

Original Research

Assessing the Toxic Potential of Insecticide and Indigenous Botanical Extract against the Stored Grain Pest *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Received: 15 June 2017

Accepted: 29 October 2017

Abstract

The current investigation was undertaken to assess the toxic potential of synthetic insecticides (K.Othrin and bio-max) and botanical extracts (*Mentha royleana* L. and *Artemisia absinthium* L.) against *Tribolium castaneum* in the laboratory. Different concentrations of insecticides and botanical extracts were used following complete randomized block design. The results indicated that the toxic effects were directly proportional to concentrations of insecticide and botanical extracts. Higher concentrations had more resilient toxicity than lower concentrations. Among the tested insecticides, Biomax (Chlorpyrifos) showed >90% and K-Othrin (Deltamethrin) <80% mean toxic effect at 2% concentrations. In the botanical extract, *Mentha royleana* toxicity at 5% concentration is > 90% as compared to *Artemisia absinthium* against *T. Castaneum*. The results could be helpful in designing an effective management plan for the control of *T. castaneum*.

Keywords: insecticides, botanical extract, toxicity, wheat flour pest, *Tribolium castaneum*

Introduction

The red flour beetle *T. castaneum* (Herbst) (Coleoptera: Terebrionidae) is a cosmopolitan species

and causes considerable loss to stored food grains [1]. Managing storage food grain pests is not a current issue. Various recommendations of grain storage techniques using different natural products to kill or keep away the infesting insect pests have been advised by previous researchers [2-3]. *T. castaneum* is a common and most destructive pest throughout the world and is generally

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found in granaries, mills, and warehouses [4]. It causes serious damages to all kinds of stored grain products [5]. Many plant preparations are studied for insecticidal actions [6-8], and these preparations showed positive actions against pests. The plant-derivative chemical activates after plant extract screening and has been used as an active protectant and antifeedant, and insecticides and natural antifeedants are used to control pests, to avoid environmental pollution, and to reduce the use of synthetic pesticides [9-10]. The use of plant pesticides is low cost, suitable to use, and environmentally friendly.

About 35% of world crops are damaged by different insects [11]. In Pakistan pest insects have been documented nourishing on grains and its foodstuffs. Most of the destruction is done by the granary weevil, lesser grain borer, rice weevil, khapra beetle, rice moth, and flour beetle, and not only damages grains but also reduces nutritional value [12]. So, worldwide environmentalists are declaring less use of tenacious insecticides.

The harmful effect of plant extracts and pure compounds on insects can be demonstrated in numerous ways, including antifeedant, growth inhibitor, toxicity, suppression of reproductive behavior, mortality, and reduction of fertility and fecundity [13-15].

Red flour beetle (Herbst) is a global insect pests of mills, food warehouses, retail stores, and urban homes [7, 16]. Therefore, this study is based on the toxicological effects of indigenous floras of Gilgit against *T. castaneum*, as there is no proper storage in Gilgit to protect food materials from insect infestation. This study will be helpful for managing the storage of flour from red flour beetle (*T. castaneum*) infestation in storage bins and control the flour beetle by using local plant extracts with comparison to chemical pesticides.

Materials and Methods

Insect Collection and Rearing

Insect *T. castaneum* was collected from naturally infected flour bins and reared in glass jars in the Research Laboratory Department of Biological Science, Karakoram International University under controlled conditions. During the nurturing period the temperature ($25\pm 1^\circ\text{C}$), relative humidity ($60\pm 5\%$), and food were provided to the test organisms. Ten-day-old adults were used for all trials.

Test Materials

The insecticides K. Othrin and Bio-max and botanical extracts *Mentha royleana* L. and *Artemisia absinthium* L. at concentration were tested against *T. castaneum*.

Plant Material and Insecticide Assay

Fresh leaves of the plants *Mentha royleana* L. and *Artemisia absinthium* L. were collected during 2014-2015 from different areas of District Gilgit. The collected plants were washed with water, dried in shade, and ground to a fine powder. The plant extracts were prepared following the method described by Valsaraj et al. [17]. One-hundred grams of powder was mixed separately in 300 mL of ethanol 99% (w/v) ratio at room temperature. The mixtures were stirred for 45 minutes in an ultrasonic bath at constant temperature of 25°C , left to stand for 72 hours, and shaken several times at certain intervals. Finally, different concentrations including 5, 2.5, 1.25, 0.63, and 0.31% of each extract were made. In order to test the insecticide toxicity against *T. castaneum* we studied two insecticides, K. Othrin and bio-max, at five concentrations: 2, 1, 0.5, 0.25. and 0.13%.

