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Toxicity of Cypermethrin and Malathion on Rice Weevil *Sitophilus oryzae* (L.) and Their Effect on Esterase Isozymes

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Abstract: Experiments were conducted to study the toxicity and esterase isozyme pattern in rice weevil (*Sitophilusoryzae*), a major pest of the stored rice. Bioassay was conducted with cypermethrin and malathion against the adult weevils and LC_{50} was found to be 110.30 ppm and 12.19 ppm respectively at 24 hours of exposure. Malathion was found to be much more toxic to *S. oryzae* than that of cypermethrin. In general, the mortality increased with an increased dose and with the time of exposure. Altogether, four esterase bands (Est-$1^{1.36}$,Est-$2^{0.99}$, Est-$3^{0.45}$ and Est-$4^{0.24}$) were detected on 7.5% polyacrylamide gels with α naphthyl acetate as substrate. Changes of esterase isozyme due to cypermethrin and malathion treatment was also observed. Est-1 was appeared only in the cypermethrin treated dead samples of 3-9 days of exposure, which was found highly intense after 5 days and the intensity gradually decreased afterwards. Esterase activity in malathion killed samples decreased with the increase of exposure period and with dose concentrations in general, however, fluctuation in esterase activity was found in cypermethrin killed samples.

Key words: Rice weevil, Insecticide toxicity, Electrophoresis, Esterase isozymes

INTRODUCTION

Stored grain pests is a great problem in Bangladesh and 5-8% of the food grains, seeds and different stored products are lost annually due to stored pests[1]. About, 13 species of stored insects have been recorded from Bangladesh[1] which loss about 15% of stored rice[2]. The rice weevil, *Sitophilus oryzae* (Coleoptera; Curculionidae) that is widely distributed, is one of the important stored pests of the many common cereals[3]

and can cause losses to grain in storage, either directly through consumption of the grain or making unfit for human consumption within eight months of storage[4]. Cypermethrin is a synthetic pyrethroid that is used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes and behaves as a fast-acting neurotoxin in insects. The synthetic pyrethroids cyfluthrin and cypermethrin may be potential to control prevalent resistant strains of many stored pests. On the other hand, Malathion is an organophosphate insecticide suited for the control of different types of pest insects.

Esterases are the lipid-hydrolyzing enzymes that could be separated on polyacrilamide gels considering different iso-electric points[5]. The term 'esterases' is usually used to describe those hydrolases that cleaves esters made up of organic acids and alcohols or phenols[6]. Several studies have shown that changes in esterase sensitivity to inhibition by organophosphorus and carbamate insecticides can confer high levels of resistance[7], particularly, arylesterases and cholinesterases that interact with insecticides either by break down of it[8] or by degradation of it[9]. These enzymes are important for insecticide breakdown and its isozymes have been amongst the most widely used molecular markers for this purpose.

Although, *S. oryzae* is recognized as an important pest of stored rice, a limited research work has been reported in Bangladesh, especially on molecular basis of toxicology. Hence, an attempt was taken to determine the toxic potency (LC_{50} and LC_{90}) of cypermethrin and malathion and its effects on esterases in above mentioned species.

EXPERIMENTAL

The adults of *Sitophilus oryzae* were collected from naturally infested stored rice of Central Storage Department, Tejgoan, Dhaka to be reared in the laboratory (Genetics and Molecular Biology Lab., Department of Zoology, University of Dhaka) for the continuous supply of experimental insects at 60-70% of relative humidity and 28-32^{0}C of temperature. Serial dilutions of formulated cypermethrin and malathion were made by adding tap water followed by Islam[10]. For each test, fresh solutions of the insecticides were prepared considering active ingredients (ppm) and were used on the same day. 5 ml of insecticides of each dose were mixed with 5 gram of parboiled polished rice, which was used as carrier. Screening test was standardized by splitting a range of doses into smaller fragments and for the explanatory test, three replica of each dose was maintained in same environmental condition. Mortality was counted either hourly or daily basis separating dead from alive and was stored on deep freeze (-20^{0}C) for molecular study. The dose response data were analyzed using Biostat 2009, SPSS and Microsoft Excel Program. To see the impact of pesticides on esterases, 5-6 pesticide killed adults (irrespective of male or female) were taken into separate eppendrof tubes and squashedseparately in TBE buffer (proportionate to the sample weight; 0.016 g ~ 40 µl), centrifuged at 12500 rpm for 15 min and aliquots from each sample (15 µl) was loaded on the gel slots for electrophoresis[11]. The entire technique for PAGE was followed by Shahjahan et al.[12] and the electrophoretic bands of esterase isozymes resulting from stained gel with napthyl acetate were assigned to increasing numbers based on decreasing mobility following Richardson[13].

