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Population control of pulse beetle *Callosobruchus chinensis* (L.) by sustainable mode of pest management

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Abstract
A laboratory trial was designed to investigate the bio-pesticide effects of acetone extracts of ten aboriginal plant species. Most of the concentrations of the extracts found effective to control the population of severe pulse beetle, *Callosobruchus chinensis* (L.).Extracts effectively reduced the number of eggs laid by female beetles on the seed surface, killed the eggs to avoid further damage to the stored seeds and as repellent reduce the appearance of the pulse beetles on the stored pulses.
Laboratory experiments were conducted at optimum conditions of temperature and relative humidity i.e. 25+/- ºC and 70+/-5 RH respectively.

Keywords: bio-pesticide, ovicidal, repellency

Introduction
It has been almost more than a century economic entomologists think about the idea of safer insecticides as traditional chemical pesticides imposed a serious threat to non- target organisms including human health. Non-biodegradable chemical compounds become health hazard by entering in to food chain through different agents like polluted water and soil.

Therefore to reduce the threat of chemical non-biodegradable pesticides various formulations of leaves, barks, flowers and seeds having potential to protect the stored products (Pulses) from serious pulse beetle *Callosobruchus chinensis* (L.). This beetle is a common pest targeting many different species of stored legumes and it is distributed across the tropical and subtropical regions of the world. *C. chinensis* is one of the most damaging crop pests to the stored legume industry due to their generalized legume diets and wide distribution. Many attempts have been made to evaluate the efficacy of plant products, i.e. citrus oil, coriander seeds, calamus oil, ricinus oil, neem leaves and seeds etc. against pulse beetle *Callosobruchus chinensis* (L.). (Garcia,1990, Giga *et al*,1990,and Su, 1985 and 1986) [3, 4, 8, 9].

Material and method
Experimental insects
The pulse beetle *Callosobruchus chinensis* (L.) is a member of Bruchidae family of Coleoptera order. It causes substantial damage in various stored pules and infests the pods in the fields also. Adult beetles of the pest are harmless because being are non-feeding stage and only grubs (larval stage) are responsible for damage. *C. chinensis* is a small insect without snout like other weevil. The adults are brown in colour with black and grey patches over the body. The abdomen of the female is slightly longer than the elytra so can not take longer flights like males. The adults are capable of flight and they can disperse to longer distances.

This species exhibits some sexual dimorphism where the female is larger and heavier than the male beetle. The antennae are pectinate in males while in females the antennae are serrate.

Adult females lay eggs singly, glued on the seed surface. On hatching of eggs larvae directly penetrate in to the seeds by making a tunnel and continue to consume the inner content of the seed till complete their development and left seed kernel only.

The culture of pulse beetle were reared in the laboratory in pre sterilized glass jars containing cowpea seeds (*Vigna sinensis*, Chawla).50 pairs of freshly emerged pulse beetles were taken from parent pure culture collected from Durgapura Agriculture Research Institute, Jaipur and released on disinfested cowpea grains. Subsequent subcultures were established on blooming of youth in the parent culture. Continuous supply of freshly emerged beetles and eggs to run the experiments was maintained by repeating the process every week. Stale grains were frequently replaced by the fresh ones to maintain the healthy conditions of stock cultures. Optimum conditions of constant temperature (27^{+}_{-} 2 º C) and relative humidity (60 % to 70 %). Cowpea was used for stock as well as sub cultures to prevent food effects, as all the experiments were carried out on cowpea grains only. Sex identification of beetle was done according to Arora (1977) [11].

Male Female

Fig 1: Adult *Callosobruchus Chinensis* (L.)

Collection and extraction of plant material

Indian soil is very rich in floratic species, still work on botanical has centered only on Neem. Inspired by this parochial attitudes around one dozen plant species (Table:1) were selected after a preliminary screening. The plant materials i.e. leave and flowers of respective plants were brought to the laboratory and were washed so as to remove dirt. Then the plant materials were dried in the shade and grounded in grinder to make 6 mesh powders. Further the extraction was done by following Soxhlet method using Acetone as solvent. The collected extract were filtered and considered as stock or mother solution.

