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The repellency, toxicity and growth regulatory effects of essential plant oils on the *Cryptolestes ferrugineus*

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Abstract
The study was carried out at the Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad, during the year 2010-2011. The repellency effects of all the essential oils i.e. *Datura inoxia*, *Nigella sativa*, *Amaranthus viridis* and *Trachyspermum ammi* at the concentration of 2, 4 and 6 percent showed repellency (as high as 89.79%) with no significant difference. Highest mean mortality (25%) was observed on *D. inoxia*. Zero larval survival percentage was observed on *N. sativa* and *T. ammi*. *N. sativa*, *A. viridis* and *T. ammi* were found effective in plummeting pupal emergence. All the essential oils except *D. inoxia* were proved to be highly effective against population build up.

Keywords: *Cryptolestes ferrugineus*, plant essential oils, toxicity, repellency, *Datura inoxia*, *Nigella sativa*, *Amaranthus viridus* and *Trachyspermum ammi*

1. Introduction

Cryptolestes ferrugineus is a significant pest of numerous stored products especially cereals [1]. The damage is caused by both the larval and adult [2]. *C. ferrugineus* does not belong to the group of economically most harmful pests, such as pests of *Sitophilus* genus. However, their rising population and occurrence of all developmental phases have direct affect on qualitative property of the stored products [3]. *Cryptolestes* spp. are not able to damage whole kernels but may be coupled with primary invaders such as Sitophilus spp. and *Rhyzopertha dominica. C. ferrugineus* (The rust red flat grain beetle) frequently happen in mixed infestations with *Tribolium castaneum* [4].

The plant based pesticide, usually called as phytopesticide, botanical pesticide, biopesticide or natural pesticides, can serve as the alternatives to insecticides [5]. In latest years, studies has paid attention on the utilization of essential oils, plant powders, plant extracts and non-volatile oils as probable substitute to fumigants or synthetic residual pesticides. Utilization of plant oils for protection of agricultural products is very significant since they have low mammalian toxicity, high biodegradability and eco friendliness when used in traces [6].

Researchers have paying attention on the likelihood of applying essential oils of plants for use in stored commodities to manage insect pests [7, 8]. About 2000 plant species are acknowledged to have several insecticidal actions [9]. These are capable of affecting insects in numerous ways: they may disrupt major metabolic paths and cause rapid death, act as attractants, deterrents, and feeding stimulants antifeedants or alter oviposition, may accelerate or deaccelerate growth or hinder the life cycle of the insects. Numerous products from a variety of plants have been established to act as repellents, toxicants and antifeedants against a number of coleopterans attacking stored commodities [10].

Extracts of Botanicals of *Zanthoxylum rhetsa* (Bazna), *Melia sempervirens* (neem), *Barringtonia acutangula* (Hijal), *Pangamia glabra* (Karunja), *Swietenia mahagoni* (Mahogani), *Tagetes patula* L. (Marigold), *Azadirachta indica* (Neem), *Vfex engundo* (Nishenda) and many others can be used as a substitute to chemical pesticides [11]. Lopez *et al.* [12] reported that the essential oils from coriander, caraway or basil that are active against the *Cryptolestes pusillus*.Obeng-Ofori [13] observed 100% of mortality in *Cryptolestes pusillus* and *Rhyzopertha dominica* resulting in absolute protection by plant oils of cottonseed, soybean, maize, groundnut and palm. They Xie *et al.* [14] signified the feasibility of extracts as a natural grain protectant and 50-98% control can be achieved by varying the concentrations of bark extracts of Melia plant.

The essential oils of *Datura inoxia*, *Nigella sativa*, *Amaranthus viridus* and *Trachyspermum ammi* were evaluated for the repellency, toxicity and growth regulatory effects on *Chryptolestes ferrugineus* to determine effective dose rate, by keeping the concentrations at minimum level, check for their direct repellency, toxicity and also effects on larval and pupal emergences and too compare the four of the plants for their effectiveness.

2. Materials and methods

The research was concluded at the Grain Research, Training and Storage Management Cell located at Agri. Entomology Department, University of Agriculture Faisalabad, during the year 2011.

2.1 Collection and Rearing/ Culturing Insects

C. ferrugineus were obtained from Grain Research, Training and Storage Management Cell cultures already maintained in the incubators plastic jars covered with muslin cloth, at temperature 28±2 ºC and 65±5% relative humidity. Flour along with crushed wheat added with yeast (1% w/w) was given to the *C. ferrugineus* as a diet. The flat grain beetle was allowed to multiply to get the F_1.

2.2 Experimental Conditions

All experimental procedures were carried out in incubators with ambient temperature 28±2 ºC, 65±5% relative humidity and L10:D14 regime.

2.3 Preparation of Essential Oils:

The fruit of *D. inoxia* and leaves of *A. viridis* were obtained from field. They were then tap washed thoroughly to be cleaned and then dried in the shade. *N. sativa* and *T ammi* seeds were purchased from local market of Faisalabad. In order to make seed free of contamination it was washed and then soaked in the water for 24 hour interval and then dried in the shade. The dry material was then grounded in the electrical grinder. A powder is formed.