RESULTS AND DISCUSSION

Insecticidal effect: The mortality rate of the insects against cypermethrin and malathion increased with the increase of dose concentration in all the experimental cases. Exposure period also increased up to certain period of exposure, after which the exposed insects either were died or tend to become resistant at the exposed doses (**Table-1 and Table- 2).** Both insecticides were compared on the basis of killing potency (LC_{50} and LC_{90}) against the adult rice weevils *S. oryzae,* where, malathion was found much more toxic than that of cypermethrin **(Table- 3).**

Table-1: Mortality rate (average of three replicas) of adult rice weevil (*S. oryzae*) against cypermethrin at 60-70% of relative humidity and 28-32^{0}C of temperature (Number of exposed insects = 10).

Average cumulative mortality (%)											
N1	**D**	**Hours**					**D**	**Days**			
		1	**2**	**3**	**4**			**1**	**2**	**3**	**4**
	11000	100	100	100	100	**N2**	4.4	10	20	23	30
	1100	100	100	100	100		3.3	0	10	20	40
	110	80	100	100	100		2.2	0	0	0	0
	11	40	90	100	100		1.1	0	0	0	0
	1.1	40	60	80	100		0.55	0	0	0	0
C	0.00	0	0	0	0		0.00	0	0	0	0

N3	**D**	**Days**									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**-**
	99	40	70	100	100	100	100	100	100	100	-
	88	23	43	53	63	66	73	93	93	93	-
	77	26	46	63	73	76	83	93	96	100	-
	66	10	20	43	50	53	76	83	86	90	
	55	3	16	46	50	60	73	80	80	83	-
	44	3	13	40	50	63	80	83	83	93	-
C	0.00	0	0	0	0	0	0	0	0	0	-

N1, N2, N3 = Screening test number, **D**= Concentration of doses (ppm), **C**= Control

Table-2: Mortality rate (average of three replicas) of adult rice weevil (*S. oryzae*) against Malathion at 60-70% of relative humidity and 28-32^{0}C of temperature (Number of exposed insects = 10).

Average cumulative mortality (%)										
N1	**D**	**Hours**					**D**	**Days**			
		1	**2**	**3**	**24**			**1**	**2**	**3**	**4**
	110000	100	100	100	100		11	20	73	100	100
	11000	100	100	100	100	**N2**	9.9	7	60	93	100
	1100	20	40	60	100		8.8	0	36	77	100
	550	0	10	20	100		7.7	0	27	63	100
	440	0	0	0	100		6.6	0	33	60	93
	330	7	10	30	100		5.5	0	30	63	100
	220	13	20	20	100	**C**	0.00	0	0	0	0
	110	13	20	37	100	**N1, N2** = Screening test number					
	11	0	0	0	100	**D**= Concentration of doses (ppm)					
C	0.00	0	0	0	0	**C**= Control					

LC_{50} values of cypermethrin, against the adults were found to be 110.30, 85.63, 65.19, 60.31, 54.88, 36.91, 38.60, 38.12 and 34.82 ppm for day 1 to day 9 respectively, which indicated that the lethal concentration decreases with the increase of exposure period **(Table 3)**. LC_{50} values of malathion against the adults were found to be 12.19, 7.54, 6.54 and 5.22 ppm for 1 to 4 day respectively that showed a much lower lethal doses than that of cypermethrin but same tendency to exposure hour **(Table 3).**

Table-3: Comparative LC_{50} and LC_{90} values (in ppm) of the test insecticides against the adults of *S. oryzae* at 95% of confidence limits.

