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Determination of the Toxicities of Fenpropathrin (Pyrethroid) and Neem Formulation (RB–a+PBO+Tx–100) Against *Alphitobius diaperinus* Adults and Their Effects on Transaminases

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Abstract: This report describes the determination of the toxicity of two pesticides, neem formulation (RB–a+PBO+Tx–100) and fenpropathrin, on *Alphitobius diaperinus* adults. The mortalities caused by the two compounds were found to be 70% for a 117.8µg/cm^2 dose of neem formulation, and 88% for a 1.96 µg/cm^2 dose of synthetic pyrethroid, fenpropathrin (Danitol). The LD_{50} value of Danitol was found to be 0.2749 µg/cm^2, and that of neem formulation was found to be 58.92 µg/cm^2. Danitol–treated *Alphitobius diaperinus* adults showed inhibitions of 44.66% and 45.91% in GPT and GOT activity, respectively, Whereas insects treated with neem formulation showed inhibitions of 52.48% and 12.15% in GPT and GOT activity, respectively.

Key Words: Toxicity, Pyrethroid, *Azadirachta indica*, Transaminases, *Alphitobius.*

Fenpropathrin (Pyrethroid) ile Neem Formülasyonunun (RB–a+PBO+Tx–100) Erişkin *Alphitobius diaperinus*'a Karşı Toksisiteleri ve Transaminaslar Üzerindeki Etkileri

Özet: Fenopathrin (Danitol) ve neem formülasyonu (RB–a+PB+Tx–100) olmak üzere iki böcek ilacının erişkin *Alphitobius diaperinus* üzerindeki etkileri araştırıldı. Danitol'ün 1.96 µg/cm^2 dozunda ölüm oranının %88 olduğu, neem formülasyonunun 117.8 µg/cm^2 dozunda ölüm oranının %70 olduğu saptandı. Danitol'ün LD_{50} değeri 0.2749 µg/cm^2, neem formülasyonunun LD_{50} değeri ise 58.92 µg/cm^2 olarak bulundu. Danitol'e maruz bırakılan erişkin böceklerde GPT etkinliğinde %44.66, GOT etkinliğinde %45.91, neem formülasyonuna maruz bırakılan böceklerde ise GPT etkinliğinde %52.48, GPT etkinliğinde %12.15 oranında inhibisyon meydana geldi.

Anahtar Sözcükler: Toksisite, Pyrethroid, *Azadirachta indica*, Transaminaslar, *Alphitobius.*

Introduction

Much work has been done on stored grain pests with respect to toxicology, but very little work has been reported on *Alphitobius diaperinus*. Among the most common insecticides used in the past for the control of insect species were DDT, dieldrin and malathion. These compounds were found to be persistent and effective, but later various inscet species developed resistance, especially *Musca dometica* and *Culex fatigans* (1, 2). Neem components are being tested now in different countries to replace conventional pesticides, which have problems of pollution, residues and resistance. On the other hand, being a natural product, neem extracts would be less pollutant to the environment. Of various neem extracts, RB–a alone has been reported to be ineffective when used in crude from, especially against stored grain pests (Khan, unpublished) but to act as a superior toxicant with a synergist (piperonylbutaoxide) (3). In addition to the toxic effect, neem extracts also disturb the enzyme pattern in the insect body. The enzyme pattern altered by neem compounds has also been reported by other researchers 4, 5, 6, 7.

Danitol has been extensively used against coleopterous insects, namely, *sitophilus oryzae, Rhyzopertha dominica* and *Tribolium castaneum* 3, 7, 8, 9, 10. The purpose of the present study on neem extract was to provide initial

data to test the provision of the use of natural products for more efficient and safer control of stored grain pests.

