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Effect of Botanicals against *Callosobruchus maculatus* (Fabricius) in Stored Mung Bean

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***Abstract*:** The study evaluated three botanical biopesticides *viz*., azadirachtin (10,000 ppm), acorus oil and acorus powder for their efficacy against the pulse beetle, *Callosobruchus maculatus* (Fabricius 1775) in stored mung bean (*Vigna radiata* L.), with spinosad as a chemical check. Experiments were conducted under controlled laboratory conditions for a storage period of five months. The botanicals completely inhibited oviposition and adult emergence, resulting in zero insect damage and seed weight loss, while spinosad and untreated seeds showed progressive increases in oviposition, adult emergence, up to 44 per cent seed damage and 9 per cent weight loss. Seed quality parameters, including germination percentage (85-87%), seedling length (22.36 -21.14 cm), seedling dry weight (0.15- 0.14 gm) and vigour indices (S.V.I I-1953.95-1833.73, S.V.I II- 13.38-12.42) were significantly better preserved in botanical treatments compared to spinosad (39%) and control (32%). The results highlight the potential of azadirachtin @ 0.6%, acorus oil @ 2.5% and acorus powder @ 5g kg^{-1} as eco-friendly alternatives to synthetic insecticides for safe and sustainable management of pulse beetles in stored mung bean.

***Keywords*:** Mung bean, Pulse beetle, Seed quality, Seedling vigour, Storability, Storage Insects

Pulses are important sources of protein, dietary fibre and other essential nutrients. However, their production is severely constrained by insect pests, which cause significant quantitative and qualitative losses both in the field and during storage. Among these, pulse beetles (*Callosobruchus* spp.; Coleoptera: Bruchidae) are the most destructive storage pests. Economically important species include the adzuki bean weevil, *Callosobruchus chinensis* (Linnaeus), the cowpea bruchid, *Callosobruchus maculatus* (Fabricius) and the pulse weevil, *Callosobruchus analis* (Fabricius) (Singh and Boopathi 2021), with hosts ranging from mung bean, adzuki bean, rice bean, cowpea, pigeon pea, lablab, common bean, lentils, chickpeas to peanuts (Tuda et al., 2005, 2006). Adult females lay eggs singly on the smooth surface of seeds (Senthilraja and Patel 2024). The larvae develop inside the seeds, consuming the endosperm and upon completing the larval stage, metamorphose into pupae and adults emerge by boring through the seed coat, completing the life cycle and initiating new infestations. Pulse beetles can cause 30–40% loss within six months, which may escalate to complete loss if left unmanaged (Paikaray et al., 2022, Bhattarai et al., 2024).

The growing incidence of insecticide resistance in storage pests, coupled with concerns over harmful residues in food and the environment, has greatly limited the dependence on chemical insecticides and fumigants. In view of these problems, along with WTO regulations, there is a pressing need to restrict their use globally and to implement safer alternative methods of insect management. Botanical products, which are already being utilized in many countries, offer a promising solution. Plant-derived essential oils have demonstrated ovicidal, larvicidal, and repellent effects against a wide spectrum of insect pests and are increasingly recognized as environmentally safe alternatives to synthetic chemicals (Isman 2000, Cetin et al., 2004). Neem-based formulations, in particular, have shown considerable potential as natural grain protectants under tropical conditions (Saxena et al., 2018). *Acorus calamus* Linn. (Acoraceae), a perennial herb with insecticidal properties, has traditionally been used for stored-product pest management (Koul et al., 2008). Its rhizome-derived products, such as essential oil and powder contain α, β and γ asarone, which contribute to its insecticidal activity (Juan et al., 2009a, 2009b, Liu et al., 2013, Swamy and Wesley 2017). Similarly, spinosad, a biopesticide derived from the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae) (Sparks et al., 1998), is increasingly adopted for the management of stored-product pests in several countries (Vayias et al., 2009, Mondal et al., 2018). Despite extensive research on botanical insecticides, comparative studies on their long-term efficacy in maintaining both pest control and seed quality in stored mung bean are limited. Hence, the present study was undertaken to evaluate the effectiveness of azadirachtin (10,000 ppm), acorus oil and acorus powder in comparison with spinosad against *C. maculatus* under laboratory storage conditions.

MATERIAL AND METHODS

The present study was carried out at the Entomology Laboratory, S.V. Agricultural College, Tirupati, Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India during 2024-2025.

Insect collection: The initial mother culture of pulse beetle, *C. maculatus* adults were collected from pulse storage godown, Regional Agricultural Research Station, Tirupati. The collected pulse beetles were brought to the laboratory, Department of Entomology, S.V. Agricultural College, Tirupati and were mass multiplied on mung bean seed under laboratory conditions at 25± 2°C and 75% RH.

