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A comparative study on changes in acetylcholinesterase activity in *Samia ricini* on exposure to the sub lethal doses of Malathion and dimethoate

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Abstract
The toxic effects of Malathion and dimethoate, the two organophosphorous pesticides, on the acetylcholinesterase activity of *Samia ricini* was studied. 4th and 5th instar larvae of eri silkworm were exposed for 72 hours in 0.2 ppm and 0.4 ppm sub-lethal doses of Malathion and dimethoate. Exposure of eri silkworm to the sub lethal doses of both the pesticides caused severe decrease in acetylcholinesterase activity. Highest deviation was observed in the brain 4th instar larvae exposed to 0.4 ppm sub lethal dose of dimethoate at 72nd hour.

Keywords: eri silkworm larvae, brain tissue, leg muscles, acetylcholinesterase, malathion, dimethoate

Introduction

A wide variety of enzymes are inhibited by organophosphorous compounds. Most of these enzymes are esterases or proteases but a few are oxidases or dehydrogenases. The toxicity of the organophosphorous compounds result from their ability to inhibit the enzyme acetylcholinesterase (AChE). This in turn disrupts the transmission of nerve impulses. Malaoxon, an oxygen analog of malathion appears to be the active part that binds to AChE (O' Brien *et al.*, 1974) [17]. However, organophosphorous insecticides are widely used in agriculture and public health due to their low chemical and thermal stability, rapid decomposition in the environment (soil, water and surface of plants) into nontoxic substance.

Organophosphorous pesticides account for a major percentage of pesticides used in domestic, agriculture and industrial application throughout the world (Chambers *et al*., 1990) [6] because they are less damaging to the environment and they are less persistant than organochlorine pesticide. The run-offs from insecticide applied fields interfere with the non-target animals and disrupt the growth and physiology of the animals. Pesticides are generally neurotoxicants and thereby paralyzing the insects thus results in their death (Casida *et al*. 1983) [4]. The basic toxicity of the organophosphorous insecticides are exerted on the central nervous system via inhibition of acetylcholinesterase by phosphorylating the hydroxyl group of serine in the active site of the enzyme. These pesticides can also indirectly interact with the biochemical receptors of acetylcholine. The inactivation of cholinesterase by cholinesterase inhibitor, pesticides allows the accumulation of large amounts of acetylcholine, with resultant widespread effects that may be separated into four categories:

1. Potentiation of post-ganglionic parasympathetic activity.
2. Persistent depolarization of skeletal muscle.
3. Initial stimulation following depression of cells of the nervous system.
4. Variable ganglionic stimulation or blockade (Dreisbach, 1987) [8]

The inhibitory effects of insecticides on fish Acetylcholinesterase activity had been reported by various authors, metacid- 50 and carbaryl in *Channa punctatus* (Gosh and Bhattacharya,1992) [11] Elsan in *C. punctatus* (Rao *et al.*, 1987) [19] cypermethrin in *Channa striatus* (Ahamed *et al.*, 2015) [1].

The toxicity of some lipophilic inhibitors on acetylcholinesterase in insects is confirmed by Chadwik and Hill (1947) [5]. They observed a relevant correlation between the sign of poisoning and acetylcholinesterase inhibition in vivo. Thus on one hand, accumulation of acetylcholine has been reported as poison and induces toxic effects in insects, but Pant and Katiyar (1982) [17] indicated it as a growth promoter and to some extent as protector against toxic effects in the lepidopteran phytophagous eri silkworm *Philosamia ricini* on exposure to the chlorofungicide hexachlorobenzene. The role of acetylcholine as a protector to insects against insecticidal toxicity could be traced to choline released from acetylcholine which tones up the nutritional status of insects (Pant and Katiyar, 1982) [17].The fact that on one hand acetylcholine proves beneficial to insects and on the other, accumulation thereof in them produces toxicity is rather intriguing.

Malathion is an organophosphorous pesticide which is extensively used to control a wide range of sucking and chewing pests of field crops, fruits and vegetables. It has many structural similarities with naturally occurring compounds, and their primary target of action in insects is the nervous system; it also inhibits the release of the acetylcholinesterase at the synaptic junction. In the control of nerve excitability at post synaptic sites acetylcholinesterase plays a key role. Malathion like other phosphorous compounds is reported to inhibit the acetylcholinesterase. Inhibition of liver acetylcholinesterase (AChE) activity is generally regarded as a useful indicator of poisoning by these pesticides. Health (1961) [12] expressed that "almost every aspect of the action of phosphorous compounds on insects is debatable as many of the techniques applied to the study of mammals are not applicable to the study of insects". The

abnormal enzyme activity induced by phosphorous compounds damages the nervous system irreparably.

