Bio-activity assay of arid region plant *Prosopis cineraria* on adult emergence of pulse beetle, *Callosobruchus chinensis* Lin.

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ABSTRACT

The powdered samples derived from the fruit, leaf, and bark of the *Prosopis cineraria* (L.) plant were evaluated for their ability to safeguard green gram seeds from damage caused by the pulse beetle *Callosobruchus chinensis*. The biological parameter examined for *C. chinensis* was "Percent adult emergence," which refers to the proportion of adult insects that emerged from the green gram seeds upon which *C. chinensis* lay its eggs. Formulations derived from plant extracts were observed to inhibit the development of the pulse beetle from the egg stage to the adult stage and eliminate it within the grain. The ovicidal and larvicidal effects of *P. cineraria* were demonstrated, as the extracts dramatically decreased the percentage of adult emergence by 56 to 93% at a 5% level of significance, compared to 96% in control groups. A minimum adult emergence rate of 56.68% was recorded in the groups treated with 10% aqueous bark extract of *P. cineraria*. Significant inhibition of emergence was seen in groups treated with 10% fruit formulations, with a range of 56 – 59%.

Keywords: Pulse beetle, *Callosobruchus chinensis*, plant-based pesticides, adult emergence, Pest emergence, ovicidal, larvicidal

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INTRODUCTION

Pulses constitute the main protein source of an average Indian vegetarian diet. Moreover, they are also high in dietary fibres and minerals (Gupta *et al.*, 2021). Half of the global production of pulses comes from India. In 2023, 23 million metric tons of pulses were produced in India (Pulses in India, 2024). However, post-harvest, these seed grains have to be stored for a long period in a warehouse or household conditions. Here, they are destroyed by several biotic factors like fungus, bacteria, rodents, and insects. The most notorious of these pests are insects like weevils, beetles, and moths, which lay eggs on the grains, after which their larvae enter the seeds, causing physical damage and deterioration in the quality of seeds, rendering them unsuitable for consumption and for use as seeds for replanting. Infestation by bruchids alone has been reported to cause grain losses that range from 4 to 60 % (Mishra *et al.,* 2017). The FAO report on the global economy of pulses has lamented the need to bridge the gap between the actual and the potential yield, especially in the small and marginal farms in South Asia and Africa (Rawal and Navarro, 2019).

Since the majority of the botanical pesticides have been found to function best against one or few pests, owing to the specific as well as diverse ecological niche, the best results can be obtained by an exhaustive focus on one pest. *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae), commonly known as Adjuki bean weevil, is one of the major pests causing significant damage to stored pulses, causing hefty losses annually. The high fecundity of the female, short development period to adult stage, long adult female longevity, and a high relative growth rate of 0.101 ± 0.006 mg/day add to the nuisance value of this pest. (Singh and Bhoopathi, 2022). It is the major pest of pulses in temperate and tropical countries under storage facilities as it severely reduces the commercial value of the seeds by way of weight loss and reduced germination potential (Kiradoo and Shrivastava, 2010; Gbaye *et al.,* 2011).

To overcome these losses, the use of synthetic pesticides becomes essential for in-field as well as storage purposes. This, in turn, has placed a huge load on the environment as the practice of using chemical-based insecticides and fertilizers has also increased in the same proportion as the food grain production. The chemicals used in pesticides have a number of side effects like toxicity, biomagnification, non-biodegradability, carcinogenicity, nutrient depletion of soil and pest and disease resurgence to name a few. (Kalliora *et al.,* 2002; Nicolopoulou-Stamati *et al.,* 2016; Tripathi *et al.,* 2020). Additionally, other human activities like mining, soil erosion and contamination of water sources have added to the environmental load. Still, owing to a lack of mindfulness and, more importantly, a dearth of viable options, there is an increase in the consumption as well as production of pesticide in the country as the government reports have shown (Consumption of Chemical Pesticides, 2024). Thus, it becomes imperative to seriously look for alternative methods and to give a thrust to the use of bio-pesticides for their host specificity, eco-friendly nature, biodegradability and no residual toxicity. From a wide range of variety that bio-pesticides have to offer like biochemical pesticides, microbial pesticides, including semiochemicals, insect growth regulators, plant incorporated protectants (PIPs) utilizing GMOs; plant-based extracts and essential oils have arisen as a multifaceted weapon owing to their wide range of action viz as repellants, attractants, respiratory and ovipositional inhibitors, decreased adult emergence (ovicidal and larvicidal effect) and anti-feedent activities (Keita *et al.,* 2001, Tapondjou *et al.,* 2002; Halder *et al.,* 2013; Ali *et al.,* 2017; Tripathi *et al.,* 2020; Kumar *et al.,* 2021).

