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Valsala KK
Department of Zoology, Govt. College Madappally, Kozhikode, Kerala, India, 673102.

Gokuldas M
Insect Physiology and Biochemistry laboratory, Department of Zoology, University of Calicut, Kerala, India 673635.

Correspondence:
Gokuldas M
Insect Physiology and Biochemistry laboratory, Department of Zoology, University of Calicut, Kerala, India 673635.

Repellent and oviposition deterrent effects of *Clerodendrum infortunatum* on the pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

Valsala KK, Gokuldas M

Abstract
Experiments were conducted to study the repellent and oviposition deterrent effects of different concentrations (0.5, 2, 4 and 6%) of leaf extract of *Clerodendrum infortunatum* on the pulse beetle, *Callosobruchus chinensis*. All the tested concentrations showed significant difference in repellent and oviposition deterrence against *C. chinensis*, when compared to the control sample. Here a dose dependent effect was observed. Repellency increases with concentration and decreases with exposure time. Among the different concentrations tested, 6% extracts showed highest repellent effect. Dose dependent effects were observed in the case of oviposition deterrence and adult emergence of insects while treating with the extracts. Maximum oviposition deterrence was exhibited in insect present in 6% concentration of extract treated sample. Minimum number of insects (mean, 0.5) emerged from the samples treated with the higher concentration (6%). Percentage of reproduction control exhibited by the extract was 97.1. It is thus inferred that *C. infortunatum* extract at 6% concentration exhibited the highest per cent repellency and oviposition deterrency index against the pulse beetle, *C.chinensis*.

Keywords: *Clerodendrum infortunatum, Callosobruchus chinensis*, oviposition deterrent, repellent.

1. Introduction

Insect pests of stored grains are a problem throughout the world, because they reduce the quantity and quality of grains. The pulse beetle, *Callosobruchus chinensis* is a major pest of economically important leguminous grains such as cowpea, green gram, lentil and black gram [1, 2, 3]. The larvae bore into the grains which become unsuitable for human consumption, or for the production of sprouts. The insect multiplies very rapidly in storage and brings in nearly 10% loss in pulses during post-harvest handling and storage. Generally, management of stored product pest is done through fumigation and also is controlled by synthetic insecticides which have many limitations and undesirable side effects [4]. Therefore, alternative methods are being used in many countries. Botanical insecticides are biodegradable, relatively specific in the mode of action and easy to use [5]. Natural plant products have traditionally been used for insect control in stored food products [6]. Some natural plant products that prevent insect damage to animals or plants by rendering them unattractive or offensive are called repellents. Their strong and pungent odour, make them unattractive to host plants or stored grains. Use of insect repellent offers a hope for protection of stored grains from insect attack. They are more effective, more persistent and more economical than the existing synthetics [7]. Extensive works have been undertaken to control the oviposition of stored grain pests by using plant materials. In earlier days, various oils have been used for the control of *Callosobruchus* species, mainly neem oil [8, 5, 9]. Study of Bhatnagar *et al.* [10] revealed that neem oil has significantly higher repellent, oviposition deterrent and ovicidal effects against the pulse beetles tested. Powdered leaves and extracts of *Vitex negundo* [11], cardamom and clove powder [12], aqueous extracts of *Calotropis gigantia, Phyllanthus amarus, Ocimum tenuiflorum* and *Catharanthus s roseus* [13] leaf extract of *Clitoria ternatea* [14] were reported to have significant oviposition deterrent, repellent and other biological activity against stored grain pest. The present study was carried out to determine the oviposition inhibition and repellent effects of a local aromatic and medicinal plant, *Clerodendrum infortunatum* on the pulse beetle *Callosobruchus chinensis.*

2. Materials and methods

Present study was undertaken during the period from August 2013 to December 2014, at the laboratory of Research and Post Graduate department of Zoology, Government College Madappally of University of Calicut.

2.1. Insect Culture

Laboratory culture of *C. chinensis* was maintained on green gram *Phaseolus radiatus* at optimum conditions of temperature (28±2 °C) and relative humidity (70±5%) in plastic containers.