The literature available deals with cholinergic transmission (Breer, 1981) [2] and the effects of neurotoxic substances on the central nervous system (CNS) of different species, mainly vertebrates (Shankaran *et al.*, 1981) [20]. Further it is known that the mode of action of neurotoxic substances on the central nervous system in insects is similar in quality with that of vertebrates (Doull, 1976) [7]. Therefore the present investigation is taken to study the comparative study on changes in acetylcholinestrase activity in *Samia ricini* on exposure to the sub lethal doses of malathion and dimethoate.

Materials and Methods

The Eri silkworm (*Samia ricini*) was selected as the test organism for the investigation. Healthy, disease free seeds of eri silkworm were collected from "Eri Silkworm Seed Production Centre, Mirza and Khanapara (Central Silk Board)" and reared up to the pupation stage. The larvae were fed with the green variety of castor leaves (*Ricinus communis*). Fresh castor leaves were supplied three to four times a day with a care so that no larvae suffer from starvation. Proper cleanliness and hygiene were maintained during the time of rearing to prevent the occurrence of any diseases in larvae. 4th and 5th instar larvae were used for experimental purpose.

After the emergence of 3rd instar larvae they were divided into two groups - control group (Group I) and Experimental group (Group II). Malathion 50 EC and Dimethoate 30 EC solution were taken as representative of organophosphorous pesticides.

The normal control group (Group I) consists of normal healthy 3rd instar larvae cultured separately, from which required number of 4th and 5th instar larvae were sacrificed on the fixed dates along with the experimental groups.

The experimental Group (Group II) was further divided into two sub groups- Gr. IIA and Gr. IIB. Larvae of the sub-group II (A) were treated with pesticide Malathion in two different doses – 0.2 ppm (Gr.II Ai) and 0.4ppm (Gr.IIAii). Sub group II (B) was treated with two different doses of dimethoate – 0.2 ppm (Gr.II Bi) and 0.4 ppm (Gr.IIBii).

The study parameter was estimated in both normal control group and pesticide treated experimental group from the day of treatment at three different time intervals (24th hour, 48th hour and 72nd hour) in two different larval instars (4th and 5th instars). A Pilot experiment was done to find out LD_{50}values (Finney, 1971) [9] of the pesticides for both 4th and 5th instar larvae of eri silkworm of which two sub-lethal doses (0.2ppm and 0.4 ppm) were selected. The selected doses of both the pesticides had been administered and periodically monitored by taking hourly changes.

The LD_{50} of Malathion for 4th and 5th instar larvae were found as 1.32 ppm and 1.66 ppm respectively. The LD_{50} of Dimethoate for 4th and 5th instar larvae were found as 0.88 ppm and 1 ppm respectively. Two sublethal doses, 0.2 ppm and 0.4 ppm of both the pesticides were selected for the experimental purpose.

4th and 5th instar larvae of Eri silkworms were dissected alive for collection of tissues i.e. brain tissues, leg muscles. The collected tissues were dried over filter paper and weighed and recorded. The tissue homogenate was prepared in deionized water with the help of homogenizer. The tissues were collected from both normal control as well as experimental groups on the desired time intervals i.e. at 24th hour, 48th hour and 72nd hour of treatment for both 4th and 5th instar larvae.

Acetylcholinesterase (AChE) activity was estimated by Cholinesterase Monokinetic trinder method (Raghuramulu *et al*., 1983) [18].

Results and Discussion

The experimental animal in the present study setup was categorized into 4 groups. The first two groups were under the supplementation with two different doses of Malathion (0.2 ppm and 0.4 ppm) and the other two groups are administered with two different doses of Dimethoate i.e. 0.2 ppm and 0.4 ppm.

Acetylcholinesterase activity, in brain (Table 1, 2) of the 4th instar larvae under 0.2 ppm malathion exposure shows an overall dampening effect in 4th instar larvae throughout the experimental period with slight fluctuations. It was more pronounced in case of higher doses of pesticides with highest decline of more than 30% in case of 0.4 ppm of both the pesticides whereas in lower dose of 0.2 ppm the inhibitory effect was observed to be only about 10% below the normal base line (Fig. 1.). But the 5th instar larvae treated with pesticides exhibit a different trend and inhibitory effect was not prominent in case of Malathion (Fig. 2.). The enzyme activity was found to increase gradually from the initial to the terminal part of the experimental period (about 25% above normal). Whereas, Dimethoate in both the concentrations was observed to be effective in decreasing the Acetylcholinesterase activity.