Table 1: Detail of Plants Used to check their Effects on *Cryptolestes ferrugineus*

Scientific name	Family	Parts used
Datura inoxia	Solanaceae	Fruits
Nigella sativa	Ranunculaceae	Seeds
Amaranthus viridus	Amaranthaceae	Leaves
Trachyspermum ammi	Umbelliferae	Seeds

2.4 Essential Oil Extraction

Hot Continuous Extraction method (Soxhlet) was used [15]. In this method, the finely 50 g of powder was placed in a porous bag of filter paper. The bag was placed in the main chamber of the Soxhlet apparatus. The 250 ml of acetone (used as an extracting solvent) was added in flask of soxhlet apparatus. By the use of power supply, it was heated and its vapors condensed in condenser. The condensed extractant dripped into the plant powder, and extracted it by contact. It took about 4-5 hours every time, while extracting the plants for oils. Essential oils thus obtained were put in clean and air tight lid bottles and stored in the refrigerator.

2.5 Preparation of Concentrations

Different concentration ranging from 2%, 4% and 6% were prepared in acetone by adding essential oils, in acetone in v/v. For the preparation of 2% concentration 2ml of stock solution was taken in the 100 ml flask and remaining flask was filled with acetone to make the volume and flask was shaken. This was 2% concentration. In the same way 4% and 6% concentrations were prepared [16].

2.6 Repellency Tests on Filter Paper

The repellent effects of the different essential oils against *C. ferrugineus* were evaluated using the area preference method [17]. 1 ml of the test solution was applied on filter paper Whatman's no. 48 (9 cm diameter) cut in half. The other half filter paper was kept untreated. Both the treated and untreated halves of filter paper was joined with staple pins and placed in petri dishes. All experiments and control was dried for 10 minutes. Twenty adults were released in the centre of each petri dish. Petri dishes were subsequently covered. The treatments were replicated three times and the number of insects on the two half paper disks will be recorded after 24-hours interval.

2.7 Bioassay

The essential oils were checked against insects for mortality. The experiment was laid out in Completely Randomized Design, consisting of three concentrations (2%, 4% and 6%) of four insecticidal plant oils and a control for each. The study was done in small plastic vials by diet incorporation method. Sterilized wheat flour was treated with respective essential oils and allowed to dry for 24 hours. The control was treated with acetone and allowed to dry. On the next day 30 adult insects were released in each vial. Each concentration of the treatment was replicated three times. The data of mortality were recorded after 24, 48 and 72 hours interval.

2.8 Growth and Fecundity Plummeting Effects

The growth regulatory effects were studied by recording the data regularly from the survivors of the treatment up to 60 days. The number of larvae and pupae was recorded weekly. The dead adult insects were counted and removed. The alive adults were also recorded. After 60 days, the adult insect population build up was calculated

2.9 Statistical Analysis

Data were corrected for mortality in the control by using Abbott's formula [18]. $\{Pt= [(Po – Pc) / 100- Pc] \times 100\}$,
Pt = corrected mortality, P0 = observed mortality and Pc = control mortality.
Similarly the corrected repellency was also calculated. Statistical analysis of the recorded data was done by computing analysis of variance using STATISTICA 10 software. Factorial ANOVA was used for calculations. Significant results of ANOVA were checked further by multiple comparison of means using Tukey's w-honestly significant difference (Tuckey-HSD).

3. Results and Discussion

The repellency effects of all the essential oils at the concentration of 2, 4 and 6 percent showed no significant difference (table 2). Results were in accordance with the findings of Hill and Schoonhoven [19] and that of Desmarchelier [20] who reported that essential oils are known to possessed repellent activities against stored-product insects

However, the maximum repellency was observed to be 89.79% in N. sativa at the 6% concentration, while minimum 64.28% in the same treatment at 2% concentration. The repellent activity was very high on *C. ferrugineus* as compared to control (Table 2). However, all tested plants were found to be equally effective and the repellency was concentration dependent. The highest concentration showed more repellent activity as reported by Ko *et al*. [21] and Cosimi *et al*. [22].

The toxic effects of plants essential oils on *C. ferrugineus* showed significant difference at various concentrations on interactional as well as on average basis (Table 3). Larval survival percentage was highest on 2% concentration of *D. inoxia* (1.26), zero larval survival percentage was observed on *N. sativa* and *T. ammi*. The pupal survival was also highest on the *D. inoxia* treated insects, *N. sativa*, *A. viridis* and *T. ammi* were found effective in plummeting pupal emergence. Overall, for population build up all the essential oils except *D. inoxia* were proved to be highly effective. (Table 4) the results were in Accordance to results of Deshmukh and Borle [23] they reports Datura seed to be toxic when used as extract on *Dactynotus carthami*, whereas *N. sativa* appeared to be less toxic. The exposure time increased the mortality in the *C. ferrugineus* (Table 3).

