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TOXICITY OF *CLERODENDRUM INERME* EXTRACT AND CYHALOTHRIN AGAINST *RHIZOPERTHA DOMINICA* PARC STRAIN AND THEIR EFFECT ON ACID PHOSPHATASE AND CHOLINESTERASE ACTIVITY

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Toxicity of *Clerodendrum inerme* (dumdum leaves extract) and cyhalothrin (Pyrethroid) was tested against adult beetles of *Rhizopertha dominica*. Filter paper impregnation method was adopted for the determination of LC_{50} dose. LC_{50} dose of *C. inerme* and cyhalothrin was found to be 1460 µg/cm^2 and 0.00063 µg/cm^2, respectively. Biochemical estimation revealed that both the products inhibited the acid phosphatase and cholinesterase activity to some extent.

Key words**:** *Rhizopertha domimica*, Cyhalothrin, Acid phosphatase, Cholinesterase, *Clerodendrum inerme.*

Introduction

Lesser grain borer, *Rhizopertha dominica* is a cosmopolitan insect and a serious pest of stored grains especially wheat and flour. According to our food requirements wheat has a prominent place in our daily diet. In Pakistan farmers store about 60% of wheat for food and for sowing requirements. Unfortunately about 5-7% of wheat is destroyed by insect pests.

The insect also attacks the germ part of the grain, feeds upon the endosperm and fills the burrows with excrement which leads to poor quality of seed. In Karachi, the lesser grain borer damages more wheat in Dockyard areas probably due to damp climate which softens the hard pericarp of wheat grain. Conventional (synthetic) pesticides are used for the control of this insect pest. But their indiscriminate use causes problems like pollution, residual toxicity and insect resistance (Zettler 1982; Holiday *et al* 1988; Saleem and Shakoori 1993). Synthetic pyrethroids are rapid in action, have low mammalian toxicity and wide controlling range (Bagherwal *et al* 1994). Several workers have reported effect of biopesticides on certain enzyme levels of insects (Bandyopadhyay 1982; Hoyaoka and Dauterman 1982; Shakoori and Saleem 1989; Shakoori *et al* 1994, Ahmad *et al* 2001).

Plant products kill insects, however, they have low mammalian toxicity, leave no toxic residues and do not pollute the environment.

Extracts from dumdum leaves (*Clerodendrum inerme*) can be used effectively to protect stored grains from insect infestations. The extract possesses anti-feedent, repellent, insecticidal and growth disrupter effects on insects. There are no estimates of dumdum leaves production in Pakistan. However, it is widely grown in Sindh and Punjab and some farmers are aware of its pest control properties.

As the synthetic insecticides are toxic and hazardous, so phytopesticides which are much less toxic and biodegradable, the present extract was used against *R. dominica*.

Materials and Methods

Rhizopertha dominica beetles were obtained from PARC, TARC, University Campus, Karachi, reared under controlled conditions i.e., 28± 3˚C and 65 ± 5% R.H. Sterilized wheat was used as rearing medium. All experiments were carried out with 7 days old adults of uniform size. Cyhalothrin was purchased from market and extraction of *Clerodendrum inerme* leaves was prepared in the laboratory.

Extraction of sample. 250g *C. inerme* leaves, collected from Karachi University Campus, were washed for the preparation of extract. Leaves were macerated in 50% methanol (1:1 H_2O: CH_3OH). The macerated leaves were left for 24 h in 250 ml of 50% methanol. Maceration of leaves was done in Ultra Turax grinder and homogenized for 30 min . Finally, it was filtered twice and stored in a refrigerator at 10˚C.

For the treatment of insects filter paper impregnation method was employed. After preliminary test 236, 475, 950, 1900 and 3800 µg/cm^2 doses of *C. inerme* and 0.000316, 0.000633, 0.00126, 0.00253 and 0.00507 µg/cm^2 doses of cyhalothrin were selected. These doses were applied on the filter paper of 2.5 cm diameter with the help of pipette. Twenty *R. dominica* adults of

same age were released in each petri dish separately. For the estimation of acid phosphatase and cholinesterase 300 adults were treated with LC_{50} dose, a day prior to enzyme assay. Thereafter, 150 surviving beetles were crushed in 2 ml double distilled water with the help of mortar and pestle and then homogenized for 10 min at 1,000 rpm at 4°C. The homogenate were centrifuged in Labofuge 15,000 at 3,500 rpm for 20 min , placed in cold chamber. Supernatants were taken in separate tubes and used for biochemical estimation of acid phosphatase activity by thymolphthalein monophosphate using QCA kit and cholinesterase activity was estimated by Clonital's Kit No. KC 060.