Although there isn't a dearth of research in this subject area, there is still a research lacuna in addressing the costliness of eco-friendly alternatives. This study was designed to address this issue and to create such biodegradable and effective pesticides that could be produced with minimal requirements and employing local plants by farmers and laymen for use at home, as well as storage houses. This research paper illustrates a lab investigation to check the potential viability of formulations made from a locally available plant, *Prosopis cineraria*, nicknamed the king of the desert as a commercial insecticide.

MATERIALS AND METHODS

Bioefficacy of plant *Prosopis cineraria* extracts prepared using different plant parts in reducing the emergence of adult insects of *C. chinensis* from seeds of green gram were assessed. This study was conducted in the Entomology Department, Dungar College, Bikaner.

The pest chosen for the assay was *C. chinensis*. A single pair of one-day-old adults was used to raise a pure line, which I used throughout the study. It was easy to differentiate the sexes as the bruchid exhibits sexual dimorphism. The insects were reared on cleaned and disinfested seeds of green gram, *Vigna unguiculata* Linn., kept in glass jars. The jars were kept in Biochemical Oxygen Demand incubator. Ambient laboratory conditions of a temperature of 28± 2^{o}C and relative humidity of 70% were maintained throughout the experiment.

The college campus has a few trees of *P. cineraria*, which were identified by experts in the subject and used for the study. The plant parts used were bark, leaf, and fruit. The plant parts were washed, shade-dried, ground, and kept in air-tight containers. The different parts of the plant (bark, leaf, and fruit) were used in two forms, *viz.*, liquid extracts and powder suspension. Two separate media – water and ether were used for preparing liquid extracts, as the solvent of the active element of *Prosopis* sp. was uncertain. One gram of pulverized plant material like bark, fruit, and leaf separately was packed in a thimble made with Whatman filter paper (size 10 X 10 cm) was boiled with 50 mL of distilled water till it reduced to 10 mL. This was designated as a 10 percent concentration, which was used as such, and also as a stock solution for 5, 2.5, and 1 percent solutions. Similarly, one gram of pulverized bark, fruit, and leaf separately were packed in a thimble made with Whatman filter paper (size 10 X 10 cm) was placed for distillation in a Soxhlet extraction unit with ether. This extract was made to a volume of 10 mL. This was designated as a 10 percent concentration and was used as a stock solution to prepare 5, 2.5, and 1 percent concentrations. For formulating suspension, 10, 5, 2.5, and 1 g powder of the bark, fruit, and leaf (separately) was weighed, added to fifty grams of grains, and the suspension formulated by just adding a few drops of distilled water.

Fifty grams of green gram were treated with 1 ml of the required extracts. 5 pairs of one-day-old beetles were placed into all investigational sets of the four concentrations (10, 5, 2.5, and 1 %), taken in three replicas, and compared with the control. The percent adult emergence was calculated by counting the insects that came out of the grains (E) and dividing by the total number of eggs (T) in the fifty grams of grains.

$$\text{Percent adult emergence} = \frac{E}{T} \times 100$$

The data obtained was subjected to analysis of variance (ANOVA) at 5% level of significance (IBM SPSS Statistics 20.0). Data obtained were presented as Mean ± SD and are represented here in Table 1 and Charts 1,2, and 3.

RESULT AND DISCUSSION

The percent adult emergence of *C. chinensis* during the current study under different treatments has been presented in Charts 1, 2, and 3. In the current study, 96% adult emergence was observed in control sets. While in sets treated with *P. cineraria* formulations, it ranged from a minimum of 56.68% (in 10% aqueous extract of bark) to a maximum of 92.84% (in 1% aqueous suspension of bark). Notable inhibition of pest emergence was found in sets treated with 10% formulations of fruit, which were in a range of 56 – 59%. These were significantly lower than the values of the control sets, which had 96% of emergence. The best treatment turned out to be 10% aqueous bark extract with an emergence of 56.68%.