Name of insecticides	Observed LC_{50} (ppm)	Calculated LC_{50} (ppm)	95% confidence limits for LC_{50}		Observed LC_{90} (ppm)	Calculated LC_{90} (ppm)
			Lower	Upper		
Malathion						
1 day	>11	12.19	11.59	13.68	>11	14.39
2 days	9.9>->8.8	7.54	7.54	10.99	>11	20.37
3 days	<6.6	6.54	2.22	7.59	≈ 9.9	10.13
4 days	<5.5	5.22	3.82	5.80	<5.5	3.93
Cypermethrin						
1 day	>99	110.30	101.38	127.16	>99	202.36
2 days	>77	85.63	74.79	116.29	>99	142.40
3 days	77>->55	65.19	42.19	100.75	>88	139.04
4 days	55	60.31	39.14	92.90	>88	125.60
5 days	66>	54.88	28.83	104.46	>88	136.50
6 days	44>	36.91	6.61	206.21	>88	129.43
7 days	44>	38.60	26.72	45.85	≈ 77	68.92
8 days	44>	38.12	26.17	45.37	≈ 66	64.41
9 days	44>	34.82	11.84	102.33	<55	52.87

LC_{90} values of cypermethrin were found to be 202.36, 142.40, 139.04, 125.60, 136.50, 129.43, 68.92, 64.41 and 52.87 ppm for day 1 to day 9 respectively that showed a more or less same tendency as like LC_{50}. LC_{90} values of malathion were found to be 14.39, 20.37, 10.13, and 3.93 ppm for day 1 to day 4 respectively **(Table 3)**. Probit graphs obtained from different dose response data were shown in **Figure 1.**

Ahsan et al.[14] studied the effectiveness of cypermethrin against *S. oryzae* (L.) where LC_{50} values were found to be 19 mug/cm^2.Thaung et al.[15] examined the joint effects of temperature and insecticides on mortality of *S. oryzae* (L.) in wheat and maize where they found that the toxicity of cypermethrin decreased with increasing temperature. But, during the present experiment, temperature was controlled within a large incubator where aeration was also not a killing factor. Thus, present data represents solely the effects of insecticides.

Toxicological study against *S. oryzae* was also reported by several toxicologist viz. essential oils, oil from eucalyptus, terpenes and benzaldehyde[16]; neem extract RB-a in comparison with a pyrethroid[17] where they found a variety of responses. Champ et al.[18] established that malathion was effective only against

newlyhatched larvae of *S. oryzae*, but present communication represents malathion as a highly active insecticide against the adult that could be due to differences in the application of pesticide and environmental conditions. When organisms were treated with the insecticides, continuous nerve impulse transmission due to inhibition of acetylcholine esterase may in turn result sudden death of organisms. The condition may occur due to low production of esterase or lack of gene that produce this enzyme.

