body with regard to water quality and zooplankton studies. Consequently, the current study aims to explore the abundance and seasonal diversity of zooplankton specifically in the Chikkere water body.

MATERIAL AND METHODS

Sira, a taluk headquarter, is situated approximately 50 kilometers away from Tumkur, a district headquarter. It is located along National Highway No. 4. The coordinates of water body fall under 130 75' 25" N latitude and 76090' 70" E longitude at about 662 meters above mean sea level (MSL). Every month water samples collected for the period from February 2020 to January 2022. The physical parameters, including air and water temperature, as well as pH, were recorded on-site during the collection of samples. Enumeration of phytoplankton and zooplankton were done with separately collected as per APHA (2005).

Modified Haron-Trantor net with a square metallic frame measuring 0.0625 m^2 was used for collecting zooplankton samples. The filtering cone consisted of a nylon bolting silk plankton net (No. 25 mesh size 50 μ) for zooplankton collection. With the help of Needham and Needham (1962), Tonopi (1980) and Battish (1992) identification process was done and same was confirmed by Zoological Survey of India.

Statistical analysis: The data was analyzed using the Past software 4.0.

RESULTS AND DISCUSSION

The highest temperatures were 25.1°C and 25.3°C in May 2020 and 2021, respectively, while the lowest was 22.1°C in October 2021. Turbidity was maximum of in May 2021 (90 NTU) and minimum in October 2021 (14 NTU). Total dissolved solids were maximum in April 2020, with the lowest of 406 mg/L in December 2021. Electrical conductivity values ranged from 616 µS/cm in November 2021 to 2290 µS/cm in April 2020.

pH values ranged from 7.2 (January 2022) to 9.3 (September 2021). Dissolved oxygen (DO) concentrations varied between 1.8 mg/L in March 2021 and 6.5 mg/L in December 2021. Total alkalinity ranged from 181 mg/L in December 2021 to 512 mg/L in May 2021. Total hardness fluctuated from 154 mg/L in December 2021 to 420 mg/L.

Calcium hardness was maximum of in May 2020 (262 mg/L) and minimum in December 2021*154 mg/L), while magnesium hardness varied from 162 mg/L in April 2020 to 92 mg/L in December 2021. Chloride concentrations ranged from 65 mg/L in December 2021 to 310 mg/L in February 2020. Biochemical oxygen demand (BOD) ranged from 3 mg/L in November 2021 to 38 mg/L in February 2020. Nitrate (NO_3^-) concentrations varied from 2.9 mg/L in November 2020 to 21.7 mg/L in September 2021. Sulfate (SO_4^{2-}) concentrations peaked at 145 mg/L in January 2021 and dropped to a minimum of 45 mg/L in October 2020. Phosphate levels ranged between 0.1 mg/L in July 2021 and 0.7 mg/L in December 2021.

All physicochemical parameters were within the permissible limits. However, during the summer and southwest monsoon (SWM) seasons, electrical conductivity, turbidity, total dissolved solids, and chloride levels exceeded BIS limits, while dissolved oxygen was lower than the recommended 5 mg/L. Overall, the water samples fall within the BIS standards, corroborated by the findings of Meride and Ayenew (2016). Among the four groups of zooplankton, rotifers were the most dominant, representing 41%, followed by Cladocera, protozoa (Fig. 1). The highest 2167 individuals/liter of zooplankton was in December 2020, and 1922 ind/liter in December 2021. Conversely, the lowest of 555 ind/liter were in June 2020 and 653 ind/liter in June 2021. The highest number of protozoa, *Centropyxis spinosa,* was 661 ind/liter(Fig. 2).

Rotifer was identified as the predominant group within the zooplankton community. Rotifers with maximum density 6312 ind./liter are considered the most considerable. The peak seasonal diversity was observed during the northeast monsoon (NEM) (15201 ind/liter), followed by summer (8939 individuals per liter) and southwest monsoon (7169 individuals/liter) seasons, respectively (Fig. 3). The dominancy and composition of 41 % of rotifers indicate the eutrophication (Jagadeeshawara et al., 2015, Sulata and Devashish 2016). Turbulent and excess flow of water in the rainy period leads to environmental stress, resulting in low density of rotifers (Edward and Ugwumba 2010). Similar findings were also reported by Anita et al. (2019). *Brachionus calyciflorus*, a pollutant-tolerant species and an indicator of

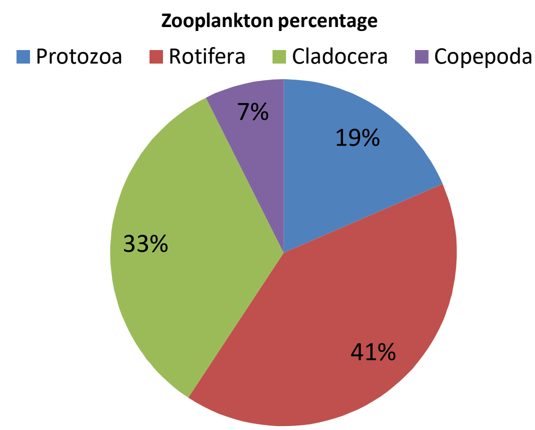

Fig. 1. Percentage of Zooplankton groups

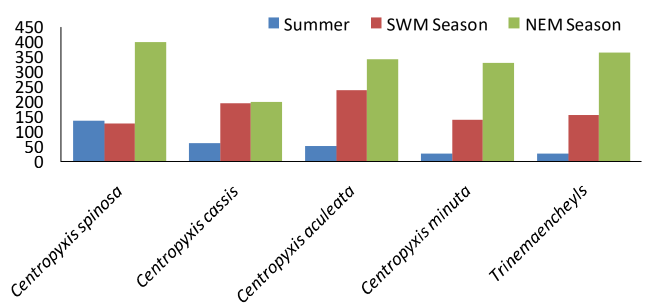

Fig. 2. Seasonal variation of protozoa of Chikkere water body (Number of individuals/lit)

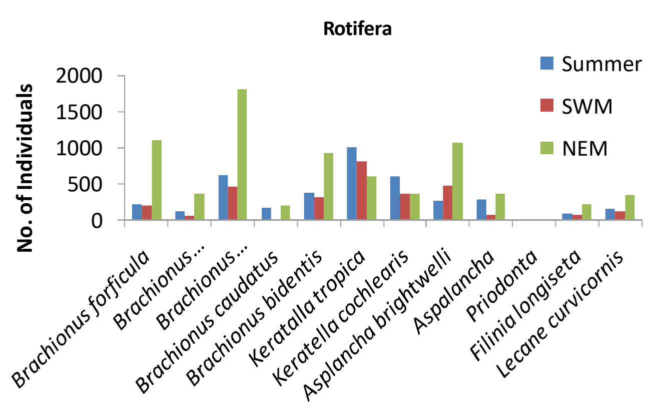

Fig. 3. Seasonal variation of Rotifera of Chikkere water body (Number of individuals/lit)

organic pollution (Pandey et al., 2014, Fatibi et al., 2017), emerged as the dominant species during the NEM and in the annual average, with counts of 1,810 and 2,888 individuals per liter, respectively. Species from the Brachionidae family, along with *Keratella tropica*, *Filinia longiseta*, *Filinia caudata*, and *Filinia diversicornis*, are recognized indicators of eutrophication (Pal et al., 2015, Somani et al., 2012, Bera 2021) and were dominant during the summer and NEM seasons.

The zooplankton community structure displays a mixture of mesotrophic to eutrophic species, with *Brachionus* species commonly found in eutrophic environments, as documented by various studies (Baloch et al., 2004). The presence of *Keratella cochlearis*, an indicator species for eutrophication, suggests a transition from mesotrophic to eutrophic conditions. Paulose and Maheshwar (2008), as well as Ezhili et al. (2013), observed a higher abundance of rotifers in correlation with elevated water temperatures, nutrient levels, and pH. Rotifers exhibit adaptability by rapidly appearing and disappearing in response to environmental conditions

The higher number of cladocerans were in the northeast monsoon season due to congenial conditions and sufficient food. The dominant species among Cladocera were *Daphnia pulex* (3178 individuals) followed by *Daphnia carinata*, *Daiphanosoma excism*, and *Macrothrix goelidii* (Fig. 4). *Daphnia* species are indicators of organic pollution (Vijayapriya et al. 2019, Shashikanth 2013). The dominance of *D. pulex* and the low relative abundance of other *Daphnia species* in ponds with fish presence imply ineffective fish predation. This leads to the dominance of large cladocerans, possibly due to the competitive superiority of larger species. Rotifers are the predominant species within the zooplankton community (Ezhili et al., 2013, Sivakami et al., 2014, Basawarajeshwari Indur et al., 2015), due to their feeding, parthenogenetic reproduction, and fecundity rates (Sampio et al., 2002). Water temperature, ranging from 20.2°C to 23.1°C, regulated the density and diversity of the dominant zooplankton population in the freshwater, moderate warming can enhance the growth and feeding rates of certain filter-feeding zooplankton species, such as some *Daphnia sp.*. Warmer temperatures can also favor smaller zooplankton during their developmental stages. Water temperature, DO, and r magnesium hardness are seasonally the most influential parameters (Vikaskumar et al., 2024).

Presence copepods are the signs of availability of diatoms and blue-green alg**ae, as** they are the food sources for all the developmental stages of cyclopoid copepods (Pandit et al., 2020). Even feeds on rotifers and cladocera (Mathur et al., 2008). The northeast monsoon season exhibited the highest density of copepoda, with *Mesocyclops leukarti* species reaching its peak at 1668 ind/l because of abiotic conditions and abundant food availability (Fig. 5). The lower copepod population indicates a food-rich environment, favoring Cladocera. Majagi and Vijaykumar (2009) observed lower abundance of copepods in monsoon season.

Highest zooplankton were in summer and lowest in the northeast monsoon season (Fig. 6), indicating the significant role of temperature in their distribution). The correlation between zooplankton groups and different physico-chemical parameters showed both positive and negative relationships

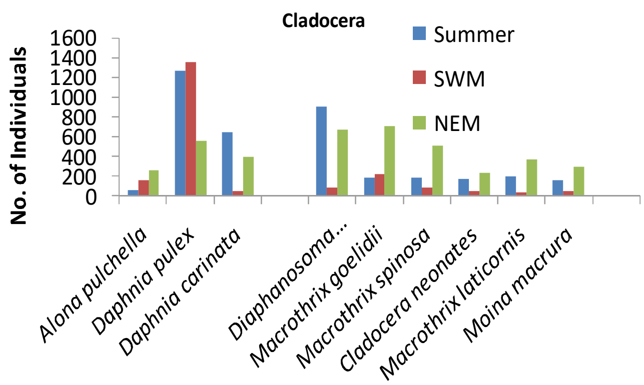

Fig. 4. Seasonal variation of Cladocera of Chikkere water body (Number of individuals/lit)

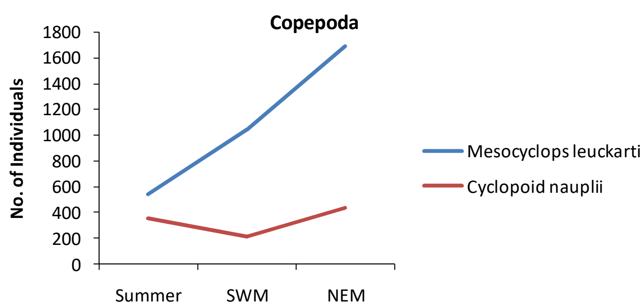

Fig. 5. Seasonal variation of Copepoda of Chikkere water body (Number of individuals/lit)

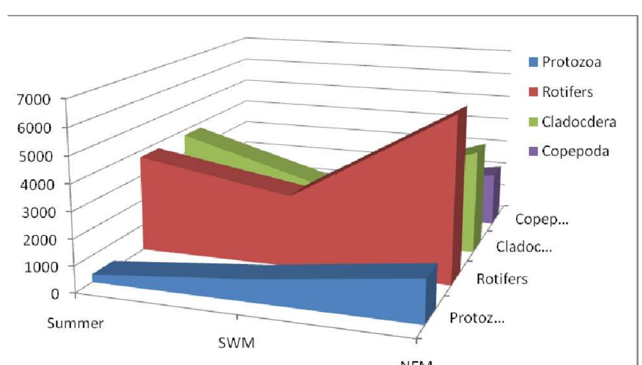

Fig. 6. Seasonal variation of Zooplankton groups (Number of individuals/lit)

Table 1. Diversity indices of zooplankton in different seasons

Diversity indices	NEM	Summer	SWM
Taxa_S	27	27	27
Individuals	8939	7169	15201
Dominance_D	0.07071	0.08958	0.05697
Simpson_1-D	0.9293	0.9104	0.943
Shannon_H	2.903	2.778	3.081
Evenness_e^H/S	0.675	0.5956	0.8064
Brillouin	2.893	2.765	3.074
Menhinick	0.2856	0.3189	0.219
Margalef	2.858	2.929	2.7
Equitability_J	0.8807	0.8428	0.9347
Fisher_alpha	3.433	3.547	3.188
Berger-Parker	0.1413	0.189	0.1191
Chao-1	27	27	27

(Fig. 7). Dominance was high during the summer season (0.0895) and low in the southwest monsoon season (0.056) Similarly, the highest is 0.062471 in the protozoa group and the lowest is 0.04843 in the cladocera. With regard to dominance index, the aquatic environment is classified as very good when H>4, good when 4-3, moderate when 3-2, and poor when <1. The Shannon index ranged from 2.918 to 3.098 in the Chikkere water body, and the maximum dicates the stability of the water body. The northeast monsoon season recorded a Shannon index of 3.081, while the summer and southwest monsoon seasons recorded 2.778 and 2.903, respectively (Tables 1, 2 Fig 7). Among these groups, cladocera exhibited the highest (3.098) diversity index, while protozoa had the lowest (2.918) Highest was in the southwest monsoon season (3.081) and lowest in the summer (2.77). The population of zooplankton showed an

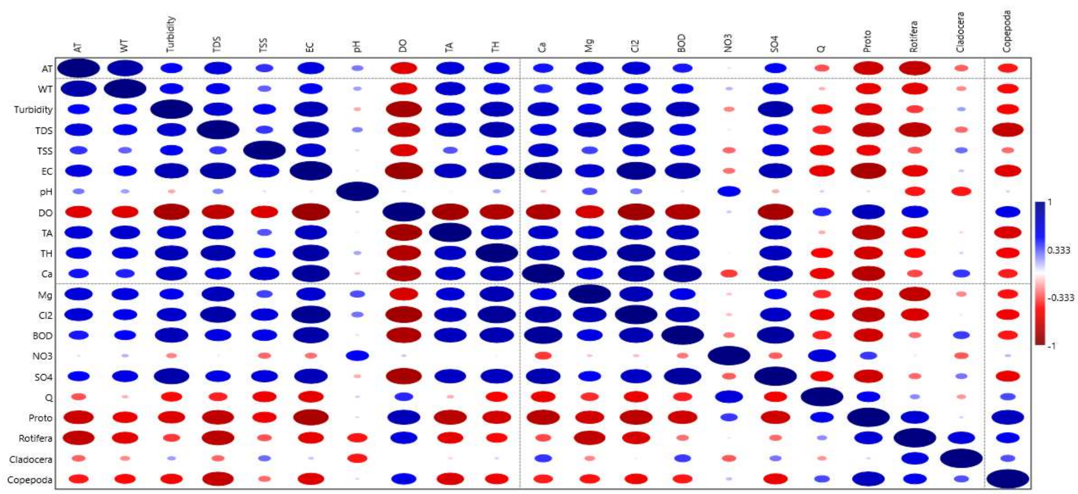

Fig. 7. Correlation between the physicochemical parameters and zooplankton groups

Table 2. Diversity indices among the different groups of Zooplankton

Indices	Protozoa	Rotifera	Cladocera	Copepoda
Taxa_S	24	24	24	24
Individuals	2780	14367	9902	4260
Dominance_D	0.06271	0.04988	0.04843	0.05722
Simpson_1-D	0.9373	0.9501	0.9516	0.9428
Shannon_H	2.918	3.079	3.098	2.979
Evenness_e^H/S	0.7712	0.9058	0.9233	0.8192
Brillouin	2.893	3.073	3.089	2.961
Menhinick	0.4552	0.2002	0.2412	0.3677
Margalef	2.9	2.403	2.5	2.752
Equitability_J	0.9182	0.9689	0.9749	0.9372
Fisher_alpha	3.61	2.81	2.957	3.358
Berger-Parker	0.1004	0.0774	0.07352	0.09272

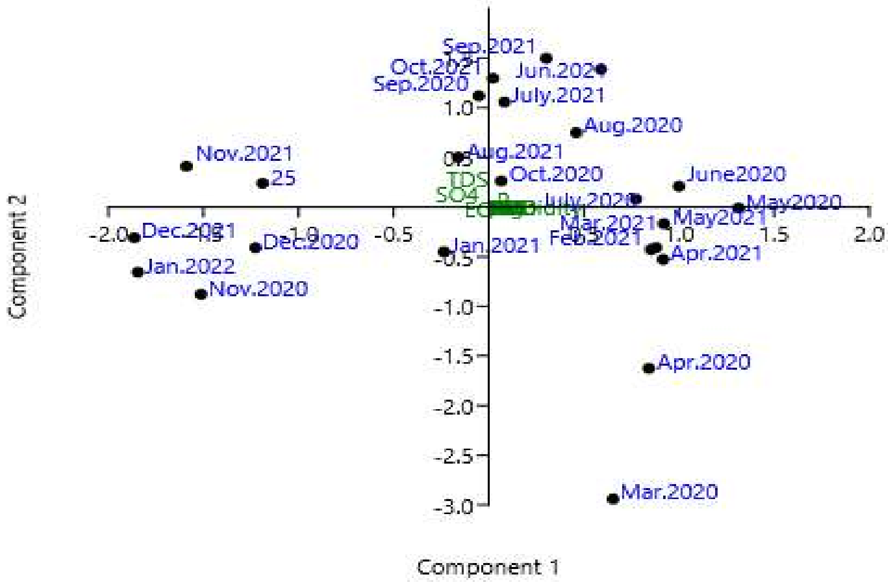

Fig. 8. PCA analysis

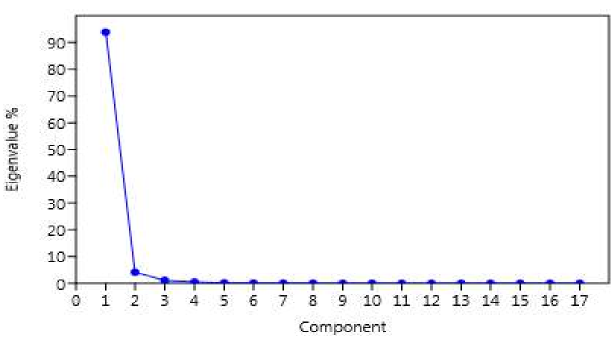

Fig. 9. Scree plot of the eigen values

upward trend during the northeast monsoon season due to favorable environmental conditions such as temperature, dissolved oxygen levels, and the availability of nutrient-rich sources like bacteria, nano-plankton, and suspended detritus. Salve and Hiware (2010) reported a higher number of zooplankton during the winter season due to favorable environmental factors. The evenness during the southwest monsoon was 0.804, indicating a moderate level of evenness. The higher value, 0.9233 was a cladocera and a lower 0.7712, in the protozoa group.

PCA has proven to be a valuable tool in explaining the variance of interrelated variables using a smaller set of independent variables. This analysis helps in accurately assessing the water quality. Although many parameters fall within permissible limits, by and large, the water quality of the Chikkere waterbody indicates that it is unsuitable for drinking or domestic purposes. Further studies are needed continuously to monitor this waterbody. The principal components (PCs), their eigenvalues, and the percentage of variance attributed to each PC 9 (Fig. 8). The scree plot illustrates the eigenvalues for each component, with 14 PCs having eigenvalues greater than 1, collectively accounting for 72% of the total variance in the dataset (Fig. 9). The change in slope is observed in the scree plot between the first and second eigenvalues. PC1 accounts for 93.9% of the variation in water quality in Chikkere, while PC2 explains 4.1%. PC3 contributes 1.4%, with the remaining PCs each contributing less than 1%.

CONCLUSIONS

The water quality was not detrimental to the survival of zooplankton rather significantly contributed to the enhancement of productivity. The concentrations of all the physicochemical parameters were within acceptable limits. The Chikkere water body indicated the presence of zooplankton groups of protozoa, rotifera, cladocera, and copepoda. Zooplankton abundance and diversity play an important role in the freshwater ecosystem. The plankton density in the water body influenced the stocking rate of commercially important fishes and contributed to the growth of the inland fishery sector.

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Distribution and Feeding Preference of Giant African snail *Lissachatina fulica* (Bowdich 1820) in Dakshina Kannada District of Karnataka

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***Abstract*:** *Lissachatina fulica* is a polyphytophagous pest effecting native biodiversity and agricultural crops. Considering this the distribution and feeding preferences of *Lissachatina fulica* in three sites of Karnataka state was observed. The snails were fed with leaves of commonly available garden plants like *Areca catechu, Basella alba, Carica papaya, Piper betel* and their consumption was recorded. Abundance of *Lissachatina fulica* was more in Arecanut plantation (68 snails/quadrate), followed by home gardens and least in river bank. Among the single types of plant leaves and combination of leaves of provided to check the preferential plants for feeding, consumption of *Areca catechu, Hibiscus rosa-sinensis, Musa parasidisiaca, Carica papaya, Basella alba* was maximum than the other plants. The abundance of these plants in the selected sites was observed and snails were found actively feeding on these plants. Thus, abundance of *Lissachatina fulica* in Dakshina Kannada district could be attributed to the presence of these plants for feeding. However, plants preferred by Gaint African snails can be good baits to trap these snails, aiding in pest control and crop protection. The thorough comprehension of the feeding behaviour relating to the crop types can enable targeted crop protection strategies such as precision-based crop protection approaches.

Keywords: Giant African snail, *Lissachatina fulica* (Bowdich 1820), Feeding behaviour, Achatinidae

The Giant African snail, *Lissachatina fulica* (Bowdich 1820) is a Gastropod mollusc belonging to the family Achatinidae. It is a native of east Africa, has invaded many countries in the world and established as a polyphytophagous pest (Raut and Barker 2002). It is the largest land snail in the world and is known for its destructive nature on cultivated plants (Jayashanker et al., 2013). This herbivore does not discriminate between living or dead plant matters. It feeds on more than 500 types of plants, including those farmed by humans (D'Souza and Shenoy 2024). These snails are carriers of *Angiostrongylus cantonensis*, a rat lungworm, causing meningitis in humans (Prashad et al., 2004). African land snails established as a pest in areca ecosystem of Shimoga, Karnataka (Ravikumara et al,. 2007). Distribution records on Giant African snail in Karnataka in general and Dakshina Kannada in particular are limited and feeding habits are not observed. Although this snail is voracious feeder, having faster multiplication capacity, their distribution accordant with the availability of host plants for feeding is not known. Due to prolific breeding and hard protective shell, the control of this pest is difficult. Hence, the present study is designed to know the distribution of Giant African snails in Dakshina Kannada district and its feeding preferences.

MATERIAL AND METHODS

Study area: The three sites viz. home garden of Kanchana village (12°45'5.00"N, 75°12'48.15"E), arecanut plantation of Savanoor (12°44'37.52"N, 75°18'27.93"E), Uppinangady river bank (12°50'27.28"N, 75°14'54.94"E) of Dakshina Kannada district were selected and visited on monthly basis from August-December 2020. The snails were collected using quadrates (50cm x 50cm) and by handpicking, and abundance was noted.

The collected Giant African snails (GAS) were placed into well-ventilated plastic container. The base of the container filled with soil for burrowing and maintaining relative humidity and snails were acclimatized to the laboratory conditions. Prior to the experiment the snails were starved for 24 hours to ensure that the snails would have been motivated to search for food by hunger. The leaves of the different plants were used for the study (Table 1). These leaves were selected on which snail was actively feeding in field. The weeds such as *Chromolaena odorata, Crotolaria juncea, Lantena camera* are also included to examine the polyphytophagous nature of the Giant African snail beyond the consumption of cultivated crops. The leaves were cut into square shape so that total area of each leaf piece was 125sq.cm. Five leaf pieces of each plant were offered for the individual leaf feeding experiment.

Based on the feeding ability of GAS on single plant species and combination of plant leaves offered to check their preference for food. In the combined leaf feeding experiment, four kinds of leaves were used in each set of experiment (Table 1). The most fed plant leaves in the individual feeding experiments were offered with the leaves of least fed plant species. The leaf pieces of equal size were kept at each corner of the container Then the snails were transferred to the experimental container and allowed to starve and acclimatised. The containers were kept undisturbed for 8 hours. After 8 hours the snails were removed from the container and the remaining leaf pieces were measured and their area fed was calculated.

RESULTS AND DISCUSSION

Lissachatina fulica was mainly abundant in the arecanut plantation in Savanoor. Abundance of *Lissachatina fulica* was observed in August (68 snails/quadrate) in all the sites and decreased thereafter (Table 2). The abundance was more in arecanut plantation (Savanoor), then in home garden (Kanchana Village) and least in the river bank.

The Gaint African Snail preferred plants viz. *Areca catechu, Hibiscus rosa-sinensis*, *Carica papaya, Crotolaria juncea, Chromolaena odorata, Musa paradisiaca, Basella alba.* This preference could be due the attraction of Gaint African snail to the odour and increased palatability. The least preferred leaves for food were *Piper beetle, Jasminium sp, Hevea brasiliensis, Piper nigrum, Prunus dulcis, Coffea arabica, Theobroma cacoa, Mussaenda erythrophylla* (Fig. 1). Least preference is due to the repellent properties of plants because of their chemical nature.

Second set of experiment, the snails consumed *Piper betel* (20%) and *Coffea arabica* (21%). Snails did not feed on *Jasminium sp.* and *Theobroma cacoa.* The snails consumed *Mussaenda erythrophylla* (19%) and *Coffea Arabica* (22%) were preferred over *Hevea brasiliensis* and *Theobroma cacoa* (Table 3).

In the fourth set of experiments the snails completely fed the *Carica papaya, Hibiscus rosa-sinensis* leaves *Cucurbita pepo* was preferred in less amount whereas leaf pieces *Solanum lycopersicum* was not eaten. In the fifth set of experiments *Crotolaria juncea* was highly consumed (40 sq. cm) than the *Musa paradisiaca, Lantena camera* and *Mussaenda erythrophylla* (9 sq. cm). The snails completely consumed *Crotolaria juncea* and *Musa paradisiaca* not as fast as *Lantena camera*. The snails ate only a small portions

Table 2. Abundance of *Lissachatina fulica* in the study area (number of individuals/quadrate)

Months	Uppinangady river bank	Savanoor	Kanchana village
August	67	70	68
September	47	55	52
October	35	26	25
November	10	14	12
December	-	-	-

Table 1. Feeding preference of Giant African snail

Scientific name	Individual (Using single type of leaves)	Combined (four types of leaves)				
		Set 1	Set 2	Set 3	Set 4	Set 5
Areca catechu	+	-	-	-	-	-
Basella alba	+	-	-	-	-	-
Carica papaya	+	-	-	+	+	
Chromolaena odorata	+	+	-	-	-	-
Coffea arabica	+	-	+	-	-	-
Crotolaria juncea	+	+	-	-	-	+
Cucurbita pepo	-	-	-	+	+	-
Hevea brasiliensis	+	-	+	-	-	-
Hibiscus rosa-sinensis	+	+	-	+	+	-
Jasminium sp.	+	-	+	-	-	-
Lantena camera	-	-	-	-	-	+
Musa paradisiaca	+	-	-	-	-	+
Mussaenda erythrophylla	+	-	-	+	-	+
Piper betel	+	-	+	-	-	-
Piper nigrum	+	-	-	-	-	-
Prunus dulcis	+	+	-	-	-	-
Solanum lycopersicum	-	-	-	+	+	-
Theobroma cacoa	+	-	-	+	-	-

+ Present, - Absent

of the *Lantena camera* and *Mussaenda erythrophylla.* During the study, the abundance of *Lissachatina fulica* was more in arecanut plantation and home gardens, and least in the river bank. This could be due to the presence of plants for feeding, palatability and favourable ecological conditions (Kurup 2018).The snails were abundant in August because the rain intensifies their population will multiply (Patiño-Montoya et al. 2022). The decrease in their number till December corresponds to the high temperature and dry season. The snails undergo the state of dormancy called aestivation, which occurs when the weather condition is harsh, either dry season or warm season (D'Souza and Shenoy 2024). During this period, the Giant African snails form epiphragm, sealing the aperture of the shell which serves as the water preservative strategies, preventing the mechanical damage of the inner soft tissues (Ademolu 2016). *Areca catechu, B. alba, C. papaya, C. odorata, C. juncea, H. rosa-sinensis, M. paradisiaca* were which indicated the non-selective feeding ability of the snail and capacity to damage variety of plants (Ogbu et al., 2014). From the combined feeding preference experiments it was confirmed that GAS prefer juicy plants such as *H. rosa sinensis, C. papaya* and *B. alba*. These findings are in line with the studies of Kant and Siaka (2016). Preference of crops such as arecanut and garden plants by the snails for feeding could be due the attraction to the odour of the plants (Kumar et al., 2018). The study shows that when the required palatable plants are available in their habitats preference to plants such as *P. dulcis, Jasminium* sp*, P. beetle,* and *Hevea* sp decreased.

The uncontrolled weed diversity of in an agricultural landscape is a food source for the Giant African snail in the absence of a cultivated crop (Chandaragi 2014). The current study provides evidence that there was preferential selectivity for the weed offered to the Giant African snail such as *Chromolaena odorata, Crotolaria juncea* and *Lantana camera*. There is apparent palatability preference for these weeds and the implications related to their distribution (Albuquerque et al., 2008). Knowledge on the vulnerabilities

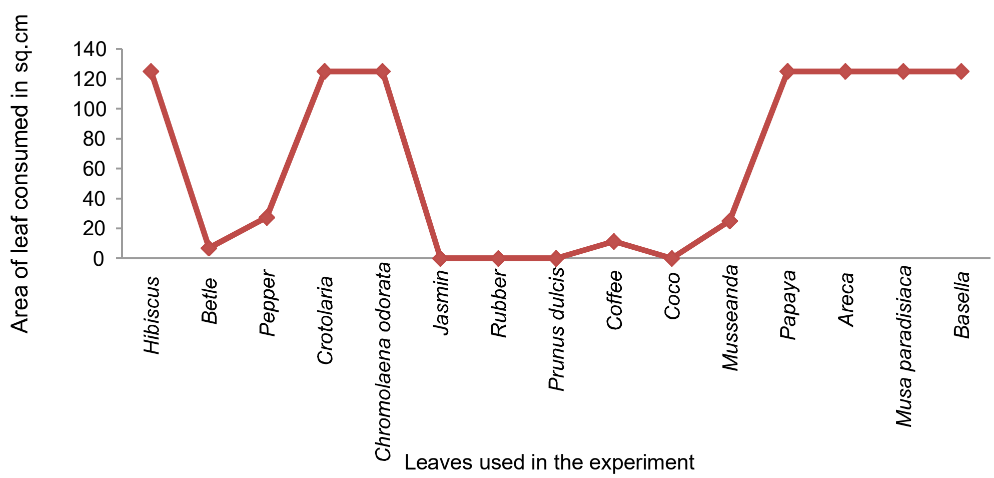

Fig. 1. Area of leaves consumed by the Gaint African snail in the experiment

Table 3. Leaves consumed by *Lissachatina fulica* in the combined feeding experiment

Plant	Area of leaves consumed (sq. cm)				
	Set 1	Set 2	Set 3	Set 4	Set 5
Carica papaya	-	-	-	35	-
Chromolaena odorata	20	-	-	-	-
Coffea arabica	-	12	13	44	-
Crotolaria juncea	42	-	-	-	45
Cucurbita pepo	-	-	-	27	-
Hevea brasiliensis	-	11	00	-	-
Hibiscus rosa-sinensis	45	-	-	45	-
Lantena camera	-	-	-	-	15
Musa paradisiaca	-	-	-	-	40
Mussaenda erythrophylla	-	-	12	-	13

of plant to preferential herbivory by Gaint African Snail is useful for the success of the crop protection efforts.

CONCLUSION

Giant African snails were abundant in arecanut plantations than the home garden and river bank. The distribution data has helped to understand the invasiveness of GAS and presence of preferred plants for feeding. The commonly available leaves of garden plants *Hibiscus rosa-sinensis, Musa paradisiaca, Carica papaya, Basella alba, Areca catechu* are the preferential plants for feeding. However, the least preference plants were *Piper betel, Jasminium* sp*, and Theobroma coca* indicates the availability most preferred plants for feeding. Consumption of weeds indicates that these could be used in control of GAS population. The plants preferred by GAS can be good baits to trap these snails thus helping in pest control and crop protection.