Toxicity of Plant Extract and Synthetic Insecticide

Plant extracts and synthetic insecticide were tested by filter paper impregnation method. The filter was dipped in different concentrations of plant extract and synthetic insecticide as described earlier. The wetted filter papers were placed in sterilized Petridis and inoculated 10 adult insect of *T. castaneum* against each concentration and maintained 3 replicates along with control. All the data obtained were subjected to one-way analysis of variance at 5% significance level, and means were separated using Duncan's multiple range tests. Mortality was calculated by the following formula:

$$MP = \frac{IC - IT}{IC} \times 100$$

MP = Mortality percentage; IC = Insect in control; IT = Insect in treatment

Results and Discussion

T. castaneum was exposed for 5 days to 2 indigenous plant extracts and two synthetic insecticides at different concentrations. The results depict dose rate having significant effects on the mortality of the tested insects.

Insecticide Biomax (Chlorpyrifos)

Different concentration of Biomax were used to assess toxicity and mortality against the flour beetle (*T. castaneum*). The selected concentrations for Biomax were 2%, 1%, 0.5%, 0.25%, and 0.125%. Mortality rate increased with higher concentrations of the tested chemical. The observed mortalities at concentrations of 2% were 100%, 100%, 90%, 80%, and 90% on days 1-5, respectively. For 1% concentrations, the observed mortalities were 100%, 90%, 80%, 60%, and 80%.

Table 1. Toxicity test of K. Othrin against *T. Castaneum* at different concentrations/hour.

Conc. %	1 st day	2 nd day	3 rd day	4 th day	5 th day
2%	7.0 ^a (70%)	8.0 ^a (80%)	7.0 ^a (70%)	7.0 ^a (70%)	8.0 ^a (80%)
1%	6.0 ^a (60%)	6.0 ^{ab} (60%)	7.0 ^a (70%)	7.0 ^a (70%)	6.0 ^{ab} (60%)
0.50%	2.0 ^b (20%)	4.0 ^{bc} (40%)	6.0 ^a (60%)	4.0 ^{ab} (40%)	4.0 ^b (40%)
0.25%	2.0 ^b (20%)	4.0 ^{bc} (40%)	4.0 ^{ab} (40%)	2.0 ^b (20%)	3.0 ^b (30%)
0.12%	1.0 ^b (10%)	2.0 ^c (20%)	1.0 ^b (10%)	1.0 ^b (10%)	3.0 ^b (30%)
STD. Err.	1.461	1.633	1.461	1.461	1.633
LSD	3.254	3.639	3.254	3.254	3.639
LC ₅₀	1.38	1.58	1.36	1.19	1.54

Means in each row followed by the same letter are not significantly different at LSD test ($P = 0.05$), while numbers in parenthesis indicate inhibition percentage over control

For 0.5% concentrations, the observed mortalities were 50%, 40%, 40%, 50%, and 40%. For 0.25% concentrations, the observed mortalities were 20%, 30%, 30%, 40%, and 30%. For 0.125% concentrations, the observed mortalities were 20%, 10%, 10%, 20%, and 10% on days 1-5, respectively (Table 1).

Insecticide K-othrin (Deltamethrin)

The mean in each row followed by the same letter are not significantly different at LSD test ($P = 0.05$), while numbers in parenthesis indicate inhibition percentage over control. Different concentrations of K-Othrin were used to evaluate toxicity and mortality against the flour beetle (*T. castaneum*). The data presented in Table 2 revealed that the higher the concentration of pesticides, the maximum mortality of test insects were observed.

The particular concentrations of K-othrin were 2%, 1%, 0.5%, 0.25%, and 0.125% for assessment. Maximum mortalities were 80% (days 2 and 5) and 70% (days 1, 3-4) showed at the concentration of 2%. Meanwhile, the decrease of the concentration to 0.125% mortality was only 10% on days 1, 3, and 4, and on day 5 the observed mortality was 30% (Table 2).

Plant Extract *Mentha royleana*

Five different concentrations of *Mentha royleana* were selected for the estimating toxicity and mortality against *T. castaneum*. The results revealed that concentration and mortality were directly proportional. At 5% concentration of extract of *Mentha royleana* the mortalities were 100%, 100%, 100%, 100%, and 70%. At 2.50% concentration of extract the mortalities

Table 2. Toxicity test of Bio-max against *T. Castaneum* at different concentrations/hour.