In the present investigation, the leg muscles (Table 3, 4) of 4th instar larvae treated with two different doses of malathion and dimethoate showed an overall and gradual decrease in the AChE activity till the end of the experimental period. The percent deviation showed a significant dose dependent decrease i.e. with the increasing doses of the pesticides the enzyme activity declines more. The highest deviation was recorded at 72nd hour on 0.4 ppm dimethoate exposure. (Fig-3).

From the present investigation it was clear that in the leg muscles of 5th instar larvae on exposure to malathion and dimethoate shows an overall decrease in AChE activity below the normal baseline except the 0.2 ppm malathion treated group which showed a different trend (Fig. 4). When the larvae were exposed to lower dose of Malathion (0.2 ppm) at 24th hour, the activity of AChE rises initially (1.44%) above the normal baseline which declines at 48th hour of exposure (-2.35%) and then again rised to 1.42% at 72nd hour of exposure. Highest deviation was observed at 0.4 ppm dimethoate exposure (-19.28%).

Between the Brain tissue and leg muscles the lowest AChE activity was observed in the leg muscles. On pesticidal exposure, the brain tissues of 4th instar larvae was more affected and shows the highest percent deviation (-32.37%) below the normal baseline at 72nd hour during dimethoate exposure. It was noted in the present study that of the two larval instars the enzyme activity in the 4th instar larvae were more affected than that of the 5th instar larvae. It was also noted from the results of the present experimental set up that between Malathion and Dimethoate, used as the

representative of the organophosphorous pesticides, here impact of Dimethoate was more in *Samia ricini* than that of Malathion.

Many pesticides exert acute toxicity by inhibiting the nervous system enzyme, acetylcholinesterase (Viarengo, 1989) [22]. This inhibition leads to the accumulation of the neurotransmitter, acetylcholine (Galloway *et al*., 2005) [10]. The measurement of AChE activity was employed as a useful dose dependent biomarker of exposure, in both vertebrates and invertebrates (Hyne and Maher, 2003) [13].

Accumulation of Acetylcholine had been shown to poison and induce toxic effects in insects (Pant and Katiyar, 1982) [17]. The reduction of AChE activity was observed in the cockroach on azadirachtin (AZA) exposure (Singh and Singh, 2000) [21]. Oral administration of highest doses of Malathion was reported to significantly decrease acetylcholinesterase activity in the whole body of *G. mellonela* larvae. The overall decreasing trend observed in the present study set up supports the findings of many previous researchers (Pant and Katiyar, 1982) [17].

Conclusion

The results obtained in the present study tallies with those of the findings of earlier studies with different animal. It may be concluded that the pesticides minimum of about 22.72 % below the lethal dose can also be effective in depressing the acetylcholinesterase activity in the particular sericogenous insect.

Table 1: Showing the mean values of Acetylcholinesterase activity (Unit/mg protein) in Brain of 4th and 5th instar larvae of *Samia ricini* in different experimental groups at different hour's interval.