Maintenance of *C. maculatus* insect culture: The mass multiplication of *C. maculatus* was carried out in the plastic containers (11cm x 8 cm) containing mung bean seed. The adult beetles of mixed sexes were released and the container was covered with muslin cloth and secured with rubber band. The plastic jars were kept undisturbed till the emergence of adults and the freshly emerged F1 population was used for storage studies. Fresh seed lot of mung bean seed with high germination of > 99% was used for storage studies. Commercial neem formulation, neem azal *i.e.,* azadirachtin (10,000 ppm), acorus oil, acorus powder and spinosad (Tracer 45% SC) were purchased from local market in Tirupati.

Mung bean seeds were treated with azadirachtin (10,000 ppm), acorus oil (2.5%) and acorus powder (5g/kg) along with spinosad (Tracer 45% SC, 0.5 ml/kg) serving as the chemical check and untreated control was maintained without any seed treatment. The experiment was laid out in a Completely Randomized Design with five treatments and four replications. To achieve equal distribution treated mung bean seed in the containers was shaken for 5 minutes. The treated mung bean seed was shade dried and then 10 adult pulse beetles of mixed sex were released into the containers with 1 kg of mung bean seed. The containers were closed with muslin cloth for sufficient ventilation and were placed in continuous dark conditions in the laboratory. Insect damage (%), weight loss (%), F1 adult emergence, germination (%), oviposition, seedling length, seedling dry weight were recorded at two months intervals up to five months. Seedling vigour indices were calculated as follows:

Seedling vigour index-I= Per cent germination x Seedling length in cm (root length+ shoot length)

Seedling vigour index-II= Per cent germination x Seedling dry weight in grams

The initial germination of mung bean seed recorded was 100 % without any insect damage and maintained good seedling vigour indices I and II at 2469.94 and 19.22 respectively.

Statistical analysis: Percentage data were angular-transformed before analysis. Data was analyzed with SPSS and treatment means compared using Duncans Multiple Range Test (p≤0.05).

RESULTS AND DISCUSSION

Effect of Botanicals on Pulse Beetle, *C. maculatus* Damage of Stored Mung Bean

Oviposition: All the plant products viz., azadirachtin (10,000 ppm), acorus oil and acorus powder were found to be effective against *C. maculatus* in treated mung bean seeds up to 5 months of storage. No oviposition was observed on mung bean seeds treated with azadirachtin (10,000 ppm), acorus oil and acorus powder indicating complete inhibition of egg laying and development of *C. maculatus*. In contrast, spinosad and untreated control recorded progressive increase in egg deposition after one (5 and 6 eggs/100 seeds), three (197 and 212 eggs/100 seeds) and five months (310 and 319 eggs/ 100 seeds) of treatment respectively during the storage period (Table 1). These results are in line with works of earlier researchers who confirmed the strong deterrent and ovicidal properties of neem and acorus oils against *Tribolium castaneum* (Rani 2022) and *Rhyzopertha dominica* in rice (Anu 2023).

Adult emergence: Significant reduction in adult emergence (0%) was observed with azadirachtin (10,000 ppm), acorus oil and acorus powder up to five months of storage as nil egg laying was observed compared to untreated control with the emergence of 986 adults/ kg seed. The chemical check

Table 1. Effect of botanicals against pulse beetle, *C. maculatus* on oviposition, F1 adult emergence, grain damage and weight loss of stored mung bean

Treatment	Dose	Oviposition * (no. of eggs laid/ 100 seeds)			F1 adult emergence * (no./ per kg seed)			Pulse beetle damage (%)*			Weight loss (%)*
		1M	3M	5M	1 M	3M	5M	1M	3M	5M	5M
Azadirachtin 0.6%	5 ml/kg	0.0 b	0.0 c	0.0 b	0.0 b	0.0 c	0.0 c	0.0 b	0.0 c	0.0 c	0.0 c
Acorus oil 2.5%	5 ml/kg	0.0 b	0.0 c	0.0 b	0.0 b	0.0 c	0.0 c	0.0 b	0.0 c	0.0 c	0.0 c
Acorus powder	5 g/kg	0.0 b	0.0 c	0.0 b	0.0 b	0.0 c	0.0 c	0.0 b	0.0 c	0.0 c	0.0 c
Spinosad 0.05 %	5 ml/kg	5.00 a	197.00 b	310.00 a	37.00 a	619.00 b	939.00 b	2.07 a	29.75 b	42.11 b	8.70 b
Untreated control		6.00 a	212.33 a	319.00 a	40.00 a	644.00 a	986.00 a	2.20 a	33.41 a	44.46 a	9.00 a

*Data was recorded at 1,3 and 5 months after storage
Initial insect damage in mung bean seed- nil
Means followed by same letters are not significantly different (p ≤ 0.05) by DMRT

spinosad @ 0.5 ml /kg was found to be ineffective in suppressing the pulse beetles with the highest number of adult emergence on par with untreated control (939 beetles/ kg). The bruchid emergence was increased with increase in storage period with 37, 619 and 939 adults per kg mung bean seed treated with spinosad recorded after 1, 3 and 5 months of storage which is on par with untreated control (40, 644 and 986 adults) at 1, 3 and 5 months, respectively indicating decrease in residual toxicity of spinosad over the storage period as reported by Ammar et al. (2024) in stored cow pea against *C. maculatus*. The results also showed increased egg laying, insect damage, decreased vigour and germination over the storage period in cow pea seed treated with spinosad.

