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Comparative Toxicological Studies of RB-a (Neem Extract) and Coopex (Permethrin+Bioallethrin) Against *Sitophilus oryzae* With Reference to Their Effects on Oxygen Consumption and Got, Gpt Activity

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Abstract: The toxicity of a neem extract RB-a in comparison with a pyrethroid, Coopex, was studied along with their effects on GPT and GOT activities and oxygen consumption in *S. oryzae.* The LD_{50} value of Coopex was found to be 6.128 µg/cm^2 whereas RB-a was found not to be an acute contact poison. Even at a dose of 1257 µg/cm^2 it could cause 34% mortality of *S. oryzae.* The oxygen consumption under the effects of Coopex was found to decline less i.e. at 6.12 µg/cm^2 from 0.0005191 to 0.0006589, as compared to RB-a where the rate of respiration declined more i.e. at 1257 µg/cm^2 from 0.0005191 to 0.0002076. Coopex brought about an inhibition of 62.77% in GPt activity and caused almost no inhibition of GOT activity in insects treated with its LD_{50}. In th case of neem extract it was noted that GPT was inhibited by about 57.47% and almost no effect on GOT was observed at a dose of 1257 µg/cm^2.

Sitophilus oryzae'de Oksijen Tüketimi ve GOT, GPT Aktivitesine Etkileri Açısından RB-a (Neem Özütü) ve Coopex (Permethrin + Bioallethrin) Üzerine Karşılaştırmalı Toksikolojik Araştırmalar

Özet: Neem özütü olan RB-a ile pyretroid olan Coopex'in, S. oryzae'de GPT ve GOT aktiviteleri ile oksijen tüketimi üzerindeki etkileri birbiriyle karşılaştırılarak incelenmiştir. Coopex'in LD_{50} değerinin 6.128 µg/cm^2 olarak tespit edilirken, RB-a'nın deri yoluyla bulaşan zehir olmadığı bulunduğu halde 1257 µg/cm^2 dozunda bile S. oryzae'de %34 mortaliteye neden olduğu saptanmıştır. Coopex'in oksijen tüketimini arttırdığı (6.12 µg/cm^2 dozunda, 0.0005191'den 0.0006589'e), RB-a'nın ise oksijen tüketimini azalttığı (1257 µg/cm^2 dozunda 0.0005191'den 0.0002076'ya) saptanmıştır. Coopex'in LD_{50}'siyle ilaçlanan böceklerde, GPT aktivitesinde %62.77'lik azalma meydana gelirken GOT aktivitesinde hemen hemen hiç azalma olmamıştır. Neem özütünün 1257 µg/cm^2 dozunda, GPT aktivitesinde %57.47'lik azalmaya neden olduğu, GOT akitvitesinde ise hemen hemen hiç azalmaya yol açmadığı bulunmuştur.

Introduction

Neem tree, known for its excellent insecticidal properties has drawn the attention of pesticide workers for years. Nowadays workers worldwide are emphasizing the neem tree and giving this plant importance in their research programmes e.g. Abraham and Ambica (1–13).

Transaminase enzymes help in the production of energy. In order to understand the effects of these compounds on respiration, the oxygen consumption and GOT and GPT values were also determined.

This study compares a neem extract, RB-a, with Coopex, a permethrin formulation, with emphasis on their effects on the rate of respiration and the GPT and GOT enzyme level in *Sitophilus oryzae*, a ubiquitous stored grain pest.

Material and Methods

The experiment was conducted on adult *Sitophilus oryzae*, using the contact method. Initially, adult *Sitophilus oryzae* were obtained courtesy of Mr. Shamim Iqbal, GSRL, PARC and subsequent generations were obtained in the laboratory via raising on rice in jam jars under 30±2°C and 70±5 RH.

One-day-old adults were used for the toxicity test with pesticide impregnated filter paper. The filter papers 90 mm in diameter were soaked with the desired compound i.e. Coopex and RB-a with doses of 1.089 µg/cm^2, 1.636 µg/cm^2, 2.455 µg/cm^2, 3.680 µg/cm^2 and 6.128 µg/cm^2 for Coopex and for RB-a the dose of application was 628.535 µg/cm^2, 785.669 µg/cm^2, 942.803 µg/cm^2, 1099.937 µg/cm^2 and 1257.071 µg/cm^2. Doses were set after preliminary testing.