Groups		Mean, SEM, SD, CV%, % deviation	Hours of treatment (4th Instar)			Hours of treatment (5th Instar)		
			24th hr	48th hr	72nd hr	24th hr	48th hr	72nd hr
Normal		Mean	11.82			24.90		
		±SEM	0.56			0.95		
		±SD	1.68			2.85		
		CV %	14.21			11.45		
Normal control (Group I)		Mean	11.82	11.06	11.11	24.90	23.56	22.90
		±SEM	0.56	0.57	0.83	0.95	0.64	0.38
		±SD	1.68	1.70	2.50	2.85	1.91	1.15
		CV %	14.21	15.37	22.50	11.45	8.11	5.02
		% deviation from Normal	0	-6.43	-6.00	0	-5.38	-8.03
Malathion treated	0.2ppm Group II (Ai)	Mean	11.04	10.96	9.76	24.12	23.40	23.06
		±SEM	0.87	0.60	0.86	0.66	0.43	0.62
		±SD	2.61	1.81	2.59	1.98	1.30	1.85
		CV %	23.64	16.51	26.54	8.21	5.56	8.02
		% deviation from Normal control	-6.60	-0.90	-12.15	-3.13	-0.68	0.70
	0.4ppm Group II (Aii)	Mean	9.66	7.52	7.78	23.19	24.45	28.28
		±SEM	0.66	0.41	0.48	0.75	0.56	0.81
		±SD	1.99	1.24	1.44	2.24	1.67	2.42
		CV %	20.60	16.49	18.51	9.65	6.83	8.55
		% deviation from Normal control	-18.27	-32.01	-29.97	-6.87	3.78	23.49
Dimethoate Treated	0.2ppm Group II (Bi)	Mean	11.28	9.82	9.40	23.94	22.90	21.51
		±SEM	0.58	0.68	0.50	0.68	0.77	0.64
		±SD	1.74	2.03	1.48	2.04	2.32	1.93
		CV %	15.43	20.67	15.74	8.52	10.13	8.97
		% deviation from Normal control	-4.57	-11.21	-15.39	-3.86	-11.29	-6.07
	0.4ppm Group II(Bii)	Mean	9.98	7.48	6.86	20.90	19.40	16.26
		±SEM	0.51	0.86	0.62	0.77	0.48	0.72
		±SD	1.53	2.59	1.86	2.30	1.43	2.16
		CV %	15.33	34.63	27.11	11.00	7.37	13.28
		% deviation from Normal control	-15.57	-32.37	-38.25	-16.06	-17.66	-29.00

Table 2: Showing the comparison of mean values of Acetylcholinesterase activity (Unit/mg protein) in the Brain between different groups of 4th and 5th instar larvae of Eri Silkworm at different hour's interval.

t between group of silkworms		Hours of treatment (4th instar)			Hours of treatment (5th instar)		
		24th hr	48th hr	72nd hr	24th hr	48th hr	72nd hr
Between Normal and Group I	t	0	0.95	0.71	0.17	1.17	1.95
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group I and Group IIA (i)	t	0.75	0.12	1.13	1.14	0.21	0.22
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group I and Group IIA (ii)	t	2.50	5.04	5.04	1.41	1.04	6.01
	p	<0.05	<0.01	<0.01	>0.05	>0.05	<0.01
	df	18	18	18	18	18	18

Between Group I and Group IIB (i)	t	0.67	2.01	1.76	0.82	0.66	1.87
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group I and Group IIB (ii)	t	2.43	3.47	4.10	3.27	5.2	8.16
	p	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01
	df	18	18	18	18	18	18
Between Group IIA (i) and Group IIA (ii)	t	1.27	4.73	2.01	0.93	1.49	5.12
	p	>0.05	<0.01	>0.05	>0.05	>0.05	<0.01
	df	18	18	18	18	18	18
Between Group IIB (i) and Group IIB (ii)	t	1.69	2.13	3.19	2.96	3.86	5.45
	p	>0.05	>0.05	<0.01	<0.01	<0.01	<0.01
	df	18	18	18	18	18	18
Between Group IIA (i)and Group IIB (i)	t	0.23	1.26	0.36	0.18	0.57	1.74
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group IIA (ii)and Group IIB (ii)	t	0.39	0.04	1.17	2.13	6.85	11.09
	p	>0.05	>0.05	>0.05	>0.05	<0.01	<0.01
	df	18	18	18	18	18	18

Table 3: Showing the mean values of Acetylcholinesterase activity (Unit/mg protein) in the Leg Muscles of 4th and 5th instar larvae of *Samia ricini* in different experimental groups.