Results were in accordance with the findings of Hill and Schoonhoven [19] and that of Desmarchelier [20] who reported that essential oils are known to possessed ovicidal and insecticidal activities against various stored-product insects. Rahman *et al*. [24] found the neem insecticide Nimbicidin to be toxic to larval and adult stages of *C. pusillus*. According to Bari *et al.* [25] the methanolic extracts of *Smilax zeylanica* are more toxic to the adults of *C. pussilus* and which could be potent controlling agent against the beetle. Minimum larvae appeared on all the other plants showing no significant difference among each other except for *D. inoxia*. The larva decreased with the increase in concentration (Table 4). No work has been seen so far upon the effect of plant oils on the larval emergence of *C. ferrugineus.*

The pupal formation decreased with time. The maximum number of papae was observed on the *D. inoxia*, while the other three plant oils remained with having no significant differences. The reduction in pupal formation was observed when compared to the control treatment (Table 4). Maximum population build up occurred on *D. inoxia* as compared to the *N. sativa*, *T. ammi* and *A. viridis*. Almost no population build up was found on higher concentrations. At the 6% of the concentrations the population became more or less zero (Table 4). According to Tapondjou *et al.* [10] all dosages of *Chenopodium ambrosioides* leaf powder caused significant reduction in F_1 progeny produced by all the test insects. No progeny were produced by the bruchids. Plants treated on cryptolestes as Xie *et al*. [14] reported that *Melia toosedan* bark extracts significantly reduced the F_1 adults of *C. ferrugineus* and *T. castaneum.*

Complete suppression of progeny was observed by the essential oils, results were in accordance with Tripathi *et al*. [26] The results of Nadra [27], Echezona[28], Owoade[29] showed that irrespective of the Insect species, plant species and concentrations showed that there is similarity in effect, i.e., mortality and suppression of F_1 adult emergence.

Conclusion

The plant extracts can be effectively utilized as repellents against the stored grain pest. These extracts show effective growth regulatory effects. The plant extracts can be recommended as effective and the safest control against the pests.

Table 2: Repellency Effects of Essential Oils of Plants on *Cryptolestes ferrugineus*

Plant	Concentration			Mean percentage repellency
	2%	4%	6%	
D. inoxia	67.68 ± 9.46 a	72.79 ±1.70 a	74.48 ± 5.10 a	71.65 ± 3.30 a
e N. sativa	64.28 ± 2.94 a	79.59 ± 2.94 a	89.79 ± 0.00 a	77.89 ± 3.89 a
A. viridis	67.68 ± 4.49 a	77.89 ± 6.80 a	79.59 ± 5.89 a	75.06 ± 3.45 a
T. ammi	72.78 ± 3.40 a	72.78 ±10.34 a	72.78 ± 7.41 a	72.79 ± 3.80 a

Table 3: Toxic Effects of Plant Essential Oils on the *Cryptolestes ferrugineus*

Concentration	Plant			
	D. inoxia	*N. sativa*	*A. viridis*	*T. ammi*
2%	25.96±2.54 def	2.80 ± 0.19 b	11.13±4.39 abc	19.67±3.01 cd
4%	32.03 ± 2.34 ef	6.93 ± 3.24 bc	13.91 ± 5.75 abc	32.99 ± 2.08 f
6%	45.96 ± 5.10 g	18.70 ± 5.16 cd	20.66± 8.56 de	66.51 ± 5.71 h
Percentage mean mortality	25.027 ± 1.81 d	4.97 ± 0.68 a	22.93 ± 0.81 c	8.46 ± 1.63 b

Means followed by same letter do not differ significantly by Tukey-HSD test at P value ≤ 0.05.

Table 4: Effects of plant essential oils on the larval and pupal survival and population buildup of *Cryptolestes ferrugineus*

Plant	Conc.	Larval survival	Pupal survival	Pop. Build up
D. inoxia	2%	1.26 ± 0.46 b	1.40 ± 0.51 bc	0.10 ± 0.03 b
	4%	0.60 ± 0.25 ab	1.47 ± 0.35 c	0.06 ± 0.04 ab
	6%	0.26 ± 0.15 ab	0.40 ± 0.19 abc	0.00 a
N. sativa	2%	0.84 ± 0.44 b	0.60 ± 0.23 abc	0.07 ± 0.02 ab
	4%	0.07 ± 0.07 a	0.067 ± 0.067 a	0.03 ± 0.02 a
	6%	0.00 a	0.00 a	0.00 a
A. viridis	2%	0.20 ± 0.11 a	0.60 ± 0.27 abc	0.00 a
	4%	0.14 ± 0.09 a	0.20 ± 0.20 ab	0.00 a
	6%	0.00 a	0.00 a	0.00 a
T. ammi	2%	0.20 ± 0.107 a	0.27 ± 0.12 abc	0.00 a
	4%	0.067 ± 0.067 a	0.00 a	0.00 a
	6%	0.00 a	0.34 ± 0.18 abc	0.00 a
Control		4.26 ± 0.46 d	2.46 ± 0.63 c	1.23 ± 0.02 bc

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