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Bioefficacy of *Annona squamosa* L. Seed Extracts as Oviposition Deterrents and Adult Emergence Inhibitors Against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae)

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ABSTRACT: The pulse beetle, *Callosobruchus maculatus* being a primary storage pest is of great menace and are being tackled by synthetic insecticides and fumigants that act as effective method of pest control. In contrary, the usage of these insecticides and fumigants brought resistance in insects. Thus to overcome this constrain, an eco-friendly and cost effective method *i.e.,* use of botanicals in managing pulse beetle population was attempted. With a basic understanding on the conventional method of using plant materials as insect repellents, the study was undertaken with the various solvent extracts of seeds of *Annona squamosa*, to manage population of the storage pest *C. maculatus.* The results revealed that, the seeds of *A. squamosa* extracts were found to show oviposition deterrent and reduced adult emergence activity. Methanolic extracts of *A. squamosa* exhibited higher reduction (90.64%) on fecundity at 20% concentration followed by ethyl acetate extract. The results of efficiency of *A. squamosa* extracts clearly indicated that botanical extracts showed good control on adult emergence (91.95%) at 20% concentration of *A. squamosa* seed extract and it protects the green gram seeds from *C. maculatus.*

Keywords: *Annona squamosa,* seed extract, *Callosobruchus maculatus,* Green gram.

INTRODUCTION

In India, pulses are the good source of protein, vitamin, mineral, fiber and also indirectly enrich the soil fertility. In the year, 2021-22, the total pulse area 28.83 million hectares with production in India was 25.75 million tonnes with productivity 892 kg/ha. India is the world's largest producer as well as consumer of green gram and India contributes 70% of world's greengram production. But, this production was lost upto 8.5% at the time of storage and postharvest handling (Kosar and Srivatsava 2016).

The genus *Callosobruchus* belongs to the order Coleoptera which have many number of species, among which *C. maculatus* is one of the major and primary storage pest of pulses. Beetle infestation begins in the field but causes serious damage to the seeds during storage (Kayode and Ileke 2019) and are known to inflict quantitative and qualitative losses to stored pulses (Soe *et al.,* 2020). The beetle lays eggs on the outer side of the pod/grains following the hatching first instar larvae enter into the seeds, developed by feeding on embryo grains and endosperm turned into pupae and complete its lifecycle by emerging as adult (Kalpna *et al.,* 2022). It attacks whole grain of the pulses, affect the seed quality, market value and can reduce cow pea seed viability up to 2% after few months of storage (Caswell and Akibu 1980). In storage condition, pest control measures are generally taken by using synthetic insecticides and fumigants which is considered as quickest and effective method of pest control which brought insecticidal resistance in insects. It is necessary to find out alternative control measures and the plant derivatives are the best choice because of their efficacy and safety to environment. It is a traditional practice of Indian farmers to use plant material as repellent for storage pests. Plant possesses secondary metabolites which can be an effective source of insecticides and use of botanicals as a pesticide can help to reduce the risks.

The Custard apple, *Annona squamosa* Linn. (Family: Annonaceae) is tropical and sub-tropical crop and the seeds, leaves, bark, twigs, fruits, flowers, roots of this plant were reported to have toxicity against insect pests of crops in field and storage. The alkaloids present in *A. squamosa* have been proved to have antioxidant, anticancerous, antidiabetic, antioxidant, vasorelaxant,

abortifacient, anti-HIV, antimicrobial, insecticidal, anti-head lice and anti-thyroidal activity. Hence to utilize the available botanical resources, the present study is undertaken to study the bioefficacy of *A. squamosa* seed extracts against *C. maculatus.*

MATERIALS AND METHODS

A. Extraction from seeds of A. squamosa

Ripened fruits of *A. squamosa* were procured from different fruit markets of Madurai district. The separated seeds from pulp were washed with tap water and shade dried for four weeks. Then the shade dried seeds were powdered thoroughly by using grinding mill. From one kg of seeds 860g of seed powder was collected. Different solvents *viz.,* methanol, ethyl acetate, hexane, chloroform and acetone were used for extraction. Soxhlet apparatus was used for extraction from 30g of seed powder using 300ml of solvent. The apparatus was made to run for 6 hrs at 60°C temperature. The crude extract was collected after evaporating the solvents by using rotary vacuum evaporator at 45°C for 30 minutes. After evaporation of solvents from seed extracts recovery percentage of each solvent extract was calculated (Khalequzzaman and Sultana 2006).

B. Mass culturing of pulse beetle, C. maculatus

Mass culturing of pulse beetle was done at the Insectary, Department of Entomology, Agricultural College and Research Institute, Madurai, by following the method reported by Ramazeame *et al.* (2014).

Two hundred gram of green gram seeds with uniform moisture content were taken in 59×21×18 cm size plastic container. Ten pair of adult beetles were released in each container and tightly covered with muslin cloth by using rubber band. The containers were kept in dark condition at a temperature of 30±5°C and 70% relative humidity. The adult beetles emerged with in 25-30 days. During culturing, biology of *C. maculates* was also noticed. The *C. maculatus* was identified based on its external features. Both the male and female of *C. maculatus* had serrate type of antenna (segments with short triangular projections on one side). Pigidium was well exposed, the female beetle had dark stripes on Pigidium and male had no stripes (Beck and Blumer 2011).