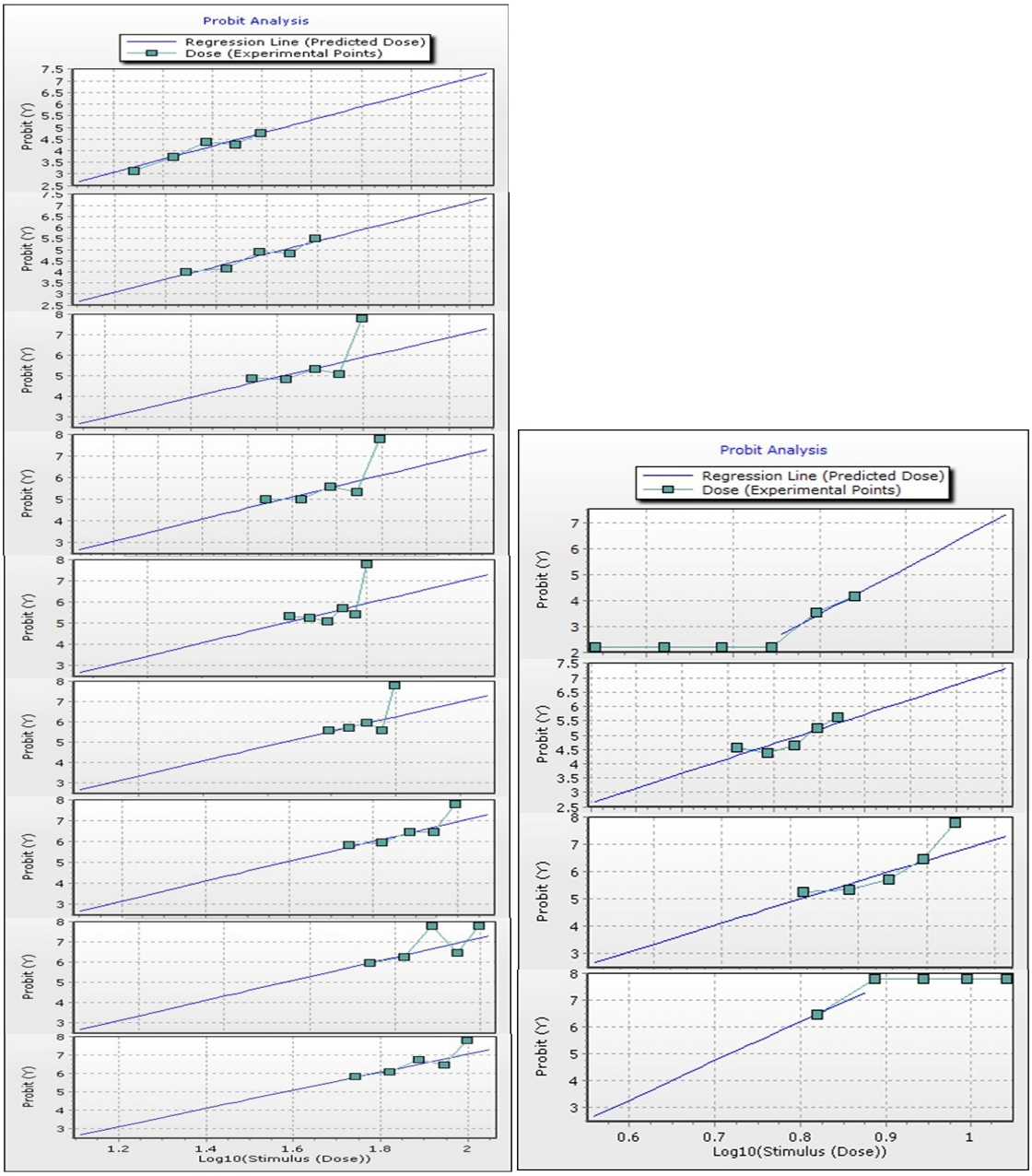

Figure 1: Adjusted probit values and predicted regression line of cypermethrin and malathion exposed dose response data in the adults of *S. oryzae*.

Esterase isozyme variability: Altogether, four (Est-1, Est-2, Est-3 and Est-4) and three (Est-2, Est-3 and Est-4) esterase bands were found in the cypermethrin killed and malathion killed samples of *S. oryzae* **(Figure 2)**, the details of which are stated bellow:

Cypermethrin-Day specific: Est-1 was found in 3rd to 8th day's cypermethrin treated dead samples, it was faintly stained in 3rd, 7th and 8th day's dead samples; medium stained in 4th & 6th day's dead samples and dark in 5th day's dead samples. This band was absent in control, cypermethrin treated live samples and in 2nd and 9th day's dead samples. **Est-2** was present in all the samples. It was dark in control, cypermethrin treated live samples and in 2nd, 6th and 8th day's dead samples. The band was medium stained in 3rd day's dead samples and faint in 4th, 5th, 7th and 9th day's dead samples. **Est-3** was absent in 2nd, 7th and 9th day's dead samples and present in all the others. It was dark in 3rd & 4th day's dead samples and in cypermethrin treated live samples; medium stained in control and in 6th day's dead samples. It was faint 5th and 8th day's dead samples. **Est-4** was present in all the samples. It was dark in control, cypermethrin treated live samples, 3rd, 6th, 7th& 8th day's dead samples and medium stained in 2nd, 4th, 5th & 9th day's dead samples. This band was faint in nowhere.