Table 1: Plants selected for extraction

S. No.	Name of plant	Vernacular name	Family	Part of plant used
1	*Lawsonia inermis*	Mehandi	*Lytheraceae*	Leaves
2	*Ricinus communis*	Arandi	*Euphorbiaceae*	Leaves
3	*Parthenium hysterophorus*	Congress weed	*Asteraceae*	Leaves
4	*Lantana camara*	Ghaneri	*Verbenaceae*	Leaves
5	*Thevetia neriifolia*	Pile Kaner	*pocynaceae*	Leaves
6	*Jasminum arborescens*	Chameli	*Oleaceae*	Leaves
7	*Dalbergia sissoo*	Shisham	*Papilionaceae*	Leaves
8	*Eucalyptus rudis*	Safeda	*Myrtaceae*	Leaves
9	*Cassia fistula*	Amaltas	*Casalpiniaceae*	Leaves & Flowers
10	*Bignonia venusta*	Cross vine	*Bignoniaceae*	Leaves & Flowers

Following were the common parameters used to assess the toxicological effects of plant extracts

- Ovicidal Action (Egg mortality)
- Oviposition deterrent (Decrease in egg laying capacity)
- Repellent Action.

Experimental design

Ovicidal action

To evaluate the ovicidal efficacy of selected extracts, four dose levels i.e. S/25,S/50,S/75 and S/100 were prepared by diluting the stock solution with varying proportion of respective solvents. For ovicidal action cowpea grains with freshly laid 20 eggs were taken in petridishes and treated with 1 ml. of various dose levels. A control was also run simultaneously treating the substrate with solvent (Acetone) only. After complete evaporation of solvent, the grains were transferred in plastic vials. Results were taken by counting the hatched and unhatched eggs in treated as well as control vials.

Egg mortality was calculated by using Abbotts formula (1925) [1].

$$= \frac{\%\ \text{kill in treated} - \%\ \text{kill in control}}{100 - \%\ \text{kill in control}}$$

Fresh cowpea seeds **Eggs laid by Pulse Beetle on seeds**

Fig 2

Ovipositional deterrent action

To assay the oviposition deterrent property of the extract three replications of 100 % concentration and control (solvent only) were run simultaneously. 5 grams of cowpea seeds treated with 1 ml. leaf extract of *R. communis* and solvent were kept in the pre-sterilised plastic vials. Three pairs of freshly emerged (0-24 hrs.) insects were released in the experimenting vials. Results were recorded by counting the number of eggs laid by the insects on the treated seeds. Percent reduction in the oviposition and Deterrent quotient was calculated by the formula given by Messina and Renwick, 1983 [5].

Percent reduction in oviposition

$$= \frac{\text{No. of eggs on control seeds} - \text{No. of eggs on treated seeds}}{\text{Total No. of eggs on control seeds}} \times 100$$

Deterrent quotient: DQ

$$= \frac{\text{No. of eggs on control seeds} - \text{No. of eggs on treated seeds}}{\text{Total No. of eggs (control + Treated seeds)}}$$

DQ ranges from -1 (eggs on treated seeds) to + 1(eggs on control seeds)

Fig 3: Infested seeds with Emergence holes

Repellent action

For repellency experiment, freshly emerged insects of 0-24 hrs. were taken from mother culture. To evaluate repellent action of the extract 'Y' shaped Olfactometer was used (Read *et al*,1970 and Ahmed and Eapen,1986) [2, 6]. A cotton ball soaked in 1 ml. of stock solution (100 %) of the extract was plugged in the experimental arm, whereas control arm plugged with the cotton ball soaked in the same amount of the solvent (Acetone). Newly emerged insects were placed in the centre of the Olfactometer from the Base arm for 30 minutes and after that, number of beetles in the Experimental Control and Base arm was counted. The experiment was run three times. Repellency results were statistically analysed by calculating Standard Deviation (S.D) and Chi square test (x^2).

