Comparative Toxic Effects of Methamidophos and Cypermethrin on Cholinesterase Enzyme of *Callosobruchus analis*

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Abstract: In the present study the cholinesterase inhibition was observed as 65% and 12, for methamidophos and cypermethrin, respectively. LC_{50} values were found as 16 and 2.2, µg/cm^2 for methamidophos, cypermethrin, respectively against *Callosobruchus analis* by the filter paper impregnation method after 24 hours of treatment. The order of efficacy was found as cypermethrin > methamidophos.

Keywords: Toxicity, Cholinesterase, *Callosobruchus analis,* Methamidophos and Cypermethrin.

INTRODUCTION

Amongst the most damaging pest of stored grains almost all of the coleopterans insect species are known damaging stored grains like paddy, wheat, corn, rice, and pulses. They cause loss to stored food grain not in developing countries only but they are also a big problem in developed countries too. According to an estimation about 5 – 10 % of world stored food grains is lost due to insect's infestation [1, 2]. In Pakistan 2-6% loss of stored grains per year is recorded in the storage bins [3, 4].

Synthetic chemicals and applicable equipments are costly and have adverse effects on our environment as they cause destructive consequences for human health [5, 6]. They not only have direct effects on human health but also indirect effects as well. Management of pests by safer means is important and by the better understanding of the mode of action of these pesticides that could be correlated with possible effects on human being.

In the present investigation an OP compound Methamidophos, and a pyrethroid Cypermethrin were tested on *Callosobruchus analis* for their toxicological effects on cholinesterase.

MATERIALS AND METHODS

All the experiments were conducted on *Callosobruchus analis*.

Rearing Technique

Initial strain of *Callosobruchus analis* was procured from PARC and further rearing was made at Toxicology Laboratory of Department of Zoology, University of Karachi where *Callosobruchus analis* were cultured at controlled laboratory conditions of known diet, temperature and humidity for the regular supply of diseased-free insects. The insects were kept in 500g glass jars covered with muslin cloth tied by means of rubber band. Grains of *Vigna radiata* were used as food and egg laying media when the eggs were laid by the adults they soon died and the new adults were transferred to the fresh grain containing jars.

I. Toxicity Determination

All the compounds were tested by means of filter paper impregnation method [7]. For this purpose six sets of Petri dishes of 2.5 cm diameter were taken and these dishes were washed, dried in air and sterilized in the oven.

Five dishes were marked for each dose whereas one was kept as control to determine the environmental effect. The filter papers were placed at the bottom of petri dishes then by the help of pipette five different containers of the Cypermethrin and Methamidophos were spread on the filter paper. After that 10 adults of same age and size were released in each petri dish separately. After 24 hours of treatment, percent mortality was observed in all petri dishes including control and the check. Data were analyzed statistically through probit analysis by Finney in 1971 and presented in Table **2** & **4** and Figure **1** & **2** respectively [8].

II. Estimation of Cholinesterase (EC 3.1.1.8) Activity

a. Preparation of Homogenate

For the preparation of homogenate or sample 100 adult beetles of same size and age of *Callosobruchus analis* were treated with LC_{50} of the under test

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Table 1: Statistical Analysis of Toxicity of Methamidophos Against *Callosobruchus analis* (After 24 Hours Treatment with Methamidophos)

Concentration µg/cm^2	Mean x (%)	S.D. (±)	S.E. (±)	Range at 95% confidence limit
Control	–	–	–	–
3.3	06	5.4	2.42	1.26 – 10.74
6.631	20	7.87	3.17	0.856 – 13.28
13.261	30	7.07	3.17	23.7 – 36.21
26.522	60	12.24	5.48	39.6 – 60.74
53.05	86	5.47	2.45	81.198 – 90.8

compounds i.e., Cypermethrin (2.2 µg/cm^2), and Methamidophos (16 µg/cm^2). After 24 hours of treatment the insects were crushed in one ml of distilled water with the help of mortar and pestle, and then homogenized for five minutes at 10,000rpm. The homogenate were centrifuged in "Labofuge 2000 Heraeus Sepatech" for 5 minutes at 5000rpm. The supernatants were taken in separate micro-tubes and were kept in refrigerator at 2-8^{o}C. This supernatant was used for the estimation.