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Seasonal Influence on Roosting Ecology of Short-Nosed Fruit Bat, *Cynopterus sphinx*

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Abstract: The current study was conducted to determine the potential impact of microclimate on the roosting ecology of short-nosed fruit bat, *Cynopterus sphinx,* between plants and building roosts in the Lucknow region. The microclimatic parameters, population, roost preference and roost shifting in both the roosting habitats at regular intervals were analysed. The roost preference was severely affected by extreme summer (46.70 °C) and the average population of plant-roosting bats declined from 46.8 in April to 29.4 in June. The building roosting bat population remained unaffected by the same weather conditions from April (11.1) to June (13.6). However, the population of building-roosting bats increased due to newborn pups. The extreme summer temperature forced the tent-roosting bats to vacate and shift to safer locations such as abandoned buildings/heat-tolerant plant roosts that were not normally inhabited by this species. Thus, *C. sphinx* is likely to respond to climate changes at plant roosts by migrating to more suitable areas. Therefore, the current study identified the habitat risks associated with roost selection of *C. sphinx* at high temperatures, and this study suggests further research is required on fruit-bat roost management.

Keywords: Chiroptera, Habitat conservation, Roost, Microclimatic parameters

Bats are one of the most speciose and ecologically diversified mammalian order, constituting 22% of all mammalian diversity living today about 1,474 species (Simmons 2005, Simmons and Cirranello 2024), next to rodents, and distributed across the world except Antarctica (Simmons, 2005). The pteropodids observed consuming food on 1072 plant species from 493 genera and 148 families. Sixteen pteropodid species have been validated as pollinators for 21 plant species and 29 pteropodid species act as seed dispersers for 311 plant species, potentially benefiting the ecosystem by long-distance pollen and seed dispersal services (Aziz et al., 2021) and maintain the plant kingdom's genetic diversity in tropical and sub-tropical ecosystems (Muscarella and Fleming 2007, Kunz et al., 2011). The short-nosed fruit bat *Cynopterus sphinx* (Vahl 1797) is an ubiquitous frugivorous bat widely distributed throughout the Indomalayan region. *C. sphinx* occupies a variety of roosts and alters different types of tree foliage, modifying it into 'tents'. Several studies suggest that *C. sphinx* also roosts in man-made structures, such as the ceilings of abandoned or unused buildings and partially enclosed porches, either individually or in harems (Hasnim et al., 2020). The use of modified roosts was recorded for 21 bats in neotropical and paleotropical regions (Kunz and Lumsden 2003, Dechmann et al., 2005). In bats, the variety of roosts used for shelter typically reflects both functional and social attributes of the species, in which mating and rearing of young ones take place and selection of optimal shelter may also have profound survival fitness consequences for the species (Campbell et al., 2006).

The roost's physical condition such as temperature, humidity, light intensity, wind velocity and roost characteristics play a significant role for bats in their reproduction, stabilizing the social structure and protection from adverse weather conditions, predator attacks, as bats spend a maximum time in their roosting environment (Kunz 2013). Many factors, such as microclimate, structural characteristics of the roost, surrounding habitat, human disturbance and risk of predation, may influence roost selection by bats (Doss et al., 2018). The majority of studies of roosting ecology of tent-making bats behaviour have been focused mainly on the physical properties and functional value of the modified roost (Stoner 2000), while relatively little attention has been paid to the relationship between roost selection concerning the microclimatic parameters. Likewise, comparative studies for *C. sphinx* opportunistic in their use of modified roosts in plants and buildings are lacking. The proper knowledge of the roosting ecology of bats is absolutely important for their conservation. Therefore, the current study was planned to understand the seasonal influence on roost preference of *C. sphinx* between building roosts and plant roosts by examining the bat population size, harem size, roost longevity, roost diversity, and roost characteristics of *C. sphinx* concerning the roost's microclimatic factors.

MATERIAL AND METHODS

Study species: *Cynopterus sphinx* is a medium-sized fruit bat listed as least concern and characterized by a deep emargination between the projecting nostrils, a short and broad muzzle structure with an average wingspan of 380 mm. It shows a socially polygynous mating system and is more agile on the wing than larger fruit bats (Garg et al., 2018, Kumar et al., 2025).

Study site: Observations on the roosting ecology of *C. sphinx* were conducted during the day time from January to June 2024 at roosting sites within Babasaheb Bhimrao Ambedkar University (80°55'10” E, 26°46'18” N) on alternate days. Additionally, three other roosting sites in the Lucknow district were monitored to study their roosting ecology (Table 1). These sites, including the National Bureau of Fish Genetic Resources, Maharaja Bijli Pasi Degree College, and Sisandi Kothi, are located in Lucknow, Uttar Pradesh, India (Fig. 1). Lucknow, positioned at an elevation of 123 meters above sea level, lies between 26.30°–27.10° North latitude and 80.30°–81.13° East longitude. The city experiences an extreme continental climate, with hot summers, cold winters, and approximately 75% of its total annual rainfall occurs during the monsoon season.

Roost characteristics and microclimatic conditions measurement: Roosting sites were identified by the presence of bolus and faecal droppings beneath roost trees or in abandoned buildings designated as roosting locations (Chan et al., 2021). The main focus was given to plant species frequently used by *C. sphinx* for roosting, including palm, creepers, mast trees, and artificial structures (Nagarajan-Radha et al., 2024). The roost characteristics include roost height (in feet), canopy area, number of roosting trees, availability of feeding trees, and proximity to water bodies. The roost microclimatic parameters, like the roost's temperature (°C) and humidity (%), with the help of a thermo-anemometer (Neumann & Miller-9818), were recorded at every roost site. Roost's light intensity was measured with a Lux meter (KUSAM – MECO- LUX-99) by placing it below the roost. The GPS coordinates of all the sites have been taken by a hand-held GPS (Montana 680, Garmin). The GPS coordinates, details of roosting sites, population, availability of food and water resources, along with roost characteristics of all the study sites were recorded (Table 1).

Bat roost observation: The roosting behaviour of *C. sphinx* was studied across five different sites at regular intervals. Of these sites, three were tree roosts, while two were located in abandoned buildings (Table 1). At each roosting site, the bat's harem size and total roost population were counted using a manual counter machine. The identification of the sex of roosting individual bats was approached with binoculars to observe the individual sex (Aculon- A211 NIKON). Roosting individuals at static positions within the roosting tents were photographed using a camera (Nikon, D5200). In harem condition, identifying the sex of individual bats is quite difficult because they typically cluster closely at the apex of tent cavities in the diurnal roosting groups (Balasingh et al., 1995), where the pelage colour of adult males can be easily distinguished from females (Bates and Harrison 1997, Storz and Kunz 1999). The population size of the roost site was used across the study months to check the impact of season on roost preferences of bats in both types of roosting habitats. The interpretations were made with the recorded microclimatic variables. Average population size was used to explain the behavioural responses through graphs and tables using Microsoft Excel.

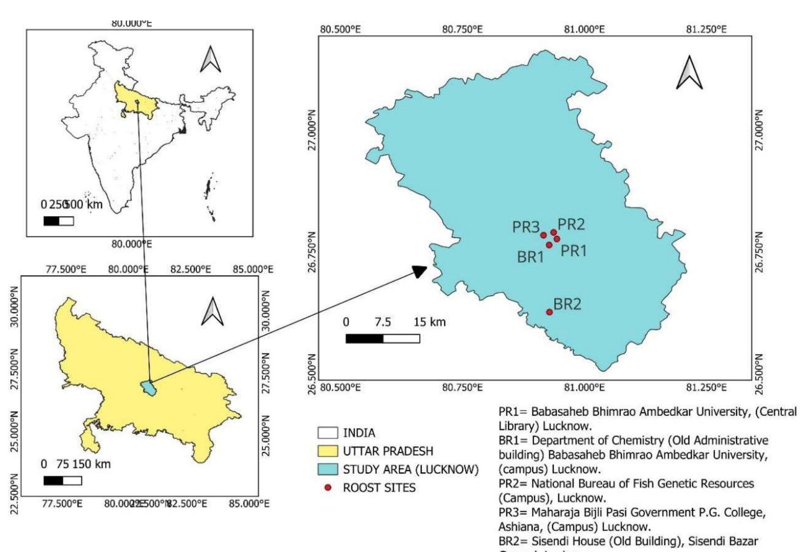

Fig. 1. Study sites at different places in plant roosts (PR) and building roosts (BR) in Lucknow district, Uttar Pradesh, India

Table 1. *Cynopterus sphinx* roost locations, details of roosts, population, available food tree, and distance from water sources

Study sites	Roosts	Study site code	GPS location	No. of roosting trees with tents	Total available bats in the roosting site (n)	Available food trees at study sites (number of trees)	Water sources distance	Study site description
Babasaheb Bhimrao Ambedkar University, (campus) Lucknow.	*Polyalthia longifolia (n=257) Caryota urens(n=2)*	PR1	26°46'168"N 080°55'597"E	28 roosting trees, with tents (n=68). 2 roosting trees of *Caryota urens* with tents (n=3)	115	*Mangifera indica* (10), *Ziziphus mauritiana* (62), *Morus alba* (120), *Syzygium cumini* (20), *Fucus racemosa* (26), *Ficus bengalensis* (10), *Ficus religiosa* (8), *Azadirachta indica (250+), Psidium guajava (50), Musa acuminata* (6), *Neolamarckia cadamba (18).*	5 water ponds Average distance 250 m.	The site comprises many trees (roosting & feeding trees), water bodies, grasslands, buildings, and a big part of the forest within the campus, spread over about a 250-acre University campus, situated in a semi-urban area.
Department of Chemistry (Old Administrative building), Babasaheb Bhimrao Ambedkar University, (campus) Lucknow.	In the old Building (n=1)	BR1	26° 45'856"N 080°55'627"E	(At fused tube light sockets and building ceiling) (n=3)	8			
National Bureau of Fish Genetic Resources (Campus), Lucknow.	*Polyalthia longifolia (n=156) Caryota urens(n=4)*	PR2	26°47'228"N 080°56'208"E	74 roosting trees with tents (n=42). 4 roosting trees of *Caryota urens* with tents (n=12)	96	*Mangifera indica* (8), *Musa acuminata* (5), *Fucus racemosa* (6), *Psidium guajava* (10), *Syzygium cumini* (11), *Azadirachta indica* (5), *Neolamarckia cadamba* (18)	12 water ponds with an average distance of 120 m.	The campus comprises several trees (roosting & feeding trees), water bodies, buildings, and agricultural fields near the campus, spread over about a 52-acre campus, situated near the Indian Institute of Sugarcane Research campus area, 186.5 ha in a semi-urban area.
Maharaja Bijli Pasi Government P.G. College, Ashiana, (Campus) Lucknow.	*Caryota urens(n=6)*	PR3	26°75'335"N 080°93'183"E	12 roosting trees with tents (n=8).	78	*Neolamarckia cadamba* (20), *Mangifera indica* (10), *Ziziphus mauritiana* (12), *Psidium guajava* (5).	2 water ponds with an average distance of 450 m.	The roosting site is present inside the college campus, on palm trees that are surrounded by buildings. Where a regular human presence is observed and the foraging routes are constantly exposed to artificial light illumination at night in semi-urban areas. Near the roosting tree, a good numbers of vegetation were observed in the nearby park areas.
Sisendi House (Old Building), Sisendi Bazar Ground, Lucknow.	Old Building	BR2	26°69'664"N 080°87'199'E	The ceiling of the well without tents occupied by bats as a harem.	20	*Mangifera indica* (20), *Ficus religiosa* (10), *Ziziphus mauritiana* (6), *Psidium guajava* (8).	3 water bodies with an average distance of 350 m.	The roosting sites were present at the ceilings of old wells near the old unused buildings. The roosting site had no other roosting tree found like *Polyalthia longifolia*, or *Caryota urens,* while several feeding tree presences were recorded near the roosting sites at agricultural fields and gardens. This roosting site was surrounded by a dense human colony in a rural area.

PR= Plant roost, BR = Building roost; n= 1,2,3… Roost serial number

RESULTS AND DISCUSSION

Roosting Ecology

Behaviour of tent-roosting bats: The roosting ecology of *C. sphinx* was surveyed in a total of 413 *P. longifolia* plants. Of these, 102 had tent structures built by male bats. Similarly, all surveyed *C. urens* plants contained bat-constructed tents, with a total of 15 tents recorded (Table 1). These findings suggest that *C. sphinx* primarily prefers *P. longifolia* for roosting, followed by *C. urens*. Despite the presence of diverse plant species, only these two tree species were predominantly used as roosting sites. In *P. longifolia*, bats modify leaves and twigs to create canopy tents, which vary in shape, size, and group size, ranging from 2 to 14 individuals per tent, with each tree usually hosting a single tent and rarely 2–3 tents per tree (Fig. 2 A, B). In *C. urens*, bats alter floral structures by carving out a bell-shaped cavity in hanging flower or fruit clusters, chewing and discarding central portions. The group sizes varied from 2 to 20 individuals per floral tent, with each tree typically supporting 3–8 tents, depending on the number and size of floral clusters (Fig. 2 C, D). Previous studies show that *C. sphinx* may utilize more than 80 species of vascular plants as their diurnal roost worldwide (Kunz et al., 1994; Balasingh et al., 1995), such as *P. longifolia*, *Musa acuminata, Borassus flabellifer, Corypha umbraculifera, Livistona chinensis, Roystonia regia, Persea gratissima,* and *Philodendron giganteum* (Garg et al., 2018). Roost tents are typically used for weeks or months, depending on ecological conditions. Bats frequently construct new tents when existing ones no longer provide adequate shelter and relocate to nearby trees to establish fresh tents. During the day, *C. sphinx* roosts in groups of around 20 individuals, though smaller groups (1–3 bats) were also observed roosting inside tents on *P. longifolia* and *C. urens*. Throughout the day, bats engage in social interactions or rest quietly in the shade, preferring well-hidden, secure roosting spots (Fig. 2 B, D). The roosting behaviour of *C. sphinx* is influenced by different types of roost characteristics, including greater transfer of information between groups, access to many mates, familiarity with diverse roost sites, easy entrances to roost sites, and the ability to limit predator attacks and parasite infections (Rajasegaran *et. al.,* 2018). However, environmental changes play a primary role in determining roost stability, often prompting bats to abandon unsuitable tents and seek alternative sites. Similar findings observed among the five studied sites; bats switch their roost destinations based on the closest availability of food and water resources from daytime roosts in plants and building roosts. Greater food resource sites possess larger populations than those with fewer food resources (Table 1). Roost structure and other physical characteristics of the roosting sites play an important role in roost selection (Digana et al., 2003).

Behaviour of abandoned building-roosting bats: In addition to plant-based roosts, *C. sphinx* was also observed utilizing old, unused buildings, the ceilings of water wells, and non-functional ceiling tubes at two roosting sites, BR1 and BR2 (Table 1, Fig. 3). Building roost provides relative flexibility and specialization in roost choices and the comprehensiveness of roost modification among closely related species has been reported in two of the seven

Fig. 2. Plant roosts (tents) of *Cynopterus sphinx* (A) *Polyalthia longifolia* tent shown in red circle, and yellow circle indicates bats' harems. (B) *C. sphinx* harem resides in the tents on *P. longifolia*. (C) *Caryota urens* a roosting plant preferred by *C. sphinx,* inside the flower cluster, each red circle indicating a harem inside the flower cluster. (D) Clear view of the harem inside the flower cluster

species of genus *Cynopterus* recognized by Simmons (2005). *C. sphinx* consistently preferred relatively enclosed roosts (well ceiling), securing themselves using their toes and claws on roof crevices, clay tiles, eaves, and other structural gaps. Bats prefer roosting in dimly lit (6.96 Lux), moderately high humidity (25.20 %), and low temperatures (28.3°C), than plants roost in undisturbed areas (Table 2). They were generally found in a harem, consisting of adult males, females, sub-adults, and pups. The social structure in harems ranged from 2 to 18 individuals, forming stable groups without tent construction. Compared to plant roosts, *C. sphinx* spends more time in man-made structures, which often serve as long-term maternity roosts, used consistently for a long period. Buildings provide stable microclimatic conditions, particularly in terms of temperature and humidity, making them ideal for maternity colonies. Female bats select specific areas within buildings to raise their young, ensuring a secure and sheltered environment for pup development. Consequently, building roosts exhibit greater longevity than plant-based roosts. At both roosting sites, BR1 and BR2, *C. sphinx* did not share roosting habitats with other bat species and predominantly occupied dark, enclosed spaces, which offered protection from predators. These bats preferentially use abandoned buildings in humid, low-light conditions near food and water sources, particularly when suitable roosting trees are unavailable in the foraging area (Table 1). Titley *et al.* (2021) observed that animals may relocate to more favourable locations, including previously unoccupied places, in response to environmental changes. This scenario is more severe in tropical nations (Kingston 2010), and estimated that the loss of bat species along this zone would approach 40% in the subsequent decades (Lane et al., 2006). The effects of global climate change indirectly influence the microclimate of their roosts (Welbergen et al., 2008), which could result in an eventual change in bats' choice of roost sites. The studies on the roosting ecology of human-associated bat species in primitive and abandoned anthropogenic habitats should be recommended to ensure the effectiveness of human-associated bat conservation programs and to facilitate human-bat conflict-solving problems (Hasnim et al., 2020).

Impact of Microclimatic Factors on Bat Roosts

Temperature: The roost temperature was measured in both types of habitats, i.e., plant roosts and building roosts. The lowest temperature was in January at both types of roosting sites, in the plant roost (12.6°C) and the building roost (9.6 °C). In winter seasons, from January onwards, roost temperature rises and reaches the highest temperature in plant roosts (46.7 °C) and building roosts (42.2 °C) in June. The plant roost's average temperature (31.1°C) was slightly higher across the study period than the building roost's (28.3 °C) (Fig. 4 A). The plant roost's higher temperature forced bats to vacate the plant roost as a result population of plants inhabited bats goes down from 46.8 in April to 29.4 individuals at site in June. The building-roosting bat population remains unaffected by the same environmental changes. Although building roosting bat populations increase from 11.1 in April to 13.6 individuals at the site in June due to the addition of newly born pups of the gravid females (Table 2, Fig. 5). Direct observations indicate a population increase associated with the parturition of newborns among bats roosting in building roost. In contrast, plant-roosting bats tend

Fig. 3. (A) Bats roosting on the ceiling of a dome above the well. (B) Bats harem inside view under well ceilings (C) Individuals of *C. sphinx* roost in an abandoned tube light frame (D) Capturing of individual bats by hoop net at roosting site. (E) *C. sphinx* captured individual bats

Table 2. Microclimatic parameters variables with the population size in both the roosting sites, building, and plant roost throughout the study periods (Mean ±SD).

Parameters variables	Building roost							Plant roost						
	January	February	March	April	May	June	Mean	January	February	March	April	May	June	Mean
Temperature (°C)	12.9 ± 3.54	19.9 ± 4.64	26.6 ± 1.87	35.8 ± 2.65	36.7 ± 3.86	38.1 ± 3.11	28.33	15.3 ± 1.92	22.5 ± 1.92	28.3 ± 5.90	37.8 ± 2.12	40.9 ± 2.66	42.0 ±4.11	31.12
Humidity (%)	82.4 ± 8.65	42.1± 16.34	39.5± 29.62	21.0 ± 7.39	31.9± 11.22	27.4± 11.78	40.71	74.3 ± 8.81	35.2 ±8.70	36.9 ±11.42	21.0 ± 7.75	30.5± 11.95	30.5± 16.30	38.89
Wind speed (Km/h)	3.63 ± 0.92	5.63 ± 3.38	7.13 ± 4.91	12.63± 6.48	7.75 ± 3.69	11.88± 5.22	8.10	5.3 ± 2.12	8.1 ±5.74	9.6 ± 5.18	13.8± 6.54	14.9 ±3.60	12.9 ± 4.58	10.75
Light intensity (Lux 1x)	5.5 ± 0.53	5.5 ± 0.53	5.5 ± 0.53	5.6 ± 0.53	5.6 ± 0.74	5.8 ± 0.71	6.96	9.4 ± 0.92	10.5 ±1.41	12.0 ± 1.41	14.0 ± 0.76	14.1 ± 1.25	15.0 ± 0.53	12.50
Tent occupancy (n)	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.00	6.0 ± 2.51	6.6 ± 2.26	6.9 ± 2.53	7.3 ± 1.83	7.5 ± 1.93	8.3 ± 2.38	10.29
Available tents (n)	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.00	16.8 ± 7.78	17.3 ± 7.25	18.3 ± 8.31	18.8 ± 7.70	20.0 ± 8.85	20.8 ± 8.31	31.60
Population (n)	5.0 ± 4.07	6.1 ± 3.76	8.3 ± 4.43	11.1 ± 7.02	12.8 ± 7.78	13.6 ± 8.18	9.48	18. ± 10.82	32.3± 13.74	44.3± 14.01	46.8 ±2.76	43.3 ±7.72	29.4 ±10.32	35.77

to abandon their tents during this period and relocate to nearby, safer roosts, either newly constructed plant tents or building structures that provide suitable microclimatic conditions, particularly optimal roosting temperatures. Similarly, another study in bats reveals that behavioral thermoregulation is a coping mechanism used by bats in general, but high temperatures during heat waves can overwhelm them, resulting in thermal stress and dehydration (Welbergen et al., 2008). Thus, high temperature in the plant roosts leads to a higher chance of roost fidelity in plant roosts from April onwards, as persistent sunlight falls on roosting plants, as a result increase in plant roost temperature. The increasing rate of temperature creates a stressful situation within the tent and residing bats were forced to vacate the newly constructed tents (existing tents) from the plant roost to safe roosting places. In building, residing bats live more comfortably due to a negligible rise in building roost temperature and no stressful effect on the bat harem. Thus, building roosts provides good suitability and saves from direct (sunlight) heat stroke than plant roosts in summer. Therefore, building roost shows a poor chance of roost fidelity due to the negligible effect of roost temperature on harem size. The *C. sphinx* roost shifting is a survival strategy in which bats migrate to shaded or cooler microhabitats, such as dense foliage, or buildings with improved thermal insulation (Bronrier et al., 1999). This pattern of behaviors enables *C. sphinx* to escape the dangers of prolonged exposure to extreme heat, allowing them to discover more ideal microclimates that aid in thermoregulation. However, the current study shows that no deaths were reported at both types of roosting sites in extreme weather conditions. The building roosts adaptation and longer roost stability could facilitate the evolutionary approaches of roost-modifying behaviour in *C. sphinx* in extreme environmental changes. *C. sphinx* switch roosts for maintaining physiological balance and preventing heat-related mortality (Velpandi et al., 2024). Thus, the building roost's microclimate provides greater support to bats than plant roosts at extreme temperature to overcome summer temperature increasement.

Humidity: The roost humidity was also an equally important parameter for understanding the roosting ecology of bat habitats. The highest humidity was in January, 82.4 %, and the lowest humidity in April, 21.0% in the building roost. Similarly, in the plant roost humidity in January was 74.3% and 21.0% in April, respectively (Fig. 4 B). In the summer, as warmth starts in March onwards, a negligible difference was recorded in the humidity at both the roosts. This was due to the roost being highly influenced by other physical parameters (roost canopy, tent type) and environmental conditions like high wind, temperature, etc. The mean variation in the roost humidity was a minor difference, 38.89 % and 40.71 % in the plants and buildings roost, respectively (Table 2). The persistent low humidity negatively affects the population size of the plant, inhibiting bats in the summer months (Fig. 5). Observation signifies that poor humidity leads to the instability of roosting bats in their habitats, resulting in roost population decline. Another study reveals that in the Mandore tunnel, bats actively select roosts based on microclimate conditions, including humidity. The observation shows a positive correlation between bat population and humidity, suggesting that bats prefer more humid environments. High fluctuation in roost humidity, due to factors like deforestation or building renovations, may lead to roost abandonment, reducing population density (Singh and Dookia 2021). In the present study building roost was mainly chosen in close proximity to water resources (well) that provide adequate humidity to the roosting harem (Fig. 3A). The high humidity in roosts helps bats to conserve water and maintain stable body temperatures. In contrast, low humidity can lead to increased water loss through respiration and skin, causing dehydration and higher energy expenditure (van Zuijlen and Groenendijk 2022).

The bats roosting in low humidity indicate that extreme temperature weather conditions show a negative impact on the population at the plant roost. Bondarenco et al. (2014). observed that fruit bat species were highly susceptible to heat-related mortality in these circumstances because their thermoregulatory systems can be rapidly overwhelmed by extended exposure to high temperatures. This is most likely because, despite their strong acclimatization capacity, bats have weak thermoregulatory approaches for dissipating heat at high temperatures due to the absence of sweat gland (Downs et al., 2012), resulting its more challenging for them to dissipate heat and making them vulnerable to high temperatures (Downs et al., 2012).

Wind: Wind speed was also recorded as an important parameter at both roosting sites (Fig. 4 C). The plant roosts were located in open areas, allowing bats to come into direct contact with the wind, which experiences higher wind velocities of 10.75 km/h compared to building roosts that are enclosed by walls and ceilings, resulting in lower wind speeds of 8.10 km/h (Table 2). In the plant roost, the highest wind speed occurred in May 14.9 km/h, while the lowest was in January, 5.3 km/h. In plant roosts, higher wind speeds combined with elevated temperatures (40.9°C) generate heat waves that adversely affect roosting individuals, prompting bats to leave the roosting tents associated with the roosting trees (Table 2). Direct observations in building roosts revealed no such impact from wind speed; bats maintain the same harem position for extended periods, and proper population growth was noted with the addition of newly born pups (Fig. 4 A, C). Furthermore, elevated wind speeds often correlate with cooler temperatures, influencing the energy balance of these thermoregulating animals, particularly during rest periods

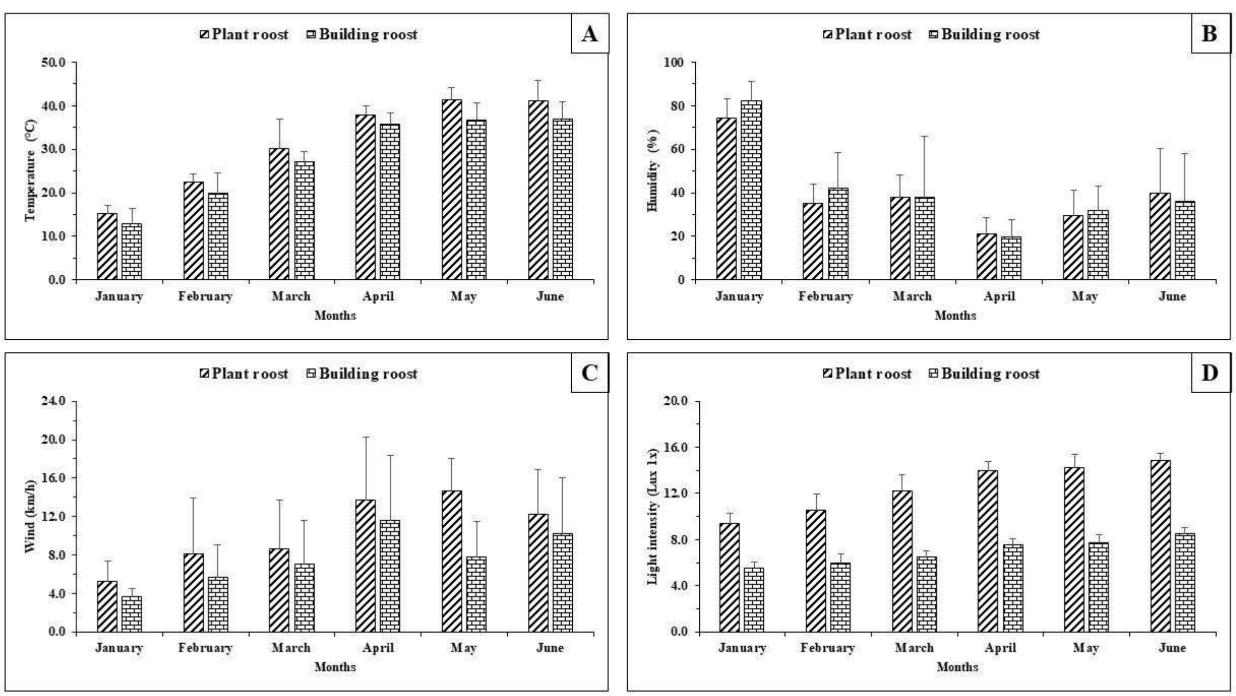

Fig. 4. *C. sphinx roost*'s microclimatic parameters in two different habitats, i.e., plant roost and building roost. (A) Temperature (B) Humidity (C) Wind speed (D) Light intensity

(Voigt and Kingston 2011). Current studies indicate that high wind conditions can lead to decreased roost site fidelity and increased movement between roosts as bats seek locations that reduce exposure to harsh environmental conditions (Kunz et al.,2003). To conserve energy and maintain thermal stability, *C. sphinx* may adjust its roosting patterns, favouring spots that offer windbreaks, especially during windy or cooler periods (Voigt et al., 2011).

Light intensity: The roosting tents were observed in both building and plant roosts. *Cynopterus sphinx* prefers to roost within the canopy region, which is altered by bats into a hollow space where the observed light intensity is consistently lower, 12.50 lx, than the normal light intensity level. During the daytime, light intensity within the tents ranges from 5-18 lx; plant-roosting bats experience an average of 9.4 lx in January and an average of 15 lx light intensity in June (Table 2, Fig. 4D). The light intensity gradually increases as the seasons change from winter to summer in all the observed roosting colonies. In extreme summer (June), increased sunlight adversely affects their roosting habitats and their population size within the plant roost. Tent-roosting bats leave the plant roost tent, leading to reduced roost fidelity due to extreme light intensity in summer. Conversely, in the building roost, bats experience an average of 5.5 lx light in January and 5.8 lx in June (Table 2). The building roost shows a slight increasement in roost light intensity, resulting in negligible changes, and no roost fidelity was observed due to light intensity. *C. sphinx* roosts in tents made of folding palm fronds and is exposed to intermediate light levels (10-15 lx within tents), while *R. leschenaultii* prefers caverns that are entirely shielded from daylight (< 0.1 lx) (Murugavel et al., 2021). Pteropodid bat species select different roosting locations based on ambient light levels. Some roost in trees with high sunlight exposure (>1000 lx), while others prefer dark caverns (< 0.1 lx) (Murugavel et al., 2021). They inhabit diverse environments; *Pteropus* and *Acerodon* roost in open trees well-exposed to strong daylight (>1000 lx), while *Rousettus, Eonycteris*, and *Latidens* prefer caves, houses, and tunnels with minimal daylight exposure (< 0.1 lx) (Bates and Harrison 1997). Previous studies projected an increase in global temperature, raising concerns regarding the frequency and intensity of extreme weather events for fruit bats (Masson- Delmotte et al., 2021). Light also influences roosting ecology by affecting the circadian rhythms of animals (Narendra et al., 2010).

Population: In winter, the presence of bats in the roosting sites was low at both types of roosting locations. Tracking hidden bats' presence in the canopy or dark buildings were highly difficult, making it challenging to determine the exact population due to mild foggy weather and low light intensity (Fig. 5). In the plant roost, the population increasement was recorded from February onwards. The rise in roost microclimatic temperature increasement results in improved visibility of bats within the roost tents. By the end of March, gravid females began parturition (March-April) of young ones (pups), consequently increasing the harem size per roosting tent, which also resulted in an increased number of bat records at both roosting habitats. The overall population peaked in April, coinciding with a rapid increase in population size, and the roost temperature rose quickly, reaching extremes of over 42°C (above the threshold). Suddenly, the plant' tent-roosting bats began vacating the roost tents or shifting to denser areas (safest places) within the canopy, close to their previous tent positions, disrupting the harem structure and causing the remaining individuals to leave the roost tents. Although the population in the plant roost declined, no bat deaths were reported due to the extreme heat, as temperatures continued to rise until June in the plant roost. Research on bat behaviour during heat waves has revealed similar patterns of roost shifting to mitigate thermal stress (Bondarenco et al., 2014). Several previous consecutive studies on bats have indicated that habitat specialist bat species are more sensitive to environmental changes than generalist species due to their narrower niche

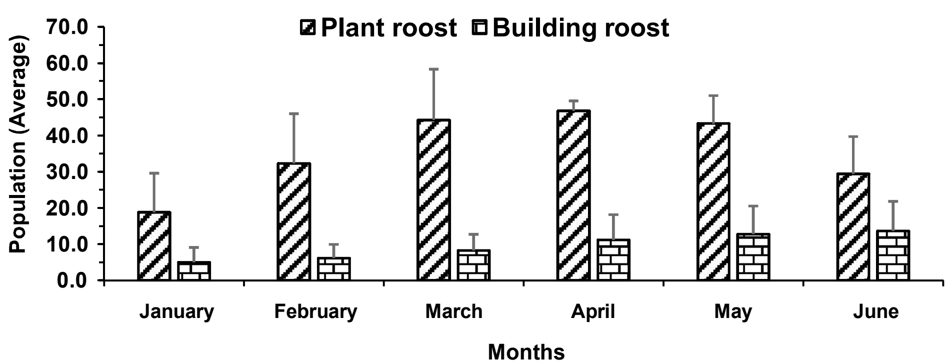

Fig. 5. Average population size in the plant and building roost throughout the study period

or stronger association with specific habitats (Novella-Fernandez et al., 2022). Larger fruit bats struggle to regulate their body temperature during extreme heat due to their roosting pattern in generally open plant roosts, which offer little protection from intense sunlight. Bats cope with extreme heat through various thermoregulatory strategies, such as increasing the area of their exposed wing surface, enhancing evaporative cooling by salivating and licking their wing membranes, and flapping their wings more during hotter afternoons than in the morning and evening, especially on sunny days rather than on cloudy ones (Anjum et al., 2024). Fruit bats are often vulnerable to high temperatures as they lack sweat glands (Downs et al., 2012).