C. Per cent reduction in oviposition

Green gram seeds (50g) was taken and treated with solvent extract of *A. squamosa* seeds at 1% concentration. Similarly, for each solvent extract and for each concentration 50g of seeds were used. The treated seeds were taken in a plastic container and newly emerged beetles of ten pairs male and female at 1:1 ratio were released. The NSKE (0.5g) were used as a standard check for the experiment. Each treatment was replicated four times. Corresponding solvents were maintained as untreated check on fecundity. The observation was taken after seven days of adult release. The eggs of the *C. maculatus* were counted by using handlens. The reduction in oviposition was calculated by using the formula reported by (Pankaj and Singh 2013).

D. Per cent reduction in adult emergence

The experimental set up was kept as such to observe the adult emergence and data on adult emergence was taken upto ten days. The per cent reduction in adult emergence of *C. maculatus* was calculated by the formula stated by Chudasama *et al.* (2015).

$$\text{Reduction in oviposition (\%)} = \frac{\text{No. of eggs laid in control} - \text{No. of eggs laid in treated seeds}}{\text{No. of eggs laid in control}} \times 100$$

$$\text{Percent reduction in adult emergence (\%)} = \frac{\text{No. of adults emerged in control} - \text{No. of emerged in treatment}}{\text{No. of adults emerged in control}} \times 100$$

RESULTS AND DISCUSSION

The experimental results of per cent oviposition reduction, clearly revealed that, all the treatments observed more than 30 per cent reduction in fecundity (Table 1). Among the five different extracts, methanol extract at 20 % concentration showed 90.64% reduction in fecundity which was followed by ethyl acetate extract that resulted in 77.92% reduction activity. The extracts hexane and chloroform at 20 per cent concentration showed 75.35% and 66.60% reduction in fecundity respectively. The least per cent oviposition deterrence was observed with acetone extract (65.26%). The standard botanical check neem seed kernel powder had shown 63.16% oviposition deterrent activity.

Among the various concentrations tested, 20% concentration of methanolic seed extract of *A. squamosa* performed well and resulted in 91.95 per cent reduction of adult emergence (Table 2). It was followed by 15% concentration (75.27%) and 10% concentration (67.35%). The per cent reduction of adult emergence at 1% concentration was 56.99%. The extracts *viz.,* ethyl acetate and hexane at 20% showed per cent reduction of adult emergence as 76.20% and 71.42% respectively. The standard botanical check neem seed kernel powder resulted in 68.94% reduction of adult emergence.

The observation on adult emergence and ovipositional deterrent activity of *A. squamosa* seed extract revealed that, the plant extracts had effective against *C. maculatus* with more than fifty per cent growth inhibition and oviposition deterrent activity at higher concentrations. At lower concentrations also 1-5 % concentration shown significant reduction of adult emergence and oviposition was observed upto 40-50%. This was in accordance with Chudasama *et al.* (2015) who reported that maximum percent pulse beetle oviposition deterrence was recorded in custard apple seed and leaf extract (67.19% and 65.95%) and adult emergence was also reduced in custard apple seed and leaf extract (78.45% and 77.14%).

The adult emergence reduction percentage also high in seed and leaf extracts of *A. squamosa.* The oviposition deterrent effect of *A. squamosa* against *C. maculatus*

was also reported by Vanmathi *et al.* (2012). Our findings is in line with Jatav *et al.* (2022) who reported that lowest (19.67, 21.33 and 23.00) number of eggs and minimum (20.67, 23.33 and 27.33 %) adults emerged in seeds was recorded on pulse grain treated with custard apple leaf powder (10 g/kg). And also the minimum seed damage of 26.67 per cent was recorded in custard apple leaf powder treated seeds, while maximum seed damage (63.33 %) was recorded in the control.

Among different aqueous extracts of plants materials, maximum percentage of oviposition deterrence was observed in custard apple seed extract (67.19%), custard apple leaf extract (65.95%), neem seed extract (65.44%), mustard seed extract (64.65%) and jatropha leaf extract (64.16%) at 5 per cent concentration. The maximum adult emergence reduction was seen in custard apple seed extract (78.45%), custard apple leaf extract (77.14%) and neem seed kernel extract (NSKE) (77.09%) at 5 per cent dose level. Minimum weight loss percentage was observed in the cowpea seeds treated with custard apple seed extract at 5% dose level, which recorded 79.20%. The results revealed that all of the tested materials with some variations had deterrent and toxic effects against the pest.

Table 1: Toxicity of *A. squamosa* seed extracts against *C. maculatus*: Ovipositional deterrent activity.