For overall comparisons between the plant parts, extracts, and concentrations, ANOVA was applied, which showed significant differences between the plant parts, concentrations, and treatments at 5% level of significance. During the current study, the formulations of *P. cineraria* were observed to result in significantly lower ($P<0.05$) adult emergence of the pest as compared to the control.

Among the plant parts, insects treated with bark showed an adult emergence of 74.14%, while the leaf formulations resulted in a mean pest emergence of 75.35%. These were significantly lower ($P<0.05$) than the emergence observed in sets treated with fruit (77.93%).

When the treatments of extracts were compared for emergence, 74.92% adult emergence was found in the aqueous extract-treated sets, and 75.01% was found in the ether extract-treated sets. The aqueous extract and ether extract differed non-significantly ($P>0.05$) from each other. Although they were significantly better than the sets treated with aqueous suspension, which recorded an adult emergence of 76.32% of the pest.

Emergence of *C. chinensis* in treatments made with different concentrations, namely, 1, 2.5, 5, and 10% differed significantly from one another. Lowest emergence was recorded with 10% formulations (63.68%); followed by 5% formulations (70.34%) and 2.5% formulations (79.68%). Maximum adult emergence of *C. chinensis* was found in 1% formulations (87.99%). The most effective treatment combating the pest emergence turned out to be concentrated aqueous bark extract of *P. cineraria*; adult emergence in the treated sets was significantly lower as compared to all the other treatments of plant parts.

The current results conform with the earlier works in which different plants/botanicals have been used against insects and have been observed to reduce adult emergence. *C. maculatus* emergence

Table 1. Adult emergence (%) of *C. chinensis* due to different concentrations (1, 2.5, 5 and 10%) of bark, fruit, and leaf of *P. cineraria.*

	Control	**1% Bark**	**1% Fruit**	**1% Leaf**	**2.5% Bark**	**2.5% Fruit**	**2.5% Leaf**	**5% Bark**	**5% Fruit**	**5% Leaf**	**10% Bark**	**10% Fruit**	**10% Leaf**
Aqueous extract	96.00±0.00	88.52 ±2.15	85.33 ±2.52	83.11 ±2.01	79.47 ±0.70	72.84 ±2.93	72.94 ±2.77	63.78 ±1.20	65.94 ±2.86	70.11 ±1.17	56.68 ±3.74	57.20 ±1.17	63.48 ±2.51
Ether extract	96.00±0.00	86.08 ±3.55	81.58 ±1.51	85.61 ±2.34	82.68 ±3.17	73.33 ±0.58	77.42 ±1.78	74.90 ±1.72	62.96 ±2.24	71.10 ±2.59	63.54 ±2.63	58.44 ±3.20	61.11 ±2.17
Aqueous suspension	96.00±0.00	92.84 ±2.36	84.57 ±1.88	88.78 ±2.37	76.06 ±6.03	75.44 ±3.36	79.33 ±4.04	73.04 ±2.62	64.54 ±0.94	66.44 ±2.88	70.94 ±2.53	56.92 ±1.67	62.05 ±0.93

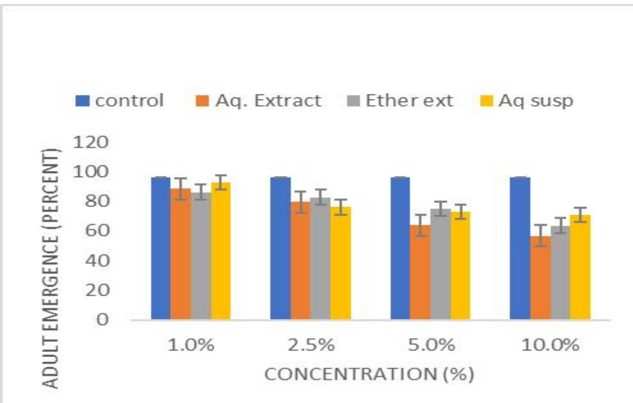

Fig. 1. Adult emergence (%) of *C. chinensis in* relation to different concentrations (1.0, 2.5, 5.0 and 10.0%) of aqueous, ether and aqueous suspension of *P. cineraria* bark