The genus *Cynopterus* roosts in foliage, which is indeed more exposed to solar radiation and thus more prone to heat stress compared to those that roost in more sheltered locations like caves or buildings. Increased exposure to sunlight makes them more vulnerable to heat stress, which can lead to dehydration. Overheating can also elevate metabolic rates, leading to a higher demand for water and food, causing energy depletion and immune suppression, making them more susceptible to infections (Voigt and Kingston 2016, O'Shea et al., 2016). Such heatwave-related issues disrupt physiology, including thermoregulation and the reproductive cycle, and consequently may lead to mortalities. Earlier findings on bat mortality based on body size and roosting nature linked with large bat species like *P. medius* and *P. conspicillatus* show that are highly susceptible to extreme temperatures, resulting in mass mortality reported in different places in northern India and Australia due to their reliance on open, exposed roosting sites such as tree canopies, where they face direct sunlight (Dey et al., 2015; Diengdoh et al., 2022).

The current findings reveal that the disturbed harem structure of plant-tent-roosting bats due to climate change may be prone to the newly born young ones, which may cause the overall population decline in subsequent years. Previous studies report fruit bats suffer imminent dangers such as habitat loss and fragmentation, climate change, and extreme weather (Frick et al., 2018). These factors can cause fruit bat populations to decline, which can have serious ramifications for the environment, especially in areas where they are keystone species (Florens et al., 2017).

CONCLUSIONS

Cynopterus sphinx roosting pattern was influenced by various factors such as temperature, wind speed, humidity, light intensity, etc., contributing to the roost preference in the available building and plant roost with a large population size. Microclimatic factors can significantly impact the roosting behaviour of *C. sphinx* and cause them to change their plant roost to a safe plant canopy or building roost in extreme weather. These life-saving strategies reduce thermal stress and mortality and help them to survive better than other fruit bats. Therefore, important to study roost characteristics, roost microclimate, and availability of resources in roosting ecology for the conservation and management of bat roosts. Bat survival is a critical part of many ecosystems as they play a key role in pollination, seed dispersal of various plant species, and forest rejuvenation. Rapid climatic changes and roost-associated threats highlight the importance of protecting bats and their natural habitats by developing conservation strategies for sustainable biodiversity. Understanding the roosting behaviour is crucial to adopt region-specific conservation initiatives against climate change impact on their roosting habitats.

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AUTHOR'S CONTRIBUTION

Vijay Kumar: Writing original draft, formal analysis, software, data curation, methodology, review and editing. Shiv Shankar Pandey: Roost identification. Subham Acharya and Dinesh Gautam: Field observation and data collection. Vadamalai Elangovan: conceptualization, supervision, validation, review and editing of final manuscript.

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Characterization of Biscuits Fortified with Shrimp (*Litopenaeus vannamei*) Waste Protein Concentrate

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***Abstract*:** Shrimp has accounted for a substantial share of crustacean utilization in recent years and processing results in the accumulation of solid trash, such as the head, shell, and tail parts. The accumulation of bio wastes without proper use has led to waste of materials, waste disposal issues, and environmental contamination. In this sequence, the present study was carried out with an aim to prepare biscuits supplemented with protein concentrate from shrimp head and shell waste to enhance their protein content. The prepared biscuits along with shrimp waste were examined for various physicochemical and biochemical parameters along with organoleptic evaluation. Mineral profiling was also carried out to assess the presence of major minerals in the shrimp waste as well as biscuits prepared from it. The shrimp waste has significantly enhanced the protein as well as mineral content of biscuits, however, also enhanced TMA and TVB-N value which affects the shelf life of biscuits. All the biochemical findings were confirmed with the FT-IR analysis. Prepared biscuits were also subjected to a storage study of 150 days at ambient temperature; and a significant variation was observed in physicochemical and biochemical composition as well as in the organoleptic properties of the biscuits within due course of time. At the end of the storage time, biscuits were quite safe. Therefore, the present investigation can revolutionize shrimp farmers as well as shrimp processors for efficient utilization of shrimp head and shell waste into value-added products and also to grasp more economic benefits.

Keywords: White shrimp, *Litopenaeus vannamei*, Shrimp waste, Protein-enriched biscuits, FT-IR spectroscopy, Waste management

The world's largest industry for producing seafood, crustacean aquaculture, provides a food source high in protein. Shrimp and shrimp products are widely consumed all over the world and their demand is increasing yearly owing to their delicacy and nutritional value. The amount of shrimp produced globally was 5.03 million tons in 2020, and by 2025, it is predicted to reach 7.28 million tons with a compound annual growth rate of 6.1% from 2020 to 2025. More than 80 percent of the world's shrimp produced in Asia and plays significant role in the shrimp farming industry (Mao et al., 2017). In Asia, Thailand is the leading exporter of farmed shrimp exporting to the USA, Europe, Canada, Japan, and South Korea. Out of all the shrimp species, *Litopenaeus vannamei*, or Pacific white shrimp, is the most widely farmed species in Thailand. Shrimp are usually frozen, either with or without a shell, and are exported or kept based on the need from the market. About 50–60% of the solid waste is produced during processing as by-products, which include the head, viscera, shell, and other components. Additionally, the washing and cooking process produces pollution and wastewater (per ton of cooked shrimp, about one gallon is created). These by-products make up to 45–50% of the catch and, because of their uncontrolled discharge, they pollute the environment and provide disposal challenges. Shrimp waste is discarded in enormous numbers, which results in the loss of important and bioactive components, even though a limited amount is used as animal feed and an element in aquaculture feed formulation.

The economics of shrimp producers as well as processors would benefit from the recovery of these valuable components from waste and their subsequent use in profitable goods. Due to the decrease in the disposal of shrimp waste, this would also help in lowering environmental contamination. Shrimp waste possesses valuable components, i.e., protein/peptides, chitin/chitosan, pigments, enzymes, lipids, minerals and vitamins. However, the sources and processing conditions affect the levels of every component. Shrimp biscuit, also known as "hae bee hiam" in Singapore, is a popular snack that originated from Southeast Asia. The biscuits are frequently consumed around the world and well preferred by all age groups. Therefore, it is very logical to opt for biscuits as a base material to develop such functional foods. Keeping these things in view, the present study was designed to utilize shrimp head and shell waste to prepare protein-supplemented functional biscuits.

MATERIAL AND METHODS

Procurement and processing of shrimp: Pacific white shrimp (*Litopenaeus vannamei*) was procured from a local Shrimp farm of Fatehabad, Haryana during the season 2022-2023. The shrimps were transported to the laboratory, washed twice, and the head and shell were separated manually. The waste material was washed and dried using a lab scale oven (Equitron stream 7051-091) at 50°C for 4 to 6 hr to a moisture content of 10 %. Then, the dried material was ground into a fine powder using a mixer (Havells vitonika, 500w) and sieved (particle size<1 mm). The prepared powders were stored in a cool and dry place till further use.

Preparation of shrimp head and shell waste protein concentrate: The protein concentrate was prepared. The shrimp head and shell waste powder was mixed with distilled water (1:10 (w/v)) and stirred while maintaining a pH of 10-11 using 0.1 N sodium hydroxide (NaOH). Then, the prepared mixture was heated at 80-90 °C for 1 to 2 h to facilitate protein extraction. The mixture was allowed to cool and filtered using filter paper to separate the liquid containing extracted proteins. Subsequently, the filtrates were acidified to pH of 4-5 using 0.1 N hydrochloric acid (HCl) that allows the precipitation as well as the separation of proteins. The precipitates were filtered and dried in an oven (Equitron stream 7051-091) at a temperature of 50-60°C up to a moisture content of 10 %. Finally, the extracted protein concentrates were stored in a PET container.

Preparation of functional biscuits: Biscuits were prepared by the modified method of Khan et al. (2014). The ingredients used for the preparation of biscuits i.e., shrimp waste protein concentrate (50g) (approximately 15 % of the total ingredients), wheat flour (150g) (approximately 50 % of the total ingredient), hydrogenated fat (40g), sugar (12g), ground sugar (12g), milk (50ml), baking powder (4g), and salt (0.010g), cardamom powder (10 g as a flavoring agent) were finely mixed and the dough was prepared using a lab scale planetary mixture (Hobart N-50). The dough so prepared was pressed into a flat sheet and were cut with help of die to give appropriate shape. Then, the biscuits were baked at 230 °C for 15-20 min, cooled for 3-5 min, and packed in LDPE pouches. The functional biscuits were evaluated up to 150 days at a 30-day interval for their physicochemical, biochemical, and organoleptic properties

Physicochemical composition: Various physicochemical parameters *viz*., moisture, crude protein, crude fat, pH, and ash content of shrimp head and shell waste, shrimp waste protein concentrate, and shrimp biscuits were determined by standard methods, on dry weight basis .

Moisture content was estimated using following formula:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W2} \times 100$$

Where, W1= Weight of sample before drying; W2= Weight of sample after drying in oven

To assess the pH of samples, 5 g of sample was ground with 50 ml distilled water using a pestle and mortar, samples were filtered and using a digital pH meter, the pH value was recorded. Crude protein determination was carried out using the micro Kjeldahl method.

$$\text{Crude protein (\%)} = \frac{N \times 6.25}{W}$$

Where, N= Nitrogen (%); W=Weight of sample; 6.25=conversion factor for protein

The Soxhlet extraction apparatus was used for the determination of crude fat content of samples. To estimate crude fiber, the fat free sample obtained after the estimation of fat was used.

$$\text{Fibre (\%)} = \frac{W2 - W1}{W} \times 100$$

Where, W=weight of the sample; W1=weight of the crucible with sample after muffle furnace; W2= weight of the crucible with sample after oven drying

Total carbohydrate content was calculated by subtracting the total of moisture, protein, crude fat, ash and crude fiber from 100. The total energy content was calculated by multiplying protein, crude fat and carbohydrate values obtained from analysis by their respective calorific value i.e., 4, 9 and 4 respectively; and expressed as Kcal/100g. To estimate ash content, one gram of the sample was collected and burned on a hot plate in a pre-weighed silica crucible. The crucible was placed in muffle furnace at 650 °C for 5 h. Using the crucible's weight differential, the ash content was calculated and represented as a percentage.

Trimethylamine (TMA) and total volatile base nitrogen (TVB-N): To determine TMA content, 10g sample was extracted with trichloroacetic acid (TCA) (10 %) and filtered using Whatman No.1. Thereafter, 1 mL of the obtained supernatant was carefully pipetted into the outer compartment of the Conway micro-diffusion unit. In the inner chamber, 1ml of 0.01N H_2SO_4 was added, followed by 1ml saturated potassium carbonate and 0.5 ml of formaldehyde solution in the outer chamber. The unit was set aside at room temperature for the night. In the inner chamber, titration was performed with 0.01N NaOH and blank in the same way without sample was also run. The results were expressed as mg of N/100 g of sample. In order to determine the TVB-N content, 10 ml of TCA (20%) was used to homogenize 5 g of material. The homogenate was then filtered through Whatman No. 1 filter paper and brought to a 100 ml volume using distilled water. The filtrate was used for further analysis. The micro-Conway diffusion unit's edges were coated with petroleum jelly, and the inner chamber was filled with 1 ml of 0.01 N H_2SO_4. Additionally, the outer chamber was filled with 0.5 ml of normal potassium carbonate and 1 ml of TCA extract. Overnight, the unit was left untouched. Tashiro's indicator was used to titrate against standard 0.01 percent NaOH in order to calculate the amount of unreachable acid in the inner chamber. Blank was simultaneously prepared with 1 ml of 2 % TCA solution. TVB-N was expressed as mg of N/100g.

Organoleptic evaluation: The samples of biscuits were evaluated for organoleptic quality on the basis of appearance, taste, texture, smell, overall acceptability on a 9-point hedonic scale by the panels of semi-trained judges consisting 10 members, where, 9-Like extremely, 8-Like very much, 7-Like moderately, 6-Like slightly, 5-Neither like nor dislike, 4-Dislike, 3-Dislike moderately, 2-Dislike very much, 1-Dislike extremely. The results of judges were recorded on a Performa and samples with highest average scores were marked as best.

Mineral analysis: Using inductively coupled atomic absorption spectrometry (ICP-AAS), the minerals in the raw materials and shrimp biscuits were calculated. Exactly 250 mg of dried sample was digested with 10 ml of diacid mixture (nitric acid and hydrogen peroxide in the ratio of 4:1) in a microwave digestion unit (Anton Paar multiwave 7501). The digested samples were dissolved in doubled distilled water and then filtered using Whatman filter paper no. 1. The volume of the filtrate was then made up to 25 ml with double distilled water and was used for the determination of minerals.

FT-IR (Fourier-transform infrared spectroscopy): Using an FT-IR spectrometer (Nicolet-6700, Thermo Electron Scientific Instruments Corporation, USA), the raw material and produced shrimp biscuits were qualitatively assessed. The spectra were recorded in the wave number range of 4000-400/cm with a maximum resolution of 0.85/cm, and all samples were evaluated at an ambient temperature of 29±3°C. The measured spectra for the samples were analyzed in accordance with the guidelines given by.

Statistical analysis: Statistical analysis was carried out using standard procedures with 16.0 (IBM, SPSS Inc., USA) software. Additionally, post hoc tests (Tukay's Multiple Range Test) were used to assess the data.

RESULTS AND DISCUSSION

Physicochemical and biochemical composition of shrimp head/shell waste and protein concentrate: The percent crude protein, crude fat, ash, moisture, crude fiber, carbohydrate and energy content of shrimp head and shell waste were 42.34, 7.31, 21.11, 13.94, 9.20, 6.10, and 259.55 kcal/100g, respectively. Protein concentrate possessed protein, ash, moisture, total carbohydrate, and total crude fat content of 76.79, 11.17, 9.11, 2.16, and 0.77, percent whereas, the energy was 322.73 kcal/100g also observed similar trend. These findings provide insights into the nutritional composition of the extracted protein concentrate from shrimp head and shell waste. The relatively low levels of crude fat and carbohydrate content endorses the suitability of protein concentrate for certain dietary needs, such as low-crude fat or low-carbohydrate diets.

The biochemical composition was assessed based on the TVB-N, TMA, and pH values of the shrimp head and shell waste. These parameters are often used as indicators of freshness and the absence of significant changes in these values suggests that the waste material is suitable for further processing. The TVB-N, TMA, and pH of the shrimp head and shell waste were 8.42, 4.20, and 7.81mg/100gm, respectively. The TVB-N, TMA and pH values for protein concentrate were 7.23, 3.72, and 7.68 mg/100g, respectively (Table 1). The TVB-N was much lower than acceptable limit (30-40 mg N/100) reported that TMA value of the thawed shrimp head were of 17.76 mg/100g in fresh head of *L. vannamei*. Lower TMA values indicate that the raw material was fresh. Besides, pH is also an important parameter that affects the shelf life and quality of seafood products. The observed pH value also confirms that the shrimp waste is in favourable condition for utilization.

Mineral composition of shrimp waste and shrimp biscuits: The phosphorous (P), potassium (K), sodium (Na), iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg) and manganese (Mn) content of shrimp waste were 1094.35, 610, 693, 19.74, 7.49, 4.38, 32.11, and 26.01mg/100g, respectively. The resulting shrimp biscuits still contain 674.61, 400 and 1603.8mg/100g phosphorus, potassium and sodium. Additionally, 30.65, 29.31, 21, 1.99 and 3.09mg/100g magnesium, iron, copper, and zinc were also enhanced due to addition of protein concentrate, showcasing the potential to create value-added food products from shrimp waste. Similar observations were reported by in the preparation of tapioca cookies by incorporation of fish carcass flour of different species.

Shelf-life evaluation of the Biscuits incorporated with shrimp head and shell waste protein concentrate

Effect of storage on physicochemical and biochemical composition of biscuits: Concerning physicochemical composition, the results showed a significant alteration in moisture, crude protein, crude fat, crude fiber, ash, carbohydrate content, and energy value; highlighting the impact of storage duration on the product. As the biscuits containing shrimp head and shell waste protein concentrate were stored, the moisture content gradually increased, suggesting that the packing material may have had a permeability error. The crude protein decreased gradually over storage period of 150 days. This gradual decrease in crude protein of shrimp waste biscuits here shown could be associated with the decrease in the available amino acids. The crude fat, crude fiber, and ash content of the biscuit exhibited negligible changes over time. These findings indicate that the biscuit maintained its dietary fiber and mineral content. The carbohydrate content demonstrated non-significant increase. Observed that in biscuits produced from breadfruit and wheat flours enriched with edible fish meal. The energy content of the biscuit remained consistent throughout the storage period.

The biochemical composition, the analysis focused on TVB-N, TMA, and pH revealed significant changes in the biochemical parameters of the biscuits over time. The TVB-N content, an indicator of protein degradation and spoilage, increased gradually throughout the storage period might be

Table 1. Physicochemical, biochemical, and mineral composition of shrimp head and shell waste, protein concentrate, wheat flour, and functional biscuits

Parameters	Shrimp head and shell waste powder	Shrimp waste protein concentrate	Shrimp biscuits	Wheat flour
Physicochemical composition				
Protein (%)	42.34	76.79	14.16	10.23
Crude fat (%)	7.31	0.77	16.14	1.33
Ash (%)	21.11	11.17	7.01	1.00
Moisture (%)	13.94	9.11	5.54	3.33
Crude fiber (%)	9.20	-	4.51	0.51
Carbohydrate (%)	6.10	2.16	52.64	83.6
Energy (kcal/100g)	259.55	322.73	331.76	364
Biochemical composition				
TVB-N (mg/100g)	8.42	7.23	4.29	-
TMA (mg/100g)	4.20	3.72	2.19	-
pH	7.81	7.68	6.50	-
Mineral composition (mg/100g)				
Phosphorous (P)	1094.35	-	674.61	108
Potassium (K)	610	-	400	107
Sodium (Na)	693	-	1603.8	2.00
Iron (Fe)	19.74	-	21.6	2.39
Copper (Cu)	7.49	-	1.99	0.22
Zinc (Zn)	4.38	-	3.09	2.22
Magnesium (Mg)	32.11	-	29.31	120.0
Manganese (Mn)	26.01	-	30.65	0.82

Table 2. Changes in physicochemical composition of biscuits during storage

Storage period (days)	Moisture (%)	Protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Carbohydrate (%)	Energy (kcal/100g)*
0	5.54	14.16	16.14	4.51	7.01	52.64	331.76
30	5.89	13.96	16.016	4.46	6.99	52.68	330.64
60	6.24	13.71	15.88	4.38	6.95	52.82	329.67
90	6.64	13.41	15.73	4.29	6.90	53.01	328.65
120	7.07	13.04	15.55	4.20	6.83	53.30	327.57
150	7.52	12.61	15.35	4.11	6.76	53.65	326.44

due to the increased microbial activity, the breakdown of large amounts of amino acids and an accelerated rate of deamination. Similarly, TMA is responsible for the characteristic "fishy" odor, also exhibited an upward trend during storage which was caused by degradation of protein and non-protein nitrogenous compound. The pH of the biscuits gradually decreased which may be due to the production of volatile compounds continuously after the baking process.

Table 3. Changes in biochemical composition of biscuits during storage

Storage period (Days)	TVB-N(mg/100g)	TMA (mg/100g)	pH
0	4.29	2.19	6.50
30	5.11	2.67	6.10
60	5.88	3.18	5.81
90	6.47	3.71	5.53
120	7.56	4.27	5.25
150	9.22	4.93	5.14

Changes in organoleptic scores of biscuits during storage: Over the course of the storage period, a significant decline was noted in every organoleptic characteristic of the biscuits. However, the overall acceptability of the biscuits remained relatively stable throughout the storage period. This finding suggests although the sensory qualities of the biscuits deteriorated during storage, consumers still were moderately acceptable. Similar results for organoleptic characteristics well observed in Biscuits fortified with Fish protein concentrate.

FTIR studies of raw material and shrimp biscuits: Fourier Transform Infrared Spectrometry (FTIR) was used to identify functional groups as well as other fingerprint groups present in raw material and produced biscuits. Bands ranging from 3675.16 to 3270.47/cm showed intramolecular bonded O-H stretching, indicating the presence of alcohol and hydroxyl groups in the sample. The bands around the 2925.55 to 2852.61/cm indicate the presence of C-H stretching which indicates the presence of alkanes. The range of 2160.69 to 2023.43/cm is known as C-C Stretching, and it indicates the

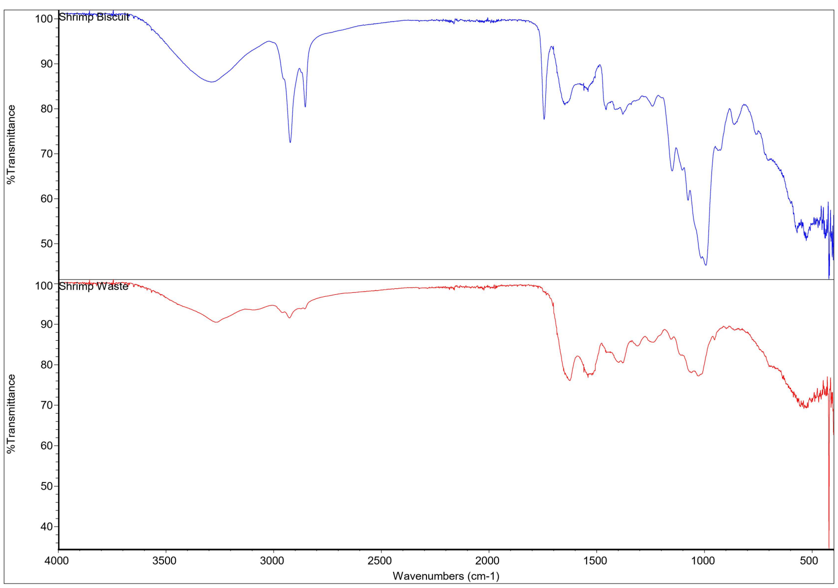

Fig. 1. FT-IR analysis of (a) shrimp biscuit and (b) shrimp head and shell waste

Table 4. Changes in organoleptic evaluation of biscuit during storage

Storage period (days)	Appearance	Taste/Flavour	Texture	Smell	Overall acceptability
0	8.49	7.50	6.52	7.50	7.50
30	7.80	7.31	6.02	7.31	7.11
60	7.50	6.81	5.73	7.01	6.76
90	7.31	6.52	5.53	6.52	6.47
120	6.91	6.12	5.33	6.42	6.20
150	6.52	5.53	5.04	5.83	5.73

Table 5. FT-IR spectral table depicting absorption band, functional groups and compound class concerning shrimp waste and shrimp biscuits

Frequency range	Absorption (cm^{-1})		Functional groups	Compound class
	Shrimp waste	Shrimp biscuits		
4000-3000	3675.16	-	O-H stretching	Alcohol
	3270.47	3288.86	O-H stretching	Alcohol
3000-2500	2925.55	2922.42	C-H stretching	Alkane
	-	2852.61	C-H stretching	Alkane
2400-2000	2160.69	-	CΞC stretching	Alkyne
	2023.43	-	CΞC stretching	Alkyne
2000-1650	1972.07	-	C-H bending	Aromatic compound
	1702.41	1743.48	C=O stretching	Conjugated acid, esters
1670-1600	1623.35	1647.31	C=C stretching	α,β-unsaturated ketone, alkane, conjugated alkane
1600-1300	1558.70	1539.50	N-O stretching	Nitro compound
	1539.62	1455.53	N-O stretching	Nitro compound
	1378.98	1377.96	C-H bending	Aldehyde
1400-1000	1307.96	-	O-H bending	Phenol
	1234.95	1238.91	C-N stretching	Amine
	-	1149.03	C-N stretching	Amine
	1025.17	1075.02	C-F stretching	Fluoro compound
1000-650	952.29	992.59	C=C bending	Alkene
	-	860.73	C-Cl stretching	Halo compound
	634.10	-	C-Br stretching	Halo compound

presence of alkynes. The bands ranging 1972.07/cm represents the C-H bending and indicates presence of aromatic compounds. The spectrum between 1702.41-1743.48/cm represents C=O stretching which indicates the presence of conjugated acids and esters respectively. The range of 1623.35 to 1647.31 cm in the spectrum reveals C=C stretching, indicating α and β-unsaturated ketone, alkane, and conjugated alkane, in that sequence. The bands between 1558.70 to 1455.53/cm and 1378.98-1377.96/cm indicate the N-O stretching and C-H bending respectively which represents nitro compounds and aldehyde, respectively. The band area representing N-O stretching has increased in biscuits in comparison to shrimp head and shell waste which indicates the enrichment of various proteins as well as amino acids in the functional biscuits. The spectrum 1307.96/cm indicates O-H bending which represents phenol and spectrum ranging from 1234.95 to 1149.03/cm indicates C-N stretching which represents Amines in sample. The spectrum 1025.17-1075.02/cm indicates C-F stretching claims the presence of fluoro compounds and peaks 952.29-992.59/cm, 860.73/cm and 634.10/cm indicates C=C bending, C-Cl stretching and C-Br stretching which represents alkene and halo compounds respectively.

CONCLUSION

The present study successfully demonstrated the potential of utilizing shrimp (*Litopenaeus vannamei*) head and shell waste as a valuable ingredient in the development of protein-enriched functional biscuits. The physicochemical analysis of Pacific white shrimp (*Litopenaeus vannamei*) head and shell waste revealed high protein, ash, and mineral content, including phosphorus ,sodium and iron . The shrimp protein concentrate showed an even higher protein content with reduced fat and moisture levels. Biscuits enriched with this shrimp waste demonstrated improved protein and fat levels compared to wheat flour along with enhanced mineral content. During 150 days of storage, the biscuits showed a significant decrease in protein and an increase in moisture, TVB-N and TMA . Despite these changes, sensory scores for overall acceptability remained within acceptable limits until day 120. FT-IR analysis confirmed the presence of functional groups such as O–H, C–H, C=O, and N–O, indicating the retention of key bioactive compounds in both waste and biscuits. These findings demonstrate the nutritional enhancement and storage stability of biscuits enriched with shrimp processing by products.

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Vulnerability Assessment of Coastal Fisher Households in Southern Districts of Tamil Nadu: A Climate Change Perspective

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***Abstract*:** Climate change has had a significant impact on both agricultural and aquatic ecosystems. However, research on the vulnerability of the fishing sector is less extensive than that on the agricultural systems. It is imperative to investigate the various climatic stresses that affect the resilience of these ecosystems, which are crucial to the livelihoods of billions of people. The study evaluated the vulnerability index (VI) of three coastal districts in southern Tamil Nadu using the Parameter, Attribute, Resilient indicator, and score method (PARS). Tirunelveli exhibited the highest vulnerability, followed by Tirunelveli and Kanyakumari. The VI ranges for different villages within these coastal districts were as follows: Tirunelveli (0.05 to 0.27), Kanyakumari (0.04 to 0.30) and Thoothukudi (0.03 to 0.31). Thoothukudi Fishing Harbour (0.31) demonstrated the highest vulnerability index, followed by Colachel (0.30) and Uvari (0.27). Additionally, an assessment of 150 fishing households in the Thoothukudi area utilising the rank-based quotient (RBQ) revealed that climate change differentially impacts environmental, fishery, and socioeconomic development factors. The results of the questionnaire survey indicated that climate change predominantly affects the environment, with subsequent effects on fisheries. This study provides foundational information for the vulnerability assessment of the southern districts of Tamil Nadu.

***Keywords*:** Climate change, Mitigation measures, PARS methodology, Southern Tamil Nadu, Vulnerability

Climate change is a significant global phenomenon, contributing to increased agricultural losses due to droughts and rising sea levels resulting from excessive rainfall (Nelson et al., 2009). More than half a billion individuals rely on fishery ecosystems for their livelihoods, highlighting the critical importance of ecosystem vulnerability (Allison et al., 2009, FAO 2010, Islam et al., 2014). The effects of climate change, including storms, floods, droughts, sea level rise, sea surface temperature, and precipitation, pose threats to populations that depend on fishing for their livelihoods (Sarch and Allison 2000, Perry et al., 2010). Numerous researchers have investigated the impacts of climate change on Indian fisheries (Vivekanandan et al., 2009, Salagrama 2012). However, there is a paucity of baseline vulnerability assessments concerning the effects of climate change on small-scale tropical fishermen, focusing on the risks faced by inland fisheries (Das et al., 2016) and the susceptibility of coastal fishermen (Patnaik and Narayanan 2009).

India ranks sixth among Asian and tropical countries in terms of coastline length (Central Intelligence Agency 2018), and is recognised as one of the 27 countries most vulnerable to sea level rise, coastal erosion, and flooding. Approximately 35% of the Indian population resides along the 7,516-km coastline (Prasad and Rao 2014). The warming of oceans and melting of glaciers significantly contribute to the Indian Ocean's role in India's susceptibility to sea level rise. Vulnerability to climate change is a complex process with coastal districts generally exhibiting heightened vulnerability. Therefore, it is essential to assess vulnerability levels by developing a vulnerability index that is specific to each coastal village. Previously, the coastal vulnerability index (CVI) for the coastal zone of Udupi, Karnataka, was calculated using various shoreline change criteria, including the rate of sea-level change, coastal slope, tidal range, and coastal geomorphology (Dwarakish et al., 2009). It was subsequently utilised as an additional parameter to calculate the CVI for Odisha (Kumar et al., 2010). Several studies have evaluated the vulnerability of coastal fishermen's households on the east and west coasts of India (Muktha et al., 2016, Johnson et al., 2016, Geetha et al., 2017, Koya et al., 2017). The east coast of India is more susceptible than the west coast to extreme events, such as cyclones and depressions (Patwardhan et al., 2003).

Tamil Nadu is a leading state in marine fish production, characterised by a coastline extending 1076 km, encompassing 13 coastal districts, and supporting a population of 7,95,708 fishermen across 575 fishing villages (CMFRI-DoF 2020). Numerous studies on sea-level rise vulnerability along the east and west coasts have utilised physical attributes to compute coastal vulnerability indices.

Research on anthropogenic and tsunami impacts on the east coast of India has examined coastal displacement (Murali et al., 2009), run-up, and inundation boundaries (Jayakumar et al., 2005). The most severely affected region is Nagapattinam, which is located in Tamil Nadu's southern state on the east coast (Ramanamurthy et al., 2005). The Thoothukudi, Kanyakumari, and Tirunelveli districts of southern Tamil Nadu are susceptible to both natural and anthropogenic disasters. The majority of inhabitants in these areas are fishermen residing in huts and face socioeconomic challenges. The southern districts of Tamil Nadu were selected as model maritime states for this study because of their significant contribution to fishery-related industries. This study was conducted to identify the susceptibility indices of coastal villages in three districts in response to climate change, felicitate the planning and implementation of disaster management and mitigation strategies.

MATERIAL AND METHODS

Selection of study location: The study was conducted in the southern districts of Tamil Nadu, namely Thoothukudi, Kanyakumari, and Tirunelveli, spanning from Vembar in the northern part of Thoothukudi (9°04'42.6"N 78°22'01.6"E) to Neerodi in the northern part of Kanyakumari (8°17'22.4"N 77°06'05.8"E). Total coastline 283.9 km, comprising 223.9 km of west coast (from Vembar the southern tip of Kanyakumari), and 60 km along the west coast i.e., from the southern tip of Kanyakumari to Neerodi (Fig. 1).

Vulnerability index: The methodology employed was adapted from Patnaik and Narayanan (2009). Parameters, including demography, occupation, infrastructure, climate components, and fishery components, were assessed for various coastal districts, forming the basis for deriving vulnerability indices for these coastal districts. The framework for constructing the vulnerability index is illustrated in Figure 2. The average index for all four impacts and the vulnerability index were calculated using Equations 1 and 2, respectively (Patnaik and Narayanan, 2009).