Conc. %	1 st day	2 nd day	3 rd day	4 th day	5 th day
2%	10.0 ^a (100%)	10.0 ^a (100%)	9.0 ^{ab} (90%)	8.0 ^b (80%)	9.0 ^{ab} (90%)
1%	10.0 ^a (90%)	9.0 ^a (90%)	8.0 ^b (80%)	6.0 ^c (60%)	8.0 ^b (80%)
0.50%	5.0 ^b (50%)	5.0 ^b (50%)	4.0 ^c (40%)	5.0 ^{cd} (50%)	4.0 ^c (40%)
0.25%	2.0 ^c (20%)	3.0 ^c (30%)	3.0 ^c (30%)	4.0 ^d (40%)	3.0 ^c (30%)
0.12%	2.0 ^c (20%)	2.0 ^c (20%)	1.0 ^d (10%)	2.0 ^c (20%)	1.0 ^d (10%)
STD. Err.	1.08	0.88	0.68	0.68	0.68
LSD	2.41	1.96	1.52	1.52	1.52
LC ₅₀	0.92	0.98	1.03	1.58	1.03

Means in each row followed by the same letter are not significantly different at LSD test ($P = 0.05$), while numbers in parenthesis indicate inhibition percentage over control

Table 3. Toxicity test of *Mentha royleana* against *T. Castaneum* at different concentrations/hour.

Conc. %	1 st day	2 nd day	3 rd day	4 th day	5 th day
5%	7.0 ^a	10.0 ^a	10.0 ^a	10.0 ^a	10.0 ^a
	(70%)	(100%)	(100%)	(100%)	(100%)
2.50%	6.0 ^a	9.0 ^a	8.0 ^{ab}	8.0 ^{ab}	9.0 ^a
	(60%)	(90%)	(80%)	(80%)	(90%)
1.25%	6.0 ^a	8.0 ^a	8.0 ^{ab}	8.0 ^{ab}	7.0 ^{ab}
	(60%)	80%	(80%)	(80%)	(70%)
0.63%	2.0 ^b	4.0 ^b	5.0 ^{bc}	6.0 ^{bc}	5.0 ^{bc}
	(20%)	(40%)	(50%)	(60%)	(50%)
0.31%	1.0 ^b	2.0 ^b	2.0 ^c	3.0 ^c	3.0 ^c
	(10%)	(20%)	(20%)	(30%)	(30%)
STD. Err.	1.461	1.633	1.633	1.633	1.633
LSD	3.254	3.639	3.639	3.639	3.639
LC ₅₀	3.18	2.28	2.51	2.99	2.94

Means in each row followed by the same letter are not significantly different at LSD test ($P = 0.05$), while numbers in parenthesis indicate inhibition percentage over control

were 90%, 80%, 80%, 90%, and 60%. And for 1.25% concentration the mortalities were 70%, 80%, 80%, 80%, and 60%. For 0.63% concentration of extract of *Mentha royleana* the mortalities were 50%, 60%, 50%, 40%, and 20%. At concentrations of 0.31% extract of *Mentha royleana*, the mortalities were 30%, 30%, 20%, 20%, and 10% on days 5, 4, 3, 2, and 1, respectively, for all five concentrations (Table 3).

Plant Extract *Artemisia absinthium*

Different concentrations of *Artemisia absinthium* were used to assess toxicity and mortality against the flour beetle (*T. castaneum*). The selected concentrations for the treatment were 5%, 2.5%, 1.25%, 0.63%, and 0.3125%. Highest mortality was shown by the extract with higher concentration, and while the concentration decreased mortality also decreased. At 5% concentration the highest mortalities detected were 90% (day 3), 80% (day 4), 70% (days 1-2) and 60% (day 5). For 2.5% concentration of extract the mortalities were 70% (days 3-4), 60% (days 2 and 5), and 50% (day 1). For 1.50% concentration the mortalities detected were 50% (days 2-3), 40% (day 5), and 30% and 20% on days 4 and 1, respectively. Likewise, at 0.625% of the extract concentration the mortality was reduced and recorded data were 40% (day 2), 30% (days 3-5), and 20% (day 1). Similar results were observed for the 0.3125% concentration, which means the lowest mortality was observed at the lowest concentration (Table 4).

