OVIPOSITION DETERRENCY AND OVICIDAL EFFECT OF SELECTED ESSENTIAL OILS AGAINST *Callosobruchus chinensis* L. IN LABORATORY CONDITIONS

Kathirvelu, C. & Mangayarkarasi S.* & Maline, A. S.*
Assistant Professor, *PG Scholars
Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu - 608 002.

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ABSTRACT: *An experiment was conducted in the Department of Entomology, Annamalai University from July to November 2018 to study the bioactivities especially oviposition deterancy and ovicidal effect of selected essential oils obtained from plant species namely, Lantana oil (Lantana camera Linnaeus), Citronella oil (Citronella naradus Linnaeus), Cinnamon oil (Cinnamomum zeylanicum Blume) and Ajwan oil (Trachyspermmum copicum Linnaeus) against pulse beetle, Callosobruchus chinensis (Linnaeus). The oviposition deterrency activities were determined by fumigating newly emerged adults with the two sub-lethal concentrations viz. 30% and 60% of 24-h LC_{50} of the test solution. The fumigation effect of essential oils on egg hatching rate of C. chinensis was also carried out with different concentrations. Among the concentrations tested for oviposition deterrency, L. camara 60 % of 24 h LC_{50} and T. copicum 60 % of 24 h LC_{50} performed the maximum Oviposition Deterrency Index (ODI) of 61.33 % and 48.54 % respectively. The maximum Hatching Inhibitory Rate (HIR) was noted on C. zeylanicum 6 µl (73.95 %) and L. Camara 6 µl (68.05 %). The above results clearly indicated that the selected essential oils were performed better at higher concentrations against the test insect, C. chinensis.*

Key Words: *Callobruchus chinensis, Deterrency, Essential oil, Oviposition and Ovicide.*

Introduction

Durring the last six years, about 62,000 tonnes of food grains mostly rice and wheat have been damaged in the godowns of Food Corporation of India (FCI). A damage of 8,679 tonnes of food grains was reported in 2016-2017. Stored grain infestation is a very severe problem as different life stages of insects which cause economic damage and destroy the quality of food grains and their products. There are many stored grain insect pests which infest food grains both in public warehouses and farmers level storage and massively surge due to abandoned environmental conditions and use of poor ware housing technology. The stored food grains are being damaged by various pests such as 355 species of mites, 70 species of moths and 600 species of beetle which results in both quantitative and qualitative losses (Sahayaraj, 2008). The most important insect pests of stored grain are *Rhyzopertha dominica* (Fabricius) (lesser grain borer), *Oryzaephilus surinamensis* (Linnaeus) (saw toothed grain beetle), *Sitophilus oryzae* (Linnaeus) (rice weevil), *Trogoderma granaria* (Everts) (khapra beetle), *Tribolium castaneum* (Herbst) (red flour beetle), *Callosobruchus maculatus* (Fabricius) (pulse beetle) and *Plodia interpunctella* (Hubner) (Indian meal moth). Among these storage pests, the cowpea beetle, *Callosobruchus chinensis* (Linnaeus) and the four spotted bean weevil, *Callosobruchus maculatus* F. (Bruchidae: Coleoptera) are the notorious pests of common legumes and pulses grown in Asia, Africa, Central and South America. It lays egg on the seed coat, the grub hollows out the grain and cause huge yield loss (Singh *et al*., 1990).

The synthetic insecticides have been used to control the agricultural pest which helps to enhance the yield and give protection to stored food products and may cause serious health hazards to consumers. Botanical insecticides have been developed as an attractive alternative to synthetic insecticides for pest management. Hence, more attention has to be paid towards the utilization of plant products in pest control (Kedia *et al.,* 2015). Plant derived biopesticides are more readily recyclable, less toxic to mammals, less likely contaminate the environment. The toxic plant compounds are present plenty. Therefore, the researchers are searching a new class of naturally available insecticides that should be easily in sync with newer pest control approaches (Dubey *et al*., 2008)

For controlling major insect pests of stored produce, essential oil based botanical insecticides have been used as a sound alternative to the persistent synthetic pesticides (Sahaf and Moharramipour, 2008). The glandular hairs or secretory cavities of plant-cell wall possess essential oils and are present in the form

of droplets of fluid in the leaves, stems, bark, flowers, roots and fruits in different plants. The aromatic characteristics of essential oils offer different functions for the plants together with (i) attractant or repellent of insects, (ii) protects themselves from heat or cold and (iii) utilizes chemical constituents of the oil as defence materials (Koul *et al.,* 2008).