2.2. Preparation of plant extracts

Fresh leaves of *C. infortunatum* were collected, washed thoroughly and shade dried for about 3 weeks, until they become crisp dry. The dried leaves were pulverized into fine powder using domestic grinder and was sifted through fine mesh of a sieve (sieve size 0.25mm). The powdered plant material was used for the preparation of extract in petroleum ether (60-80 °C). Fifty grams of plant powder was mixed with 200 ml of petroleum ether taken in a conical flask and the mixture was agitated on an automatic shaker for 24 h. The extract was filtered through Whatman No.1 filter paper by negative pressure using a Büchner funnel and a suction pump. The residue was re-extracted with another 200 ml petroleum ether and filtered after 24 h. The filtrates were combined and allowed to dry in a hot air oven maintained at 35-40 °C until constant weight was obtained. From the dried extract, 10% stock solution was prepared by dissolving 1 g extract in 10 ml acetone. From the stock solution appropriate concentrations (0.5, 2, 4, and 6%) of the extracts were prepared by diluting with acetone.

2.3. Repellent activity

For conducting bioassay on repellent effect of the plant extract, 'Y' shaped glass tube as described by Read *et al*. (1970) [15], Ahmed and Eapen (1986) [16] and Appel (1994) [17] was employed. Four samples of five grams each of green gram were smeared with different concentrations (0.5, 2, 4 and 6%) of petroleum ether extract of *C. infortunatum* separately and allowed to evaporate off the solvent. The treated grains were placed in one of the paired arms of the Y tube (experimental arm) and the other arm contained the grain treated with the solvent alone (control arm). Twenty adult insects of *C. chinensis* were introduced through the median arm of the Y-tube. The open ends of the tube were plugged with cotton wool and the experimental set up was kept for observation. Number of insects present in the experimental, control and median arms were counted after 30 min, 1h, 3h, 24 h, 48h and 72 h of treatment. Experiments were replicated five times with each concentration.

Percentage of repellency was calculated by using the formula:

$$\text{Percentage of repellency} = \frac{NC - NT}{NC + NT} \times 100$$

NC - number of insects in the control area

NT- number of insects in the test area

From these values, percentage of repellency and overall averages were calculated

2.4. Oviposition deterrent activity

To determine the oviposition deterrent effects, five sets of 20 grains each of green gram were taken and grains in four sets were smeared with 0.2 ml each of different concentrations (0.5, 2, 4 and 6%) of petroleum ether extracts of *C. infortunatum*. In the fifth set, the control, grains were treated with acetone alone. All the grains were freed from any trace of the solvent. After drying, each lot was taken in individual vials and introduced a pair of (1 male and 1 female) newly emerged pulse beetle, *C. chinensis* adults separately. Covered the mouth of the glass vials with muslin cloth and tied tightly. The experiment was replicated 10 times. The experimental set up was kept undisturbed at optimum temperature (28±2 °C) and RH (75±5%). After 5 days, the insects were removed from the vials and the total number of eggs attached on all the grains of each set was counted by using a hand lens. Oviposition deterrence was calculated by using the discrimination quotient (DQ) (Messina and Renwick, 1983) [18].

$$DQ = \frac{\text{No. of eggs in the control seeds - no. of eggs on the treated seeds}}{\text{Total no. of eggs on control + treated seeds}}$$

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

Percentage of reduction in oviposition was calculated by the formula;

$$\text{Percentage of reduction in oviposition} = \frac{NC - NT}{NC + NT} \times 100$$

2.5. Statistical analysis

The data obtained were analysed statistically by one way ANOVA and differences in repellent effects due to different concentrations were compared by DMRT (Duncun's multiple range test) at 5% level.