Average Index$_i$ = (Indicator 1 + Indicator 2+……………..+ Indicator J)/J …………………..1

$$\text{Vulnerability Index} = [\sum_{i=1}^{n} (\text{Average Index}_i)^{\alpha}]^{1/\alpha} / n \qquad …..2$$

J denotes the number of indicators in each source of vulnerability and n denotes the number of sources of vulnerability.

In selecting coastal villages within the designated coastal district, several factors were considered, including socioeconomic parameters, the number of families living below the poverty line, adult-child ratio, average family size, gender ratio, literacy rate, dependence on fishing activities, inventories of crafts and gear, participation in cooperatives, and ancillary activities. The sampling strategy prioritises the distribution of coastal households to establish vulnerability indicators. Data were collected from various villages using a predefined schedule encompassing multiple aspects such as family characteristics, education, asset details, savings, farming practices, and livestock, primarily focusing on perceptions of climate change awareness and its underlying causes.

Selection of coastal districts*:* Based on Patnaik and Narayanan’s (2009) model, the coastal district vulnerability index was calculated, and the districts were chosen based on an evaluation of the coastal district vulnerability index calculated for each coastal district of Tamil Nadu. The PARS (Parameter, Attribute, Resilient Indicator, and Score) approach was employed to produce vulnerability indicators, and a conceptual framework was created to evaluate the susceptibility of coastal livelihoods to climate change (Koya et al., 2017). The fishermen were asked to rate their vulnerability on a scale ranging from 1 to 5, representing how serious the vulnerability was - five was very high, four was high, three was medium, two was low, and one was negligible/marginal., Thus, every parameter would result in a distinct attribute, which would result in a different statement or resilient indicator that would be determined by a different score. This approach is highly helpful in determining which parameter, or attribute of the parameter, is the area most vulnerable to climate change. The IDLAM study was conducted over a 12-month period in the Threspuram fishing village. Villages were selected based on a comprehensive index developed using the aforementioned parameters. A total of 150 fishing households distributed across the three fishing villages were selected using proportionate random sampling, and the PARS methodology was employed to assess the vulnerability of coastal livelihoods.

Validation: The scores were analyzed using the rank-based quotient technique, and the ranks were assigned such that the attribute that was most impacted was ranked highest. The values of each statement were measured, and 125 statements in the schedule that dealt with climate change were analyzed. The following Rank Based Quotient (RBQ) formula (Sabarathnam 1988) was used to assess the PARS approach.

$$\text{Rank Based Quotient} = \sum_{i=1}^{n} (Fi)(n+1-i) X \frac{100}{Nn}$$

where,

Fi = Frequency of respondents for the ith rank; N = Total number of respondents; n = Total number of ranks/factors.

This methodology is useful for determining which parameter or attribute of the parameter is the most vulnerable factor of the area in terms of climate change via first-hand analysis. Thoothukudi fishermen's perspectives on climate change vulnerability were generated through various participatory programs aimed at raising awareness and encouraging preparation and mitigation measures.

Mapping: The vulnerability indices of different coastal villages integrate various elements into a spatial representation at the village level, enabling the quantification of vulnerability and its contributing factors while also identifying indicators that require further investigation and suitable adaptive measures.

RESULTS AND DISCUSSION

The VI results of the three coastal districts, based on the PARS methodology, revealed that Tirunelveli had the highest average vulnerability (0.15) followed by Kanyakumari (0.13) and Tuticorin had the least average VI values with 5 taluks ranging from 0.03 to 0.31. However, Thoothukudi Fishing Harbour (0.31) is known for its highest value among all villages in 3 districts. The coastal villages and taluks selected for the vulnerability index assessment are shown (Fig. 3) and the detailed VI values for each coastal community and extremely susceptible villages for all three 3 districts are explained in the following sections.

Vulnerability index for the Thoothukudi district: The average vulnerability Index of the six Taluks of Thoothukudi was estimated to be approximately 0.11 and Sathankulam Taluk had the highest VI (0.14). Among the 32 coastal fishing villages in the district, Thoothukudi Fishing Harbor (0.31) had the highest Vulnerability Index, followed by Therespuram (0.22) (Table 1).

Vulnerability indices for the Tirunelveli districts: The vulnerability index (VI) of the fishing villages in the Tirunelveli district ranged from 0.05 - 0.27. The VI was highest in Uvari (0.27), followed by Idinthakarai, Thomaiyar Puram and Radhapuram Taluk (Table 2).

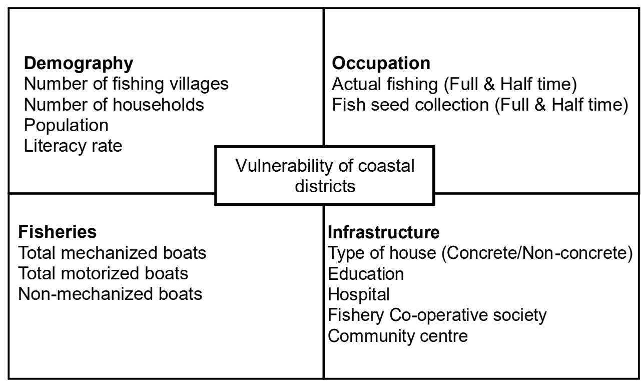


Fig. 2. Framework for constructing the vulnerability index (VI), as modified and adapted from Koya et al. (2017)

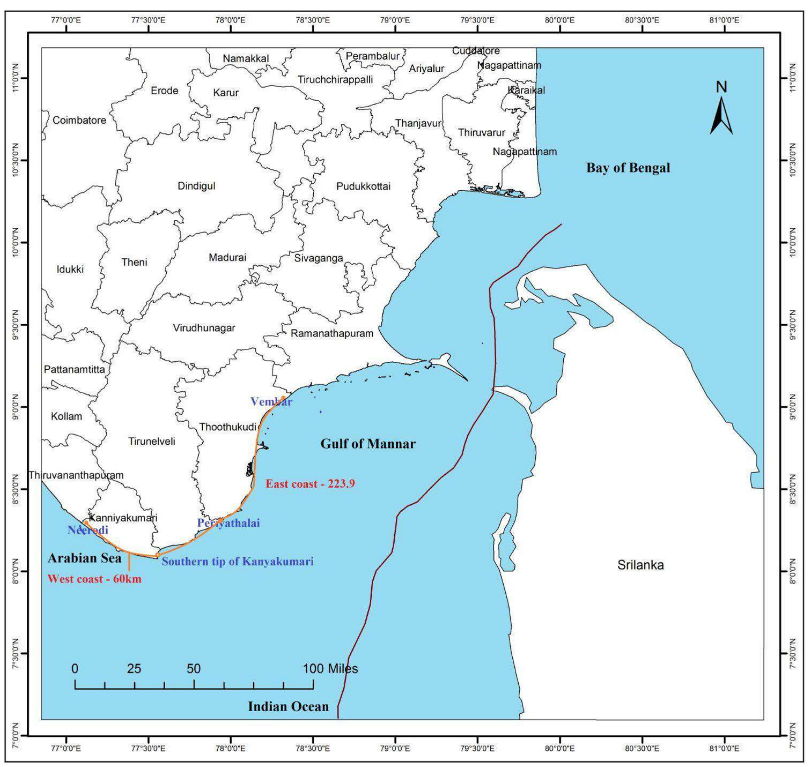
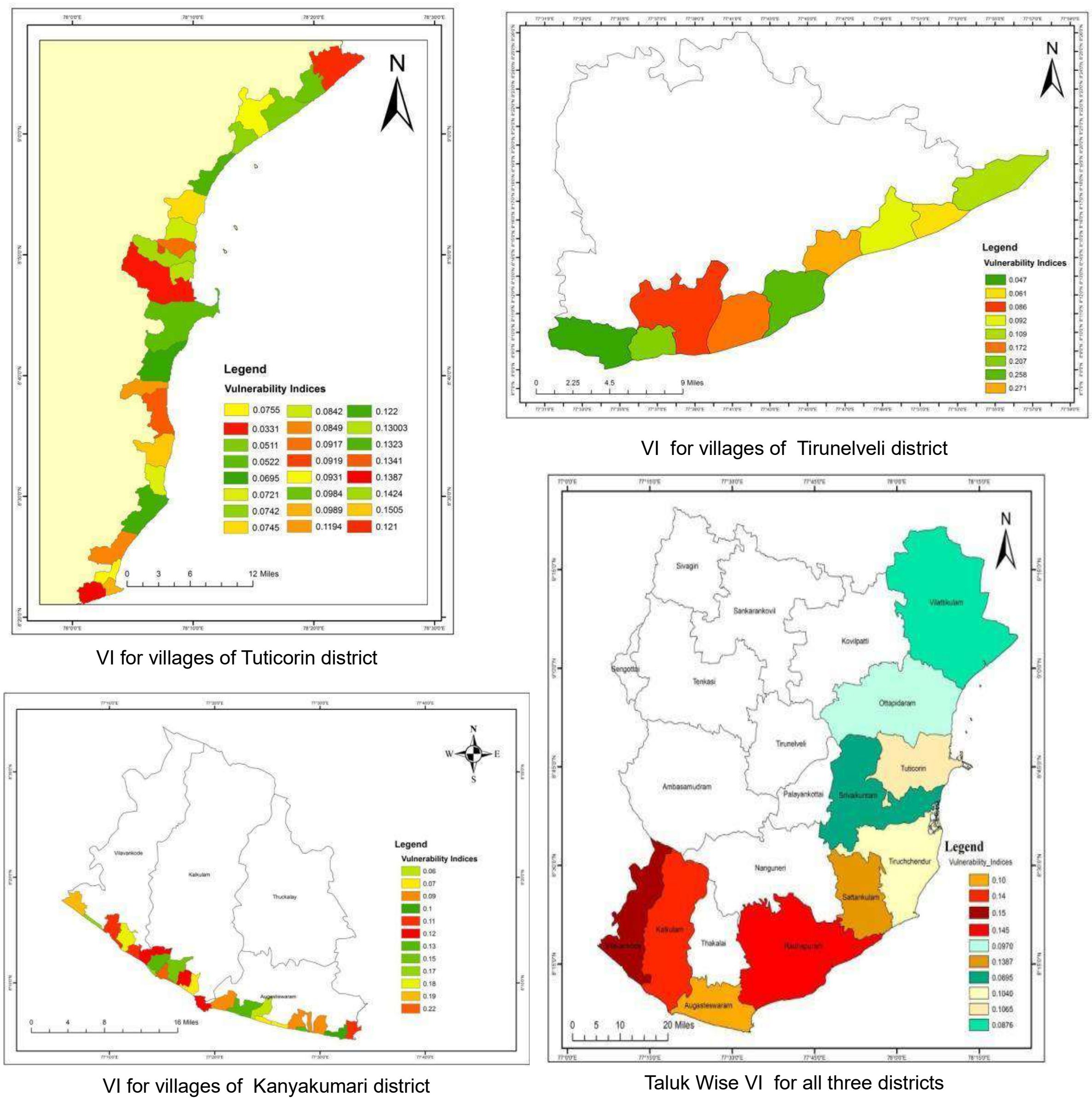


Fig. 1. Study location from Vembar to Neerodi along 3 southern districts of Tamil Nadu

Vulnerability indices for the Kanyakumari districts: The Kanyakumari district comprises three taluks, with an average VI of 0.13, with Vilavancode as the first place, with an average of 0.15. These taluks extend from the southeast coast of Arockiapuram to Neerodi in Southwest India. The vulnerability index (VI) of the 47 fishing villages in the Kanyakumari district ranged from 0.05 0.30 (Table 3). The VI was highest in Colachel (0.30), followed by Eraviputhenthurai (0.25), and lowest in Puthugramam (0.05).

Fishers' perception of the effect of Climate change: Using the PARS methodology, five parameters with different attributes were selected to assess the impact of climate change on the selected study areas. The data perceived from Threpuram fishing village revealed that climate change is most negatively impacted by the environment, with fishery as the second, and social and economic issues coming under the third followed by fishery and economic parameters. Development drivers are known to have the least impact parameter, according to fishermen's perceptions (Fig. 4).

Effects of casual factors on Climate change: The leading factors were temperature (75.95), wind (70.41), industrialization (69.59), and orbital variations (69.73) (Fig. 5). The biggest contributor to climate change is global warming, because rising temperatures cause sea levels and



VI for villages of Tuticorin district

VI for villages of Tirunelveli district

VI for villages of Kanyakumari district

Taluk Wise VI for all three districts

Fig. 3. Maps of vulnerability indices (VIs) for the fishing villages of the Thoothukudi, Tirunelveli, and Kanyakumari districts and the combined Taluk Wise VI map for all three districts

Table 1. Vulnerability indices for the fishing villages (Taluk Wise) in the Thoothukudi district

Taluk - I Thoothukudi	VI	Taluk -II Tiruchendur	VI	Taluk -VI Ottapidaram	VI
Alangarathatu	0.07	Alanthalai	0.08	Vellapatti	0.08
Ananda Nagar	0.03	Amalinagar	0.10	Pattinamaruthur	0.07
Inico Nagar	0.07	Jeevanagar	0.08	Tharuvaikulam	0.13
Loorthammalpuram	0.08	Komputhurai	0.12	Average	0.10
Meenavar Colony	0.09	Kulasekara Patnam	0.07	Taluk - V Vilathikulam	
Mettupatti	0.09	Manapad	0.13	Keezhavaipar	0.07
Rajapalayam	0.03	Punnakayal	0.15	Periyasamypuram	0.09
Siluvaipatti	0.05	Singithurai	0.12	Sippikulam	0.05
Thalamuthu Nagar	0.09	Tsunami Nagar (Anna Nagar)	0.13	Vembar (South)	0.10
Therespuram	0.22	Veerapandian pattinam	0.06	Vembar (North)	0.12
Thoothukudi Fishing Harbor	0.31	Average	0.10	Average	0.09
VOC Statue	0.13	Taluk - III Sathankulam		Taluk - VI Srivaikundam	
Average	0.11	Periyathalai	0.14	Pazhayakayal	0.07
Total average of 6 Taluks					0.11

Table 2. Vulnerability indices for fishing villages (Taluk wise) in the Tirunelveli district

Village	VI	Village	VI	Village	VI
Taluk: Radhapuram					
Idinthakarai	0.26	Kooduthalai	0.11	Koottapannai	0.06
Kuttapuli	0.21	Kuthenkuli	0.17	Periyathalai	0.09
Perumanal	0.09	Thomaiyarpuram	0.05	Uvari	0.27
Average					0.15

Table 3. Vulnerability indices for fishing villages (Taluk wise) in the Kanyakumari district

Taluk-I Agasteeswaram	VI	Taluk-II Kalkulam	VI	Taluk-III Vilavancode	VI
Annai Nagar	0.11	Chinnavalai	0.07	Enayam	0.16
Arockiapuram	0.10	Colachel	0.30	Enayamputhenthurai	0.19
Azhikkal	0.09	Keezhakadiapattinam	0.14	Eraviputhenthurai	0.25
Chinnamuttom	0.12	Keezhamuttom	0.10	Eraymanthurai	0.11
Kanyakumari	0.18	Kodimunai	0.17	Ezhudesam	0.17
Keezhamannakkudy	0.09	Kottilpadu	0.15	Helen colony	0.10
Kesavanputhenthurai	0.08	Kurumpanai	0.14	Keezhamidalam	0.12
Kovalam	0.14	Melakadiapattinam	0.15	Kurumpannai	0.11
Manakudy	0.13	Melamuttom	0.11	Marthandanthurai	0.20
Pallam	0.09	Periyavilai	0.07	Melmidalam	0.11
Periyakadu	0.06	Puthoor	0.12	Mullurthurai	0.09
Pillaithoppu	0.09	Simon Colony	0.14	Neerodi	0.18
Pozhikkarai	0.07	Vaniyakudi	0.12	Poothurai	0.12
Puthenthurai	0.07			Ramanthurai	0.11
Puthugramam	0.05			Thoothoor	0.17
Rajakkamangalam	0.13			Vallavillai	0.18
Siluvai Nagar	0.04				
Vavathurai	0.09				
Average	0.1	Average	0.14	Average	0.15
Total average for 3 Taluks					0.13

ocean currents to shift. One of the main causes of the increase in global temperature is pollution from farming practices, industries, and transportation. This perspective demonstrates how all components are related to one another. Figure 6 explains fishers' perceptions of climate change impacts with detailed attributes.

Analysis of climate change impacts on fisheries: According to fishers' perceptions, fishery impacts come next to environmental impacts. From the study of Threspuram village fishermen, fishery parameters are mostly affected by species composition, followed by catch and loss in the fishery. Fishermen claim that, as a result of climate change, important fish breeding seasons along the coast have shifted, and coastal species have migrated to open waters. The fishing season also experienced a significant shift. In recent years, equipment and craft have significantly damaged by harsh weather events. In response to climate change, more than 50 fishing families from the tsunami colony are growing seaweed in the Thoothukudi area. Over the past ten years, heat waves and flooding have periodically destroyed crops in some seasons. Johnson et al. (2016) conducted an attribute analysis of different parameters and found that the fishery was impacted mostly by species composition and catch. The attribute loss in the fishery inventory and aquaculture was followed by the species composition and catch attributes in the Ramanathapuram district of Tamil Nadu. Shyam et al. (2014) indicated that the fishery was impacted mostly by bycatch in the Alappuzha district of Kerala. The analysis of the resilience indicator for this attribute indicated that fish catch has decreased

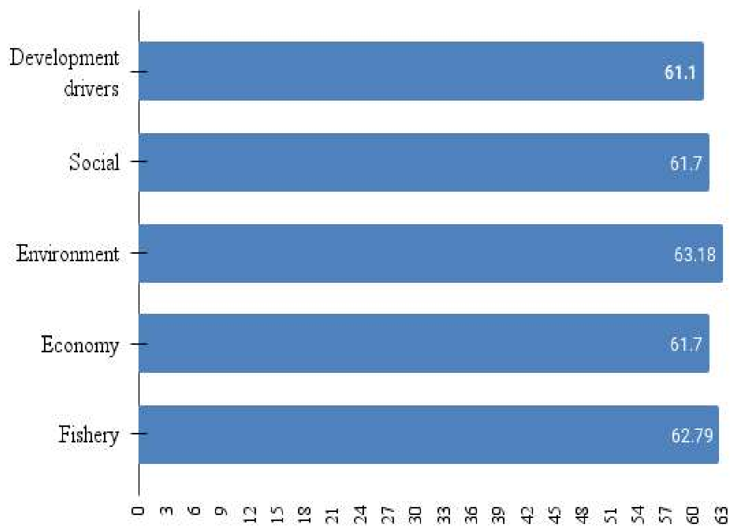


Fig. 4. Fisher's perception of climate change impacts (in percentage)

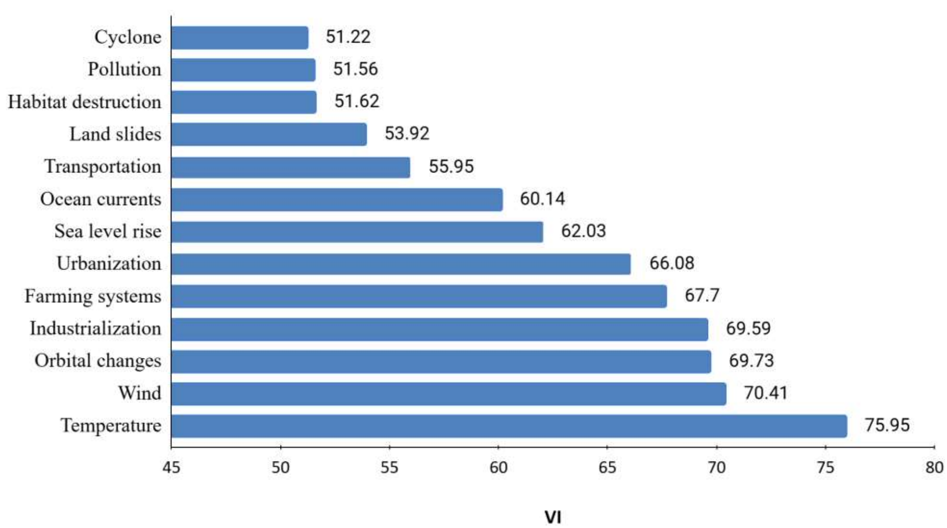


Fig. 5. Causal factors perceived by the fishermen for climate change (in percentage)

drastically over the years, and that effort has increased fairly. The attributes of phenology, distribution, and species composition followed those of catch.

Analysis of the impacts of climate change on the economy: According to fishers' perceptions, economic attributes were impacted next to the fishery in the context of climate change, where livestock and crops were the most limiting factors, followed by income effects, losses in fishing days, and migration. Owing to the shifting fishing grounds and rising fuel prices, fishing has become increasingly expensive. The Food and Agricultural Organization (FAO 2007) found that the doubling diesel price led to a doubling of the proportion of fishers' revenue that they spent on fuel and rendered many individual fishing operations unprofitable, which lends credence to this claim. Reduced income levels, rising living expenses, employment seasonality, and a lack of other pursuits all have an impact on income. Fishermen believe that over the past ten years, there has been a significant decrease in fishery resources. The increased number of boats, unrestricted fishing methods, and the adoption of horsepower-rich engines in boats have caused this drastic decrease. Therefore, there has been an increase in the number of crops and cattle. The least affected feature was the fishing cost. Johnson et al. (2016) and Shyam et al. (2014) revealed that the cost of fishing is the limiting factor for Ramanathapuram and Alappuzha.

Analysis of the impacts of climate change on the environment: According to fishermen's perceptions of Threspuram village, the environment is the most affected by climate change. Sea level rise was the most important attribute, followed by extreme weather, while habitat destruction was the least important environmental factor from the data gathered. According to an analysis of the attribute statements, Thoothukudi fishing communities have experienced significant increases in sea level and coastline erosion, which have affected the fishing and related activities of local fishermen. Similarly, previous reports have noted that coastal erosion and rising sea levels have affected fishing and other related activities (Johnson et al., 2016, Shyam et al., 2014). The respondents also felt that there was a noticeable decline in the number of rainy days over time, unpredictable monsoons, significant damage to freshwater sources, and challenging groundwater use as a result of seawater flooding.

Analysis of the impacts of climate change on social factors: The fishermen's households had an equal level of conception of the social and economic factors of climate change. The most affected attribute was demography, followed by social standards, and community orientation. Infrastructure sensitivity was the least affected by social participation. Low awareness, limited technical information exchange, insufficient training programs, and declining social participation among fishers have an impact on social participation. Community group mobilization is extremely uncommon, and there is a dearth of community-based grassroots plans. Social factors strongly impact climate change through infrastructure sensitivity, followed by social participation, community orientation, demography, and social standards of fishers in the Ramanathapuram district of

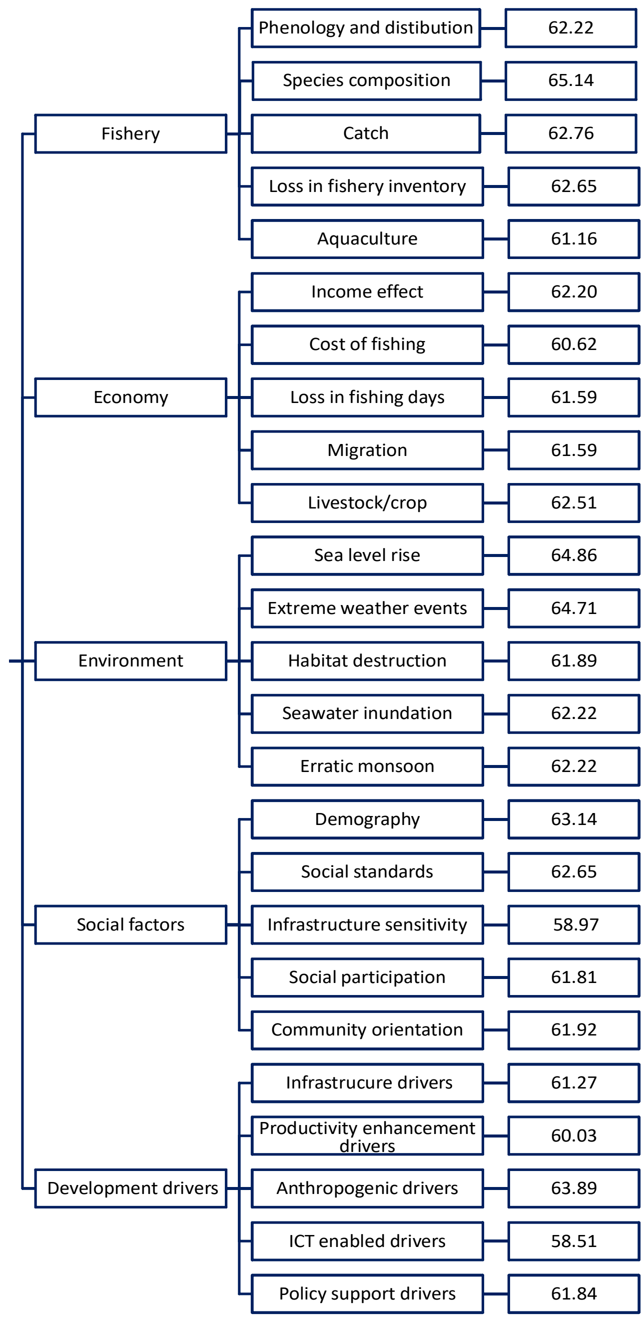


Fig. 6. Attribute analysis of climate change impacts on different parameters (Fishery, economy, environment, social factors and development drivers) (in percentage)

Tamil Nadu (Johnson et al., 2016) while The results from Alapuzha of Kerala have the most impact on social participation, followed by community orientation, social standards, demography, and infrastructure sensitivity (Shyam et al., 2014)

Analysis of climate change impacts on development drivers: In Threpuram village that fishermen had the least perception of the development drivers impacting climate change. At the attribute level, anthropogenic drivers scored highest, followed by policy drivers productivity enhancement and ICT-enabled drivers were the least affected in study. The analysis of the statements showed that the majority of the impacts were caused by human activity because of the rise in plastic use associated with coastal tourism and related activities, as well as the growth of residential areas and the disregard for sustainability issues displayed by development activities. In the context of climate change, Ramanathapuram fishermen believed that anthropogenic drivers had the greatest impact on fisheries and productivity enhancement drivers had the least impact (Johnson et al., 2016). Fishermen from Alappuzha believed that policy support drivers had the greatest impact and infrastructure drivers had the least impact on development drivers. Fishers perceived that inadequate cumulative relief and unplanned rehabilitation measures made this district more vulnerable. The study also considered the degree of community involvement and mobilization, alternative career possibilities, awareness, preparedness, and mitigation, as well as the extent of governmental support and requirements. This study underscores the significant impacts of climate change on various attributes, including fisheries, environment, development drivers, economics, and social standards.

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CONCLUSION

The fishermen perceive fishery, economic, and environmental parameters as important in climate change adaptation and mitigation plans. Fishermen's awareness level was moderate to poor, indicating that they were unable to relate the environmental changes caused by climate change to their livelihood. Fishermen were vulnerable to unpredictable monsoons and lost fishing days. By incorporating fishermen into planning and preparing for disasters, it is necessary to increase their awareness of climate change. Additionally, it is suggested that the governance of fisheries must address overfishing and overcapacity, while accounting for socioeconomic characteristics, uncertainty, and sustainability. Resilient fisheries can be achieved by adopting strategies such as co-management or participatory techniques that address the unpredictability, expertise, and dedication of fishermen in diverse places.

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Seasonal Distribution and Diversity of Fish Community in Anthropogenic Prone Zone of Ganga River

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Abstract: This study investigates fish biodiversity in the midstream region between Kanpur and Ramnagar, focusing on the effects of anthropogenic pressures like as pollution and habitat fragmentation on species diversity and distribution Seasonal sampling at nine sites during three years (2022-2024) documented 109 fish species from 71 taxa and 31 families, with Cyprinidae being the most abundant. The Shannon-Wiener Diversity Index (H') peaked at 4.25 at site Prayagraj while the Evenness Index (E^H/S) reached 0.52 at site Varanasi post-winter and the Margalef Index peaked at 4.67 at site Varanasi post-summer. Relative abundance (RA) data revealed seasonal dynamics, with *Channa striata* having consistent RA values of 7.92 (post-summer), 6.57 (post-winter), and 5.82 (post-monsoon), whereas *Acanthocobitis botia* had lower, inconsistent values of 0.20 (post-summer), 0.34 (post-winter), and 0.12 (post-monsoon). Sites Prayagraj and Varanasi were classified as biodiversity hotspots, while Bahupura and Chunar had lower richness. The findings emphasize the impact of environmental and anthropogenic variables on fish biodiversity. Sustainable fishing and habitat restoration are critical conservation techniques for maintaining biodiversity and the Ganga River's ecological health.

Keywords: Ganga River, Fish biodiversity, Biodiversity indices, Threats, Conservation

Fish variety is important for local food security, income, and cultural traditions (Garcia et al., 2021), as well as the survival of endangered species like dolphins (*Platanista gangetica*) and gharials (*Gavialis gangeticus*) (Chaudhary et al., 2023). Fish health is critical for maintaining ecological stability and resilience (Singh and Das 2022). However, anthropogenic activities such as overfishing, habitat fragmentation, pollution, and climate change pose a serious danger to freshwater fish diversity by degrading habitats and reducing populations (Kumar et al., 2023, Roy and Sharma 2023). Conservation methods such as sustainable fishing, ecosystem restoration, and pollution reduction are critical for ecological balance and long-term community livelihoods (Sharma et al., 2022).

Fish diversity studies employing measures such as Species Richness, Shannon-Wiener Diversity Index (H'), Simpson's Diversity Index (D), and Evenness Index (J') provide information about biodiversity and ecological complexity (Magurran, 2013; Shannon 1948, Pielou 1966). The Fish Community Index (FCI) is an effective measure for correlating fish community health to environmental factors (Bouvier et al., 2022). Monitoring these metrics aids in developing effective conservation strategies for sustainable fisheries management (Patel and Joshi 2024).

The Ganga River, which stretches over 2,500 kilometres, is ecologically, culturally, and historically significant. Its midstream portion between Kanpur and Varanasi supports around 140 native fish species, including economically important carps that benefit local fishing populations (Sarkar et al., 2011). This area is crucial for balancing upstream purity and downstream stresses, but it confronts difficulties from temperature changes, changed flow regimes, and invasive species including *Cyprinus carpio* and *Oreochromis mossambicus* (Krishnamurti et al., 2021, Lakra et al., 2022).

The key objectives are to measure seasonal fish diversity, analyse the effects of pollution and habitat fragmentation, investigate community conservation activities, and propose strategies for sustainable fishing and habitat restoration in the Ganga River.

MATERIAL AND METHODS

The study was carried out over the 340-kilometer main stretch of the Ganga River from Kanpur to Ramnagar, which included areas upstream and downstream of Prayagraj which, because of their high levels of urbanization, industrial discharge, agricultural runoff, and religious activities, are acknowledged as anthropogenic prone zones. Nine sampling sites were strategically selected to represent varied land use patterns and anthropogenic pressures-including industrial, agricultural, semi-urban, urban, pilgrimage, and residential areas (Table 1, Fig. 1). Seasonal fish migratory and spawning cycles, which are important for riverine fish biodiversity, were investigated from 2022 to 2024 during the post-monsoon, post-winter, and post-summer seasons.

Sampling design and frequency: For representative sample, several local fishing methods were utilized, such as weirs, trap nets, gillnets, cast nets, hook and line, and lift nets. Trap nets had the largest species diversity, validating the idea that gear type effects freshwater fish composition (Pandey et al., 2023). Standardized fishing with varied mesh sizes, as well as cooperation from local fishermen, enabled a wide range of species representation.