The Coopex was provided by Mr. Babur Sultan of Wellcome (Pakistan) Ltd. and the RB-a was procured from Dr. Beena Siddiqui of the H.E.J. Research Institute of Chemistry, University of Karachi.

Toxicity tests were carried out by using impregnated filter papers at the desired doses for both the test compounds. The filter papers were set into petri dishes in triplicate with a check (methanol impregnated for RB-a to understand the effect of the solution) and an untreated control, to determine the environmental effects. Thereafter, 20, one-day-old insects were released in each petri dish and left overnight. After 24 hours of treatment the mortality count was noted. To avoid any experimental error each experiment was repeated five times and the data was analysed statistically.

To understand the effects of the compounds on respiration, the oxygen consumption and the effect on GOT and GPT values were also determined. GOT and GPT are transaminases which help in the transfer of amino groups and play an important role in the Kreb's cycle or the high energy producing cycle. Therefore, GPT and GOT were determined, with the respiration rate. The rate of respiration and oxygen consumption were measured manometrically using Warburg's apparatus. The insects were treated with LD_{30} of Coopex and RB-a. Potassium permangnate $KMnO_4$ was used as a filling agent in the manometer's arms. Observations were made after 1 and 3 hours.

The GOT activity was determined spectrophotometrically using a kit i.e. Merieux Marcy 1 Etoite No.69260, where 2,4-dinitrophenyl-hydrazine asparate was provided for the reaction and L-asparate and 2-oxoglutarate were provided as the substrates. Oxaloacetate was formed by the enzymatic effect and the colour was determined at 560 nm. The GPT was determined with the same kit except that the substrate solution contained L-alanine and glutarate. Only 0.1 ml of supernatant was used as a sample. The treated insects were ground and homogenized in 2 ml distilled water and centrifuged for 20 minutes at 3000 rpm. The supernatant was taken out, 0.5 ml of substrate was added and kept in a bath for 5 minutes and a 0.2 ml sample was added and incubated for 1 hour. Then 0.5 ml of colouring agent was added and after 20 minutes 5 ml of 0.4 N NaOH was added and the reading was taken after 5 minutes. A standard curve was also prepared with standard reagents provided in the kit for comparison.

Results and Discussion

Contact toxicities of both test compounds as shown in Tables 1 and 2, using impregnated paper, was found to be 34% with a 1257.071 µg/cm^2 dose of RB-a while 24%, 28%, 32% and 50% mortalities were observed by applying 1.089 µg/cm^2, 2.455 µg/cm^2, 3.680 µg/cm^2 and 6.128 µg/cm^2 Coopex against *Sitophilus oryzae*, respectively. Ivbijaro (14) used neem powder and ethanolic neem extract using 2-5 g per 20 g flour to control *Tribolium castaneum.* He observed up to 55% mortality. In this study the neem extract was found to be effective at very high doses as in Ivbijaro's findings. The small difference between the two findings may be due to the difference in the method of application or might be due to the difference in the insect species. However, the two studies are in agreement with each other as far as the toxicity and grain protectant effect of the neem is concerned.

Table 1. Estimation of toxicity of Coopex (25 E.C.) against the adults of *Sitophilus oryzae* after 24 hours of treatment showing mortality range at 95% confidence limit.

Dose in µg/cm^2	% Mean mortality	S.D.	S.E.	Range at 95% confidence limit
Control	–	–	–	–
1.08	24	5.4770	2.4561	19.1861–28.8139
1.63	24	11.4017	5.1128	13.9789-34.0210
2.45	28	10.9544	4.9122	18.3720–37.6279
3.68	32	13.0384	5.8468	20.5402–43.4597
6.12	50	7.0710	3.1708	43.7852–56.2147

Ketkar (15) reported that with a dose of 2.5 ml/kg cowpea, neem oil produced 90% mortality in *Callosobruchus maculatus*. Contrary to the present findings he observed high mortalities at very low doses i.e. 2.5 ml/kg caused 90% versus 1.257 mg/cm^2 with

34% in the present results. The difference between two studies could be due to the different species and mode of applications and compound extraction. Probably the oil he had extracted had more toxic compounds therein.

Table 2. Estimation of toxicity of RB-a against the adults of *Stiphilus oryzae* after 24 hours of treatment showing mortality range at 95% confidence limit.