Materials and Methods

Doses of the insecticides to be tested were selected after preliminary trials. Danitol 10 EC, a commercial formulation of fenpropathin, was obtained locally and was dissolved in water, and different concentrations were prepared for application in desired doses i.e., 0.122 µg/cm^2, 0.245 µg/cm^2, 0.49 µg/cm^2, 0.98 µg/cm^2 and 1.96 µg/cm^2. The neem extract RB–a was obtained from Dr. Beena Shaheen Siddiqui of H.E.J. Research Institute of Chemistry, University of Karachi. RB–a was dissolved in 80% methanol along with 0.01% PBO (piperonyl butaoxide) and 0.05% Triton x–100. Different concentrations were obtained by diluting the stock solution of neem formulation in 80% methanol to make required doses, i.e., 3.92 µg/cm^2, 19.64 µg/cm^2, 39.2 µg/cm^2, 78.5 µg/cm^2 and 117.8 µg/cm^2. Stock culture of *A. diaperinus* was grown in the Insectory of the Zoology Department, University of Karachi, in jars containing wheat bran at 30±2°C and 60%±5 RH.

Different doses were applied in 1 ml of each dilution in petri dishes 4.5 cm in diameter. Volume was kept constant and dose variation was achieved by using different concentrations, for an even distribution in formation of insecticide film. Higher volumes never dry up easily, While low volumes may not form even films. These plates were exposed to air for drying for 1 hour. The adults (*Alphitobius diaperinus*) were then released, and after 24 hours readings of mortality were noted. Each experiment was repeated five times and the mean values were taken for analysis. The mortality observed against each dose was plotted on log–probit graph paper, and a free–hand regression line was drawn to determine LD_{50} from the curve, as previous researchers (1–3, 6, 7). have done according to the method described by Matsumura[11]. After determination of LD_{50}, twenty insects were treated with the LD_{50} of each compound, and transaminase (GOT, GPT) activity was estimated according to Reitman and Frankel's method (12). The assay procedurs used for GOT and GPT were the same, except for the buffer substrate solution, which was L–alanine and 2–glutarate for GPT and L–asparate and 2–oxoglutarate for GOT. GOT and GPT activities were determined with a colorimetric kit (Merckotest Diagnostic no. 14329), which is based on the method of Reitman and Frankel (12). Entire insect bodies were used for GOT and GPT activity estimation.

Results and Discussion

Toxicity Determination

The LD_{50} of Danitol and that of neem formulation (RB–a+PBO+Tx–100) against *Alpitobius diaperinus* were determined, and are interpreted in Figures 1 and 2, which indicate the LD_{50} of Danitol to be 0.2749 µg/cm^2, and that of neem formulation to be 58.92 µg/cm^2. The highest mortality caused by Danitol was 88%, and the highest mortality caused by neem formulation was 70%, as indicated in Table I. Danitol was effective in a 0.003125% concentration, whereas RB–a (synergized with piperonly butaoxide) was effective in a 0.5% concentraiton.

Table 1. Estimation of toxicity of RB–a+PBO+Tx–100 against *Alphitobius diaperinus* after 24 hours of treatment showing mortality percentage and statistical analysis.

S.No.	Dose in ug/cm^2	$\bar{X}$	S.D. ±	S.E. ±	Range
1.	Zero*	–	–	–	–
2.	3.92	4	5.47	2.45	2.33–10.33
3.	19.64	8	4.47	2.005	2.82–13.17
4.	39.20	36	13.41	6.01	20.47–51.52
5.	78.50	64	8.94	4.01	43.65–64.34
6.	117.80	70	7.07	3.17	61.81–78.18

* Untreated control.

As far as the activity of transaminase is concerned, both compounds were found to have inhibitory effects: only 55.34% and 54% activity remained after Danitol treatment in GPT and GOT, respectively, whereas 46% and 87% activity remained in GPT and GOT after neem formulation treatment. In both cases the control (untreated insects) activity was taken as 100%.

Table 2. Estimation of toxicity of Danitol against *Alphitobius diaperinus* after 24 hours of treatment showing mortality percentage and statistical analysis.

S.No.	Dose in ug/cm^2	$\bar{X}$	S.D. ±	S.E. ±	Range
1.	Zero*	–	–	–	–
2.	0.122	30	–	–	–
3.	0.245	46	5.47	2.45	39.66–52.33
4.	0.49	52	447	2.005	46.82–57.17
5.	0.98	68	4.47	2.005	62.82–73.17
6.	1.98	88	4.47	2.005	82.82–93.17

* Untreated control.