Sampling was recorded seasonally at each location, with one sampling session per season. Each session included systematic experimental fishing from 07:00 to 13:00 hours to account for diel fluctuations in fish activity. Standard fishing gear with varied mesh sizes were utilized, including:

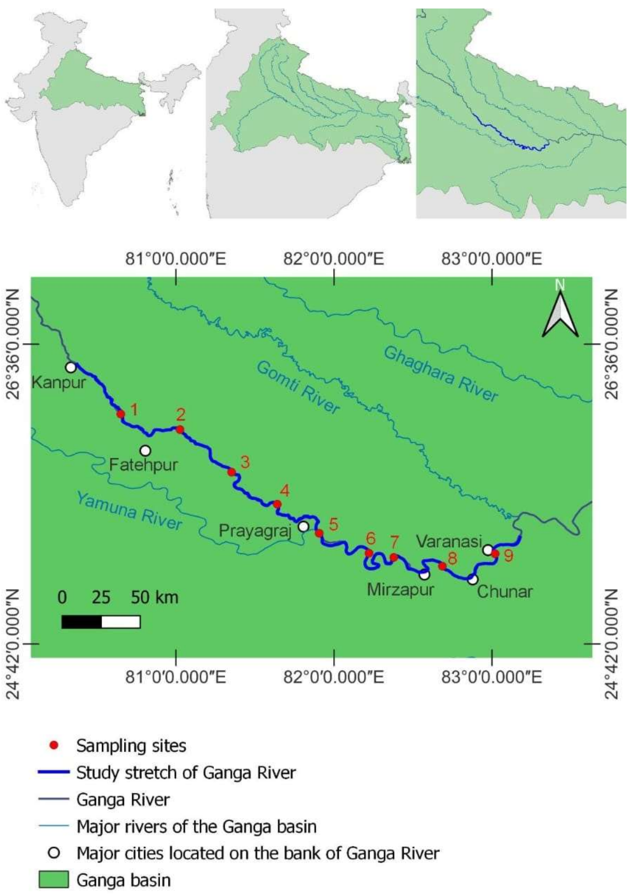

Fig. 1. Study area showing study sites, Ganga River, India

Gill nets: 2.5×2.5 cm, 3×3 cm, and 7×7 cm mesh sizes, with dimensions 75×1.3 m and 50×1 m.

Cast nets: 6×6 mm mesh, deployed 30 times per session with a settling time of 3 to 5 minutes, covering approximately 100 m² per river reach as per Bain and Knight (1996).

Drag nets: 7×7 mm mesh, measuring 80×2.5 m.

Fry collection nets*:* Nylon mosquito mesh for capturing juvenile and smaller fish species.

Approximately 10% of specimens were kept in 10% formaldehyde for identification (Talwar and Jhingran 1991, Jayaram 1999). Fish market trips helped to identify commercially valuable species (Ramesh and Gupta 2023). Primary and secondary data were used to examine fish biodiversity and ecological health under anthropogenic stress at nine sites (Kanpur- Ramnagar) (S1-S9) throughout the post summer, post winter and post monsoon seasons. The analysis uses three ecological metrics:

Shannon-Wiener Diversity Index (H'):

$H = -\Sigma\, (ni/N) \log_2 (ni/N)$

Where: H = Shannon–Wiener index of diversity, ni = Total numbers of individuals of species, N = Total number of individuals of all species.

Pielou's Evenness Index (E^H/S):

$E = H/\ln S$

Where: H =Diversity index, S = Total number of species

Margalef's Richness Index (D_Mg):

$d = S-1/\ln N$

Where: S = Total No. of species, N = Total No. of individuals of all species

RESULTS AND DISCUSSION

Fish diversity and taxonomic composition: The 109 fish species in nine Ganga River localities, representing 71 taxa and 31 families were recorded. Five families (Bagridae, Channidae, Siluridae, Schilbeidae, and Cyprinidae) were observed at every location, indicating widespread dispersion. Midstream zones had increased species

Table 1. Details of the sampling sites

Site	Location	Coordinates (Latitude, Longitude)	Altitude (ft)	Land use pattern
S1	Kanpur	26.4499° N, 80.3319° E	~410	Industrial, urban
S2	Dalerganj	25.6470° N, 81.9245° E	~300	Agricultural, rural
S3	Fatehpur	25.9273° N, 80.8135° E	~331	Agricultural, semi-urban
S4	Prayagraj	25.4358° N, 81.8463° E	~299	Urban, pilgrimage, cultural
S5	Bahupura	25.1330° N, 82.5654° E	~260	Agricultural, rural
S6	Mirzapur	25.1450° N, 82.5698° E	~266	Urban, semi-industrial
S7	Chunar	25.1263° N, 82.8839° E	~275	Agricultural, rural
S8	Varanasi	25.3176° N, 82.9739° E	~266	Urban, pilgrimage, cultural
S9	Ramnagar	25.2766° N, 82.9950° E	~275	Urban, residential

richness owing to steady flow and nutrients, which supported piscivorous and omnivorous fish such as Bagridae and Schilbeidae (Rai and Singh 2022, Sharma and Singh 2022). Cyprinidae, known for their ecological endurance, accounted for 45% of the fauna (43 species). Cyprinidae accounted for 39%, followed by Bagridae, Sisoridae and Channidae (Sinha et al., 2023). The majority of species were classified as Least Concern (LC), with *Ailia coila* and *Pangasius pangasius* listed as Vulnerable (VU). The RA suggested seasonal fluctuation; *Puntius terio* peaked in PS (12.50) but declined in PM (3.42), but *Bagarius bagarius* had consistently low RA, indicating the need for conservation (Table 2).

Spatial and seasonal variations in species richness The Shannon-Wiener diversity map (Fig. 3) revealed hotspots in Prayagraj (4.253) and Varanasi (4.175), whereas Bahupura (3.69) had the least diversity. Contour lines demonstrated biodiversity shifts, linking pollution and habitat degradation to

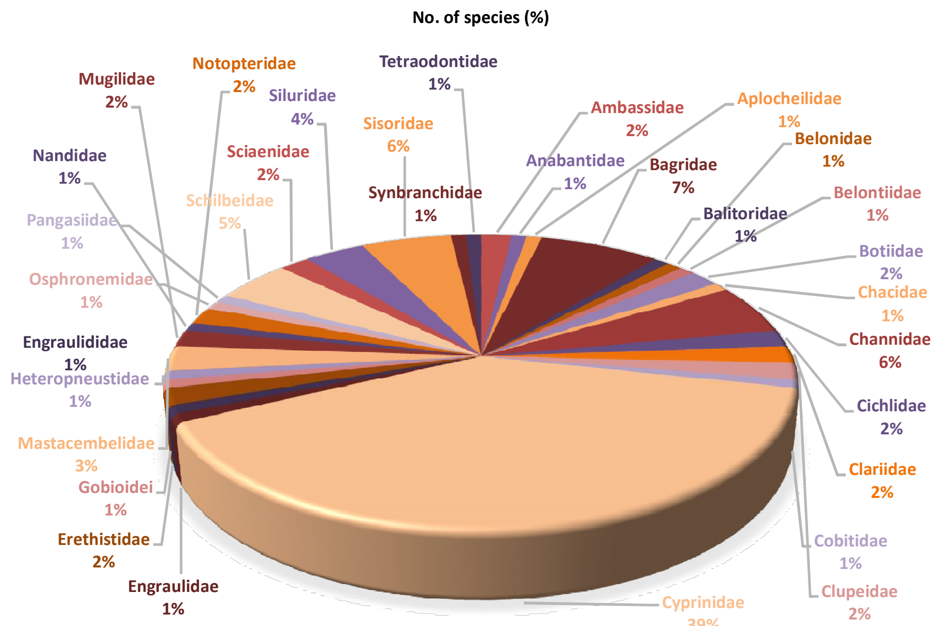

Fig. 2. Percentage composition of different fish species (families), Ganga River, India

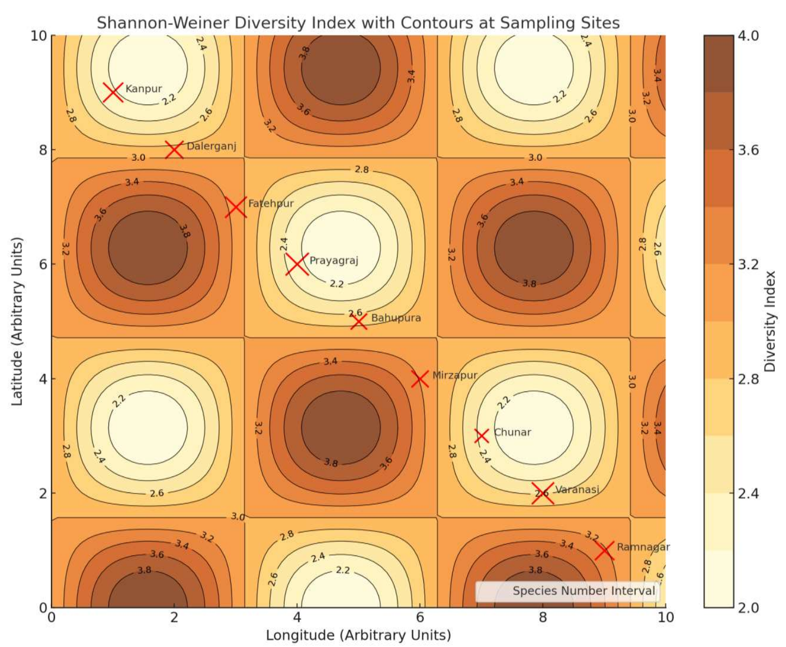

Fig. 3. Shannon-Weiner diversity index at different sampling sites of Ganga River

Table 2. Native and non-native fish species in the middle stretch of the Ganga River with conservation status and seasonal relative abundance (RA)

Scientific name	Order	Family	Abbreviation	iucn status	RA (PS)	RA (PW)	RA (PM)	Nativity
Acanthocobitis botia	Cypriniformes	Balitoridae	ABC	(LC)	0.20	0.34	0.12	Native
Ailia coila	Siluriformes	Schilbeidae	ACS	(VU)	3.52	0.34	3.04	Native
Ambastaia lohachata	Cypriniformes	Botiidae	ALC	(LC)	2.84	2.97	2.22	Native
Amblipharyngodon mola	Cypriniformes	Cyprinidae	AMC	(LC)	2.21	3.85	2.19	Native
Anabas testudineus	Anabantiformes	Anabantidae	ATA	(LC)	0.45	2.00	1.14	Native
Aplocheilus panchax	Cyprinodontiformes	Aplocheilidae	APC	(LC)	3.52	1.56	0.83	Native
Aspidoparia jaya	Cypriniformes	Cyprinidae	AJC	(LC)	2.02	2.32	2.43	Native
A. morar	Cypriniformes	Cyprinidae	AOC	(LC)	0.44	1.29	1.14	Native
Bagarius bagarius	Siluriformes	Sisoridae	ABS	(EN)	0.58	0.68	0.25	Native
Batasio batasio	Siluriformes	Bagridae	BBS	(LC)	1.59	1.66	0.70	Native
Bengala elanga	Cypriniformes	Cyprinidae	BEC	(DD)	1.81	4.58	1.93	Native
Botia dario	Cypriniformes	Botiidae	BDC	(LC)	3.36	5.42	2.56	Native
Brachydanio rerio	Cypriniformes	Cyprinidae	BRC	(LC)	1.80	0.66	1.11	Native
Chaca chaca	Siluriformes	Chacidae	CCS	(LC)	0.48	1.56	0.25	Native
Chagunius chagunio	Cypriniformes	Cyprinidae	CHY	(LC)	1.34	2.48	1.12	Native
Chanda nama	Perciformes	Channidae	CNP	(LC)	1.03	1.23	0.49	Native
Channa marulius	Anabantiformes	Channidae	CMA	(LC)	4.66	7.47	3.46	Native
C. orientalis	Perciformes	Channidae	COP	(LC)	1.05	1.39	0.74	Native
C. punctatus	Perciformes	Channidae	CPP	(LC)	5.26	1.89	3.39	Native
C. stewartii	Perciformes	Channidae	CSP	(LC)	0.55	1.34	0.49	Native
C. striata	Perciformes	Channidae	CTP	(LC)	7.92	6.57	5.82	Native
Chela cachius	Cypriniformes	Cyprinidae	CAC	(LC)	0.64	1.68	0.49	Native
C. laubuca	Cypriniformes	Cyprinidae	CLC	(LC)	0.68	1.29	0.25	Native
Chitala chitala	Osteoglossiformes	Notopteridae	CCO	(LC)	0.48	2.05	0.00	Native
Cirrhinus mrigala	Cypriniformes	Cyprinidae	CMC	(LC)	1.02	1.16	0.97	Native
C. reba	Cypriniformes	Cyprinidae	CRC	(LC)	6.35	1.00	4.25	Native
Clarias batrachus	Siluriformes	Clariidae	CBS	(LC)	4.88	8.10	3.18	Native
C. gariepinus	Siluriformes	Clariidae	CGS	(LC)	3.38	3.95	3.11	Non-Native
Clupisoma garua	Siluriformes	Schilbeidae	CAS	(LC)	2.56	1.16	2.47	Native
Colisa fasciatus	Perciformes	Belontiidae	CFP	(LC)	4.03	5.68	3.81	Native
Crossocheilus latius	Cypriniformes	Cyprinidae	CLP	(LC)	0.90	2.32	0.84	Native
Ctenopharyngodon idella	Cypriniformes	Cyprinidae	CIC	(LC)	0.41	1.56	1.49	Non-Native
Cyprinus carpio	Cypriniformes	Cyprinidae	CPC	(LC)	2.67	2.68	3.13	Non-Native
Danio devario	Cypriniformes	Cyprinidae	DDC	(LC)	4.07	3.71	2.89	Native
Erethistes pusilus	Siluriformes	Erethistidae	EPS	(LC)	2.11	2.07	1.10	Native
Eutropiichthys murius	Siluriformes	Schilbeidae	EMS	(VU)	0.49	1.39	0.84	Native
E. vacha	Siluriformes	Schilbeidae	EVS	(LC)	0.68	0.34	0.76	Native
Gagata cenia	Siluriformes	Sisoridae	GCS	(LC)	3.02	1.63	1.66	Native
G. gagata	Siluriformes	Sisoridae	GGS	(LC)	0.65	3.29	0.77	Native
Garra gotyla	Cypriniformes	Cyprinidae	GGC	(LC)	3.54	2.05	1.19	Native
Glossogobius giuris	Perciformes	Gobioidei	GGP	(LC)	1.54	4.05	0.36	Native
Gonialosa manmina	Clupeiformes	Clupeidae	GMC	(LC)	0.39	0.95	0.64	Native

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Table 2. Native and non-native fish species in the middle stretch of the Ganga River with conservation status and seasonal relative abundance (RA)

Scientific name	Order	Family	Abbreviation	iucn status	RA (PS)	RA (PW)	RA (PM)	Nativity
Gudusia chapra	Clupeiformes	Clupeidae	GCC	(LC)	0.94	3.70	2.04	Native
Hara hara	Siluriformes	Erethistidae	HHS	(LC)	5.32	9.44	2.53	Native
Heteropneustes fossilis	Siluriformes	Heteropneustidae	HFS	(LC)	0.49	5.18	2.09	Native
Hypophthalmichthys molitrix	Cypriniformes	Cyprinidae	HMC	(LC)	1.83	4.76	2.03	Non-Native
H. nobilis	Cypriniformes	Cyprinidae	HNC	(LC)	0.76	2.44	1.11	Non-Native
Johnius coitor	Perciformes	Sciaenidae	JCP	(LC)	1.84	1.39	1.08	Native
J. gangeticus	Perciformes	Sciaenidae	JGP	(LC)	1.34	4.54	0.88	Native
Labeo angra	Cypriniformes	Cyprinidae	LCC	(LC)	2.23	0.89	0.96	Native
L. bata	Cypriniformes	Cyprinidae	LBC	(LC)	5.55	1.66	1.13	Native
L. boga	Cypriniformes	Cyprinidae	LOC	(LC)	2.36	0.27	1.07	Native
L. boggut	Cypriniformes	Cyprinidae	LGC	(LC)	0.68	1.69	1.78	Native
L. calbasu	Cypriniformes	Cyprinidae	LAC	(LC)	2.54	3.32	1.61	Native
L. catla	Cypriniformes	Cyprinidae	LTC	(LC)	5.63	3.26	3.54	Native
L. dero	Cypriniformes	Cyprinidae	LDC	(LC)	5.67	1.23	2.74	Native
L. gonius	Cypriniformes	Cyprinidae	LNC	(LC)	1.95	1.00	0.49	Native
L. pangusia	Cypriniformes	Cyprinidae	LPC	(LC)	1.18	0.89	0.61	Native
L. rohita	Cypriniformes	Cyprinidae	LRC	(LC)	0.44	1.23	1.03	Native
Lepidocephalichthys guntea	Cypriniformes	Cobitidae	LUC	(LC)	0.65	2.53	1.49	Native
Macrognathus aral	Perciformes	Mastacembelidae	MAP	(LC)	1.25	1.71	1.62	Native
M. pancalus	Perciformes	Mastacembelidae	MPP	(LC)	5.35	2.10	7.41	Native
Mastacembelus armatus	Perciformes	Mastacembelidae	MRP	(LC)	16.50	0.95	8.06	Native
Monopterus cuchia	Synbranchiformes	Synbranchidae	MCS	(LC)	8.86	3.29	3.89	Native
Mystus bleekeri	Siluriformes	Bagridae	MBS	(LC)	6.19	3.74	2.00	Native
M. cavasius	Siluriformes	Bagridae	MAS	(LC)	1.18	2.76	4.14	Native
M. tengara	Siluriformes	Bagridae	MTS	(LC)	7.32	1.23	3.30	Native
M. vittatus	Siluriformes	Bagridae	MVS	(LC)	1.47	2.57	1.77	Native
Nandus nandus	Perciformes	Nandidae	NNP	(LC)	4.24	5.33	1.30	Native
Nangra nangra	Siluriformes	Sisoridae	NNS	(VU)	0.54	2.11	0.74	Native
N. punctata	Siluriformes	Sisoridae	NPS	(LC)	1.25	3.87	0.62	Native
Notopterus notopterus	Osteoglossiformes	Notopteridae	NNO	(LC)	1.97	1.00	0.48	Native
Ompok bimaculatus	Siluriformes	Siluridae	OBS	(LC)	0.99	3.06	1.12	Native
O. pabda	Siluriformes	Siluridae	OPS	(LC)	2.74	4.81	1.93	Native
O. pabo	Siluriformes	Siluridae	OAS	(LC)	1.38	1.73	1.12	Native
Oreochromis mossambicus	Cichliformes	Cichlidae	OMC	(LC)	0.59	1.55	0.83	Non-Native
O. niloticus niloticus	Cichliformes	Cichlidae	ONC	(LC)	1.44	4.39	2.30	Non-Native
Osteobrama cotio	Cypriniformes	Cyprinidae	OCC	(LC)	3.47	5.93	3.53	Native
Pangasius pangasius	Siluriformes	Pangasiidae	PPS	(VU)	13.00	5.00	1.36	Native
Parambassis baculis	Perciformes	Ambassidae	PBP	(LC)	5.15	1.34	4.26	Native
P. ranga	Perciformes	Ambassidae	PRP	(LC)	0.99	1.86	4.16	Native
Pseudeutropius atherinoides	Cypriniformes	Cyprinidae	PAC	(LC)	2.50	0.95	7.23	Native
Puntius chola	Cypriniformes	Cyprinidae	PCC	(LC)	2.97	2.88	4.86	Native

Cont...

Table 2. Native and non-native fish species in the middle stretch of the Ganga River with conservation status and seasonal relative abundance (RA)

Scientific name	Order	Family	Abbreviation	iucn status	RA (PS)	RA (PW)	RA (PM)	Nativity
P. conchonius	Cypriniformes	Cyprinidae	POC	(LC)	7.22	1.76	1.27	Native
P. gelius	Cypriniformes	Cyprinidae	PGC	(LC)	0.39	1.78	2.90	Native
P. puntio	Cypriniformes	Cyprinidae	PPC	(LC)	1.53	2.25	2.99	Native
P. sarana	Cypriniformes	Cyprinidae	PSC	(LC)	0.54	0.62	1.34	Native
P. sophore	Cypriniformes	Cyprinidae	PHC	(LC)	1.24	2.63	4.66	Native
P. terio	Cypriniformes	Cyprinidae	PTC	(LC)	12.50	3.42	9.39	Native
P. ticto	Cypriniformes	Cyprinidae	PIC	(LC)	0.44	1.76	6.42	Native
Raiamas bola	Cypriniformes	Cyprinidae	PBC	(LC)	2.24	3.71	7.86	Native
Rasbora rasbora	Cypriniformes	Cyprinidae	RRC	(LC)	0.89	0.60	3.87	Native
daniconius	Cypriniformes	Cyprinidae	RDC	(LC)	0.58	1.52	1.68	Native
Rhinomugil corsula	Perciformes	Mugilidae	RCP	(LC)	0.87	1.32	2.81	Native
Rita rita	Siluriformes	Bagridae	RRS	(LC)	2.08	1.36	0.68	Native
Salmophasia phulo	Cypriniformes	Cyprinidae	SPC	(LC)	1.89	1.23	1.29	Native
Salmostoma bacaila	Cypriniformes	Cyprinidae	SBC	(LC)	1.95	2.77	3.76	Native
Securicula gora	Cypriniformes	Cyprinidae	SGC	(LC)	2.79	2.66	3.83	Native
Setipinna brevifilis	Clupeiformes	Engraulidae	SRC	(LC)	2.80	2.29	3.11	Native
S. phasa	Clupeiformes	Engraulidae	SHC	(LC)	4.14	2.18	6.78	Native
Sicamugil cascasia	Perciformes	Mugilidae	SCP	(LC)	3.94	3.62	0.34	Native
Silonia silondia	Siluriformes	Schilbeidae	SSS	(VU)	4.96	1.64	0.00	Native
Sisora rhabdophorus	Siluriformes	Sisoridae	SRS	(LC)	0.63	0.25	0.68	Native
Sperata aor	Siluriformes	Bagridae	SAS	(VU)	0.44	1.27	0.34	Native
S. seenghala	Siluriformes	Bagridae	SES	(LC)	5.92	3.80	1.34	Native
Tetraodon cutcutia	Tetraodontiformes	Tetraodontidae	TCT	(LC)	4.71	3.34	1.02	Native
Trichogaster lalius	Anabantiformes	Osphronemidae	TLA	(LC)	1.31	0.63	2.50	Native
Wallago attu	Siluriformes	Siluridae	WAS	(VU)	2.89	0.97	1.02	Native
Xenentodon cancila	Cyprinodontiformes	Belonidae	XAC	(LC)	7.60	2.89	3.39	Native

changes in diversity (Bhatt et al., 2023).Temporal diversity varies by region between 2022 and 2024 (Fig. 4). Kanpur (S1) and Dalerganj (S2) remained steady, but Fatehpur (S3), Mirzapur (S6), and Chunar (S7) exhibited fluctuation. Prayagraj (S4) peaked at 66 species in 2024. Bahupura (S5) fluctuated, whereas Varanasi (S8) increased from 48 species in 2022 to 59 in 2023 before stabilizing. Ramnagar (S9) has remained consistent at 35 species. Increases in Prayagraj (S4) and Varanasi (S8) may indicate the National Mission for Clean Ganga's efforts. The post-summer variety (Fig. 5) varied greatly. Prayagraj (S4) peaked at 56 species in 2022, declined to 38 in 2023, then returned to 51 in 2024. S7 increased to 59 species by 2024, showing habitat improvement. Varanasi (S8) peaked at 59 in 2023 and declined to 25 in 2024, most likely owing to habitat changes. Bahupura (S5) rose from 20 in 2023 to 42 in 2024, suggesting better environmental circumstances. Cypriniformes (47), Siluriformes (30), and Perciformes (17) accounted for the majority of the 109 species. Of these, 102 were native and seven were non-native, introduced through aquaculture or habitat disturbance, including *Clarias gariepinus* and *Cyprinus carpio*.

Temporal patterns in post-winter and post-monsoon seasons: In post winter season biodiversity decreased in 2023, but recovered in 2024 (Fig. 6). Kanpur-Fatehpur (S1-S3) dropped in 2023, but rebounded by 2024. Prayagraj (S4) varied the greatest, decreasing to 21 species in 2023 before increasing to 65 in 2024 as a result of habitat restoration. Bahupura (S5) and Mirzapur (S6) exhibited small increases, whereas Ramnagar (S9) remained stable. In 2023 dip was most likely caused by pollution or climate change, but the 2024 comeback demonstrated conservation success. In post monsoon season composition trends varied according to location. S3 (Fatehpur), S5 (Bahupura), and S6 (Mirzapur) exhibited increasing variety by 2024, however S7 (Chunar) dropped. S4 (Prayagraj) maintained a high level of variety

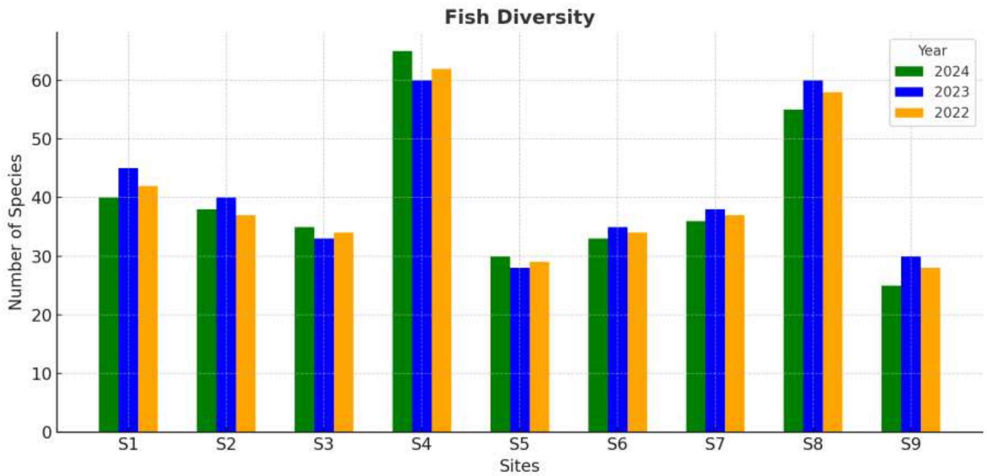

Fig. 4. Annual variation in fish species diversity across sampling sites (2022-2024)

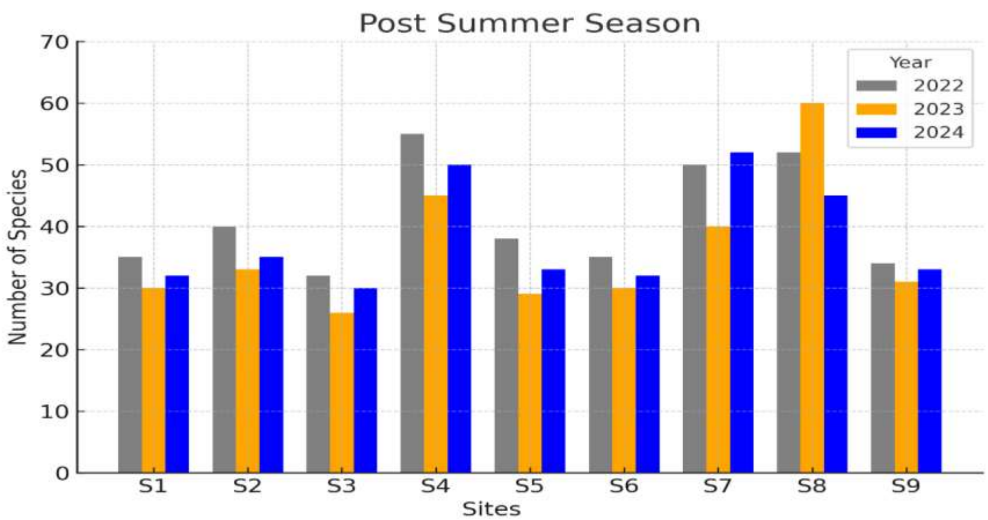

Fig. 5. Yearly fluctuations in species count post summer season

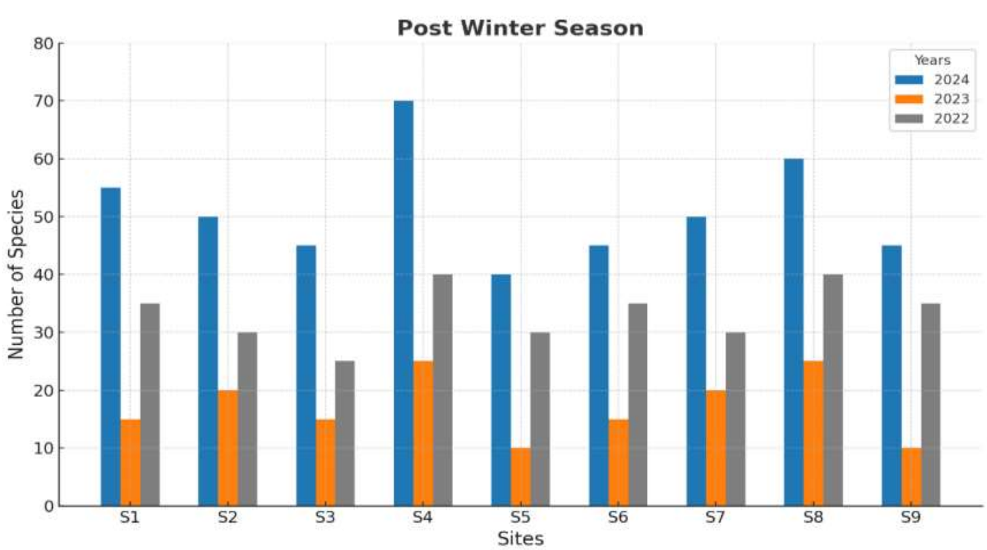

Fig. 6. Yearly fluctuations in species count post winter season

despite oscillations. Changes reflect environmental circumstances or conservation initiatives (Fig. 7). Hierarchical clustering with Jaccard indices indicated two ecological clusters: S1 (Kanpur), S2 (Dalerganj), S4 (Prayagraj) (Fig. 8). S5 (Bahupura), and S8 (Varanasi) formed a cohesive group, whereas S3 (Fatehpur), S6 (Mirzapur), S7 (Chunar), and S9 (Ramnagar) diverged due to site-specific environmental impacts.

Ecological indices, trends, and management implications: Seasonal biodiversity indices differed substantially (Table 3). The Shannon Diversity Index (H) reached peak in S4 (Prayagraj) (1.46, post monsoon) and S8 (Varanasi) (1.56, post winter), showing substantial diversity. The Evenness Index (E^H/S) was maximum in S8 (Varanasi) (0.52, post winter) and S4 (Prayagraj) (0.48, post winter), showing a balanced distribution. S8 (Varanasi) has the highest level of richness (4.67, post summer), demonstrating the value of biodiversity. S5 (Bahupura) and S7 (Chunar) exhibited the lowest markers, indicating ecological stress.

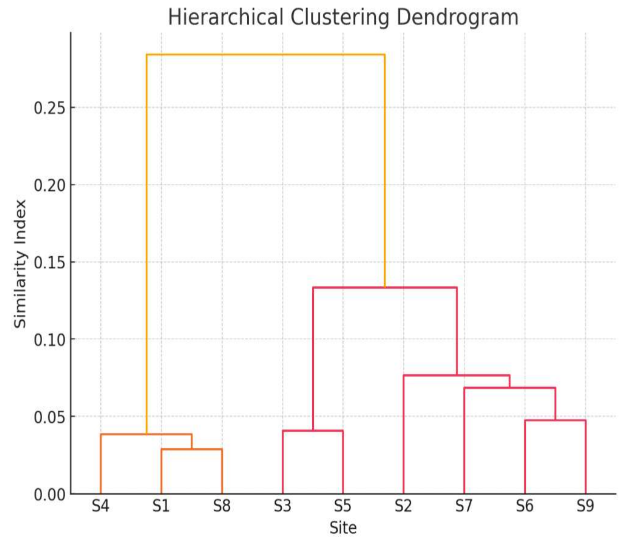

Fig. 8. Hierarchical clustering of nine different sites (S1 to S9) based on their similarity, measured through the Jaccard index across three parameters: PS, PW, and PM

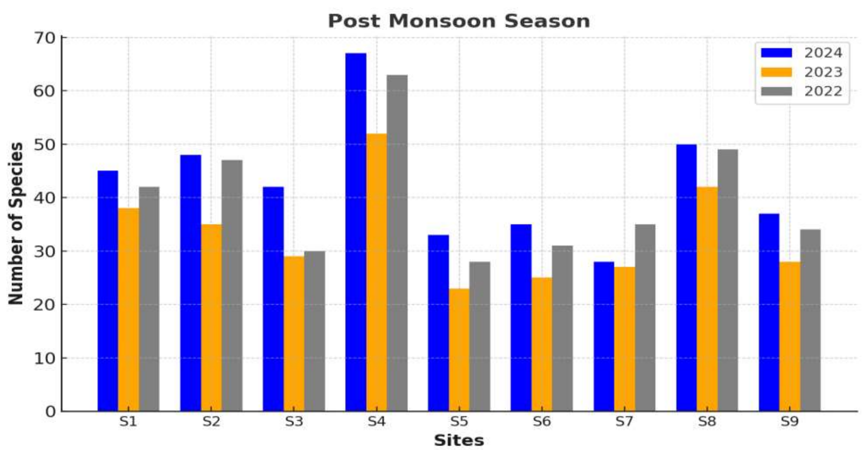

Fig. 7. Yearly fluctuations in species count post monsoon season

Table 3. Seasonal variations in biodiversity indices (Shannon diversity, evenness and species richness) across different sites

Site	Post -summer (PS)			Post-winter (PW)			Post-monsoon (PM)		
	Shannon	Evenness (E^H/S)	Richness (Margalef)	Shannon	Evenness (E^H/S)	Richness (Margalef)	Shannon	Evenness (E^H/S)	Richness (Margalef)
S1	1.36	0.27	4.16	1.39	0.46	0.15	1.39	0.29	4.21
S2	1.30	0.23	3.96	1.32	0.44	0.15	1.34	0.26	3.99
S3	1.38	0.33	4.06	1.35	0.45	0.15	1.39	0.35	4.04
S4	1.42	0.28	4.35	1.45	0.48	0.16	1.46	0.29	4.65
S5	1.23	0.27	3.14	1.05	0.35	0.12	1.28	0.29	3.37
S6	1.28	0.27	3.46	1.15	0.38	0.13	1.29	0.29	3.40
S7	1.21	0.22	3.31	1.10	0.37	0.12	1.33	0.29	3.78
S8	1.39	0.26	4.67	1.56	0.52	0.17	1.41	0.28	4.54
S9	1.36	0.33	3.79	1.26	0.42	0.14	1.40	0.33	4.30

Shannon = Shannon Diversity Index, Evenness/S = Pielou's Evenness Index, Richness = Margalef's Richness Index

The most biodiversity was seen after winter, emphasizing the necessity of conservation. The Ganga supports both top-down (predator-driven) and bottom-up (resource-driven) processes.