Sr. No.	Treatments	Per cent oviposition deterrent at different concentrations				
		1%	5%	10%	15%	20%
1.	T1- Methanolic seed extract	67.27 (55.10)^{a}	75.35 (60.23)^{a}	79.28 (62.92)^{a}	84.61 (66.90)^{a}	90.64 (72.18)^{a}
2.	T2- Ethyl acetate seed extract	57.95 (49.57)^{b}	62.68 (52.35)^{b}	67.24 (55.08)^{b}	69.29 (56.34)^{b}	77.92 (61.97)^{b}
3.	T3- Hexane seed extract	51.28 (45.73)^{c}	59.90 (50.71)^{b}	63.36 (52.75)^{b}	69.27 (56.33)^{b}	75.35 (60.23)^{b}
4.	T4- Chloroform seed extract	42.60 (40.74)^{d}	51.30 (45.74)^{c}	55.92 (48.40)^{c}	62.65 (52.32)^{c}	66.60 (54.69)^{c}
5.	T5- Acetone seed extract	33.31 (35.25)^{e}	42.60 (40.74)^{d}	51.97 (46.13)^{c}	58.59 (49.95)^{c}	65.26 (53.89)^{c}
6.	T6 – NSKE 0.5g	63.16 (52.63)^{ab}	63.16 (52.63)^{b}	63.16 (52.63)^{b}	63.16 (52.63)^{c}	63.16 (52.63)^{c}
7.	T7- Untreated check	0.00 (0.64)^{f}	0.00 (0.64)^{e}	0.00 (0.64)^{d}	0.00 (0.64)^{d}	0.00 (0.64)^{d}
SEd		1.64	1.75	1.64	1.65	1.78
CD (0.05)		3.42	3.65	3.41	3.44	3.71

Data are mean values of four replications

Figures in parenthesis are arcsine transformed values.

In the column, the means followed by the same letter are not significantly different from each other by DMRT at (P≤0.05) SEd standard error of the difference.

Table 2: Toxicity of *A. squamosa* seed extracts against *C. maculatus*: Growth regulation activity.

Sr. No.	Treatments	Per cent reduction in adult emergence at different concentrations				
		1%	5%	10%	15%	20%
1.	T_1- Methanolic seed extract	56.99 (49.02)^{b}	61.75 (51.80)^{ab}	67.35 (55.15)^{a}	75.27 (60.18)^{a}	91.95 (73.51)^{a}
2.	T_2 - Ethyl acetate seed extract	50.60 (45.34)^{c}	54.62 (47.65)^{c}	60.19 (50.88)^{b}	68.94 (56.13)^{b}	76.20 (60.80)^{b}
3.	T_3 - Hexane seed extract	46.59 (43.04)^{c}	52.12 (46.21)^{c}	57.71 (49.44)^{b}	64.03 (53.15)^{c}	71.42 (57.68)^{bc}
4.	T_4 - Chloroform seed extract	39.35 (38.85)^{d}	39.35 (38.85)^{d}	46.54 (43.02)^{c}	53.68 (47.11)^{d}	64.25 (53.28)^{cd}
5.	T_5 - Acetone seed extract	35.34 (36.47)^{d}	36.94 (37.43)^{d}	44.26 (41.71)^{c}	48.89 (44.36)^{d}	60.43 (51.02)^{d}
6.	T_6 – NSKE 0.5g	68.94 (56.13)^{a}	68.94 (56.13)^{a}	68.94 (56.13)^{a}	68.94 (56.13)^{b}	68.94 (56.13)^{c}
7.	T_7 - Untreated check	0.00 (0.64)^{e}	0.00 (0.64)^{e}	0.00 (0.64)^{d}	0.00 (0.64)^{e}	0.00 (0.64)^{e}
SEd		3.39	3.21	2.84	3.14	2.07
CD (0.05)		7.05	6.69	5.91	6.53	4.30

Data are mean values of four replications

Figures in parenthesis are arcsine transformed values.

In the column, the means followed by the same letter are not significantly different from each other by DMRT at (P≤0.05) SEd standard error of the difference.

CONCLUSIONS

The results of this study showed that *Annona squamosa* seed extracts, particularly those derived from methanol, exhibit significant potential as an eco-friendly and cost-effective alternative for managing *Callosobruchus maculatus*, a major storage pest of pulses with strong oviposition deterrence and growth inhibition effects. Ethyl acetate extracts also showed promising results, with notable reductions in both fecundity and adult emergence. The use of *A. squamosa* seed extracts can thus provide an effective, sustainable solution for controlling *C. maculatus* populations in stored pulses, helping to reduce the reliance on synthetic insecticides and mitigate the risk of insecticide resistance. The findings support the potential of plant-based pesticides as a viable component of integrated pest management strategies for postharvest pest control, contributing to the preservation of seed quality and reducing storage losses in pulse crops. Further research into optimizing the extraction methods and exploring the broader application of these extracts in different storage conditions could further enhance their practical utility.

FUTURE SCOPE

This research lies in refining the application of *A. squamosa* seed extracts for pest control, exploring their broader use in IPM strategies, and ensuring their safety, efficacy, and commercial viability for sustainable agricultural practices. It also highlights the potential of *Annona squamosa* seed extracts as a natural alternative for controlling *Callosobruchus maculatus*, but several avenues *viz*., formulation, commercialization, exploring other effects remain to be explored for maximizing their effectiveness and expanding their application.

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Conflict of Interest. None.

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