significantly diminished with extracts of neem, Drumstick castor beans, desert dates (*Balanites aegyptiaca*), yellow oleander, and African Mahogany as recorded by Mbaiguinam *et al.,* (2006). Similarly, Udo *et al.* (2004) reported a substantial drop in *S. zeamais* and *C. maculatus* emergence with botanicals extracted from *Zanthoxylum xanthoxyloides*. A comparative efficacy of three plants, *viz., Ocimum gratissinum*, *A. indica*, and *Hiptis suaveolens* on *S. zeamais* and *C. maculatus* were evaluated by Iloba and Ekrakene (2006), who observed that the emergence-controlling property of *H. suaveolens* was best out of the three plants used. A drop in adult emergence of *C. maculatus* was detected by Adedire and Akinneye (2004) with the extracts of tree Marigold, Tithonia diversifolia. Barre and Jenber (2022) evaluated the effectiveness of leaves and seeds of *A. indica*, *Zingiber officinale*, *Capsicum annuum*, *Lantana camara*, and *Allium sativum* against *S. zeamais* and found that seeds of *A. indica* and *Allium sativum* proved to be the best in controlling emergence. In a connected assay Cortese *et al.* (2022) found effects of three species of Ludwigia (sericea, nervosa, and longifolia) along with *Schinus terebinthifolius*, in managing adult emergence of *S. oryzae*. They found that *L. nervosa* extracts substantially reduced the emergence to 11.50. Ngegba *et al.* (2022) used Plant-based pesticides obtained from Neem, Red sage, Physic Nut, blue gum, gopo berry, and Uziza seeds to manage *Spodoptera frugiperda* and got promising results. Botanical oil extracts of citronella, fennel, garlic, lemon grass, patchouli, peppermint, geranium, neem, and thyme were employed for the control of whiteflies and other pests by Amour *et al.* (2023). They found Patchouli to be maximally effective as a repellent and ovipositional deterrent, while Neem was good at dropping egg laying by 50%, and adult emergence by 80%.

During the present study, aqueous extracts and ether extracts were found to be significantly better than aqueous suspensions in controlling the emergence of the beetle. Lale *et al.* (1999) detected that the *C. frutescence* fruit extract prepared in acetone could decrease *C. maculatus* emergence.

During the present study, the extract concentration seemed to have an inverse relationship with the adult emergence, being lowest with formulations of the highest concentration. Ivania *et al.* (1995) conducted an assay in which they used castor oil and soyabeen oil to manage the emergence of *C. maculatus* from chickpea seeds.

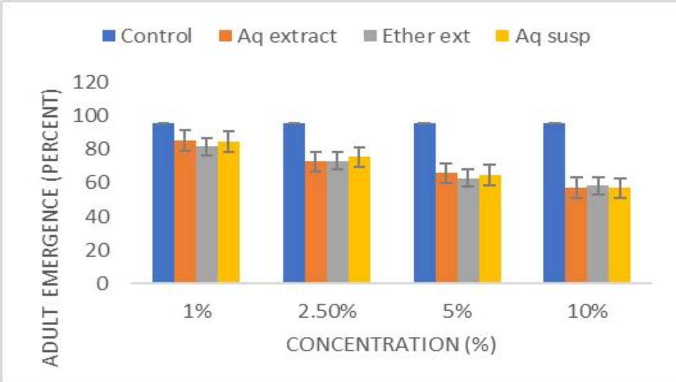

Fig. 2. Adult emergence (%)*C. chinensis in* relation to different concentrations (1.0, 2.5, 5.0 and 10.0%) of aqueous, ether and aqueous suspension of *P. cineraria* fruit

Similar results were reported in an experimental study with Pongam oil by Negi *et al.,* (1997), where dose concentration and percent adult emergence of *C. chinensis* showed an inverse relationship. *Acorus calamus*, *Solanum nigrum*, *Leucas aspera*, *Cardiospermum helicacabum*, and *Achranthes aspera* were studied for efficacy against *C. maculatus*. Low egg hatchability, along with zero emergence of beetles, was found in the treatments of 1, 1.5, and 2 percent *A. calamus*, where the control showed an emergence of 148. (Govindan *et al.,* 2020). What could be the possible mode of action of the extracts has also been a matter of research. The reason behind low emergence could be egg or larval mortality, or some hindrances to egg hatching. Abdullahi *et al.* (2011) accredited the toxicity of certain compounds present in the botanicals to be the reason behind the egg mortality.