b. Estimation Procedure

Based upon the method of Knedel and Bottger [9], cholinesterase activity was determined by the colorimetric method of RandoxCat No. CE 190, as follows:

Two test tubes were taken and labeled them as control and treated then 1.5 ml of buffer was added to each test tubes, then 0.01 ml of supernatant of control and treated was added to their respective test tubes. Finally 0.05 ml of substrate was added to each test tube. Mixed and read the initial absorbance started the timer simultaneously. The Readings were noted again after 30, 60 and 90 sec., against blank at 405 nm on Schimadzu spectrophotometer UV-120. With these values the cholinesterase activity was calculated according to the kit instructions.

RESULTS

Following are the percentage mortality at 24 hours post treatment were:

Methamidophos

LC_{50} for methamidophos was found to be 16 µg/cm^2 after 24 hours of treatment. The selected dilutions for this compound were 3.3, 6.631, 13.261, 26.522 and 53.05 µg/cm^2. The average percent mortalities were 06%, 20%, 30%, 50% and 86% respectively (Table **1** & **2**, Figure **1**). Probit Analysis of Toxicity of Methamidophos Against Callosobruchus analis (After 24 hours treatment) was carried out after Finney (1971) the log dose and probit regration equation was calculated as Y=2.313+2.111 X while a heterogenicity factor χ^2 was found to be 0.999 [8].

Cholinesterase level after 24 hours of Treatment was found as 16 µg/cm^2 of methamidophos showed 65% inhibition of cholinesterase level in *Callosobruchus analis* after 24 hours of treatment (Table **3**).

Cypermethrin

LC_{50} for cypermethrin was found to be 2.2 µg/cm^2. The selected dilutions for this compound were 0.33156, 0.663, 1.326, 2.652 and 5.305 µg/cm^2 and the average

Table 2: Probit Analysis of Toxicity of Methamidophos Against Callosobruchus analis (After 24 Hours Treatment)

Concentration	Mean % Mortality	Log Con.	Probit Value	Mortility=2.313+2.111 log Con.
3.3	6	0.5185139	3.4452	3.407885031
6.631	20	0.821579	4.1584	4.047811163
13.261	30	1.1225763	4.4756	4.683371021
26.522	60	1.4236063	5.2533	5.319000028
53.05	86	1.7246854	6.0803	5.954732756

Chi-Square value = 0.999.