3. Results & discussion

3.1. Repellent effect

In the present experiment, the exposure period given was 72h. Petroleum ether extract of *C. infortunatum* exhibited strong repellent effect at different concentrations tested (0.5, 2, 4 and 6%) against *C.chinensis*. Highest level of repellency occurred during 30 min of exposure of the insects. Here a dose dependent effect was observed. With 0.5% concentration, an average of 58.05% repellency and with 2% and 4% concentrations 61.60 and 73.20% repellency was exhibited. In the sample treated with 6% concentration of extract, about 92.57% insects were repelled. A gradual decrease in repellency was observed after 1 h and 3 h exposures. In the case of 6% concentration of extract, about 76.2% and 55.5% insects were repelled after 1 h and 3 h respectively (Table.1). Similar decrease was observed in the case of other concentrations also with different exposure periods (24 h, 48 h and 72 h) respectively.

From this analysis, it was revealed that difference in repellent effect due to different concentrations during different time durations were highly significant ($P<0.001$). However, perusal of Table 1 reveals that maximum repellency occurred immediately after the exposure (30 min) to the extracts. Then there was a gradual decline in repellency. Repellency increased with concentration and decreased with time. Dose dependent repellent effect of sweet flag, sowa, clove and cedar wood oil along with citronella and eucalyptus on rice moth *Corcyra cephalonica* was reported by Behal [19]. Similarly Jilani *et al.* [20] reported the repellent effect of sweet flag (*Acorus calamus*) and neem (*Azadirachta indica*) against the red flour beetle.

Table 1: Repellent effect of different concentrations of *C. infortunatum* against *C. chinensis.*

Conc.	30 min	1 h	3 h	24 h	48 h	72 h
0.5%	3.500^{b}	5.500^{c}	6.500^{b}	8.000^{b}	10.750^{c}	12.750^{c}
2.0%	3.500^{b}	4.250^{b}	5.250^{b}	7.750^{b}	9.500^{bc}	9.500^{b}
4.0%	2.500^{ab}	3.500^{b}	5.000^{b}	6.750^{b}	7.500^{ab}	8.000^{a}
6.0%	1.250^{a}	1.500^{a}	2.750^{a}	4.500^{a}	7.250^{a}	7.750^{a}
P Value	0.001 **	0.0016 **	00.0793 *	0.2452 NS		

Data presented in the table are the mean number of insects present in 10 replicates.
Within the vertical column, means having same superscript are not significantly different at 5 Level of DMRT.
*significant (P<0.05); ** highly significant (P < 0.001); NS – non significant at 5% level of One Way ANOVA

Table 2: Percentage of repellent effect shown by *C. infortunatum* against *C.chinensis* at different concentrations

Conc.	Treatment after					
	30 min	1h	3h	24h	48h	72h
0.5%	58.05	35.9	29.6	10.72	3.9	-4.2
2.0%	61.60	46.3	32.5	16.45	5.0	-10.8
4.0%	73.20	51.8	38.6	25.02	14.3	2.93
6.0%	92.57	76.2	55.5	45.5	20.7	5.9

Data presented in the table are the percentage of repellency presented as a means of 5 replicates

The result of the present study showed that all the tested concentrations of the extracts have considerable repellent activity against adult *C. chinensis*. Pungent and unpleasant smell of the extract may be due to the presence of certain highly volatile chemical components, which cause the immediate repellent effect against the tested insect.

3.2. Oviposition deterrent effect

Treatment of green gram seeds with crude petroleum ether extract of *C. infortunatum* at various concentrations (0.5, 2, 4 and 6%) significantly reduced the egg production, which in turn influenced the number of adults emerged and resulted in seed damage and loss in seed weight. Among the different concentrations tested, 6% concentration was the most effective oviposition deterrent, in which significantly less number of eggs (mean 1.5) was deposited on treated grains. A dose dependent decrease in number of eggs was observed in other concentrations also. Average number of eggs laid was 36.9, 31.2 and 20.7 respectively on seeds treated with 0.5%, 2 and 4% of extracts. (Table 3). The present results are thus in conformity with the results of Negi *et al.* [21] and Sharma *et al.* [22] obtained with various plant extracts against *Callosobruchus chinensis*.