As compared to synthetic pesticides, natural products are preferred for use against pest infestation due to less mammalian toxicity. The purpose of our studies was to use indigenous plants having potential to cause mortality

of the storage grain pests. For preparation of extracts of *Mentha royleana* and *Artemisia absinthium*, methanol was used as a solvent while water as a solvent was used for preparing different concentrations of pesticides (Biomax and K-Othrin). Five different concentrations of *M. royleana* and *A. absinthium* extract were studied for toxicity against *T. Castaneum*. Both the pesticides and plants extracts showed a toxic effect. At higher concentration, the mortality rate of *T. Castaneum* was increased, which means that the mortality and concentrations were directly proportional and at the lowest concentration the mortality rate also decreased. The results of the current study are in consonance with the results of other scientists. They used different plant extracts with different concentrations, but the relationships between concentration and mortality were the same. In the present study, pyrethrin, a contact poison from the pyrethroid group, was used to evaluate the toxic effect against *T. Castaneum*. The effect was maximum at the higher concentration against *T. Castaneum*, but was reduced by lowering its concentration (Tables 1-2). Similar work has been reported by Toews et al. [18], and mortality was more than 80% on all progressive stages of *T. castaneum* exposed to pyrethrin aerosol. Arthur [19] also reported the efficacy of pyrethrin aerosol to control *Tribolium confusum* in food storage facilities. Islam and Talukder [20] evaluated the residual and direct toxicities of commercial insecticides carbaryl and Malathion along with leaf powder and seed extracts of *Cynodon dactylon* (durba), *Azadirachta indica* (neem), and *Tagetes erecta* (marigold), which were tested against *T. castaneum* (red flour beetle). Their results concluded that as compared with carbaryl and Malathion (commercial insecticide),

Table 4. Toxicity test of *Artemisia absinthium* against *T. Castaneum* at different concentrations/hour.

Conc. %	1 st day	2 nd day	3 rd day	4 th day	5 th day
5%	7.0a	7.0a	9.0a	8.0a	6.0a
	70%	70%	90%	80%	60%
2.50%	5.0ab	6.0a	7.0ab	7.0a	6.0a
	50%	60%	70%	70%	60%
1.25%	2.0bc	5.0a	5.0bc	3.0b	4.0a
	20%	50%	50%	30%	40%
0.63%	2.0bc	4.0a	3.0c	3.0b	3.0a
	20%	40%	30%	30%	30%
0.31%	1.0c	4.0a	3.0c	2.0b	3.0a
	10%	40%	30%	20%	30%
STD. Err.	1.461	1.633	1.633	1.633	1.633
LSD	3.254	3.639	3.639	3.639	3.639
LC ₅₀	3.85	4.91	3.51	3.27	3.59

Means in each row followed by the same letter are not significantly different at LSD test ($P = 0.05$), while numbers in parenthesis indicate inhibition percentage over control

marigold leaf powder and neem seed extracts possess ability and are used as an alternative natural insecticide toward *T. castaneum* in stored products. Five different concentrations of *Mentha royleana* were selected for the estimation of toxicity level and mortality against *T. castaneum*.

The results revealed that concentration and mortality were directly proportional. At the highest concentration of 5% extract of *Mentha royleana*, mortality of 100% was observed. At 2.50%, 1.25%, 0.63%, and 0.31% concentration the mortalities observed were 90%, 70%, 50%, and 30%, respectively. The same type of plant was used by other scientists, but the species was different. They used it as repellent as well as toxicant against *T. castaneum*. The results were almost the same but the little difference might be due to climatic conditional changes, topography of area, and species difference. Manzoori et al. [21] found that the wild plant species contained the properties of pest control. The 5 ethanolic extracts of plant leaves – *Cymbopogon citratus* (Lemongrass), *Melia azedarach* (Bakain), *Myrtus communis* (Habulas), *Mentha longifolia* (mint), and *Datura stramonium* (Datura) – were used against pests viz., red flour beetle (*T. castaneum*. Herbst), gram dhora (*Callosobruchus chinensis*. L), and saw-toothed grain beetle (*Oryzaephilus surinamensis*. L). The results showed that the extracts had lethal as well as repellent actions against the test insects. Nadeem et al. [22] conducted studies on *Azadirachta indica* A. Juss (Neem) seed extracts and *Citrullus colocynthis* L. (Tumha) fruit ethanol extract to assess the effects of doses of 10%, 7.5%, 5%, and 2.5% against *T. Castaneum*. The result showed that the seed extract of neem against *T. Castaneum* were dose-dependent,

and at concentrations of 2.5% the mortality record was 35.93%, at concentration of 5%, 7.5%, and at 10%