CONCLUSIONS

The Ganga River's fish diversity differed by site, with Bahupura and Chunar experiencing ecological stress and Prayagraj and Varanasi serving as hotspots for biodiversity. The growth of non-native species and the decrease of sensitive species demonstrate the growing influence of humans. Management of invasive species, pollution prevention, and conservation initiatives are essential. Stricter laws, ongoing monitoring, and habitat restoration should be the main focuses of future plans.

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Algae Farming: Alternative in Aquaculture Sector

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Abstract: Algae farming has emerged as a promising alternative within the aquaculture sector, offering multifaceted benefits ranging from sustainable feed production to environmental remediation. This practice involves the cultivation of various species of algae in controlled settings, providing a renewable source of biomass rich in proteins, lipids, and carbohydrates. Algal cultivation presents opportunities for reducing dependence on traditional fish feed sources, mitigating environmental pollution through nutrient absorption, and serving as a viable solution for carbon sequestration. Moreover, algae farming showcases potential applications in pharmaceuticals, biofuels, and wastewater treatment, emphasizing its significance in fostering a more sustainable future for the aquaculture industry. This review discusses seaweed farming practices, different species of seaweed under cultivation practices, and their economic importance, especially in the Indian aquaculture system. In addition, this review focuses on commercial uses of seaweed and its byproducts. Finally, challenges and opportunities in seaweed farming are also discussed.

Keywords: Algae farming, Aquaculture, Biofuels, Pharmaceutical applications, Sustainable feed

Algae are diverse plant-like organisms that are autotrophic, primarily found in aquatic regions, and few are present on land. Algae vary in size from unicellular structures known as microalgae to multicellular systems called macroalgae, with marine macroalgae (commonly referred to as seaweed) explored for their commercial applications. For centuries, seaweed has been used in Asian cuisines and medicines, but in recent years, other industrial applications have been discovered and continue to be explored. Seaweeds can be broadly classified based on their pigmentation: Chlorophyta or green algae, Rhodophyta or Red algae, and Ochrophyta or brown algae (Leandro et al., 2019). Seaweeds have become increasingly popular to meet the growing need for sustainable bioresources. All three classes of seaweeds have multifunctional properties, making them economically important. Brown algae such as *Laminaria sp, Saccharina sp.,* and *Undaria sp.* and red algae species such as *Grateloupia* sp. and *Porphyra sp.* are low-caloric edible seaweeds with high content of proteins, vitamins, minerals, dietary fiber, and trace minerals (Leandro et al., 2019).

In addition, alginates, agar, and carrageenan extracted brown and red seaweed species, respectively, have various applications, especially in food industries – utilized in sorbets, breads and pastries. In addition, these hydrocolloids are used in livestock feed, plant biofertilizers, and healthcare textiles such as wound dressing, vascular grafts, sanitary towels, and sutures (Janarthanan and Senthil Kumar 2018). In 2019, algae production contributed nearly 30% of the global aquaculture production, with red and brown seaweeds accounting for the second and third-largest groups in world aquaculture, respectively (Cai et al., 2021). Global Market Insight predicts the commercial seaweed market will be USD 95.8 billion by 2027. East and Southeast Asian countries contributed to most of the global seaweed production, estimated at 97.4%, while America and Europe contributed 2.2% (Cai et al., 2021). According to an FAO survey, China, Indonesia, Korea, Philippines, Japan, and Malaysia were the largest seaweed producers in 2019. Interestingly, more than 99% of the seaweeds produced in these countries were from seaweed farms, except for Japan, where ~83% were produced from seaweed farms (Cai et al., 2021).

Seaweed farming does not compete with arable land and does not require fertilizers, feed, or antibiotics. Typically, seaweed is cultivated in open marine water, either near-shore or off-shore, by attaching propagules (or baby seaweeds) to nets while optimizing culture parameters (Radulovich et al., 2015). India has an extensive coastline stretching up to 8100 km, including the coastlines of the Arabian Sea, Bay of Bengal, Lakshadweep, and Andaman and Nicobar Islands, with an exclusive economic zone of 2.17 million km^2 (Ganesan et al., 2019). The Indian coastline is home to around 865 seaweed species inhabiting deep waters, shallow waters, and low and high tidal regions (Shetye, 2024). Seaweed industries in India mainly depend on wild harvests, leading to the overexploitation and depletion of native species such as *Gelidiella sp, Sargassum sp, and Gracilaria sp*. (Mantri et al., 2019).

Central Salt and Marine Chemicals Research Institute (CSMCRI) and Central Marine Fisheries Research Institute

(CMFRI) attempted the first experimental seaweed cultivation in India in 1967. The two organizations jointly developed sustainable cultivation technology and trained local fish farmers to experiment with *Gelidiella and Gracilaria* sp. evaluated the economic performance of seaweed farming in Ramanathapuram district, Tamil Nadu (Rameshkumar et al., 2019). The authors reported that the average annual income from a three-year farming project was Rs. 6,76,300, with the net cash flow increasing to Rs. 9,83,000 in the second and third years. Seaweed farming is a promising alternative to fish farming communities in India and a more sustainable practice in the aquaculture sector.

Farming System and Methods of Seaweed Cultivation

Seaweed cultivation depends on several factors, including the type of species (and strains), the location of the farms, the cultivation methods, and the resources available for the maintenance of the farms (Radulovich et al., 2015, Jagtap et al., 2022). Seaweed can be cultivated extensively in natural water utilizing natural resources such as sunlight, natural water currents, and organic and inorganic nutrients from marine water. Naturally occurring seaweed species in extensive systems are cultivated with specific farming techniques to improve productivity. In addition, based on the suitability of culture parameters, non-native species may be introduced; however, risks of decimation of native species must be considered.

For large-scale commercial production, farming areas in the sea beds are supported with natural or artificial substrates such as ropes or nets for seaweeds to absorb the nutrients to the maximum and prevent them from washing offshore or being carried away by waves (Sijl 2015). Furthermore, these open-water farming systems are practiced as (a) offshore and (b) near-shore cultivation. In the case of offshore cultivation, seaweed farms are located at a certain distance from the coastline (>2 km), either submerged by attachment to a rigid substrate or floating on anchored lines or nets. The significant issue with offshore cultivation includes the exposure of seaweeds to the harsh effects of oceans and unfavorable environmental conditions. However, installing robust substrates that can withstand the adverse effects of the sea will minimize the loss of biomass (Fernand et al., 2017, García-Poza et al., 2020). The near-shore cultivation is a traditional yet widely practiced technique, where seaweed farms are easily accessible as they are located close to the coast and have shallow waters with a depth of not more than 40 m. (Soto and Wurmann 2019). The largest seaweed-producing countries, such as China, Korea, and Japan, adopted near-shore cultivation methods in the late 1940s and significantly contributed to global seaweed production (Hwang et al., 2019). In general, these systems require minimal capital investments and are labor-intensive.

In comparison, an intensive system is practiced in enclosed systems, such as in ponds or tanks with constant water agitation and supplied with natural or artificial lights, organic or inorganic fertilizers, and phytohormones. This land-based system, known as on-shore cultivation, can cultivate many seaweed genera except the largest seaweeds, such as kelps. In addition, land-based cultivation systems allow farmers to control, monitor, and optimize environmental conditions for enhanced production and efficient use of resources. In general, seawater is pumped onshore; however, secondary sources such as effluent water from aquaculture ponds are also utilized to avoid the addition of external nutrients, thereby having the advantage of sustainability (Ahmad et al., 2022). Although land-based systems, especially tank systems, produce the highest yield compared to other cultivation systems, high capital investments, and high power inputs are limiting factors for the large-scale production of seaweeds (Tullberg et al., 2022). Radulovich et al., (2015) summarize all the possible cultivation methods that apply to extensive and intensive systems. Most of the methods described in the review, such as line cultivation, net cultivation, floating raft system, and tank or pond culture system, use a substrate to support the seaweed propagules (seedlings) during cultivation. While the cultivation method varies based on the species, the most popular and suitable methods for Indian seaweed farming include fixed-off bottom, floating raft, and integrated multitrophic aquaculture (IMTA) methods.

Fixed, bottom-line method: In this method, wooden stakes are hammered into the substrate and arranged in rows at 1 m intervals. Next, monofilament nylon lines or propylene ropes are tied to the wooden stakes, each row 10 m apart. Farmers have easy access to the lines during low tide, allowing them to monitor until harvest. Farmers in Tanzania and Mexico employ an off-bottom-line method for cultivating *Kappaphycus spp*. (Valderrama et al., 2015). *Eucheuma cottonii, Eucheuma denticulatum, and Eucheuma spp* are other species cultivated using this technique (Gavino 2022).

Bamboo raft method: Bamboo rafts of an area of 3×3 m^2 with a diameter of 7 to 8 cm and knots measuring 15 cm are built by tying the bamboo diagonally with each other with the length of the raft reaching up to 1.3 m; size may vary based on the species selected. Lower portions of rafts are covered with fishing nets to protect the rafts from open sea conditions and prevent seaweed grazing and biomass washing away. Approximately 100 g of fresh cuttings (plantings) or 400 seedlings per raft (150 g $knot^{-1}$) are added, bringing the initial weight of the rafts between 40 to 60 kg. The rafts are then anchored to stones (~30 kg) to allow the rafts to float in the seawater. Farmers can regularly monitor the rafts to ensure they are safe from biofouling and may alter the position of rafts during unfavorable weather conditions (Ganesan et al., 2019). Both off-bottom line and floating raft methods are susceptible to biofouling, where fouling species, such as bryozoans, tunicates, and epiphytes, compete with growing seaweeds for dissolved nutrients, sunlight, or cause physical damage to the infrastructure by weighing down the seaweeds, especially kelps (Bannister et al., 2019).

The floating raft method is a popular method for the cultivation of carrageenophytes such as *Kappaphycus spp, Sarconema spp, Hypnea spp, Euchema spp*; agarophytes such as *Gelidium spp and Gracilaria spp* (Ganesan et al., 2019). In addition, the Central Salt and Marine Chemical Research Institute (CSMCRI) Marine Algal Research Station (MARS), developed the single rope floating raft system (SRFR) for culturing agarophytes in a broader and deeper area in the open water where wooden stakes are attached to synthetic anchor cables to keep them afloat. The CSMCRI suggests that the floating raft technology is suitable for seaweed cultivation on the Kerala coast and a few areas in the Gulf of Kutch. (Mohamed 2015)

Integrated mariculture: In East Asian (China) and Southeast Asian (Vietnam, Philippines, and Taiwan) countries, co-culturing methods of fish and seaweeds are widely explored for their cost-effectiveness and high yield potential, and for the reduction of pollutants from the aquaculture wastewater. Seaweeds behave as biofilters – absorb excessive inorganic nutrients such as nitrogen and phosphorus from shrimp culture and finfish effluents (Arumugam et al., 2018). Several studies found similar results with the integration of seaweed with shrimp or finfish cultures (Kang et al., 2021) and the culture techniques can be distinguished as seaweeds are cultivated in tanks or cages along with the fish and in tanks receive effluents from the adjacent fish ponds or tanks.

Furthermore, to increase the efficiency of the culture system, selecting seaweed species and optimizing environmental conditions such as dissolved oxygen, water temperature, and light intensity is crucial. Compared to the traditional polyculture system, Integrated Multitrophic system (IMTA) is a novel approach that offers a better monitoring system where the culture parameters can be easily manipulated to increase the biomass of multispecies (fish, seaweeds, invertebrates, and microbes). The shrimp aquaculture industry in India is rising; however, 95% of the shrimp farms in India discharge effluents into nearby water bodies without treating them (FIAPO 2020). IMTA is an effective tool for establishing nutrient recirculation in land-based intensive farms and reducing the environmental pollution arising from effluents. Several countries, such as Canada and Portugal, are promoting the adoption of the IMTA system for kelps and fish polyculture and red algae, fish, and mollusks (Buck et al., 2018).

Seaweed Species of Economic Importance

Selection of species is of utmost importance for selecting the optimal farm site with suitable environmental conditions and the best farming technique. Generally, seaweed species are chosen based on their application and profitability, such as human food supplement, feed supplement, phycolloid production, bioactive compound extraction, biofuel production (Jagtap et al., 2022) or to mitigate environmental stress such as sequester carbon and to improve water quality in marine waters. Introducing non-native species may invade native species, alter the biodiversity of the coastal ecosystem, and affect the productivity of native species. (Eggertsen and Halling 2021). Since there is limited data on the consequences of cultivating alien species in the Indian coastal system to increase biomass production, initial seaweed farming initiatives in the country can focus on utilizing wild species for selective breeding, certifying seaweed varieties, hybridizing species, and increasing genetic variations among them. Similar methods have been successful in the world's largest seaweed-producing countries, such as China, Korea, and Japan (Hwang, Yotsukura et al*.,* 2019). Survey conducted by the Botanical Survey of India records 865 seaweed species from the intertidal and deep-water region of Indian waters. Red algae are the most diverse group, followed by green and brown algae (Mantri et al., 2019). However, only 60 species are found to be commercially significant, and fewer species are dominant on the international market (Mohamed 2015).

***Porphyra* spp.:** *Pyropia* (or *Porphyra*) spp. are among the most popular red seaweed species cultivated in Korea, Japan, and China, with the highest commercial value of USD 0.89/kg. (Cai et al., 2021). Korea exported USD 525 million of *Pyropia* products to 110 countries, making it the world's top exporter in 2018 (Hwang and Park 2020). In 2019, the global annual production of farmed *Porphyra* was about 3 million tonnes, which generated a first-sale value of USD 2.7 billion (Cai et al., 2021). The most common species cultivated in top-producing countries are *P. yezonensis, P. tenera,* and *P. haitanenisi*. At the same time, *Porphyra vietnamensis* and *P. indica* are the two most abundant species found in India, especially on the west coast, including Maharashtra, Goa, and Karnataka (Kavale et al., 2021). However, *Porphyra* spp is currently not commercially cultivated in India. *Pyropia* is primarily used as human food for distinct flavor and nutrient profile, including high levels of protein and vitamins. Kaur

(2025) reported that *P. vietnamensis* and *P. indica* have excellent antioxidant properties and are suitable for application as nutraceuticals. The growth of wild *Porphyra* spp commences at the beginning of the monsoon in June and lasts till September. Due to heavy rainfall during the monsoon season, the temperature and salinity of the water gradually decrease, favoring Porphyra sp's growth. Further, the thickness of the seaweed blade is affected by the motion of the water, and a calmer environment is preferable.

For large-scale production of *Pyropia spp.* farmers in Korea use artificial seeding to culture seaweed propagules. In this method, free conchocelis filaments (a growth stage in the life cycle of *Pyropia spp*) are allowed to attach to the oyster shells, which serve as a substrate. After a couple of weeks, the conchocelis mature within the shells to release conchospores. Seedlings of *Pyropia* are produced by outdoor seeding and indoor seeding. In both methods, cultivation nets are immersed in seawater or tank water for the conchospores to attach to the substrate. Controlling conchospore density is more feasible in indoor seeding but requires sophisticated infrastructure. Seaweed farmers in Korea periodically expose the nets to the air as a control measure to reduce or kill epiphytes and enhance seaweed growth (Hwang and Park 2020).

Kappaphycus alvarezii: *Kappaphycus alvarezii* is the most widely cultured and traded red seaweed, with a net global production of 11.6 million tonnes (33.6% of all seaweed) in 2019, which generated a first sale value of USD 2.4 billion (Cai et al., 2021). Most of the production was from Asian countries, with Indonesia (9,795,400 tonnes) being the highest producer, followed by the Philippines (1,498,788 tonnes) (Cai et al., 2021). *K. alvarezii* is a source of κ-carrageenan, a phycocolloid with industrial applications as an emulsifier, thickening agent, stabilizer, and gelling agent in food, beverages, cosmetics, and pharmaceutical products. CSIR-CSMCRI imported *K. alvarezii* biomass from Japan in 1996, which was indigenous to the Philippines, and initial cultivation was performed in Okha, on the west coast of India. Further, researchers introduced the seaweed into the coastal waters of Mandapam coast of Tamil Nadu for acclimatization and large-scale cultivation. *K. alvarezii* was also cultured in Kurusadai Island, at the Gulf of Mannar Marine Biosphere Reserve (Arasamuthu et al., 2023). Pepsico India Holdings (P) Ltd., Gurgaon, played a pivotal part in the commercial cultivation of *K. alvarezii* in India. The company provided 45 rafts to each women's Self-help Group member, distributed to farmers. The ropes with seaweed seedlings were tied to these rafts and planted on the coastal water. It is to be noted that most Southeast Asian countries produce seedlings by vegetative or self-propagation, where healthy branches from young, robust seaweed are cut and tied to ropes (Carabantes 2020). Farmers followed either fixed off-bottom or floating raft cultivation methods with a 45-day farming period. Farming cycles were designed to allow each farmer to plant and harvest one raft daily. The average yield per raft ranged between 240 kg and 280 kg of live seaweed, of which 40 kg was used as seed for the successive farming cycle. One hectare of farm could support 900 rafts with a net annual income of Rs. 4,60,000 (Ganesan et al., 2019). *K. alvarezii* farms are susceptible to grazing herbivores such as signanids, puffers, turtles, and sea urchins; therefore, it is essential to select appropriate farm sites.

In addition, ice-ice disease is a common problem observed in these seaweeds that causes a whitish appearance on the thalli, causing the thalli to break. It is unclear whether a bacterial or viral infection causes the disease. Hence, developing new disease-resistant varieties tolerant to high light intensity and temperature is imperative (Kim et al., 2017). In Kerala, cultivating *K. alvarezii* by following a nylon hook method for a farming period of 86 days and 63 days post-monsoon season increased the yield 34.42-fold and 30-fold, respectively. Also, integrated farming of *K. alvarezii* with green mussels resulted in a 20-fold increase in yield (Mohamed 2015).

Gracilaria edulis* and *G. dura: *Gracilaria spp.* is the second most cultivated red seaweed species, with a global production of 3.6 million tonnes in 2019 with USD 2 billion as the first-sale value (Cai et al., 2021). *Gracilaria* is the major source of superior-quality food-grade agar and agarose, which have various applications in the food and pharmaceutical industries. According to a survey conducted by FAO, the global seaweed production in 2019 was contributed by six East and Southeast Asian countries: China, Indonesia, Vietnam, Korea, Taiwan and Philippines (Cai et al., 2021). China comprises 30 *Gracilaria* species, but the two most widely cultivated species are *G. tenuistipitata* and *G. lemaneiformis* with pond-scattering and floating raft technique being the primary cultivation method. India is home to 32 species of *Gracilaria*, of which *G. edulis* is the most abundant and grows along the Gulf of Mannar and the southeast coast (Veeragurunathan et al., 2019).

Indian agar industries primarily sourced agar from wild stocks of *G. edulis* by harvesting 100 to 200 dry tonnes yearly, leading to over-exploitation of these seaweeds. To bridge agar's supply and demand gap in the country, industries began harvesting other species of *Gracilaria* wild stocks, Therefore, developing cost-effective high-yield methodologies with technological interventions is crucial for the self-sustained production of *Gracilaria* spp. The increased biomass production from July to August, with the maximum yield obtained from November to December. In addition, during the 60-day harvest period, the sub-tidal cultivation system showed a significant increase in biomass with the period removal of epiphytes (Islam et al., 2021). Due to the depletion of wild *G. edulis,* sourcing seedlings from wild stocks has been more challenging. Several studies found *G. dura* to be a viable substitute for large-scale production of the *Gracilaria* seaweed. The polypropylene net and floating bamboo raft methods have proven to be the highest-yielding cultivation methods on the southeastern coast of India, with daily growth rates of 3.748 day-1 and 2.61- 3.17, respectively (Veeragurunathan et al., 2015). The studies suggest that the cultivation period between October and March is ideal for increased productivity, while the seaweed is susceptible to grazing herbivores between April and August and epiphyte growth due to increased temperature.

Sargassum spp and Turbinaria spp: Globally, brown seaweed serves as a food source. However, in India, it is a major source for the extraction of alginates, which have industrial applications as thickeners, gelling agents, emulsifiers, and stabilizers. In 2019, brown seaweed production contributed to 47.3% of world seaweed production, among which two genera - *Laminaria* or *Saccharina* and *Undaria* – were the most widely produced, with East Asian countries being the largest producers (Cai et al., 2021). In India, naturally occurring genera include *Sargassum, Turbinaria, Cystoseira, Spatoglossum, Hormophysa, Chnoospora,* and *Rosenvingea*, of which *Sargassum sp.* and *Turbinaria sp.* are of commercial importance (Mantri et al., 2019). In general, industries in India rarely indicate the specific species utilized, but *Sargassum wightii* and *Turbinaria conoides* are believed to be the most predominant species (Mantri et al., 2020). In 1966, artisanal fishermen began harvesting wild stocks of *Sargassum* sp and decade later 1975, *Turbinaria* sp. was also gathered from the coastal belt extending between Kanyakumari and the Gulf of Mannar. The fishermen, predominantly women, collect seaweed from the intertidal and sub-tidal regions of the coastal belt for 10-12 days per month throughout the year (Mantri et al., 2019). *Sargassum* sp. and *Turbinaria* sp. are valued at approximately USD 800 per dry tonne; despite the steady increase of alginate production in India, brown seaweeds are not cultivated in seaweed farms (Ganesan et al., 2019). Continued harvest of wild stocks leads to their depletion and threatens the marine ecosystem. To minimize the overexploitation of brown seaweed, further studies should focus on examining competent strains, developing effective farming methods, and selecting farm sites with favorable environmental conditions.

Traditionally, in Korea, *Sargassum spp.* Seedlings were collected from wild stocks and inserted into a seedling rope. Next, the seedling rope was attached to a main longline and planted near the shore at 2-3 m depth. Alternatively, seaweed farmers preserve the attached holdfasts obtained from the seedlings and reuse them in the next cultivation cycle. However, biomass productivity decreases after every year, resulting in less yield. Recently, farmers have started using a more sustainable technique by obtaining seeding produced through sexual reproduction. This method collects fertilized eggs from vegetative fronds (stem-like), and the germinating rhizoids are attached to seeding ropes. The seedling strings are transferred from nursery tanks to open seawater, where they are attached to the seabed by the fixed-off-bottom method for mass production. Furthermore, the holdfasts may be re-used for the subsequent growing season without the loss of biomass (Kim et al., 2017).

Uses of Seaweed and Seaweed Products

For centuries, East Asian and South Asian countries have used seaweed as a staple in their traditional cuisines, making seaweed a popular food commodity (Lu and Chen 2022). In addition, local communities utilize seaweeds to treat several diseases without genuinely understanding their underlying mechanisms (Shannon and Abu-Ghannam 2019). Since seaweed farming requires minimal to no feed or fertilizers, or additional water input and seaweed tends to have high biomass productivity and has proven more sustainable than fish aquaculture or agricultural practices. Consequently, researchers developed a keen interest in examining numerous seaweed species' biochemical composition and bioactive properties and exploring their potential uses in the food and non-food sectors (Cotas et al., 2023). Globally, seaweed-derived products are now in great demand; thus, seaweed farming has tremendous potential to provide socio-economic benefits to fish farming communities in India (Ganesan et al., 2019).

Seaweed as a food source: The nutrient profile of seaweeds varies from species to species, but generally, seaweeds are rich in protein, carbohydrates, dietary fibers, and minerals but are low in lipids and fats. Padam and Chye (2020) reported that the protein content in seaweed is comparable to high-protein foods such as soybeans, lentils, chickpeas, and mung beans. Although seaweeds are considered low-energy food due to the presence of lower lipids and fatty acid content, seaweeds comprise nutritionally essential fatty acids, including polyunsaturated fatty acids (PUFA) that protect heart health due to their potential anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic effects and offers other health benefits such as anti-cancer effect (Kapoor et al., 2021). In Japan, *Nori* or *Pyropia* is consumed in sushi by wrapping dried seaweed sheets in boiled rice. In Korea, premenopausal women who consumed dried *Porphyra* had a lower risk of developing breast cancer (Kumar and Sharma 2021). In Hawaii, Indonesia, the Philippines, Malaysia, and Vietnam, *Gracilaria spp.* is considered a salad vegetable. Although most seaweed production is utilized as human food (Mahadevan 2015), Indians rarely incorporate seaweed into their diet. However, food manufacturers can use seaweed as an ingredient to formulate snacks, pasta, and soups to introduce seaweed as a food product in the Indian markets Wendin et al., (2020), found that Swedish consumers preferred to eat seaweed from store-bought products or in restaurants and were willing to try seaweed if added in breads or snacks. Most respondents knew of seaweed's sustainability advantage as a food source. Similar studies on Indian consumers would provide deeper insight into their perception of seaweed and its products.

Phycocolloids and functional ingredients: Besides protein, seaweeds are rich in polysaccharides, which can be found as storage carbohydrates that serve as a photosynthetic reserve or present in the cell wall or extracellular matrix, which acts as a mechanical barrier (Rioux and Turgeon 2015). These polysaccharides produce hydrocolloids, often called phycocolloids, with several food and non-food applications. Alginate, extracted from brown algae, while agar and carrageenan, extracted from red algae, have high commercial importance. On average, the annual global production of phycocolloids is estimated to be around 100,000 tons, with a net market value of 1 billion USD annually. Food industries are the most significant users of phycocolloids as raw materials, accounting for 80% of global agar and carrageenan production and 30% of global alginate production (Pangestuti et al., 2015).

Agar, alginates, and carrageenan are excellent emulsifiers, stabilizers, thickening, gelling, and foaming agents and are used to produce bakery products, confectionery, and beverages. Besides the rheological properties contributing to the elevation of textural and sensory attributes of food products, these natural ingredients provide many health benefits. For instance, the incorporation of agar in daily diet aids in weight management by reducing total cholesterol levels and improves gut health by increasing probiotic-bacterial survival (Rubaab et al., 2020). Reported that micro-dosing sodium alginate to a beverage formula decreased blood glucose and insulin levels in diabetic patients. Furthermore, the techno-functional properties of carrageenan have attracted meat industries to formulate low-fat, low-sodium meat products (Kerry et al., 2023). Besides food applications, agar is used in nutrient medium formulations essential for multiple microbiological and cell culture studies. Both agar and alginates are used in pharmaceutical and medical applications and the cosmetic, paper, and textile industries (Pirsa et al., 2023). Many bioactive compounds can be extracted from seaweed, which has excellent therapeutic properties. For instance, seaweed-derived carotenoids have antioxidant and protective activity against cancer and cardiovascular diseases (Kumar et al., 2021). Macro- and micro-algae-derived pigments such as beta-carotene, phycocyanin, and phycoerythrin can be used as natural food colorants.

Livestock feed supplement: Conventional livestock feed production consists primarily of high protein and high energy feeds like cereal grains, corn meal, soybean meal, and grass pastures, which compete with crop production for human consumption. The addition of seaweed feeds as an alternative feed reduces the stress on conventional crop production, and several studies suggest that the bioactive compounds in seaweed provide health benefits – improve the gut health of animals (Maheswari et al., 2021).

In addition, earlier studies reported that seaweed supplementation, especially red seaweed *Asparagopsis spp.* reduces enteric methane emissions in ruminants by 50-98% (Roque et al., 2019, Abbott et al., 2020, Kinley et al., 2020, Vijn et al., 2020). Using regenerative aquaculture technology, a Hawaiian start-up, Symbrosia, successfully cultivated and produced a low-cost ruminant feed additive called SeaGraze™ from *Asparagopsis taxiformis*. Their pilot-scale studies observed a 75% reduction in enteric methane emissions; further scaling up is in progress (Gillman 2022, Symbrosia 2022).

Besides ruminants, seaweed feed supplementation to poultry, laying hens, and fish provides comparable nutrition to conventional feed (Kulshreshtha et al., 2020). Adding 2% of raw brown seaweed *Sargassum sp.* in a regular broiler chicken diet reduces the total cholesterol levels and increases high-density lipoproteins; however, the final body weight gain was slightly lower than the conventional diet (Morais et al., 2020). Seaweed-based feed production can be achieved using clean technologies to reduce environmental implications further, thereby making them a more sustainable feed source.

Bio-fertilizer and bio-stimulants: Seaweeds have proven to be beneficial in human and livestock diets, and their uses extend to providing excellent sources of nutrients for plants and soil, making seaweeds a better replacement for chemical fertilizers. Due to the similar biochemical composition of plants and seaweed, they are biocompatible (Hassan et al., 2021), reducing the risk of developing adverse effects on plant health upon seaweed extract application. Studies report that seaweed consists of growth-promoting compounds that increase seed germination rate, plant root, and shoot length, enhancing plant productivity and overall crop yield (Nanda et al., 2021). Deepana et al. (2021) observed that in rice soil supplemented with 12.5 kg/ha of seaweed extract and 0.5% foliar spray of seaweed liquid extract increased plant height, leaf area, root length and volume, and yield. In addition, soil fertility improved as the biofertilizer had higher soil nutrients available, including N, P, K, and trace mineral compounds. Seaweeds can serve dual purposes as bio-fertilizers and bio-stimulants in agriculture. Biostimulants enhance plant nutrient and water uptake efficiency, abiotic stress tolerance, and crop quality by increasing antioxidant activity, fruit length, and diameter (Du Jardin 2015, Hassan et al., 2021). Di Stasio et al. (2020) added two combinations of seaweed extract to cultivate tomato plants under salt stress conditions. The authors observed decreased leaf water potential in treated plants due to the high concentration of osmotically active molecules and ions. In addition, the nutrient use efficiency was increased, resulting in higher yield. Overall, seaweed as biofertilizers may qualify as biologically safe and environmentally friendly.

Biofuel and biogas production: In light of the rising demand for fossil fuels and fuel price inflation, the need to develop sustainable alternative fuels is becoming increasingly important. Seaweed yields high biomass with abundant carbohydrate content with relatively minimal inputs, making it an ideal choice for cost-effective biofuel production (Twigg et al., 2024). Biofuels may be broadly classified into liquid biofuels such as bioethanol, biodiesel, biomethane, and Biogas. The biofuel extraction and yield differ from species to species due to the variability of biochemical composition; green seaweed, such as *Chaetomorpha antennina,* and red seaweed *Gracilaria corticata* comprises high lipid content, suitable for biodiesel production, while brown seaweed, such as *Sargassum angustifolium* and red seaweed such as *G. salicornia* comprises of high carbohydrate content making them ideal for bioethanol production (Sudhakar et al., 2024). Liquid biofuel production from seaweed is primarily achieved by fermentation by converting sugar molecules into alcohol. To make complex polysaccharides such as cellulose more accessible to microbes as simple sugars during fermentation, pre-treatments of algal biomass by hydrothermal treatment with acid or alkali, followed by enzymatic degradation, is feasible and cost-effective for industrial-scale production (Jiang et al., 2016). However, studies suggest that acid-hydrolysis of seaweed produces furfural as a by-product, inhibiting biofuel yield. Implementing species-specific thermal treatment coupled with hydrolysis is believed to increase biofuel production efficiency (Maneein et al., 2018). Biogas production is achieved by a process called anaerobic digestion. Pantis (2024) studied the effectiveness of co-digesting bovine slurry, an organic waste product produced from animal farms with five seaweed varieties: brown seaweed spp, including *Laminaria digitata, Saccharina latissima, Saccorhiza polyschid Fucus serratus* and green seaweed *Ulva sp.* The co-digestion of *S. latissima* resulted in the highest biogas yield with a 35% production during the first three days, followed by *S. polyschid* with an increase in production rate from the 13thday.