Certain plant species were tested for their insecticidal properties in the formulation of tablets in laboratory conditions against Rice weevil, *Sitophilus oryzae* (Linnaeus) and red flour beetle *Tribolium castaneum*, (Herbst) by Kathirvelu *et al.* (2019) reported that the formulation manifested the control over test insects. Although the tablet formulation offer fumigant and repellent action against the above pests in the laboratory condition and superior than the traditional formulations like dust and powder, the Plant essential oils and their constituents have been well confirmed against stored product pests and their main compounds are monoterpenoids, offer promising alternatives to classical fumigants (Papachristos and Stamopoulos, 2003). Considering safety, eco-friendly and cheaper insecticide for management of stored product insect pests, the present study was conducted to study the bioactivity of essential oils against, *C. chinensis* in laboratory conditions.

Materials and methods

The test insect namely pulse beetle, *Callosobruchus chinensis* L. adults were procured from pure culture maintained in the Department of Entomology and the insects were mass reared in the glass jars of size 15×10 cm with 1 kg capacity containing pulse grains (500g) as a nutritional source and maintained at 60-70 per cent relative humidity and temperature range from 30-35^0 C. To facilitate aeration, the glass jars were enclosed with a fine muslin cloth and secured with a rubber band. Periodically remove the infested grains and replaced with the same quantity of uninfested healthy materials. Thus, a continuous culture was maintained throughout the study period. Based on the fumigation action of essential oil of plant species from the literature surveyed, the essential oils namely, Lantana oil (*Lantana camera*), Citronella oil (*Citronella naradus*), Cinnamon oil (*Cinnamomum zeylanicum*) and Ajwan oil (*Trachyspermmum copicum*) were selected. The oils of *Lantana* and *Citronella* were extracted from leaves, Cinnamon and Ajwain from barks and seeds repectively. The essential oils used for this study were purchased from the Allins Exports (P) Ltd., New Delhi.

Oviposition deterrency activities of essential oil were determined by fumigating freshly emerged *C. chinensis* adults with the two sub-lethal concentrations *viz.* 30% and 60% of 24-h LC_{50}, of the test solution. Ten grams of food sources were placed at the base of the vial and ten adults of mixed sex were transferred to the vial. The conditions were maintained as in insect culture, the number of eggs laid over the grains was counted after 96 hours of treatment. For each concentration of essential oil as well as control three replicates were set. Two controls were set, one is standard check, filter paper strip was treated with acetone only and another one was untreated check.

Percent Oviposition Deterrence Index (%ODI) was calculated as using the following formula (Chaubey, 2008).

$$\% \text{ ODI} = \text{C-T/C+T X } 100$$

Where

C – Number of eggs in control
T – Number of eggs in treatment.

The ovicidal bio-assay was carried out against the test insect, *C. chinensis*, as the eggs are adhered on the seed surface and can easily be counted under microscope. In each glass vial, 100 eggs were fumigated with 2µl, 4µl and 6µl of essential oil prepared by diluting with acetone. It should be maintained for 96 hours. After fumigation, eggs were allowed to hatch and number of eggs hatched was recorded after 14 days of treatment. For each concentration of essential oil and control three replicates were set (Chaubey, 2008). Percent Hatching Inhibition Rate (% HIR) was calculated as follows:

$$\% \text{ HIR} = C_n\text{-}T_n/C_n \text{ X } 100$$

Where

C_n – number of adults in control
T_n – number of adults in treatment

The LC_{50} values for selected essential oils were calculated by using POLO programme analysis, (Probit Analysis software). The data obtained from the laboratory experiments were analysed statistically by using Completely Randomised Block Design (CRBD). Based on the Analysis of Variance (ANOVA) and

Least Significant Difference test (LSD), treatment effects were compared and ranked (Gomez and Gomez, 1976 and Rangaswamy, 1995) and documented.

Results and discussion

The effect of fumigation of essential oils on oviposition of *C. chinensis* is furnished in Table 1. The maximum Oviposition Deterrency Index (ODI) was observed in the treatment, *L. camara* 60 % of 24 h LC_{50} with 61.33 % ODI and it differs significantly from other treatments, followed by *T. copicum* 60 % of 24 h LC_{50} with 48.54 % while 38.91 % ODI was recorded in the treatment, *C. nardus* 60 % of 24 h LC_{50}. The minimum ODI was noticed as 13.93 % and 17.37 % in the treatments of *C. zeylanicum* 30 % of 24 h LC_{50} and *C. nardus* 30 % of 24 h LC_{50} respectively. The treatments *T. copicum* 30 % of 24 h LC_{50} and *L. camara* 30 % of 24 h LC_{50} were found statistically on par with each other registering 27.14 % of ODI. All other treatments were statistically differed significantly and had its own effect in maintaining ODI. This showed that *L. camara* 60 % of 24 h LC_{50} and *T. copicum* 60 % of 24 h LC_{50} had better oviposition deterrency effects among the others. The results of the current investigation is supported with the findings of Zandi-Sohani *et al*. (2012) who identified the major constituent of essential oil of *L. camara* by GC-MS analysis. The chemical composition of the essential oil of Iranian *L. camara* was α-humelene, ciscaryophyllene, germacrene-D, bicyclogermacrene, aromadendrene, and β-curcumine. Other important component includes humulene oxide, sabinene, α-terpineol, caryophyllene oxide, zingeberene, α-pinene, geranyl acetate and β-elemene may exhibit such effects.