Cypermethrin-Dose specific: Est-2 was absent in samples dead at 66 ppm of dose concentration. It was dark in positive control and in samples dead at 99 ppm of dose concentration, medium stained in samples dead at 88, 77 & 55 ppm of dose concentrations. **Est-3** was absent in 66 & 55 ppm of dose concentrations. This band was dark in 99 ppm of dose concentration, medium stained in positive control, 88 & 44 ppm of dose concentrations. It was faint in 77 ppm dose concentration. **Est-4** became dark at 99, 88, 55 & 44 ppm of dose concentrations and faint in 66 ppm of dose concentration.

Malathion-Day specific: Est-2 was absent in 3rd and 4th day's malathion treated dead samples. It was medium stained in control and in 2nd day's malathion killed samples. This band was neither dark nor faint elsewhere. **Est-3** was absent in 4th day's malathion treated dead samples. It was dark in nowhere, medium stained in control and in 2nd day's dead samples. The band was faint in 3rd day's malathion treated dead samples. **Est-4** was dark in 2nd day's malathion killed samples, medium stained in control and in 3rd & 4th day's malathion treated dead samples.

Malathion-Dose specific: Est-2 was absent in dead samples treated withmalathion at 11, 9.9 & 8.8 ppm of dose concentrations. The band was medium stained in dead samples treated with malathion at 6.6 & 5.5 ppm of dose concentrations and in control. It was faintly stained at 7.7 ppm of dose concentration. **Est-3** was absent in malathion treated dead samples of 11 & 9.9 ppm of dose concentrations. In the remaining samples with the control it was medium stained. The band was dark in nowhere. **Est-4** was medium stained at 11 & 9.9 ppm of dose concentrations and in control. It was heavily stained in dead samples treated with 8.8, 7.7, 6.6 & 5.5 ppm of dose concentrations of malathion**.**

Altogether, four esterase bands were found in the studied species indicating the presence of four loci within the genome that was similar to Pintureau et al.[19]. Esterase enzymes play an important role in conferring or contributing to insecticide resistance in insects which has been shown in *Myzus persiace*[20], *Culex quinquefasciatus* and *C. pipiens*[21], *Lucilia cuprina*[22]and *Musca domestica*[23]. A new band Est-1 appeared after three days of exposure with cypermethrin which was not present in the untreated and treated alive samples **(Figure 2)**. As like present study, a supplementary band was also found in the resistant and revertant cells of *Crypthecodinium cohnii*[6]. This band also showed variation in terms of presence or absence and staining intensity. Maximum intense band was found after five days of exposure, after which the intensity gradually decreased and diminished after nine days of exposure. Elevated esterase banding patterns were also found in resistant populations of *Schizaphis graminum*[24], *Myzus persicae*[25], *Bemisia tabaci*[26] and *Anopheles albimanus*[27]. The correlation between the resistance response of insects after pesticides treatment and the change in protein expression level was found to be limited, but, the specific changes in the structure of acetylcholinesterase could lead to resistance[28].