Similarly, further research should be performed to evaluate the co-digestion of aquaculture effluents and seaweed species for biogas production. Applying this hurdle method would allow small-scale and marginal-scale fish farmers to have renewable and affordable energy sources and treat the effluents. In addition, the biochar produced from biogas production can be used for aquaculture pond sediment management as a mitigation strategy for greenhouse gas emissions (Raul et al., 2020). Comparatively, bioethanol is the most promising alternative fuel for transportation; further research efforts on improving production technology will promote the commercialization of biofuels.

Challenges and Prospects of Seaweed Farming

Seaweed farming and producing seaweed-derived products offer many opportunities and socio-economic growth for stakeholders throughout the food supply chain and other industries, including pharmaceutical, textile, and biofuel sectors (Ismail and Zokm 2025). In addition, seaweed farming provides a positive ecological impact, including carbon sequestration, control of coastal eutrophication, and minimization of ocean acidification (Kim and Kim 2024). Despite the many advantages and the ease of seaweed farming with minimal input and a high output rate, future research efforts and policy interventions should focus on addressing issues highlighted in several studies. Some challenges and future opportunities are discussed below:

Challenges

1. Biofouling is a significant concern in seaweed farming. Epiphyte growth in the cultivation nets competes with the nutrients required for seaweed growth (Sahu et al., 2020). In addition, grazing herbivores reduce the net biomass yield. Thus, strategies must be developed for controlling the biofouling effect.
2. The vast Indian coastline includes both low-tidal and high-tidal regions. New cultivation technologies should be designed and tested to tolerate the harsh effects of the ocean and minimize biomass drifting.
3. With the global temperature increase, seaweed species' survival rate in the open marine water is hindered.

Thermo-tolerant variety development is a plausible strategy to improve biomass yield. In addition, minimal efforts are made to enhance the native species for increasing productivity or specific bioactive compounds with commercial importance. Further research on seaweed variety improvement is recommended.

4. Besides the demand and supply gap of algin and agar in India, the profitability of these products in the international market is limited due to low seaweed extract quality. Government organizations such as FSSAI should re-examine and perform amendments to Indian standards of phycocolloids to qualify them as food-grade with superior quality (Behera et al., 2022).

Future Prospects

1. The Indian coastline is enriched with a wide range of native species of seaweed, but their biochemical composition is seldom documented. In addition, no efforts have been made to investigate the techno-functional properties of these seaweed extracts. By identifying commercially profitable species, local farmers can be encouraged to cultivate native species without introducing invasive species into the coastline.
2. Introducing training programs in marginal communities, especially for women, will empower them to contribute to their families' financial stability, provide quality education, and more.
3. The Indian diet is diverse and versatile, yet food options with nutrient-dense seaweed are uncommon. Food manufacturers and product developers should explore innovative ways to incorporate seaweed-derived ingredients and bioactive compounds in packaged food to increase seaweed market demand.
4. Applying seaweed-derived bio-fertilizers and bio-stimulants in the agricultural sector is economically feasible for farmers and a safer environmental choice (Divakar et al., 2024).

Although the current report discusses the cultivation methods and potential uses of seaweed (macroalgae), microalgae are also an excellent nutrient source and contain valuable bioactive compounds. Identifying economically significant varieties and developing cultivation technologies in fishponds and tanks is paramount. In addition, optimization of farming methods and culture parameters will improve net productivity.

CONCLUSION

This review sheds light on the promising prospects of seaweed farming, particularly within the coastal/brackish water aquaculture sector, and its diverse benefits. Cultivating algae in controlled environments offers a sustainable solution for producing biomass rich in essential nutrients, potentially reducing reliance on traditional aquatic animal feed sources. Furthermore, seaweed farming demonstrates its potential in environmental remediation, particularly in nutrient absorption and carbon sequestration, and various other industries such as pharmaceuticals, biofuels, and wastewater treatment. The present snapshot document emphasizes the relevance of seaweed farming practices in India, highlighting the economic importance of different seaweed species and their commercial uses. It underscores the multifaceted applications of seaweed and seaweed-derived byproducts in various industries, emphasizing the pivotal role of this farming in promoting sustainability within the aquaculture sector and beyond. However, challenges and opportunities in seaweed farming signify the need for continued research and innovation to address hurdles and maximize the potential of this burgeoning industry. Overall, this work encapsulates the significance of algae and seaweed farming as transformative practices that hold the key to a more sustainable future for the aquaculture industry and offer promising solutions to global environmental challenges.

AUTHORS' CONTRIBUTION

Conceptualization of research work and designing of experiments (NKT,VM); Preparation of manuscript (DC, AK).

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Exploring the Role of Carotenoids in Human Health: Therapeutic Applications and Mechanistic Insights

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Abstract: Tetraterpene pigments, known as carotenoids, are found in several organisms, including some types of algae, plants, mammals, and some species of archaea and fungi. Until 2018, 850 naturally occurring carotenoids had been reported. Photosynthetic bacteria, fungi, algae, and plants can make carotenoids. Levels of carotenoids in the human body have been strongly associated with preventing and treating numerous diseases because of their protective characteristics. A substantial body of research has been amassed about the possible health benefits of carotenoids, which have long been known for their antioxidant capabilities. Due to their antioxidant properties, the primary carotenoids such as carotene, lycopene, lutein, zeaxanthin, crocin (crocetin), and curcumin are responsible for the positive health benefits of carotenoid-rich vegetables and fruits and for lowering the risk of certain diseases. Neurodegenerative processes might be stopped or delayed by strategies, such as consuming carotenoid-rich food that disrupts apoptotic pathways. Also, it explores the novelty of green technology separation techniques for isolating carotenoids. This review briefly discusses the chemistry, classification, biological functions, potential mechanisms, and environmental and commercial outcomes of some important carotenoids used to treat various disorders.

Keywords: Carotenoids, Pigments, Antioxidants, Ageing, Green technology, Neurodegeneration

Colourful liposoluble pigments, called carotenoids, are found in various foods, such as fruits, vegetables, plants, fish, fungi, bacteria, and algae. More than 600 different carotenoids, broken down into carotenes, xanthophylls, and lycopene, have natural structural variations. Approximately 20 carotenoids have been found in human blood and tissues, and 40 are in a typical human diet (Milani et al., 2017). A proton is lost during the creation of the tetraterpene skeleton (phytoene), which leads to the establishment of a double bond in the middle of the molecule. Cooking and chopping carotenoid-rich foods increases the potency of their nutrients when they enter the bloodstream, unlike other protein-rich foods and vegetables. Xanthophylls and carotenes are the two main types of carotenoids. Plants, fungi, and bacteria produce carotenoids that are terpenoid colours made up of eight isoprene units. Carotenoids of both types have antioxidant effects (Engelmann et al., 2011). Furthermore, some carotenoids are converted into vitamin A, which is necessary for human health and growth. The role of carotenoids in human health is being extensively studied due to epidemiological research suggesting that those who consume more carotenoid-rich food have a lower chance of developing various chronic diseases. Vitamin A insufficiency and various degenerative disorders, such as cardiovascular disease, cognitive deficits, and age-related macular degeneration, are linked to low levels of total plasma carotenoids and individual carotenoids (Meléndez-Martnez et al., 2019). Alpha-carotene, beta-carotene, and beta-cryptoxanthin are examples of provitamin A carotenoids, and lutein, zeaxanthin, and lycopene are non-provitamin A carotenoids.

Until 2018, the number of xanthophyll species reported in nature was around 850. The human body may be able to absorb, transport, distribute to tissues, metabolize, and utilize roughly 50 of them (Maoka, 2020). In order to ascertain the physiological significance of red and colourless tomato carotenoids, recent developments in our knowledge of their bioavailability (the percentage of intact carotenoid consumed that appears in the circulation), bioaccumulation (the amount of intact carotenoid consumed found in tissues), metabolism (the chemical modifications to a carotenoid for utilization as a participant in biological processes, or clearance), and bioactivity have been made (Milani et al., 2017).

Classification and Types of Carotenoids

Carotenoids are divided into two categories: carotenes, which include phytofluene, phytoene, lycopene and β-carotene, and xanthophylls, such as lutein, zeaxanthin, astaxanthin, fucoxanthin, and violaxanthin (von Lintig et al., 2020) (Fig. 1). Another carotenoid frequently found in food, beta-carotene, contains provitamin A activity. According to its structure, following central cleavage, it is transformed into one molecule of physiologically active retinol, and twice the molar amount is equivalent to beta-carotene. It contains antioxidant, anti-carcinogenic, and possibly immune-boosting characteristics like other carotenoids. Some epidemiological studies, although not all of them, found that eating more beta-carotene was linked to a lower risk of developing cancer and cardiovascular disease. There have not been any human clinical trials to investigate the effects of β-carotene yet, likely due to the difficulty in identifying β-carotene and the fact that it is frequently combined with significant amounts of β-carotene in fruits and vegetables.

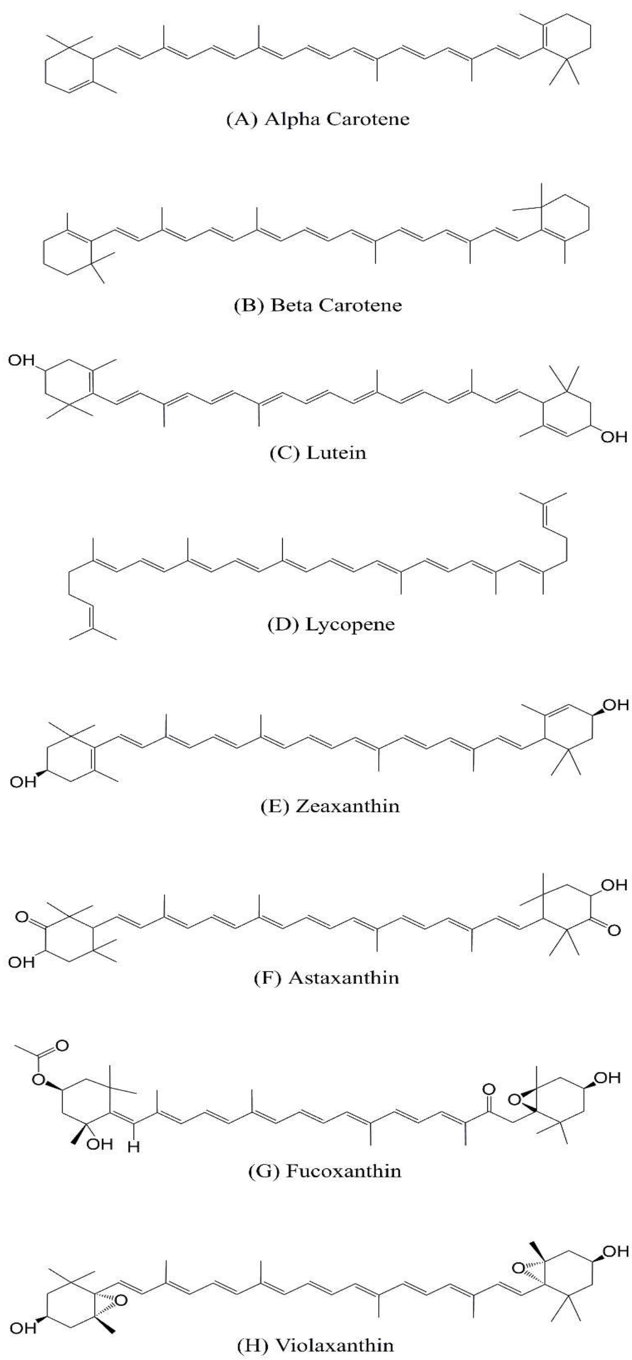

Fig. 1. The chemical structures of the most common carotenoids

The concentration of β-carotene is exceptionally high (Milani et al., 2017). Phytoene and phytofluene are colourless carotenoids found naturally in tomatoes and other crops. These are carotenoid precursors frequently discovered before the final carotenoid products in plants (Engelmann et al., 2011). These are also available in human fluids and tissues in respective concentrations of 0-2 mol/L (plasma) and 0-1 nmol/g (tissues). Regarding geometrical isomerisation, 15Z is the actual isomer for phytoene and phytofluene. They have anti-inflammatory, biomolecule stabilisation, augmentation, and protective properties and are effective against skin pigmentation (Maoka 2020).

Canthaxanthin, a red keto-carotenoid and one of the primary dietary carotenoids permitted as a food additive in many countries, has shown promise in treating erythropoietic protoporphyria, despite concerns regarding its accumulation in the retina with long-term use (Rebelo et al., 2020). It is naturally present in bacteria, algae, and some fungi. Since it is a ketocarotenoid, canthaxanthin has more potent anti-inflammatory and free radical-scavenging abilities than other carotenes and xanthophylls. Also, it is currently utilized widely as a natural skin-tanning ingredient in cosmetics and enhances the nutritional value of foods obtained from animals, as well as the health of both humans and animals who consume them (Coelho et al., 2023). Lutein is a dietary carotenoid found in many foods, including green vegetables, fruits, and egg yolk (Wackerbarth et al., 2009). A recent placebo-controlled, double-blinded, randomized, crossover study found that taking lutein capsules containing free lutein stabilized by 10% carnosic acid can protect against photodamage by lowering the expression of UVR-modulated genes like heme-oxygenase 1, intercellular adhesion molecule 1, and matrix metallopeptidase 1 (Wang et al., 2018). The distribution of lutein in different human tissues is inconsistent, with the macula having the highest concentration. Because of its high concentration of photoreceptor cells, the macula is responsible for visual acuity and central vision. It is located in the central retina, the posterior region of the eye. Zeaxanthin (a stereoisomer of lutein received from the diet) and meso-zeaxanthin (a lutein metabolite generated at the macula via metabolic transformation) are two different forms of carotenoids found in the macula (Li et al., 2020). Lycopene is an acyclic carotenoid in various foods, including tomato, watermelon, guava, papaya, apricot, and grapefruit (Mumu et al., 2022). As a dietary supplement, lycopene is a natural material that may be used in high doses without endangering the health or physiology of people. Numerous in vitro, ex vivo, and in vivo studies have shown an inverse relationship between lycopene-rich diets and heart diseases and cancer (Wang et al., 2018); several studies indicate that lycopene may provide photoprotection (Demmig-Adams et al., 2020). Zeaxanthin is a moderately polar carotenoid pigment abundant in parsley, spinach, kale, egg yolk, and meals enriched with lutein. Due to its capacity to connect with physiological proteins in people and as a scavenger for reactive oxygen species has exhibited several positive health impacts (TujJohra et al., 2020). The prevalence of AMD is inversely correlated with the amount of macular pigment (TujJohra et al., 2020). This is 1000 times more powerful than vitamin E and has the most significant ability for absorbing oxygen free radicals. It also has much more potent antioxidant activity than carotene. It has the chemical formula $C_{40}H_{52}O_4$ and is soluble in fatty acids and most organic solvents but insoluble in water. The conjugated double bond structure of astaxanthin, which also contributes to its reddish-orange hue by providing electrons for free radical reactions that produce more stable compounds, is a crucial defense mechanism against the free extreme chain reaction. Astaxanthin has garnered a lot of attention because of its possible pharmacological effects, which include a strong antioxidant property, DNA repair, stress tolerance, cell regeneration, neuroprotective, antiproliferative, anti-inflammatory, antiapoptotic, antidiabetic, anticancer, and skin-protective effects (Sluijs et al., 2015). Marine brown seaweeds, macroalgae, and diatoms, microalgae contain the marine carotenoid fucoxanthin, which is notable biologically. Numerous research studies have demonstrated fucoxanthin's significant potential and promising applications in human health. Fucoxanthin, a powerful carotenoid found in the chloroplasts of brown algae, accounts for more than 10% of the estimated total natural production of carotenoids, making it the most prevalent of all carotenoids (Yang et al., 2019).

Violaxanthin and other carotenoids are available in abundance in red, yellow and orange-coloured fruits, for example, Capsicum 5,6,50,60 -diepoxy-5,6,50,60 -tetrahydro-β, β-carotene-3,30 -diol, also called violaxanthin, is a natural orange xanthophyll, which may enzymatically be transformed into zeaxanthin when the light energy absorbed by plants exceeds the photosynthesis capacity. When exposed to low light or darkness, zeaxanthin is transformed into violaxanthin by zeaxanthin epoxidase; when exposed to intense sunlight, violaxanthin is de-epoxidized back to zeaxanthin by violaxanthin de epoxidase (Mumu et al., 2022).

Other Sources of Carotenoids

Pepper is a non-leafy vegetable that is high in carotenoids. In particular, β-carotene, β-cryptoxanthin, zeaxanthin, and capsorubin of *C. annuum* are the primary mixed carotenoids found in paprika and its oleoresin. Both are non-carcinogenic, have known biological functions linked to disease prevention, and are natural colours with fewer side effects. Also, they are secure substitutes for synthetic colouring compounds that give pharmaceutical, cosmeceutical, and nutraceutical goods their red colour. As a significant source of carotenoids, capsicum is also studied in terms of its main domesticated species, biosynthesis, carotenoid profile, antioxidant action, and safety (Imran et al., 2020).

Carotenogenesis

Cyanobacteria, mammals, plants, and microalgae produce isoprenoids called carotenoids. Carotenoids are pigments that absorb visible light during photosynthesis in photosynthetic systems. They also serve additional functions, such as signalling fruit development and luring insect pollinators, in addition to shielding photosynthetic bacteria, microalgae, and plants from light-induced cell damage. Carotenoids are found in many different types of food and are known to include over 1100 different types. Efficient mechanisms that regulate the rate of carotenoid synthesis in response to environmental stimuli are responsible for the diversity of carotenoid structures and biosynthetic pathways. Environmental factors that impact the carotenoid pathway's regulation include pH, temperature, salinity, dissolved O2, and light. Changes in the surrounding environment are known to initiate carotenogenesis, which in turn influences the accessibility and bioavailability of the pigments. Nowadays, only a small percentage of commercially sold carotenoids are extracted using microorganisms; the majority are either chemically synthesised or extracted from vegetables. According to reports, 80–90% of carotenoids are made artificially using chemicals. Toxic waste is produced when carotenoids are synthesised chemically. This prompt worries about the synthesis process' unintended consequences for human health. Carotenoids are the products of dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP), the universal C5 precursors. created through the cloning of genes that produce carotenoids.

Carotenoid Production Using Microorganisms

Bacteria: The production of carotenoids has great promise for bacteria. Heterotrophic bacteria create carotenoids as secondary metabolites, which are essential for a cell's capacity to adapt to its surroundings. It is well known that they shield cells from UV radiation. They are also thought to be responsible for the fluidity of cell membranes. Several common bacterial species have been linked to the production of carotenoids (Carlos et al., 2021).

Yeast and fungus: Different types of fungus are scattered based on their ecological roles. It is found that filamentous fungi produce colourful wide range of pigments including β-carotene, melanin, quinones, flavins, Anka Flavin. These fungal pigments have some useful roles such as, antioxidant, anticancer, cytotoxic activities enlarge the scope of their practical use. Based on the phylogenetics studies it is said some fungi can be considered as role model in the human system. Currently, industrial production of β-carotene is based on the use of heterothallic fungus *Blakeslea trispora*. The market value of carotenoid produced by *B. trispora* is expected to reach $2.0 billion in 2026. It is found that *B. trispora* is highly potentially active in for carotenoid production in industry. In case of Yeast, a big picture is basidiomycetes are a high source of microbial carotenoids. Also other yeast genera like Rhodotorula, Sporobolomyces produces highly antimicrobial carotenoids (Saubenova et al., 2024).

Green Technologies for the Separation of Carotenoids

As a possible replacement for synthetic carotenoids, the extraction of carotenoids from wastes and agro-industrial byproducts has attracted a lot of attention lately. Additionally, as consumers seek for more natural products with biological qualities and health advantages, the extraction of bioactive components is becoming more and more crucial. It has been shown that using natural monoterpenes instead of conventional solvents to extract carotenoids is more environmentally friendly. To extract lutein and xanthophylls from green Japanese knotweed leaves and avocado peels, βpinene was utilised. Furthermore, xanthophyll esters were synthesised using it as a bio-solvent (Papapostolou et al., 2023). Conversely, green extraction entails identifying and developing techniques that minimise energy consumption, facilitate the use of renewable natural resources and substitute solvents, and ensure the extraction of safe and high-quality products. The concepts of green extraction highlight the utilisation of numerous and sustainable plant varieties, innovation in variety selection, and the utilisation of renewable plant resources. Green extraction encourages using bio-based and substitute solvents, such as water or agro-solvents, instead of toxic and dangerous solvents like benzene or chlorinated solvents (Ferrando et al., 2024).

Technology Suitable for Green Extraction

Ultrasounds: A sound wave known as ultrasound has a frequency higher than what humans normally perceive as audible, usually exceeding 20 kHz. There are two types of ultrasound: low-frequency and high-frequency. High-frequency ultrasound is utilised in imaging, diagnostic, and therapeutic applications in medicine. It usually operates at frequencies higher than 1 MHz. The rapid formation and implosion of tiny bubbles in a liquid, known as "cavitation," is a phenomenon brought on by low-frequency ultrasound. This process can disrupt cellular structures and make it easier for compounds to be released from the sample, improving the extraction efficiency of compounds from solid matrices. Ultrasound-assisted extraction (UAE) raises the extracted yield while decreasing extraction time and energy in comparison to traditional extraction methods (maceration, Soxhlet, etc.). The most often used ultrasound devices in the food business are the bath and probe models. The probe ultrasound method uses a handheld probe that is submerged in the sample, whereas the bath approach immerses the sample in a liquid through which the ultrasound is applied. Both techniques have the potential to be successful in producing cavitation and breaking down cell walls to release bioactive substances; however, in order to maximise extraction efficiency and minimise any negative effects, it is important to optimise the choice of an appropriate solvent and other factors (Joshi et al., 2023).

Microwave-Assisted Extraction (MAE): Another documented green technique is the use of microwaves to extract carotenoids from microbes. Similar to sonication, this method breaks down the microbial cell wall by using the energy of microwave radiation in an effort to extract solvents more effectively. A rise in both temperature and pressure happens during microwave extraction, which causes the cell membrane to burst. Carotenoids are then liberated into the solvent, which is then diluted throughout the cell matrix. The foundation of this technique is the quick heating of the intracellular constituents, which puts a lot of strain on the cell wall. Either ionic conductivity or the use of a polarisation dipole are used to transfer the energy of the microwave radiation to the components. Because of the tremendous complexity and robust cell wall construction of microalgae, MAE may be very useful in this situation (Viñas et al., 2023). MAE's low cost and versatility in a range of matrices are further benefits. The technique's drawback is the possibility of damaging thermolabile substances and cis-trans isomerisation of carotenoids. Because of this, studies using sporadic radiation for three minutes were conducted, and the results demonstrated the effectiveness of this approach in recovering carotenoids. ADD a specific section on Green extraction technologies for carotene from plants, and microbial sources.

Biological roles and applications of carotenoids: Carotenoids have different kinds of biological roles and activities in photosynthetic organisms, including plants and microalgae, as well as humans, including Antioxidant, Anti-inflammatory, Anticancer, Anti−hyperglycemia, etc. (Fig. 3 and Table 1). Carotenoids' most critical biological roles in humans are tied to their antioxidant qualities, which are directly derived from their chemical structure (Vílchez et al., 2011).

Anti-oxidation: Free radicals are responsible for cancer, cardiovascular, ophthalmic, and neurological diseases (Meléndez-Martínez et al., 2015). Carotenoids can scavenge free oxygen radicals from the body, which aids in treating cancer and preventing tumor growth in cancer patients. Furthermore, specific carotenoids have been discovered to trigger antioxidant gene expression via the transcriptional factor Nuclear factor erythroid two related factor 2 (Nrf2), which aids in reducing neurological disorders and diabetes (Basu and Imrhan 2007). Astaxanthin has shown that it has

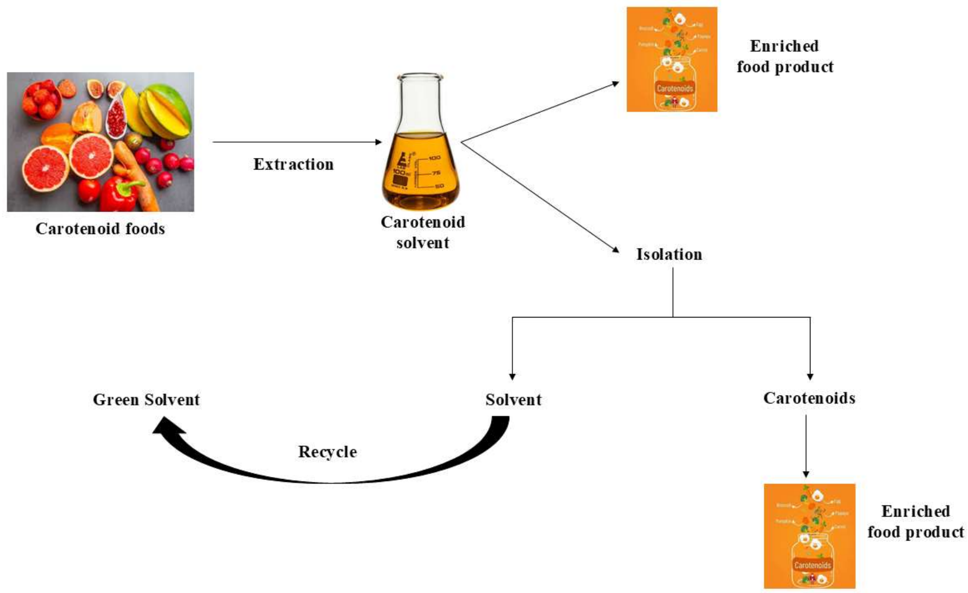

Fig. 2. Green solvents are used in the process to create a food product that is enhanced with carotenoids

Table 1. Source, biological functions and applications of carotenoids

Carotenoids	Pharmacological activity	Biological source	References
Astaxanthin	Antioxidant activity, anti-inflammatory properties, effects on skin damage, effects on DNA repair, and treatment of Colon cancer.	Soyabean, corn, olive, grape seed.	Yang et al. 2013
		Green sulfur bacteria *Xanthophyll omycesdendrorhous, Phaffia rhodozyma.*	Mapelli-Brahm et al. 2023
Canthaxanthin	1. Antioxidant activity 2. Anti-inflammatory effects 3. Food coloring agent 4. Effects on photosensitive disorders	Mushrooms, .eggs and fish.	Rebelo et al. 2020
Beta-Carotene	1. Antioxidant 2. Anticancer properties 3. Responsible for vision 4.Provides a strong immune system 5. Effects on healthy skin and mucous membrane	1. Carrots 2. Spinach 3. Lettuce 4. Tomatoes 5. Broccoli. *Rhodosporidium kratochvilovae*	Milani et al. 2017
Lutein	1.Anti-inflammatory effects 2.Responsiblefor eye health 3. Effects against age-related macular diseases.	1. Spinach 2. Broccoli 3. Peas 4. Egg yolks. *Chlorella saccharophila*	Abdel-Aal et al. 2013
Lycopene	1. Antihypertensive 2. Anticancer properties 3. Antioxidant 4. Anti-inflammatory effects 5. Cardiovascular effects 6. Neurobiological effects	1. Tomatoes 2. Apricots 3. Watermelons 4. Papaya 5. Grapes 6. Cranberries	Khan et al. 2021
Zeaxanthin	1. Anti-inflammatory effects 2. Effects on eye health 3. Antioxidant	Grapes,Oranges, Corns Mango ,*Chlorella zofingiensis*	Abdel-Aal et al. 2013
Fucoxanthine	1. Antioxidant 2. Anti neuroinflammation	Brown seaweeds	Mumu et al. 2022

antioxidant properties, such as the ability to quench singlet oxygen, effectively scavenge superoxide, hydrogen peroxide, and hydroxyl radicals, and inhibit lipid peroxidation, owing to its unique molecular structure-based chemical characteristics. Each ionone ring's hydroxyl and keto moieties are responsible for its enhanced antioxidant activity. Radicals are captured by astaxanthin both at the conjugated polyene chain and in the terminal ring moiety (Ambati et al., 2014). Fucoxanthin also successfully suppress the development of intracellular reactive oxygen species, DNA damage and apoptosis brought on by H_2O_2 (Epplein et al., 2011, Fiedor et al., 2014). It is possible to use violaxanthin as a natural antioxidant for medicinal or functional adjuvant purposes because it is highly effective at scavenging DPPH and ABTS+ radicals (Mumu et al., 2022).

Anti-inflammatory: Fucoxanthin and astaxanthin, two carotenoids with oxygen in their structure, have been shown to decrease the expression of the cytokines IL-6, TNF-α, and IL-1β and serve as pro and anti-inflammatory chemicals (von Lintig et al., 2020). Carotenoids strengthened the anti-inflammatory response by decreasing TNF-α and IL-1β gene expression in vascular cells. (Aust et al., 2005). Carotenoids inhibited inflammation by lowering NF-κB and IL-1β levels. Furthermore, in individuals with stable angina, this drug inhibited LPS-induced TNF-α, IL-6, and IL-1β production in peripheral blood mononuclear cells. Additionally, β-carotene supplementation reduced IL-1, IL-6, and IL-12 p40 cytokine transcription. Due to its capacity to decrease cytokine expression in Suid herpes virus-induced inflammation via NF-κB inactivation, it could be thought of as a possible anti-inflammatory agent for DNA-virus infections and, precisely, for the human herpes simplex virus (Milani et al., 2017). Finally, decreased levels of β-carotene have been found in individuals with inflammatory illnesses such as nonalcoholic fatty liver disease (Si and Zhu, 2022), chronic obstructive pulmonary disease (Kumar et al., 2020), acute myocardial infarction (Mohana et al., 2013), *H. pylori* infection (Fiedor and Burda 2014), and advanced coronary artery disease (Wojtasiewicz and Stoń-Egiert 2016). These findings support the theory thatβ-carotene protects the body by inhibiting inflammatory processes. Similarly, fucoxanthin plays a significant role in suppressing colitis caused by dextran sulphate sodium. It can manage the inflammatory bowel illness ulcerative colitis (UC) (Demmig-Adams et al., 2020). Astaxanthin efficiently warded off UV-induced inflammation in human keratinocytes by lowering iNOS and COX-2 mRNA and protein levels(Freitas et al., 2014).

Anticancer: Carotenoids have been shown to have anticancer properties in several studies. In most cases, carotenoids are known to stop the cell cycle, related to decreased expression of cyclin D1, cyclin D2, CDK4, and CDK6. As a result, it upregulates GADD45, which prevents the cell from entering the S phase (Grether-Beck et al., 2017). Furthermore, chemicals isolated from saffron, such as crocin and crocetin in combination, displayed anti-metastasis activities on the 4T1 cell line in breast cancer, including anti-

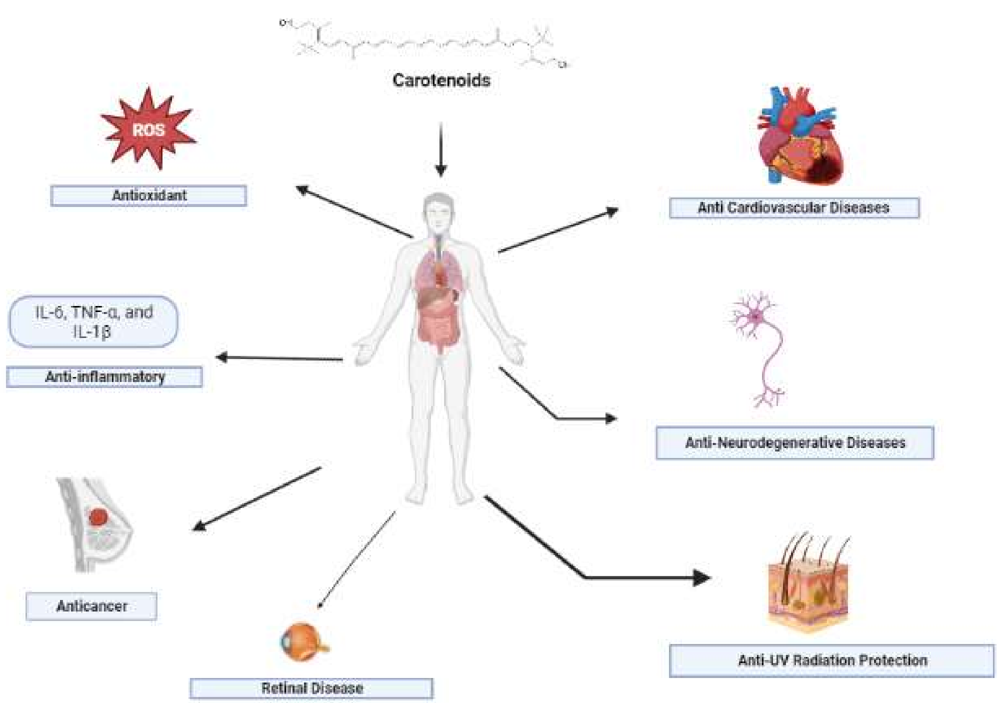

Fig. 3. Overview of carotenoids related to their biological roles for human health

migration, anti-invasive, and anti-nonadhesive effects (Biehler et al., 2012). Carotenoids such as β-cryptoxanthin and lycopene have been shown to decrease the NF-κB, which is effective in treating lung and prostate cancer (Abdel-Aal et al., 2013b). β-carotene has been discovered to have anti-angiogenic activity, which means it helps to prevent the formation of blood vessels. Zeaxanthin is inversely linked in the blood related to increasing the risk of pancreatic (Relevy et al., 2015) and ovarian cancer (Wang et al., 2019). In human epidemiological research, the benefits of dietary lutein and zeaxanthin in the prevention of cancer were discovered, with consumption of these carotenoids lowering the risk of various malignancies such as bladder cancer (Chambaneau et al., 2016), breast cancer (Epplein et al., 2011), and prostate cancer (Mužáková et al., 2010), Non-Hodgkin lymphoma (Shih et al., 2021), renal cell carcinoma (Freitas et al., 2014), head and neck cancer (Freitas et al., 2014). Similarly, intake of lutein and zeaxanthin was found to be inversely related to a lower incidence of gastrointestinal malignancies, such as oral, pharyngeal, oesophagus (Mužáková et al., 2010), colon (Shih et al., 2021) and pancreatic cancers (Shokri-Mashhad et al., 2021).