Dose in µg/cm^2	% Mean mortality	S.D.	S.E.	Range at 95% confidence limit
Control	–	–	–	–
Check	–	–	–	–
628.5	–	–	–	–
785.6	–	–	–	–
942.8	2	4.470	2.005	1.930–5.930
1009.9	6	4.477	2.456	1.860–10.810
1257.0	34	8.944	4.011	26.138–41.861

Naqvi (16), Nurulain and Naqvi (10) reported toxicity of various neem fractions against the housefly, *Blattella germanica* and the dusky cotton bug. They reported LD_{50} as 1.4 µg NFA/fly, 0.5 µg NFB/roach and 58%, 92% and 0.0171% for RB-a, RB-A and Margosan-O against the dusky cotton bug respectively. The difference between the present findings i.e. 1.257 mg/cm^2 for *Sitophilus* sp. in comparison with the studies of aforementioned authors is obviously due to the difference in compound or specis. Only the housefly is a holometabolus insect, but despite the difference in order, it is comparable with *Sitophilus*. However, the mode of application of RB-a was different in the two studies, resulting in different effects.

In order to determine the extent of the lethal effects on the respiratory system, the rate of respiration and the effects on GOT and GPT were determined post treatment. As shown in Tables 3 and 4 the consumption of O_2 in RB-a treated (with LD_{30}) insect was very low i.e. 0.0002076 µl/insect at 3 hours in Coopex treated insects in comparison with the control where it was 0.0004499 µl/insect. This indicates that RB-a reduced O_2 consumption whereas Coopex increased it. In the RB-a treated insects,

Table 3. Mean Vf values in ml at 1 and 3 hours post treatment

Compounds	Dose in µg/cm^2	1 hour	3 hours
Control	–	0.100	0.325
Check (methanol)	19.64.17	0.675	1.025
Coopex	6.12	0.125	0.475
RB-a	1257.00	0.125	0.150

K values = 1.246 µl in all three experiments.

Table 4. Amount of O_2 exchanged (in µl/insect) at 1 and 3 hours post treatment*

Compounds	Dose in µg/cm^2	1 hour	3 hours
Control	–	0.0004153	0.0004499
Check (methanol)	19.64.17	0.0028035	0.0014190
Coopex	6.12	0.0005191	0.0006589
RB-a	1257.00	0.0051910	0.0002076

*O_2 exchanged = hxk/insect/min.

Table 5. Estimation of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity in adult *Sitophilus oryzae* when treated with Coopex (25 E.C.) after 24 hours.

Enzyme	Mean of enzyme units/ml		% inhibition	S.D.	S.E.	Range at 95% confidence limit
	Control	Treated				
GOT	176	142	19.23	488.180	218.320	232.507–623.32
GPT	94	35	62.77	42.316	18.925	2.307–76.493

Table 6. Estimation of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity in adult *Sitophilus oryzae* when treated with RB-a after 24 hours

Enzyme	Mean of enzyme units/ml			% inhibition	S.D.	S.E.	Range at 95% confidence limit
	Control	Check	Treated				
GOT	176	132	175	0.57	24.4800	10.9306	1866.902–2335.702
GPT	94	96	40	57.47	20.7050	9.2433	40.364–42.035

oxygen consumption was almost half that of the control. RB-a acted as respiratory depressant as compared to Coopex which increased O_2 consumption to approximately three times that of RB-a and 33% more than the control. Being a pyrethroid it could be suggested that with nervous disturbance it increased oxygen consumption, whereas the neem is a feeding deterrent and thus reduced the metabolic rate and caused a lesser requirement of oxygen than normal.

As shown in Tables 5 and 6, the GPT and GOT activities under the effects of Coopex and RB-a were determined, at LD_{30} values post treatment. Both the compounds caused negligible effects on GOT activities i.e. 80.68% and 99.43% under the effects of Coopex and RB-a, respectively. Whereas RB-a inhibited 57.47% of the activity of GPT, and Coopex inhibited 62.77% of the activity of GPT. The two compounds though, inhibited both enzymes. However, the extent of inhibition under the effect of Coopex was more than RB-a. Moreover, the GPT was inhibitited more, possibly because pyruvate is the precursor of Kreb's cycle compounds, concerned with the mitochondrial oxidation phenomenon and ATP production.

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