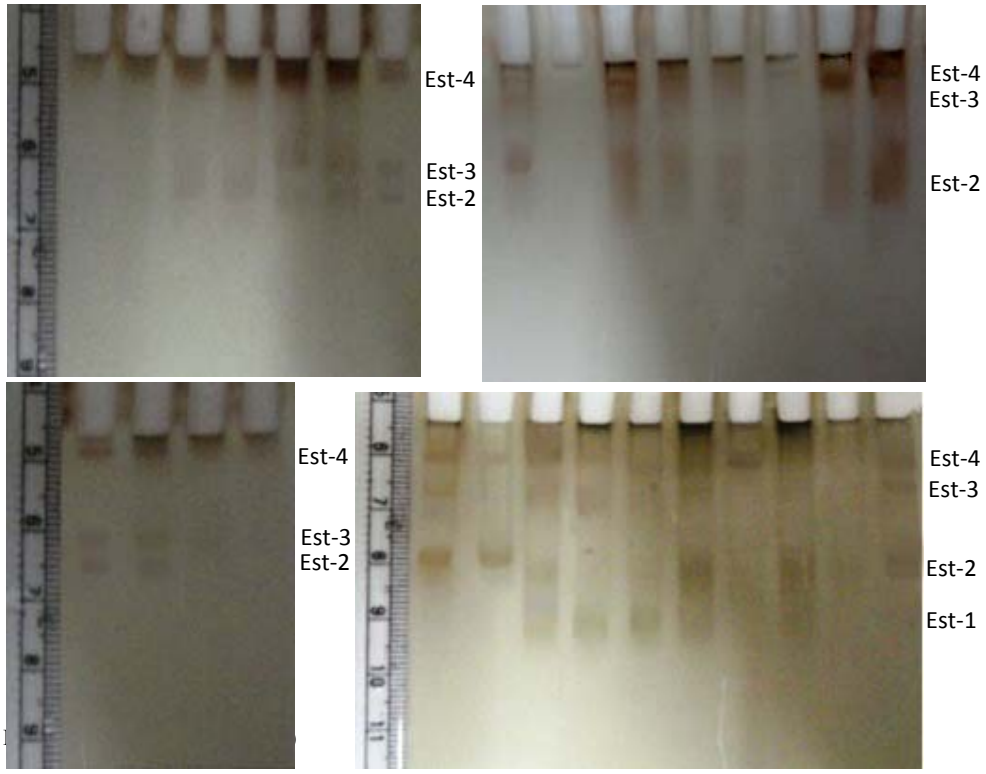

Figure 2: **Esterase isozyme banding pattern of *S. oryzae* stained in α naphthyl acetate on 7.5% polyacrylamide gels where, lane 1-6 denotes malathion treated dead samples at 11, 9.9, 8.8, 7.7, 6.6 and 5.5 ppm respectively and 7 denotes control (Plate A); 1 denotes positive control; 2 denotes negative control; 3-8 denotes cypermethrin treated dead samples at 99, 88, 77, 66, 55 and 44 ppm respectively (Plate B); 1 denotes control and 2-4 denotes 2nd to 4th day's malathion treated dead samples respectively (Plate C); 1 denotes control; 2-9 denotes cypermethrin treated dead samples of 2nd to 9th day's respectively and 10 denotes cypermethrin treated alive samples.**

At 99 ppm of dose concentration (cypermethrin), maximum staining intensity was found in all 3 bands (Est-2, Est-3 and Est-4) **(Figure 2)**. In general, the staining intensity decreased with declined dose concentration. At 66 ppm, Est-2 and Est-3 were absent and Est-4 was present as a faintly stained band. Esterase activity again increased from dose concentration of 55 ppm to 44 ppm. Flactuation in esterase activity was also observed in the pesticide treated dead samples of *Macrobrachium lamarrei*[11]. This variation in esterase production might be due to the sensitivity of pesticides. Samples treated with malathion showed that esterase activity of different isozymes decreased with the exposure time and some of the bands disappeared totally.

Those esterases were inhibited by malathion (organophosphate) could be grouped as carboxylesterases[29] but, it was difficult to represent any straight forward conclusion regarding the biochemical properties of these isozymes and need further investigation. Inhibiting action of cypermethrin and malathion on esterases was also found in *Bactrocera dorsalis* and *B. tau*[30]. Callaghan and Holloway[31] investigated increased variance about mean enzyme activity levels as an alternative biomarker of environmentally induced stress using *S. oryzae,* and *Culex pipiens.* When reared on a toxic food, *S. oryzae* showed very little change in mean activity in two detoxifying enzyme systems compared with enzyme activity on a relatively nontoxic food. When

transferred between food types, again there was very little effect on mean enzyme activity, but the variance about mean activity increased significantly, especially when the insects were transferred to the more toxic food.

CONCLUSION

Three esterase bands (Est-2, Est-3, Est-4) were detected in untreated and malathion treated samples of *S. oryzae,* but an extra band (Est-1) appeared only in the cypermethrin killed samples of 3-9 days of exposure, the staining intensity of which gradually decreased after five days of exposure. Samples treated with malathion showed that, esterase activity of different isozymes decreased with the exposure time and dose concentration. It was difficult to represent any straight forward conclusion regarding the susceptibility levels of insecticides based on present esterase study, as other pesticide inhibiting enzymes like glutathiontranferase, monoxigenase may contribute to detoxification activity.

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