S.D. = $\sqrt{\Sigma d^2/N-1}$

Where: d= X - $\bar{X}$

Chi square test: $(O-E)^2/E$

Where

E= Expected Value

O= Observational Value

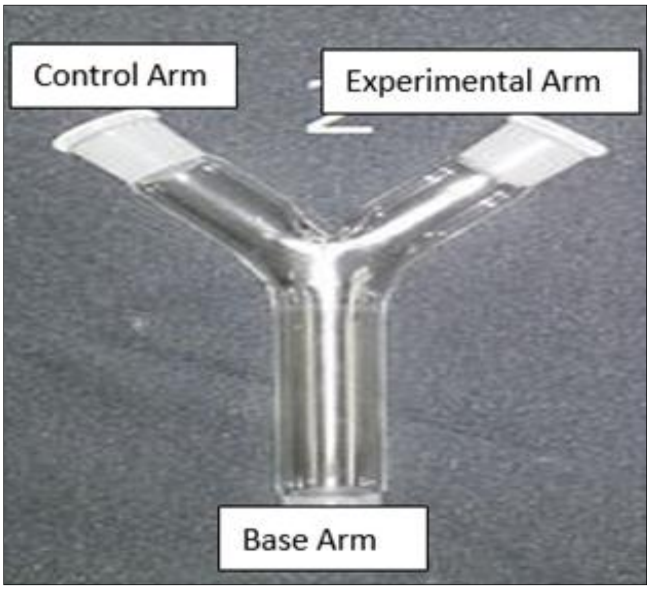

Fig 4: Y Shaped Olfatomemter

Result and Discussion

Table 2: Ovicidal, Oviposition Deterrency and Repellent effect of plant extracts

S.No.	Name of Plant	Ovicidal Effect	Oviposition Deterrency	Repellent Effect
1	*Lawsonia inermis*	85.00%	70.20 %	30.00 %
2	*Ricinus communis*	81.67 %	61.18 %	36.67 %
3	*Parthenium hysterophorus*	50.00 %	75.75 %	46.67 %
4	*Lantana camara*	87.61 %	62.38 %	43.33 %
5	*Thevetia neriifolia*	29.82 %	81.67 %	28.33 %
6	*Jasminum arborescens*	53.35 %	34.36 %	18.33 %
7	*Dalbergia sissoo*	55.00 %	49.08 %	41.67 %
8	*Eucalyptus rudis*	20.00 %	35.85 %	41.67 %
9	*Cassia fistula*	68.33 %	62.70 %	43.33 %
10	*Bignonia venusta*	55.00 %	29.36 %	36.67 %

*Results of different parameters(Table:2) depicted that Acetone extracts of *Lawsonia* and *Ricinus* leaves and flower extract of *Lantana* given promising egg mortality above 80 %,as 87.61 %, 85.00 % and 81.67 % in *Lantana Lawsonia* and *Ricinus* respectively. Results indicating that only 2 leaf extracts of *Thevetia* and *Eucalyptus* shown egg mortality less than 50 %.

**Thevetia* leaf extract in acetone found insignificant to kill eggs but effectively prohibited the egg laying capacity of female beetle on seed surface with 81.67 % oviposition deterrency. *Parthenium* (75.75%), *Lawsonia* (70%),*Ricinus* (61.81%),*Lantana* (62.38%) and *Cassia* (62.30%) acetone extracts reduced the egg laying capacity above 50 %.

*Repellency action was studied on adult beetles. Acetone extracts of selected plants repell the adults from treated arm but results found less than 50 % in all the extracts.

Discussion

Acetone extracts of plants used were significant in oviposition inhibition property in *Callosobruchus chinensis*. Results of *Lantana* and *Thevetia* supports the studies of Pandey *et al* in 1986 [19] who also indicated oviposition deterrent properties in the same plants. The result of this study are in confirmation with the trial of Olaifa and Erhun(1988) [7] and Prakash and Rao (1989) [20] to reduce the oviposition and emergence of *Callosobruchus* with *Oryza sativa* oil and leaf powder of *Vitex negundo* respectively.

Plant extracts containing oil contents (*Ricinus* and *Lawsonia*) find good support with the findings of Chander and Ahamed (1986) who reported anti ovipositional efficacy in some medicinal plant oils.

Results of *Cassia*, *Lantana* and *Ricinus* find good support from the studies of Dwivedi and Maheshwary (1995,97) [14, 15]; Rjapakse and Senanayke (1997),who have reported these plant extracts as oviposition deterrent against *Callosobruchus* sp. In 2000 Kamakshi [16] *et al* reported reduction in egg laying in *C. chinensis* by the use of *Mentha*, *Seabania* and *Ocimum*.

Pesticidal properties like egg mortality, repellent action from food surface in plant extracts is due to the chemical ingredients i.e. saponin of *Cassia* (Parmar and Devkumar, 1993) [18], Terpenes and Terpenol of *Parthenium* (Bokadia,1987) [12],essential oils in *Lawsonia*, *Lantana* and *Eucalyptus* (Zaire *et al* 1982), Alkaloids in *Eucalyptus* and *Ricinus* (Kwon *et al*,1991) [17] and Tecomine and tecostamine alkaloids in *Bignonia* (Amorpart and Harborne,1993).

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