Table 3: Oviposition deterrent effect of petroleum ether extract of *C. infortunatum* against *C. chinensis*

Conc.	Mean no. of Eggs on treated seeds	Reproduction Control (%)	DQ	Chi-square	P value
0.5%	36.9	27.6	0.165	3.90	NS
2.0%	31.2	38.4	0.245	7.69	<0.01
4.0%	20.7	59.4	0.430	18.00	<0.001
6.0%	1.5	97.1	0.947	48.04	<0.001
control	51	-	-	-	-

DQ = discrimination quotient; NS = not significant

Comparing the DQ values, the highest value (near to +1) was obtained in the case of insects exposed to 6% concentration of the extract (0.947). The DQ values for the concentrations 0.5%, 2% and 4% were 0.165, 0.245 and 0.430 respectively. DQ values revealed that there is no marked difference between different concentrations (0.5% and 2%and 4%). However, these differ considerably with 6% concentration (Table 3). The decline in oviposition at higher doses of plant extracts have been attributed to the interruption of vitellogenesis and damage to the egg chambers during various life stages of *C.chinensis* [23]. Dhar *et al.* [24] reported that oviposition was possibly regulated by the volatile compounds absorbed through cuticle. Oviposition deterrency may be due to the changes induced in physiology and behavior of the insect [13].

3.3. Adult emergence

By observing the number of adults emerged from the treated sample for 150 days, it was revealed that there is a dose dependent decrease in the emergence of adults. Here, minimum number of insects emerged from grains treated with 6% extract (mean 0.5). In other concentrations, (0.5, 2 and 4%) the mean number adults emerged were 12.15, 11.4 and 9.3 respectively (Table 4).

Table 4: Effect of petroleum ether extract of *C.infortunatum* on feeding and adult emergence of *C. chinensis*.

Concn.	Mean no. of eggs	No. of insects emerged after 150 days	Initial grain weightwt	Final grain Wt.	Weight loss	Weight loss (%)
0.5%	36.9	12.15	1.0669	0.64	0.470	44.7
2.0%	31.2	11.4	1.1337	0.77	0.335	33.6
4.0%	20.7	9.3	1.0434	0.74	0.293	29.9
6.0%	1.5	0.5	1.0946	1.06	0.037	3.4
control	51	42.7	1.1118	0.15	0.975	86.7

Data presented in the table are mean value of 10 replicates

In the present experiment, it was also observed that the number of insects emerged was proportional to the number of eggs deposited on the grains within a period of one week. Srivasthava and Mann [25] have observed this type of reduction in adult emergence of *C. chinensis* from grains treated with extract of *Peganum harma*. Remarkable decrease in the adult emergence of *C. chinensis* with increase in concentration of Pongam oil was also reported by Prakash and Rao [26] and Negi *et al.* [21]. Haridasan and Gokuldas [27] reported the effects of *Vitex negundo* leaf extracts on adult emergence of the stored product pest, *Tribolium castaneum*. Their studies revealed that there was a significant reduction in adult emergence with increased exposure period and concentration of the extract. These earlier findings are in conformation with the results of the present study. The data shown in the Table 4 reveals the effect of leaf extracts on adult emergence of pulse beetle. Considerable reduction in adult emergence was found in different concentrations. Jayakumar *et al.* [28] reported that plant extract has obvious effect on post embryonic survival of the insects and reduction in adult emergence. Annie Bright [29] and Raja *et al.* [30] reported that when the eggs were laid on treated seeds, the toxic substance present in the extract may enter into the egg through chorion and suppress their embryonic development. The results thus are in agreement with the earlier studies thus suggesting that adult emergence was greatly reduced in treated seeds than control seeds.

4. Conclusion

From the present investigation it has been concluded that the locally available aromatic plant, *C. infortunatum* can be used as a promising protectant against the attack of pulses by the pulse beetle, *C. chinensis*. It acts as an effective repellent and oviposition deterrent against the insects. This study therefore opens a new line of investigation for the management of stored grain pests using indigenous plant materials in a very safe way, without operational and residual hazards that are usually part of the use of synthetic insecticides.

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