Insect damage: There was no insect damage with different plant products like azadirachtin, acorus oil and acorus powder and in mung bean seed treated with spinosad 42.11% damage was observed five months of storage of treated mung bean which is almost equal in untreated control (44.46 %). The lower efficacy of the spinosad might be due to the resistance developed by pulse beetle to the spinosad. Sarada et al. (2021) also reported the lower efficacy of malathion 50% EC against pulse beetle. The lower persistence of malathion against pulse beetle was reported because of high degradation of malathion by hydrolysis, photolysis and high temperature (Raghu et al., 2016).

Weight loss: The five months after storage about 9 % weight loss was recorded with untreated control followed by spinosad (8.7%). All the plant products *viz*., azadirachtin (10,000 ppm), acorus oil and acorus powder under study protected the mung bean seed for prolonged periods of storage without any insect damage and weight loss of mung bean seed.

Effect of Botanicals on Seed Viability and Vigour of Stored Mung Bean

Germination percent: Irrespective of the seed treatment, germination percentage declined with the increase in storage period. The seed quality parameters*, viz.* seed germination, seed vigour, seed moisture and seed storability declined progressively with the increase in storage period (Sarkar et al., 2012). But the germination was maintained above minimum seed certification standards (MSCS) of 80 per cent in mung bean seed treated with plant products *viz*., acorus oil (85.67%), azadirachtin (87.33%) and acorus powder (86.67%) after five months of storage. The chemical check, spinosad recorded very poor germination of mung bean (38.67%) which was on par with the untreated control (32.00%) which could be due to the high pulse beetle damage to mung bean seed (Table 3). Sanon et al. (2010) also revealed that spinosad (seed coating) reduced adult emergence and seed perforation in cowpea for up to 6 months; >80% reduction in emerging insects in contrary to current study. Raheem (2011) evaluated the residual and relative toxicity of spinosad against *C. chinensis* in mung bean seeds and after one month of storage, the germination percentage is high (98%), but later on it is decreased to 50.33% after five months of storage which is similar to the current study. They also observed that adult emergence is low during first month of storage but after five months of storage there is an increased adult emergence. The seed damage recorded was around 28.66% after five months of storage. They concluded that the spinosad is effective only for shorter periods but was ineffective during longer storage periods.

Seedling shoot length and root length: Seedling shoot length of mung bean was decreased with increase in storage period (Table 3). After five months of storage the highest shoot length was observed in azadirachtin (14.96 cm) and there are no significant differences found between the other treatments. The root length recorded during the different storage intervals exhibited significant reduction with the increased period of storage. After fifth month, highest root length was recorded with azadirachtin (7.40 cm) followed by acorus powder which was on par with acorus oil compared to untreated control (5.38 cm) and chemical check, spinosad (5.83 cm). Similar results were also reported by Rajasri and

Table 2. Effect of botanicals on root length, shoot length and total seedling length of mung bean seedlings at different storage intervals

Treatment	Dose	Root length (cm)*			Shoot length (cm)*			Seedling length (cm)*		
		1M	3M	5M	1 M	3M	5M	1M	3M	5M
Azadirachtin 0.6%	5 ml/ kg	8.96 [ab]	7.90 [a]	7.40 [a]	17.08	16.57 [a]	14.96	26.03	24.47 [a]	22.36 [a]
Acorus oil 2.5%	5 ml/ kg	8.06 [b]	7.30 [b]	6.70 [b]	16.46	16.00 [b]	13.76	24.87	23.57 [c]	21.64 [a]
Acorus powder	5 g/ kg	10.01 [a]	7.93 [a]	6.86 [ab]	16.92	16.03 [b]	14.28	26.93	23.97 [b]	21.14 [a]
Spinosad 0.05%	5 ml/ kg	9.55 [a]	6.70 [c]	5.83 [c]	17.62	14.83 [c]	12.69	27.17	21.53 [d]	18.51 [b]
Untreated control		7.80 [b]	6.67 [c]	5.38 [c]	16.80	14.73 [c]	13.71	24.60	21.40 [d]	19.09 [b]

See Table 1 for details

Table 3. Effect of botanicals on germination percentage, dry weight and seedling vigour indices of stored mung bean at different storage intervals