Results and Discussion

Toxicity of *C. inerme* extract and cyhalothrin was determined by using five different doses of each compound against *R. dominica*. It was observed that the rate of mortality gradually increased with the increase in dose of each pesticide (Table 1-2). LC_{50} was calculated by using log-log graph paper. By plotting the mean mortality values against the dose of the compound. LC_{50} of *C. inerme* was found to be 1460 µg/cm^2.

R. dominica treated with cyhalothrin showed mean mortality of 32, 48, 60, 72 and 92%, respectively after 24 h . By plotting the mean mortality value against the dose, the LC_{50} of cyhalothrin was found to be 0.00063 µg/cm^2.

Activity of acid phosphatase was found to be inhibited by 20.9% (when treated with *C. inerme* at dose of LC_{50} i.e 1460 µg/cm^2) and 41% (when treated with cyhalothrin at dose of LC_{50} i.e. 0.00063 µg/cm^2, Table 3).

Cholinesterase activity was reduced upto 23.61% with *C. inerme* at a dose of 1460 µg/cm^2 and upto 30.53% with cyhalothrin treatment at a dose of 0.00063 µg/cm^2 (Table 4).

Table 1
Toxicity of *C. inerme* against *R. dominica* beetles

Dose in µg/cm^2	Mean mortality %	S.D. (±)	S.E. (±)	Range at 95% confidence limit
Control	--	--	--	–
0237	12	4.47	2.00	08.08 - 15.92
0475	26	5.47	2.45	21.20 - 30.80
0950	40	7.07	3.17	33.80 - 46.20
1900	62	4.47	2.00	58.08 -65.92
3800	82	4.47	2.00	78.85- 92.41

Table 2
Toxicity of cyhalothrin against *R. dominica* beetles

Dose in µg/cm^2	Mean mortality %	S.D (±)	S.E. (±)	Range at 95% confidence limit
Control	-	-	-	-
0.000316	32	10.90	04.88	22.44 - 41.56
0.000633	48	10.90	04.88	38.44 - 57.56
0.00126	60	14.14	06.34	47.58 - 72.42
0.00253	72	22.80	10.22	51.21 - 92.31
0.00507	95	17.88	08.02	76.28 - 107.72

Table 3
Acid phosphatase inhibition in *R. dominica* treated with *C. inerme* and cyhalothrin

Compound	Mean of unit (µ/l)	S.D. (±)	S.E. (±)	Range at 95% confidence limit	Inhibition %
Control	502.53	565.80	327.0	138.3 - 114.3	00.0
C. inerme	397.30	403.40	233.2	59.7 - 854.3	20.9
Cyhalothrin	296.33	247.20	143.1	16.3 - 576.3	41.0

In present study LC_{50} of cyhalothrin and *C. inerme* was found to be 0.00063 µg/cm^2 and 1460 µg/cm^2 for *R. dominica* beetles. These results are in close conformity with those of Bengeston *et al* (1981) and Ahmad *et al* (2001) for pyrethroids and Islam (1987), Naqvi (1987), Vollinger (1987), Azmi *et al* (1998) and Ahmad *et al* (2000) who used plant products (Safer pesticides).

In the present investigations cyhalothrin was found to be more effective than *C. inerme* against *R. dominica* adults. Bengeston (1981) also found that fenvalerate, permethrin and diphenothrin were not effective against malathion resistant *T. castaneum* and *S. oryzae*, whereas, cypermethrin and decamethrin showed satisfactory effectiveness.

Treatment of *C. inerme* and cyhalothrin inhibited acid phosphatase and cholinesterase enzymes in *R. dominica* adults.

Many workers reported inhibition of acid phosphatase and cholinesterase enzymes in storage insects after pesticide treatments (Ahmad *et al* 2000; Rizwan *et al* 2000; Azmi *et al* 2001 and Rizvi *et al* 2001).

The enzyme cholinesterase is involved in detoxification of insecticide (Saleem and Shakoori 1993; and Shakoori *et al*

Table 4
Cholinesterase inhibition in *R. dominica* treated with *C. inerme* and cyhalothrin

Compound	Mean of enzyme unit (μ/l)	S.D (±)	S.E (±)	Range at 95% confidence limit	Inhibition %
Control	58.44	45.62	26.36	6.78-110.21	00.0
C. inerme	53.57	12.61	7.31	14.92 - 43.54	23.61
Cyhalothrin	36.52	12.60	7.28	22.3 - 50.72	30.53

1998). However, enzyme inhibition in any insect by pesticide use exhibits potency of the poison.

Conclusion

It could be concluded that *C. inerme* extract prove better alternative pesticide for controlling insect pests. They have low mammalian toxicity, less persistent and do not pollute the environment.

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