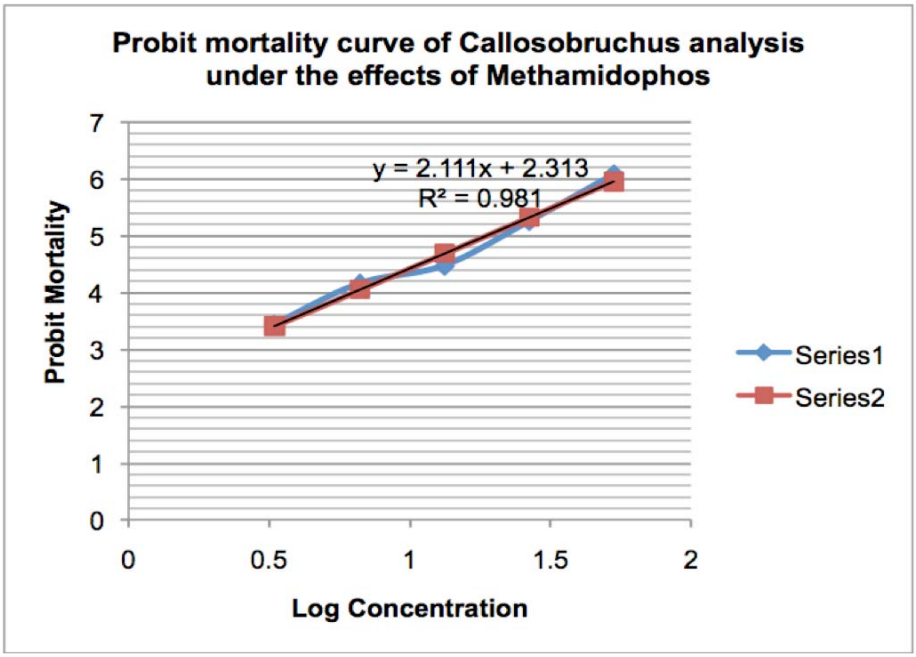

Figure 1:

Table 3: Level of Cholinesterase In *Callosobruchus Analis* (After The Treatment With Methamidophos)

Treatment	Mean µ/L	S.D. (±)	S.E. (±)	Range at 95% confidence limit	% Inhibition
Control	156.00	22.2	12.83	130.86 – 181.14	–
Treated	54.65	9.3	5.3	44.26 – 65.03	65

percent mortalities were found to be 12%, 22%, 32%, 64% and 72%, respectively (Table **4** & **5**, Figure **2**).

Probit Analysis of Toxicity of cypermethrin Against Callosobruchus analis (After 24 hours treatment) was

Table 4: Toxicity Of Cypermethrin By Filter Paper Impregnation Method Against *Callosobruchus Analis* (After 24 Hours Of Treatment)

Concentration µg/cm^2	Mean x (%)	S.D. (±)	S.E. (±)	Range at 95% confidence limit
Control	–	–	–	–
0.33156	12	4.47	2.00	8.00 – 16.00
0.663	22	4.4	1.97	18.14 – 25.86
1.326	32	4.4	1.97	28.14 – 35.86
2.652	64	5.4	2.45	59.2 – 68.8
5.305	72	4.4	2.00	68.08 – 75.92

Table 5: Probit Analysis of Toxicity of Cypermethrin Against *Callosobruchus analis (After 24 hours of treatment)*

Concentration µg/cm^2	Mean % Mortality	Log Con.	Probit Value	Mortility=4.516089+1.543464 log Con.
0.33156	12	-0.47944	3.825	3.776094
0.663	22	-0.17849	4.2278	4.240602
1.326	32	0.122544	4.5323	4.70523
2.652	64	0.423574	5.3585	5.169859
5.305	72	0.724685	5.5828	5.634615

Chi-Square value = 0.999.

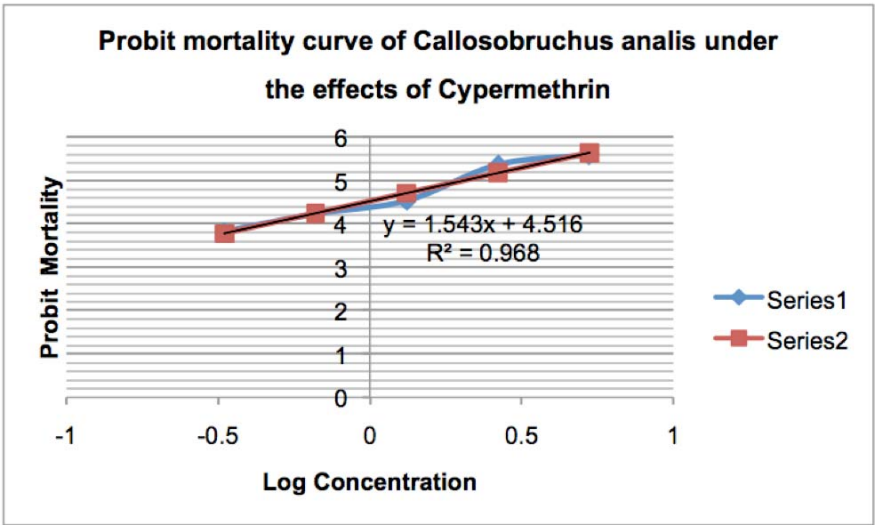

Figure 2:

carried out after Finney (1971) the log dose and probit regration equation was calculated as Y=4.516089+1.543464X while a heterogenicity factor χ^2 was found to be 0.999 [8].