Anti Cardiovascular Diseases (CVD): Clinical and epidemiological research has demonstrated a negative relationship between eating fruits and vegetables and the likelihood of developing chronic conditions like cardiovascular illnesses (CVDs). One of the significant risk factors for stroke, heart attacks, renal failure and many other consequences is high blood pressure. Because carotenoids have such strong antioxidant qualities, eating a diet high in them significantly reduces the risk of developing the disease (Jansen et al., 2013). Astaxanthin has been shown to positively benefit the heart by lowering atherosclerosis-related inflammation and altering LDL- and HDL-C levels in the blood. Compared to healthy individuals, patients with coronary artery disease had lower plasma levels of lutein, zeaxanthin, β -cryptoxanthin, β -carotene and lycopene. Furthermore, the lower plasma levels of lutein, zeaxanthin, and β -cryptoxanthin were linked to smoking, a high body mass index, and low levels of high-density lipoprotein cholesterol (HDL-C) (Broekmans et al., 2002). In addition, it can also be utilised as a food supplement to help prevent obesity-related heart dysfunction. However, further studies are necessary, particularly *in vivo* experimental approaches for cardiovascular protection (Ciccone et al., 2013)

Anti-neurodegenerative diseases: Neurodegenerative diseases such as Alzheimer's, Huntington's, Parkinson's, and amyotrophic lateral sclerosis (ALS) are brought on by increased oxidative stress in the nervous system. Proper dietary carotenoids can decrease dysfunction caused by improper signalling. Carotenoids, including astaxanthin, carotene, and lycopene, are implicated in Ca2+ ion transportation in the brain (Bhatt and Patel 2020). The antioxidant qualities, blood-brain barrier-crossing ability, and cell mitochondrial membrane integrity of carotenoid astaxanthin may all help reduce the risk of diseases of the nervous system (neurodegenerative). Astaxanthin also has antiapoptotic properties and lowers free radical damage, glutamate release, and cerebral infarction.(Aziz et al., 2020). Lycopene has also been shown to make the blood-brain barrier permeable, lessening certain disorders' severity (Fiedor and Burda 2014). ROS increases caspase activation and Akt/GSK-3β signaling in Alzheimer's disease. Carotenoids aid in restoring normal signaling and reduce caspase activation. Another well-known xanthophyll, fucoxanthin, has also shown neuroprotective properties by triggering Nrf2/HO-1 signaling and causing Nrf2 nuclear translocation to protect against brain ischemic/reperfusion injury (Almeida et al., 2019).

Anti−hyperglycemia: According to statistical analysis and assessment conducted by the European Prospective Investigation into Cancer and Nutrition-Netherlands, human use of carotenoids in their diet can reduce the incidence of type 2 diabetes. Despite this, after incorporating a few criteria such as age, sexual orientation, risk factors, and nutrition, the Hazard percentage for β-carotene was determined to be 0.78, while for α-carotene, it was 0.85. Furthermore, research shows that using carotenoids can reduce the incidence of type 2 diabetes in healthy women and men (Arzi et al., 2020). The leading cause of hyperglycemia is a person's lifestyle and eating habits. Hypertension causes oxidative stress linked to obesity, diabetes, dyslipidemia, and hyper homocysteinemia. Fatty acid radicals and ROS are crucial in raising GR, GPx and other hormones. By scavenging this fatty acid radical and ROS, carotenoids restore regulatory signals and lower illnesses by 40 to 79 per cent (Bhatt and Patel 2020).

Anti-tuberculosis: The fatty acids-carotenoid complex (FACC) has anti-TB properties that can affect MDR strains of M. tuberculosis. A promising anti-TB medication was created by using the sole biomass of the marine microalgae Chlorella vulgaris to produce CGF, lipid, lutein, and its geometric isomers. (Wojtasiewicz and Stoń-Egiert 2016).

Anti-UV Radiation Protection: The efficacy of UV-C protective activity of carotenoid pigments isolated from *M. roseus* and *M. luteus* on the growth of *S. faecalis* proved that 21 colonies out of 31 colonies were stable and resistant against UV exposure with approximately 70 to 95 percentage of coefficient of variation at 120 min. β-carotene and canthaxanthin are two carotenoids that have photoprotective characteristics. Patients with erythropoietic protoporphyria have lower serum levels of carotene, so they must take it as a dietary supplement. Furthermore, lycopene and β-carotene have been found to lessen skin redness and damage from UV radiation. This acts as a calming agent when exposed to UV radiation from the sun (Galasso et al., 2017).

Antiaging: Age-related macular degeneration (AMD), the leading cause of blindness in developed nations, is one of the most severe age-related diseases. Due to its antioxidant characteristics, GSH, a reducing molecule found in high concentrations in the lens, is crucial for maintaining tissue transparency. In ageing lenses, this substance diminishes, resulting in oxidative and degenerative processes. Zeaxanthin and lutein play essential roles in preventing AMD because their consumption is linked to an increase in macular pigment density, which affects intracellular GSH levels (Mrowicka et al., 2022).

Retinal disease: Notably, identified AMD risk factors also entail biological mechanisms that significantly lower the bioavailability of lutein and zeaxanthin. The cumulative impact of diminished antioxidant capacity due to protracted oxidative injury is believed to create a toxic, neurodegenerative environment. The proliferation of early cellular senescence in the retinal pigment epithelium (RPE), which in turn sets off the pathogenic chain of events that leads to AMD development, is known to be triggered by mitochondrial malfunction and photo-oxidation (Olufunmilayo et al., 2023). Furthermore, it has been demonstrated that the metabolic syndrome's root causes seriously impair the digestion and transport of dietary carotenoids in diabetic retinopathy. Obesity, insulin resistance, and chronic hyperglycemia lead to atherogenic metabolic imbalance, furthering the loss of macular pigment. Therefore, low macular pigment optical density (MPOD) levels probably play a crucial role in AMD development (de Carvalho and Caramujo 2017).

Miscellaneous applications: Carotenoids are used in vegetable oils as a solution or suspension in margarine coloring, baked goods, and some prepared foods as emulsions or microencapsulated beadles. It is also used in drinks like orange juice, confectionery, and other ready-to-eat meals (Epplein et al., 2011). Carotenoids were first coupled to bovine serum albumin (BSA) in a new way, and then this carotenoid-protein combination was employed to make fortified food emulsions (Terlikowska et al., 2021).

The global market for carotenoids was assessed to be close to USD 1.5 billion in 2017, and it is anticipated to increase to $2.0 billion in 2022, with a compound annual growth rate (CAGR) of 5.7% from 2017 to 2022. The following compounds account for over 90% of the market's value: capsanthin, astaxanthin, β-carotene, lutein, annatto, lycopene and canthaxanthin. By itself, capsanthin made up USD 300 million in 2017 (20% of the market). Similarly, the astaxanthin market contributed USD 288.7 million (Leoncini et al., 2015). Astaxanthin leads the carotenoids market for the anticipated period, followed by β-carotene and lutein. Because of their well-established applications, carotene, lycopene, astaxanthin, zeaxanthin, and lutein are the most critical current carotenoids. However, the colorless carotenoid precursor phytoene, fucoxanthin, and canthaxanthin are approaching the market with the potential to be economically significant (Wackerbarth et al., 2009). Recent developments in systems biology, genetic engineering, and ways to profit from biomass leftover fractions present new scenarios for making microalgae-based biofuel production financially viable in around 15 years. Combining the production of microalgae-based biodiesel and other bioproducts can increase efficiency and profitability (Vílchez et al, 2011).

CONCLUSION

Microalgae have garnered the attention of scientists owing to their rich supply of bioactive compounds. Their straightforward and economical development needs render them attractive for widespread employment in the pharmaceutical, culinary, and cosmetic sectors to enhance health. Carotenoids are one of the most frequent components of microalgae, and research has demonstrated significant health advantages. Microalgal carotenoids have demonstrated anti-inflammatory, antioxidant, and anticancer activities in various in vitro studies, animal tests, and human trials. In this regard, they have been reported to be useful in treating several inflammatory conditions including colitis and nonalcoholic fatty liver disease. Owing to the increasing demand for natural products, the identification of novel carotenoids, advancements in upstream and downstream methodologies, and broadening market opportunities, the microalgal carotenoid market is anticipated to persist in its growth.Microalgal-derived carotenoids are utilized in the food, feed, nutraceutical, and cosmetics sectors; nevertheless, legal and regulatory frameworks are lagging behind scientific progress. This study examined the possibility of several green solvents and innovative techniques for extracting carotenoids from fruit and vegetable by-products. Due to their attributes and low toxicity, green solvents are really a superior alternative to organic solvents. Cost-effective techniques for the production of microbial pigments are required to substitute synthetic alternatives.

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Identification of Groundwater Potential Zones Using GIS and Remote Sensing Techniques Combined with Analytical Hierarchy Process (AHP), in Telangana State

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Abstract: Geographic information system (GIS), remote sensing (RS), and multi-criteria decision analysis (MCDA) techniques were integrated to delineate groundwater potential zones in Telangana State, India. Ten thematic layers; slope, elevation, geology, lineament density, line density, land use/land cover (LULC), soil, drainage density, rainfall, and topographic wetness index (TWI) were generated and analysed. The analytic hierarchy process (AHP) was employed to assign appropriate weights to each thematic layer based on their relative influence on groundwater occurrence. These weighted layers were overlaid using a GIS-based weighted overlay analysis to produce a groundwater potential zone (GWPZ) map. The final output was classified into five categories: poor (56.63%, 65,095 km^2) fair (20.64%, 23,692.18 km²), good (5.32%, 6,096.20 km²), very good (4.71%, 5,409.16 km²), and excellent (12.70%, 14,546.77 km²). The results demonstrate the effectiveness of combining geospatial technologies with AHP for identifying groundwater potential zones. The AHP-derived weights assigned to each thematic layer are rainfall (22.83), Geology (16.49), slope (10.36), drainage density (7.47), LULC (8.53), lineament density (5.59), soil (5.60), line density (7.55), elevation (7.03), and TWI (8.53).This approach provides valuable insights for sustainable groundwater resource management and site selection for new bore well development in the region.

Keywords: Groundwater potential zone, Geographic information system (GIS), Remote sensing, Weighted overlay, AHP

Groundwater is a vital yet concealed natural resource, and its exploration poses considerable challenges, especially in regions like Telangana. Traditional methods such as drilling and geophysical surveys, while effective, are often time-consuming and costly. In this context, geospatial technologies offer a more efficient and systematic approach. The integration of geographic information systems (GIS) and remote sensing (RS) with the analytical hierarchy process (AHP) provides a powerful framework for delineating groundwater potential zones. GIS enables the spatial analysis of multiple thematic layers such as land use/land cover, geology, slope, soil type, and distance from water bodies. Remote sensing data, particularly from multispectral satellite sensors, allows the derivation of indices like the normalized difference vegetation index (NDVI) and normalized difference water index (NDWI), which are critical for understanding land surface characteristics influencing groundwater recharge. These indices help identify zones with favorable infiltration potential, vegetation cover, and surface water availability The AHP technique further enhances this analysis by assigning relative weights to each thematic factor based on expert judgment through a pairwise comparison matrix. This structured decision-making process ensures that each factor's contribution to groundwater potential is quantified objectively. The resulting weighted overlay analysis provides a groundwater potential map, identifying high, moderate, and low potential zones with greater accuracy. Recent studies have demonstrated the effectiveness of combining GIS, RS, and AHP in groundwater exploration, particularly in semi-arid regions. Kom et al. (2024) successfully applied this methodology in South India, and Upadhyay et al. (2023) highlighted its utility in delineating recharge zones with high spatial precision. Similarly, Ifediegwu et al. (2022) showcased the robustness of AHP-GIS integration in identifying potential zones in data-scarce environments. This integrated approach not only improves the accuracy of groundwater mapping but also supports sustainable water resource planning and management, which is crucial for regions experiencing increasing water stress. The objectives of the study are to integrate GIS and remote sensing techniques with the Analytical Hierarchy Process (AHP) for evaluating multiple thematic factors influencing groundwater potential in Telangana State., to assign weights and ranks to key groundwater indicators and to generate a comprehensive groundwater potential zone map for Telangana State that supports sustainable water resource planning and management.

MATERIAL AND METHODS

Study area: The current research was focused on the 33 districts of Telangana, located in southern India between 15° 55′ to 19° 55′ latitude and 77° 10′ to 81° 50′ longitude, covering a total area of 1,14,840 km^2. The region is irrigated by two primary rivers, containing approximately 79% of the Godavari River catchment area and around 69% of the Krishna River catchment area. The state's average annual rainfall is between 900 and 1500 mm in northern Telangana and 700 to 900 mm in southern Telangana, 80% of which comes from the South-West monsoon (June to September). The source of data collected for the study is indicated in Table 1, and the overall flow chart of the study is presented in Figure 1.

Thematic layers

Slope map: The landscape of Telangana state is an undulating terrain, ranging from flat plains to steep hillsides. The slope characteristics of the study area were derived from an ASTER digital elevation model with a resolution of 30 x 30 meters. Slope percentages across the Telangana landscape vary from 2 to 22 degrees. The study area was divided into five slope classes, and the predominant portions of the landscape fall within slope class (very low to very high).

Elevation map: The elevation map for the study area was generated using the ASTER Digital Elevation Model (DEM) and categorized into five groups (very low to very high)

Line density and Drainage density: Line density in hydrology quantifies the length of linear features per unit area, while drainage density represents the total length of stream segments of all orders per unit area (Andualem et al., 2019).

$$D_d = \frac{\sum_1^n L}{A}$$

where drainage density is denoted by D_d, the length of waterways is signified by L, and the total area of the basin is signified with symbol A.

Topographic wetness index (TWI): Mapping flood susceptibility relies significantly on the TWI, a crucial factor in hydrology and geomorphology. Areas with TWI values exceeding the average are more prone to saturation and wetness, correlating directly with flood vulnerability (Lee et al., 2017, Samanta 2018, Swain 2020). High TWI regions face elevated flood risk, whereas low TWI areas are less susceptible. The TWI calculation employed the ASTER DEM with the specified formula.

$$\mathrm{TWI} = \mathrm{In}\frac{\mathrm{AS}}{\tan(\beta)}$$

where the upstream contributing area is denoted by AS, and the gradient of the slope is denoted by β. The final TWI map was divided into five classes, showing very low to very high.

Land use land cover: The land use and land cover map was obtained from the Esri land cover map of 10m×10m. Within the study area, six distinct land cover types were identified, namely water, tree, grassland, flooded vegetation, and built-up area.

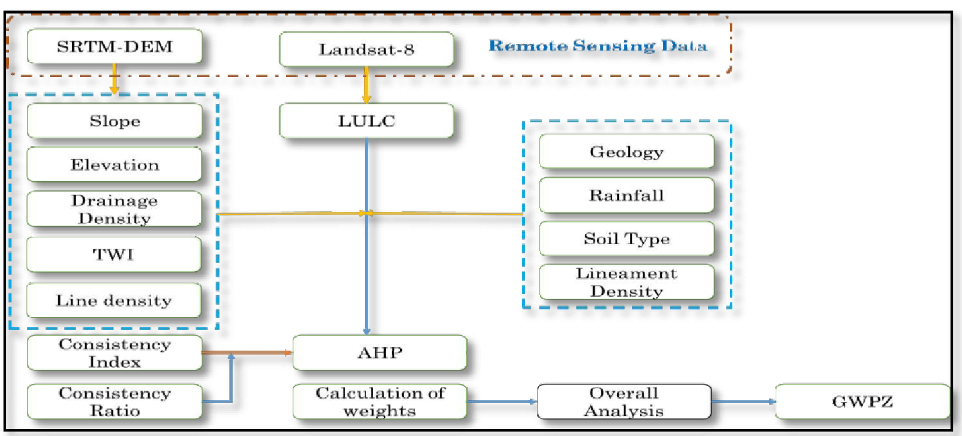

Fig . 1. Flow chart of methodology used in the study

Table 1. Sources of data used for study

Data Type	Sources of data collected	Extracted data
ASTER DEM (Grid) 30 m × 30 m resolution	NASA's official website https://search.earthdata.nasa.gov	Slope, Elevation, Soil type, Drainage Density, geology, and TWI
LULC data (Grid) 10 m× 10 m resolution	ESRI 2020 data, https://livingatlas.arcgis.com/landcover/	Land use/land cover map
Precipitation (TRMM data)	NASA's official website https://giovanni.gsfc.nasa.gov/	Rainfall map

Geology: Groundwater potential is significantly influenced by the occurrence of lithological features. The geology of Telangana, primarily characterized by the presence of the precambrian rocks of the deccan Plateau, dictates the occurrence, distribution, and quality of groundwater in the region. This type of rock predominantly covers the middle and lower courses of the study watershed.

Soil type: Soil texture is a crucial factor in agricultural production and groundwater recharge. It significantly influences water infiltration into aquifers and surface run-off. The study area has predominantly black, red, and alluvial soil, which is primarily categorized for the study.

Rainfall: Rainfall is an important parameter for delineating groundwater potential and identifying hydrological sources of groundwater storage. Approximately 85% of the rainfall occurs during the rainy season, which starts from June to October. The distribution of rainfall varies across the study area, with the upper part receiving higher amounts of rainfall compared to the southern part. The rainfall map of the study area is divided into five (1-5) categories, from deficient to very high rainfall, respectively.

Lineament density: Lineaments represent the total length of linear geological features per unit area (Andualem et al., 2019).

$$L_d = \frac{\sum_{j=1}^{i=n} Li}{A}$$

Where $\sum_{j=1}^{i=n} Li$ represents the total length of lineaments (L) and A represents a unit area.

Groundwater Potential Zone (GWPZ): The groundwater recharge potential map was generated by evaluating the comparative importance of various thematic layers and their corresponding classes. To delineate groundwater potential zones, the Groundwater Potential Zone (GWPZ), a dimensionless quantitative approach, was adopted. The GWPZ is calculated by integrating all relevant themes and features into a single layer, using the following equation (4):

$$GWPZ=Ge_rGe_w+Rf_rRf_w+Sl_rSl_w+So_rSo_w+Ld_rLd_w+DD_rDD_w+LULC_rLULC_w+LD_rLD_w+TWI_rTWI_w+El_rEl_w \quad (4)$$

where, Ge_r: Geology rank, Ge_w: Weight assigned to geology; Rf_r: Rainfall rank, Rf_w: Weight assigned to rainfall; Sl_r: Slope rank, Sl_w: Weight assigned to slope; So_r: Soil rank, So_w: Weight assigned to soil type; Ld_r: Line density rank, Ld_w: Weight assigned to lineament density; DD_r: Drainage density rank, DD_w: Weight assigned to drainage density; $LULC_r$: Land use /land cover rank, $LULC_w$: Weight assigned to land use/land cover; LD_r: Lineament density rank, LD_w: Weight assigned to lithology; TWI_r: Topographic index rank, TWI_w: Weight assigned to the topographic wetness index; El_r: Elevation rank. El_w: Weight assigned to elevation; GWPZ is the groundwater potential zone, and the suffixes r and w represent the rank and weight of each layer.

Analytical hierarchical process (AHP): The analytical hierarchical process (AHP) model is a multi-criteria decision-making (MCDM) tool used to solve complex decision-making problems, initially. It is widely accepted for assigning normalized weights to each thematic layer of groundwater prospecting factors. The final weight for each thematic layer is derived from the principal eigenvalue of the generated matrix. The reliability of the output was determined by calculating the consistency index (CI) and consistency ratio (CR) values.

$$CR = \frac{CI}{RI}$$

where CR indicates consistency ratio, RI indicates random consistency index whose values depend on the order of the matrix, and CI indicates consistency index, which can also be calculated.

$$CI= \frac{\lambda\ max-n}{n-1}$$

where λ indicates the principal eigenvalue of the matrix and n is the number of groundwater prospecting factors. The value of CR must be <0.1.

RESULTS AND DISCUSSION

The slope of the study area with slope values reclassified into categories: very steep (> 22°), steep (13°-22°), moderate (6°-13°), gentle (2°-6°), and flat (<2°). Flat slopes dominate the higher recharge rate, suggesting that most of the region is conducive to water retention (Fig. 3a). Areas with flat and gentle slopes are ideal for effective groundwater recharge, resulting in low runoff and more time for surface water to percolate. Presents the elevation profile of the study area, which is a key topographic factor and serves as a surface indicator for exploring groundwater potential. The study area elevation was classified into five classes: very low (14-188 m), low (188-297 m), moderate (297-416 m), high (416-535 m), and very high (535-949 m) (Fig. 3b).

The map has been reclassified and categorized into five classes: very low, low, moderate, high, and very high. This study found that very high drainage density is found in the eastern, northern, and southern regions. These hilly regions have steep slopes, favoring high runoff and low infiltration, indicating a low GWPZ (Fig. 2c and 2d). TWI values ranged from 10.1 to 20.1 and were reclassified into five categories: very low (10.1 to 12.5), low (12.5 to 15.6), moderate (15.6 to 16.5), high (16.5 to 17.7), and very high (17.7 to 20.1) (Fig. 3e). The low and very low TWI classes are located along river basins and low-lying regions, indicating a higher potential for groundwater availability. Despite the predominance of high and very high TWI classes in the research area, infiltration

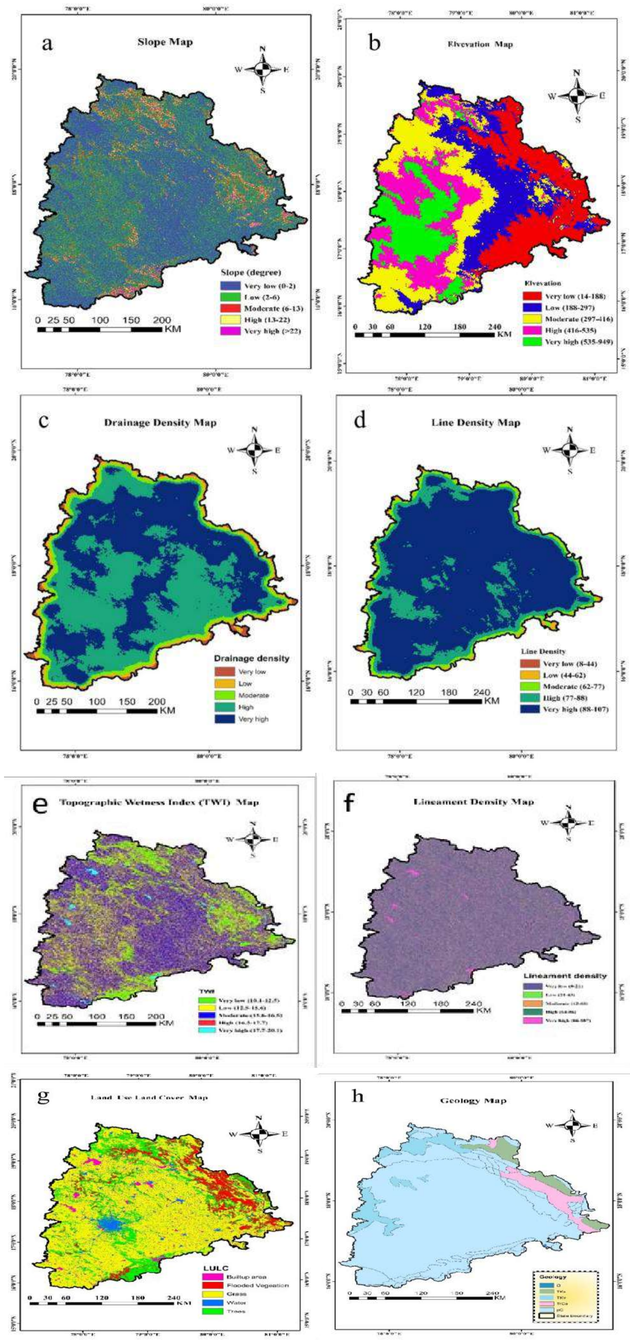

Fig. 2. Generated thematic layers (a) Slope map (b) Elevation map (c) Drainage density map (d) Line density map (e) TWI map (f) Lineament density map (g) LULC map (h) Geology (i) Soil type map (j) Rainfall map of the study area

Table 2. Groundwater potential zone in the study area

Potential zones	Area (km^2)	Percentage (%)
Fair	23,692.18	20.64
Poor	65,095.69	56.63
Good	6,096.20	5.32
Very good	5,409.16	4.71
Excellent	14,546.77	12.70

and accumulation are less common. Lineaments with densities ranging from low to very highindicating that lineament density is directly proportional to groundwater potential. Areas exhibiting a lineament density between moderate to high were identified as excellent groundwater prospect zones, encompassing major landscapes.

The LULC map of the study area was divided into six categories: water bodies, greens, trees, flooded vegetation, and built area. Among these classes, trees occupy the majority (62%) of the area. Infiltration rates increase as vegetation retains rainwater longer, aiding system recharge. Evergreen forests, cropland, and plantations have moderate runoff and high infiltration rates, contributing to increased groundwater recharge. The area comprises five different types of lithological features, including debre tabor basalts and trachyte, quaternary lacustrine sediment, middle basalt flows, upper basalts and trachyte, and guna tuff (Fig. 2h).

Soil predominantly falls into two categories: clay and sandy loam. In comparison, sandy loam soil predominates across most of the areas (Fig. 2i). Sandy loam soil exhibits superior groundwater potential compared to loam soil. Rainfall is one of the primary factors contributing to floods. The classification of the map was reclassified into five classes: very low (802-1,042), low (1,042-1,195), moderate (1,195-1,347), high (1,347-1,515), and very high (1,515-1,774) (Fig.3h).

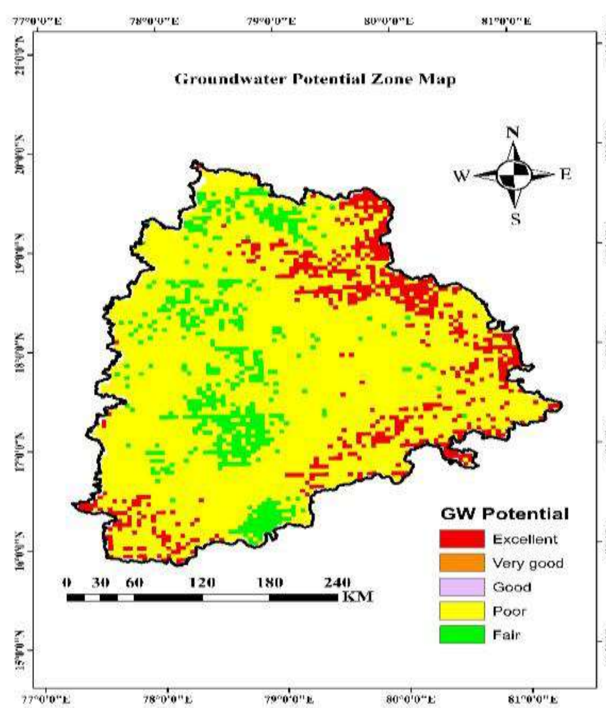

Fig. 3. Groundwater potential zone map of the study area

Table 3. Pair wise comparison matrix for the analytic hierarchy process (AHP) based on ground water potential (GWP) zoning

Criteria	Rainfall	Geology	Slope	Drainage density	LULC	Lineament density	Line density	Weight (%)
Rainfall	1	3	3	5	5	7	7	22.83
Geology	1/3	1	3	3	5	5	3	16.49
Slope	1/3	1/3	1	3	3	5	1	10.36
Drainage density	1/5	1/3	1/3	1	3	2	1	7.47
LULC	1/5	1/5	1/3	1/3	1	2	2	8.53
Lineament density	1/7	1/5	1/5	1/2	1/2	1	1	5.59
Soil	1	1	1	1	1	1	1	5.60
Line density	1/7	1/3	1	1	1/2	1	1	7.55
Elevation	1	1	1	1	1	1	1	7.03
TWI	1	1	1	1	1	1	1	8.53

Soil, Elevation, and TWI Criteria value is equal to 1

The analytic hierarchy process (AHP) was employed to assign weights to ten thematic layers; rainfall, geology, slope, drainage density, land use/land cover (LULC), lineament density, soil, line density, elevation, and topographic wetness index (TWI) based on their relative importance in influencing the target phenomenon. A pairwise comparison matrix was constructed using Saaty's 1–9 scale, where each criterion was compared against others based on expert judgment. The matrix was then normalized, and the average of each row was calculated to derive the final weights. The resulting weights revealed that rainfall (22.83%), geology (16.49%), and slope (10.36%) were the most influential factors, while lineament density (5.59%) and soil (5.60%) contributed less. The consistency check was also performed to ensure the reliability of the judgments used in the comparison matrix.

The principal eigenvalue of the matrix was 10.39 for the ten criteria considered. CI= 0.0433, and the random index (RI) of 1.49. Consistency ratio was computed was 0.029. Since the CR well below the acceptable threshold of 10%, the pairwise comparisons are considered consistent, validating the reliability of the weight assignment in the AHP process. The groundwater potential of the study area has been categorized into five distinct zones: poor, fair, good, very good, and excellent (Fig. 3 and Table 2). The poor groundwater potential zone occupies the largest portion of the study area, covering approximately 56.63%, which corresponds to 65,095.69 km². This indicates that more than half of the region has limited groundwater availability, possibly due to unfavourable geological conditions, low infiltration capacity, or reduced recharge rates. The fair groundwater potential zone is the next most extensive, accounting for 20.64% of the area (23,692.18 km²). This zone represents areas with moderate groundwater availability, where aquifer characteristics may support limited extraction depending on local hydrological conditions. In contrast, the excellent groundwater potential zone covers about 12.70% of the total area (14,546.77 km²), indicating regions with highly favourable conditions for groundwater recharge and storage. These zones are predominantly located in the northern and southern parts of the study area. The high groundwater potential in these regions can be attributed to dense forest cover, open land surfaces, and the presence of coarse-grained sediments, all of which enhance infiltration and aquifer recharge. The good and very good groundwater potential zones occupy relatively smaller portions of the study area, covering 5.31% (6,096.20 km²) and 4.71% (5,409.16 km²) respectively. Despite their limited spatial extent, these zones are still important, as they indicate areas with reasonably favorable hydrogeological conditions that can support sustainable groundwater development with proper management. Overall, while a significant portion of the study area exhibits low to moderate groundwater potential, the presence of excellent and very good zones in specific regions offers opportunities for targeted groundwater resource planning and sustainable water management practices.

CONCLUSION

The study delineated groundwater potential zones (GWPZ) in Telangana state using an integrated GIS, remote sensing, and AHP-based approach. Ten hydrologically relevant thematic layers were weighted using Saaty's AHP method and integrated through weighted overlay analysis in a GIS environment. The resulting GWPZ map classified the area into five categories excellent, very good, good, fair, and poor of the total area, respectively. High groundwater potential zones were mainly located in the northern and southern regions, associated with forest cover, open land, sandy loam soils, high rainfall, and dense lineament networks. Low potential zones were linked to rugged terrain, hard rock geology, steep slopes, urbanization, and high drainage density. Validation with observed groundwater fluctuation data showed good agreement with the classified zones. Despite potential subjectivity in AHP, the approach maintained consistency and demonstrated strong applicability for groundwater resource planning and well site selection.

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