The effect of tannic acids on adult emergence was studied by Ogigiangbe and Onigbinde (1996) on *C. maculatus*. They proposed that tannic acid content of the cotyledons checked growth of grub inside seed whereas tannins in the seed coat checked the penetration of the 1st instar larvae into the kernel. Hematpoor (2017) studied the toxic action and the mode of action of phenylpropanoids obtained from the *Piper sarmentosum* roots against *P. interpunctella. R. dominica* and *S. oryzae*. The biochemical assessment revealed that the possible mechanism of action was inhibition of acetylcholinesterase. The toxicity of some natural compounds like oil from mustard and horseradish, d-carvone and l-carvone, against *Lasioderma serricorne* was evaluated by Kim *et al.* (2003) proposed toxicity in case of insects ingesting and digesting the compounds in the stomach. However, toxicity was not observed on the cuticle.

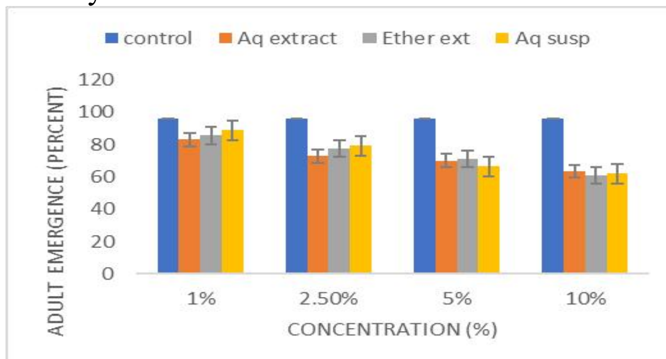

Fig. 3. Adult emergence (%) *C. chinensis in* relation to different concentrations (1.0, 2.5, 5.0 and 10.0%) of aqueous, ether and aqueous suspension of *P. cineraria* leaf.

P. cineraria is an important arid-region tree. It is used in the Ayurvedic system of medicine for the treatment of leprosy, leukoderma, dysentery, jaundice, tremors of the muscles, asthma, bronchitis, and piles. Phytochemical studies have indicated the occurrence of several categories of phytochemicals, like derivatives of flavone, tannins (gallic acid), fatty acids, alkaloids like prosophylline and spicigerine, steroids like campesterol, sitosterol, stigmasterol, and amino acids etc. (Chaudhary *et al.,* 2018). Khan *et al.* (2006) proposed that the *P. cineraria* extract made in methanol contained seven compounds, namely heneicosanoic acid, methyl heptacosanoate, 4-hydroxy-benzoic acid,1-0-coumaroylglycerol methyl 2-methoxy-5-hydroxycinnamate, methyl 2,5-dihydroxy- cinnamate and methyl 4-hydroxycinnamate, all of which presented toxicity.

C. chinensis is a serious pest of stored pulses in India. The study was set up to find local botanicals that could be used to manage this pest. The botanicals used were obtained from *P. cineraria*, which is locally known as Khejri and is a useful and easily available tree. To assess the effect of the botanical, the parameter of the pest life cycle that was taken into consideration was the adult emergence. It was observed that the extracts prepared using bark, leaf, and fruit of the *P. cineraria* were able to prolong the development of the insect, sometimes even stop it altogether by killing the growing insect stage inside the seed. This could be inferred from past research that this effect could be due to the alkaloids present in *P. cineraria.* The best treatment was found to be 10% aqueous bark extract, which could reduce the emergence of the adults to as low as 56.68% as compared to 96% in the control category. Based on the encouraging results obtained and comparing them with the control, it can be deduced that concentrated extract of parts of *P. cineraria*, especially bark, can be used as an eco-safe and sustainable replacement for harmful chemical-based pesticides in storage conditions in households and even in large warehouses. The botanicals can be easily obtained, and extracts can be easily prepared at home, too. The study was successful in achieving its aims of providing easy DIY solutions to storage problems in India.

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Declarations

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Conflict of interest

The present study involves no conflict of interest.

Authors' contribution statement

This study was done entirely by Shailja Rawat.

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