In respect of Cholinesterase enzyme, 2.2 µg/cm^2 of cypermethrin showed 12% inhibition of cholinesterase activity in *Callosobruchus analis* after 24 hours of treatment (Table **6**).

DISCUSSION

On *Callosobruchus analis* (F.) the mortality percentage due to Margosan-O^{TM} and Cyfluthrin (pyrethroid) was reported by Tabassum *et al*., [7]. LC_{50} for Margosan was found to be 2.2 µg/cm^2 and for Cyfluthrin it was found to be 0.09 µg/cm^2 [7]. LC_{50} for cypermethrin was 2.2 µg/cm^2 which co-relates the previous results to the present results. A comparative toxicity study of Margosan-O^{TM}, neem compound Cyfluthrin and organophosphate against *Musca domestica* has showed that OP pesticides were found more toxic than neem fraction and Pyrethroid, the order of efficacy was DDVP > dimethoate> Cyfluthrin > Margosan-O^{TM} > nimolicine > H-34 [10]. In the present investigation cypermethrin , pyrethroid was found better than methamidophos, OP compound against *Callosobruchus analis* that could be difference of compounds as reportedly cypermethrin's metabolites cause cholinesterase inhibition along with effects on nerve sheath [11].

The efficacy of cypermethrin and methyl parathion (OP) was studied by Ahmed *et al*., [12] against the *Tribolium castaneum*. The LC_{50} were found to be 1300 µg/cm^2 for neem extract, 2.3 µg/cm^2 for cypermethrin and 0.19 µg/cm^2 for methyl parathion. LC_{50}s for cypermethrin in the previous study (2.3 µg/cm^2) and present study (2.2 µg/cm^2) are similar although the insects were different. Toxicity also has been reported as 0.29, 0.056, 0.032, 0.45 and 0.08 µg/cm^2 for *Acorus calamus*, lorsban, cypermethrin, methamidophos and shogun respectively. According to LC_{50} the order of efficacy was found to be cypermethrin > lorsban > shogun > *Acorus calamus* > methamidophos [13], while in the present study cypermethrin was found more toxic than methamidophos their LC_{50}s were found to be 2.2 and 16 µg/cm^2. The order of efficacy of both studies are similar though LC_{50s} are different. This difference is due to the difference of test insects.

Azmi *et al*. reported toxicological effects along with biochemical estimations of *Clerodendrum inerme* and cyhalothrin against adult beetles of *Rhizopertha dominica*, biochemical estimations showed that both the products decreased the acid phosphatase and cholinesterase activity to a little value [14]. In the

Table 6: Level Of Cholinesterase In *Callosobruchus Analis* (After The Treatment With Cypermethrin)

Treatment	Mean u/L	S.D. (±)	S.E. (±)	Range at 95% confidence limit	% Inhibition
Control	195.5	4.9	2.8	190.01– 200.9	–
Treated	172.04	4.8	2.7	166.74 – 177.33	12

present case cypermethrin and methamidophos both showed inhibition of cholinestease to different levels as well as the previous findings showed.

Presently, Cholinesterase activity inhibition has been found as 65%, 12%, for methamidophos and cypermethrin, respectively. Methamidophos showed higher inhibition of cholinesterase where as cypermethrin showed inhibition of cholinesterase to a lower level this is because of the fact that methamidophos is an organo-phosphate (OP) compound and OP compounds could effect cholinesterase to highest levels and pyrethroids could not effect cholinesterase activity too much only their metabolites are expected to inhibit [11].

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