The fumigation effect of essential oils on egg hatching rate of
C. chinensis is given in Table 2. The maximum Hatching Inhibition Rate (HIR) was achieved in the treatment, *C. zeylanicum* with 73.95 % followed by *L. camara* with 71.17 % and *T. copicum* (68.05 %) was noticed when treated with 6 µl. Similar effects of treatments were also recorded in *T. copicum*, *C. nardus* and *C. zeylanicum* @ 6 µl and 4 µl treatments respectively. The least per cent HIR was observed as 30.69 in the *C. nardus* treatment with 2 µl. This was followed by 37.93 % and 40.34 % in the treatments, *T. copicum* and *L. camara* 2 µl each and found on par as per statistical analysis. Among the lowest concentrations *i.e.,* 2 µl of *C. zeylanicum* found to be the highest per cent HIR with 44.48 %. The result revealed that the essential oils, *C. zeylanicum* and *L. camara* showed highest hatching inhibition rate than other treatments. Essential oil extracted from the seeds of ajwan exhibited insecticidal activity against *C. chinensis* oviposition as well as egg hatching and developmental inhibitory activities are similar to the current findings was reported by Chaubey (2008) and Kostyukovsky *et al.* (2002).

Conclusion

It is evident that the selected essential oils were performed better at higher concentrations. This might be due to more amounts of active ingredients or insecticidal components present in higher concentrations, which were responsible for altering the normal physiological process in test insect. The secondary metabolites present in the essential oils disturbed the ovipositional behaviour and also egg hatching of
C. chinensis.

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Table 1. Fumigant effect of essential oils on oviposition of adult females of *C. chinensis*

Essential Oils	LC_{50} (µl/l of air)	Treatments	Per cent ODI
T. copicum	23.39	T_1 - 30% of 24-h LC_{50}	27.14 (31.39)^{e}
		T_2 - 60% of 24-h LC_{50}	48.54 (44.17)^{b}
C. zeylanicum	23.90	T_3 - 30% of 24-h LC_{50}	13.93 (21.89)^{g}
		T_4 - 60% of 24-h LC_{50}	33.95 (35.63)^{d}
C. nardus	31.83	T_5 - 30% of 24-h LC_{50}	17.37 (24.62)^{f}
		T_6 - 60% of 24-h LC_{50}	38.91 (38.59)^{c}

L. camara	20.81	T_7 - 30% of 24-h LC_{50}	27.14 (31.39)[e]
		T_8 - 60% of 24-h LC_{50}	61.33 (51.56)[a]
		T_9 – Acetone	5.66 (13.76)[h]
		T_{10} – Control	00.00 (2.87)[i]
SEd			0.72
CD (0.05)			1.45

*Mean of three replications
*ODI – Oviposition Deterrency Index
*Values in parenthesis are arc sine transformed
*Values with different alphabets differ significantly according to LSD

Table 2. Fumigation effect of essential oils on egg hatching rate of *C. chinensis*

T. No.	Treatments	% HIR
1.	*T. copicum* - 2µl	37.93 (38.02)[f]
2.	*T. copicum* - 4µl	57.64 (49.40)[d]
3.	*T. copicum* - 6µl	68.05 (55.58)[b]
4.	*C. zeylanicum* - 2µl	44.48 (41.83)[e]
5.	*C. zeylanicum* - 4µl	66.14 (54.44)[b]
6.	*C. zeylanicum* - 6µl	73.95 (59.32)[a]
7.	*C. nardus* - 2µl	30.69 (33.64)[g]
8.	*C. nardus* - 4µl	47.39 (43.51)[e]
9.	*C. nardus* - 6µl	65.96 (54.32)[b]
10.	*L. camara* - 2µl	40.34 (39.43)[f]
11.	*L. camara* - 4µl	61.97 (51.94)[c]
12.	*L. camara* - 6µl	71.17 (57.53)[a]
13.	Acetone	07.39 (15.77)[h]
14.	Control	00.00 (2.87)[i]
SEd		0.92
CD (0.05)		1.88

*Mean of three replications
*HIR – Hatching Inhibition Rate
*Values in parenthesis are arc sine transformed.
*Values with different alphabets differ significantly according to LSD

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