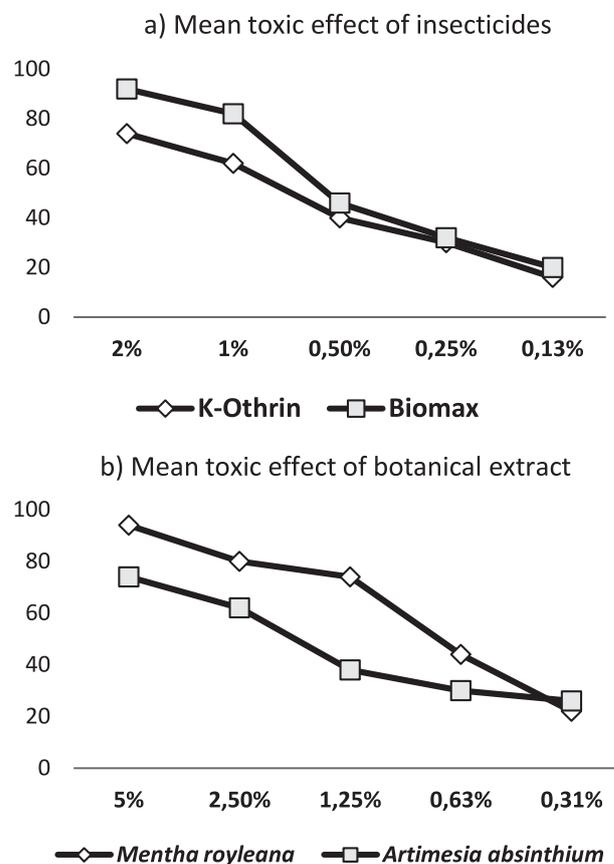


Fig. 1. Mean toxic effect of insecticides and botanical extracts against *T. Castaneum*.

the mortality recorded was 47.77%, 55.92%, and 64.44%, respectively.

In contrast, during the current studies the botanical extract of *Artemisia absinthium* in various concentrations was used as insecticide against *T. castaneum*, which showed a very low to medium toxicity against the said stored grain pest. Sagheer et al. [23] examined the toxicological and repellent effects four plant acetone extracts at different concentrations viz 10% Khar booti (*Salsola baryosma*), 7.5% kust-e-shireen (*saussurea costus*), 5% hermal (*Pegnum harmala*) and 2.5% tobacco (*Nicotiana tabacum*) against *T. castaneum* Herbst. The above research showed the effects of different wild, medicinal, and cultivated plants against the tested storage grain pest. This means there are some compounds present in the plants that are useful to human beings but effective against other organisms. The results of the present study are related to the current work due to the relationship with the concentration, but in the present study wild plant species were used as repellents – not as medicinal plants.

Likewise, in current research different concentrations of *Artemisia absinthium* were used to assess the toxicity and mortality against the flour beetle (*T. castaneum*). The selected concentrations for the treatment were 5%, 2.5%, 1.25%, 0.625%, and 0.3125%. The highest mortality was shown by the extract with higher concentrations, and while concentration decreased mortality decreased, and vice versa. At 5% concentration the highest mortality detected was 90%. This plant has many medicinal values but was used against the storage pest as a botanical insecticide for the first time in the study area. As Table 4 shows, these plants contain the toxin for flour beetle. The results might be due to the strong smell or its bitter taste, or may be due to other compounds present in it. This plant has many medicinal values in this area.

Dastagir and Hussain [24] reported on the insecticidal activity of plant extracts. Popovic et al. [25] studied the mortality rate and insecticidal activity of 9 important plant oils with known concentrations against *T. castaneum* (Herbst). Hameed et al. [26] studied 2 plant extracts, namely *Azadirachta indica* (Neem) and *Nerium oleander* (Kanair), fermented products *Actinomyces bacterium* and *Saccharopolyspora spinosa*, and commercial Spinosad biological insecticide were used to evaluate the insecticidal and persistence against *T. castaneum* Herbst. Kalita and Bhola [27] studied Dichloromethane extract of *Jatropha curcus* L, *Datura metel*, *Lantana camara*, and *Calotropis procera* A. for mortality and repellent effects on *T. castaneum* Herbst. The results were relatively the same in that all the botanical plants somehow contain toxin for storage pests but have very less mammalian toxicity. Plants in the present study used against other storage pest might be effective. These plants may have repellent effects like other bio pesticides. More than 80% mortality shown by tested plants revealed that they contain toxic compounds as well as repellent components.

Acknowledgements

This study is a part of the M.Phil. degree program and was possible due to the technical support and guidance of officials from the Agriculture Department at Gilgit-Baltistan.

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