Treatment	Dose	Germination percentage*			Seedling vigour index I *			Dry weight (g)*			Seedling vigour index II *		
		1M	3M	5M	1 M	3M	5M	1M	3M	5M	1M	3M	5M
Azadirachtin 0.6%	5 ml/ kg	97.67 [b]	91.33 [a]	87.33 [a]	2543.45 [ab]	2234.60 [a]	1953.95 [a]	0.19 [b]	0.17 [a]	0.15 [a]	18.26 [b]	15.83 [a]	13.38 [a]
Acorus oil 2.5%	5 ml/ kg	97.00 [b]	90.00 [a]	85.67 [a]	2412.97 [b]	2121.27 [b]	1855.00 [a]	0.18 [b]	0.16 [b]	0.15 [a]	17.65 [c]	14.70 [b]	13.13 [a]
Acorus powder	5 g/ kg	99.33 [a]	91.00 [a]	86.67 [a]	2675.11 [a]	2180.97 [a]	1833.73 [a]	0.19 [a]	0.16 [b]	0.14 [b]	18.87 [a]	14.86 [b]	12.42 [a]
Spinosad 0.05%	5 ml/ kg	97.67 [b]	55.33 [b]	38.67 [b]	2653.19 [a]	1191.43 [c]	713.11 [b]	0.17 [b]	0.13 [c]	0.10 [c]	16.60 [d]	7.37 [c]	3.87 [b]
Untreated control		96.33 [b]	50.67 [c]	32.00 [c]	2368.89 [b]	1084.27 [d]	608.81 [c]	0.17 [b]	0.13 [c]	0.10 [b]	16.05 [e]	6.59 [d]	3.20 [b]

*Data was recorded at 1, 3 and 5 months after storage
Initial germination percentage- 100%, S.V.I 1-2469.94, S.V.I II- 19.22, Dry weight- 0.21g
Means followed by same letters are not significantly different ($p \leq 0.05$) by DMRT

Rao (2012) with chick pea treated with neem formulations protected the seed from pulse beetle damage for longer period without any loss in seed germinability and vigour.

Seedling vigour indices: In general, with increase in storage duration, the seedling dry weight was decreased among different treatments. After five months of storage, highest seedling dry weight of 0.15gm was recorded with azadirachtin (10,000 ppm), acorus oil followed by acorus powder (0.14 gm) compared to lowest dry weight of 0.10 gm with chemical check spinosad and untreated control. Irrespective of the seed protectants used for seed treatment, seedling vigour index I declined progressively over the period of storage. Gradual decrease in vigour was observed from first to fifth month after storage; azadirachtin (2543.45 to 1953.95), acorus oil (2412.97 to 1855.00), acorus powder (2675.11 to 1833.73), spinosad (2653.19 to 713.11) and control (2368.89 to 608.81). After five months of storage higher seedling vigour was observed in seeds treated with azadirachtin (1953.95) followed by acorus oil and acorus powder. The least was in spinosad (713.11) on par with the control (608.81) (Table 3). Similarly, the seedling vigour index II was also declined with increase in storage period. Five months after the storage, azadirachtin (13.38), acorus oil (13.13) and acorus powder (12.42) treated mung bean maintained highest vigour. All the bio pesticide treatments were found superior to untreated control (3.20). The chemical check spinosad has shown least seedling vigour (3.87) which is on par with the untreated control. Similar studies were conducted by Rajasri and Rao (2012) with neem products like neem oil and commercially available neem formulations *viz*., Econeem plus®, Neemindia ® and Neemazal ® for the control of pulse beetle, *C. chinensis* in stored Bengal gram and were compared with deltamethrin treatment as a chemical check and the results indicated that the neem formulations *viz*., Neemazal, Econeem plus and Neemindia were found to be very effective against *C. chinensis* in stored Bengal gram maintaining high viability and vigour of seed up to twelve months of storage. The lower germination and vigour in spinosad treatment may be due to higher seed perforations caused due to higher number of pulse beetles and spinosad @ 0.05% was found ineffective in managing the pulse beetle damage in mung bean whereas botanicals found to be superior to control the bruchid damage in mung bean and also maintained the vigour and viability of seed up to five months of storage.

CONCLUSION

Botanical biopesticides *viz.,* azadirachtin, acorus oil and acorus powder completely inhibited oviposition and adult emergence of *C. maculatus* in stored mung bean up to five months. These treatments also maintained seed germination and seedling vigour above minimum certification standards, whereas spinosad and untreated control recorded heavy seed damage and loss in quality. The safer and ecofriendly botanical pesticides *viz.,* Azadirachtin (10000 ppm), acorus oil @ 2.5% and acorus powder 5g/kg can be used for long term storage of pulses without any quantitative and qualitative losses due to hidden bruchid infestation. Further studies on large-scale validation under farmer storage conditions and seed godowns are warranted.

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