Table 3. Percent inhibition of GOT and GPT activity in *Alphitobius diaperinus* after LD_{50} of Danitol (fenpropathrin) and neem formulation (RB–a+PBO+Tx–100) treatment.

	% Inhibition in GOT	% Inhibition in GPT
Control	0.00%	0.00%
Danitol	45.91%	44.66%
Neem formulation	12.15%	52.48%

Bengeston (4) reported pyrethroid LC_{50} values as 0.19% against *Sitophilus oryzae*, 0.18% against *Rhyzopertha dominica* and 1.2% against *Tribolium castaneum*. In the present study, the LD_{50} value of Danitol was 58.92 µg/cm^2, which is lower and may be due to differences in the insects. An earlier report (8) showed that pyrethroid usage against stored grain pests is effective, and in the present report it is confirmed that pyrethroids are still effective over time. In addition to neem components, other plant products have also been reported to have pesticidal potential against stored grain pests, e.g., the toxic effects of *Nerium indicum* in crude from against *Tribolium castaneum* (10). The LD_{50} for *Nerium indicum* has been reported as 9.43 µl/cm^2, and for Coopex (pyrethroid) 11.0 µl/cm^2. In our results, with Danitol as pyrethroid in comparison with neem formulation, we obtained a lower LD_{50} for pyrethroid (0.274 µg/cm^2) than for RB–a formulation (58.92 µg/cm^2) which indicates that pyrethroid is more effective than neem formulation. From the present and previous reports (10) it appears that different plant materials exhibit different behaviour against stored grain pests and that the *Nerium indicum* component may act as a better toxicant than neem formulation. In the same year (3), the toxicities of Danitol, methoprene and neem formulation (RB–b+PBO+Tx–100) against *Sitophilus oryzae* were reported (3). Different concentrations were applied by the same method as in the present case. The LD_{50} of Danitol was found to be 0.39 µg/cm^2, that of neem formulation was found to be 6.28 µg/cm^2, and that of methoprene was found to be 18.85 µg/cm^2. In the present study, Danitol caused 50% mortality in *Alphitobius diaperinus* in a dose of 0.2749 µg/cm^2, and neem formulation (RB–a+PBO+Tx–100) caused 50% mortality in a dose of 58.92 µg/cm^2. Danitol activity was similar to that previously reported (3), while the neem extract dose differed, although the components of neem used both in the previous and in the present case were synergized with the same compound, i.e., piperonyl butaoxide. Thus, it appears that RB–b and RB–a themselves differ in their effects. This may be due to the fact that PBO activates RB–b more, while RB–a was not synergized as much by PBO. In the toxicology laboratory in the Zoology Department, University of Karachi, comparative studies on Solfac and RB–A, a neem component in crude form, have been carried out on *Alphitobius diaperinus* adults (13). So far, this is the only report available on *Alphitobius.* It was reported that the LC_{50} of RB–A was higher than that of Solfac. In the present report, RB–a was used in synergistic formulation because it has no effect when used alone, as reported by Khan, (unpublished). This means that RB–A and RB–a (methanolic extract of ripe drupes of neem) are different in their activity but less effective than pyrethroid. In a recent on *Tribolium castaneum* adults (9), a number of enzymes were found to be sensitive to pyrethroids. In addition, it was reported that various organic compounds, namely carbohydrates, proteins, lipids, nucleic acids, etc., were affected by treatment with pyrethroids. Transaminase activities were found to increase significantly (9), whereas in the present report Danitol was found to inhibit GOT and GPT activity. This may be due to differences in strain, as resistant strains were used in the study in question. Transaminase inhibition was also reported in *T. castaneum* adults (7) after insects were exposed to LC_{50} of Sisthion and RB–a (synergistic formulation), which inhibited the GOT level by 27.72% whereas GPT inhibition was 8.48% GOT inhibition by RB–a (in synergistic formulation) was recorded as 54%, and that of GPT as 13%. These findings are similar to those of Tabassum *et al.* (7), who also reported more inhibition of GOT than of GPT. From the present findings, it may be concluded that among alternate methods being investigated as substitutes for the conventional chemicals against insects, the use of neem extract is a promising alternative, either in crude form or with a synergist.

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