Groups		Mean, SEM, SD, CV%, % deviation	Hours of treatment (4th Instar)			Hours of treatment (5th Instar)		
			24th hr	48th hr	72nd hr	24th hr	48th hr	72nd hr
Normal		Mean	13.08			26.32		
		±SEM	0.53			0.79		
		±SD	0.60			2.38		
		CV %	4.59			9.04		
Normal control (Group I)		Mean	13.08	13.06	13.16	26.32	25.99	26.36
		±SEM	0.53	0.79	0.78	0.79	0.73	0.62
		±SD	0.60	2.37	2.34	2.38	2.20	1.85
		CV %	4.59	18.15	17.78	9.04	8.46	7.01
		% deviation from Normal	0	-0.15	0.61	0	-1.25	0.15
Malathion Treated	0.2ppm Group II (Ai)	Mean	13.44	12.14	11.32	26.70	25.38	25.12
		±SEM	0.72	0.59	0.57	0.83	0.72	0.77
		±SD	2.16	1.78	1.71	2.48	2.15	2.31
		CV %	16.07	14.66	15.11	9.29	8.47	9.20
		% deviation from Normal control	2.75	-7.04	-13.98	1.44	-2.35	1.42
	0.4ppm Group II (Aii)	Mean	12.26	11.12	10.10	24.40	24.10	23.22
		±SEM	0.64	0.20	0.73	0.87	0.88	0.84
		±SD	1.92	0.59	2.19	2.62	2.64	2.54
		CV %	15.66	5.31	21.68	10.74	10.95	10.94
		% deviation from Normal control	-6.27	-14.85	-23.25	-7.29	-7.27	-10.66
Dimethoate Treated	0.2ppm Group II (Bi)	Mean	13.42	12.30	10.90	25.83	25.44	24.44
		±SEM	0.71	0.63	0.32	0.71	0.82	0.63
		±SD	2.14	1.89	0.97	2.12	2.47	1.89
		CV %	15.95	15.36	8.26	8.21	9.70	7.73
		% deviation from Normal control	2.60	-5.82	-17.17	-1.86	-2.12	-5.96
	0.4ppm Group II(Bii)	Mean	10.38	9.88	8.90	24.08	22.40	20.98
		±SEM	0.54	0.69	0.76	0.73	0.69	0.57
		±SD	1.63	2.08	2.27	2.19	2.08	1.72
		CV %	15.70	21.05	25.51	9.09	9.28	8.19
		% deviation from Normal control	-20.64	-24.35	-32.37	-8.51	-13.81	-19.28

Table 4: Showing the comparison of mean values of Acetylcholinesterase activity (Unit/mg protein) in the leg muscles between different groups of 4th and 5th instar larvae of Eri Silkworm at different hours interval.

t between group of silkworms		Hours of treatment (4th instar)			Hours of treatment (5th instar)		
		24th hr	48thhr	72ndhr	24th hr	48th hr	72nd hr
Between Normal and Group I	t	0	0.21	0.08	0	0.31	0.41
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group I and Group IIA (i)	t	0.80	0.93	1.90	0.33	0.59	1.25
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18

Between Group I and Group IIA (ii)	t	0.99	2.38	2.86	1.63	1.59	3.01
	p	>0.05	<0.05	<0.05	>0.05	>0.05	<0.05
	df	18	18	18	18	18	18
Between Group I and Group IIB (i)	t	0.38	0.75	2.68	0.46	0.50	2.17
	p	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group I and Group IIB (ii)	t	3.57	3.03	3.91	2.08	3.57	6.39
	p	<0.01	<0.05	<0.01	>0.05	<0.01	<0.01
	df	18	18	18	18	18	18
Between Group IIA (i) and Group IIA (ii)	t	1.22	1.64	1.32	1.91	0.05	1.67
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group IIB (i) and Group IIB (ii)	t	3.41	2.59	2.43	1.72	2.84	4.07
	p	<0.01	<0.05	<0.05	>0.05	<0.05	<0.01
	df	18	18	18	18	18	18
Between Group IIA (i)and Group IIB (i)	t	0.02	0.19	0.64	0.80	0.05	0.68
	p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group IIA (ii)and Group IIB (ii)	t	2.24	1.74	1.14	0.28	1.52	2.21
	p	<0.05	>0.05	>0.05	>0.05	>0.05	<0.05
	df	18	18	18	18	18	18

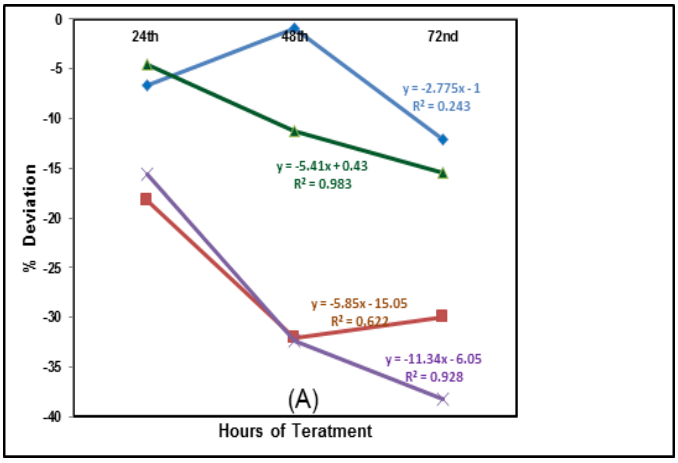

Fig 1

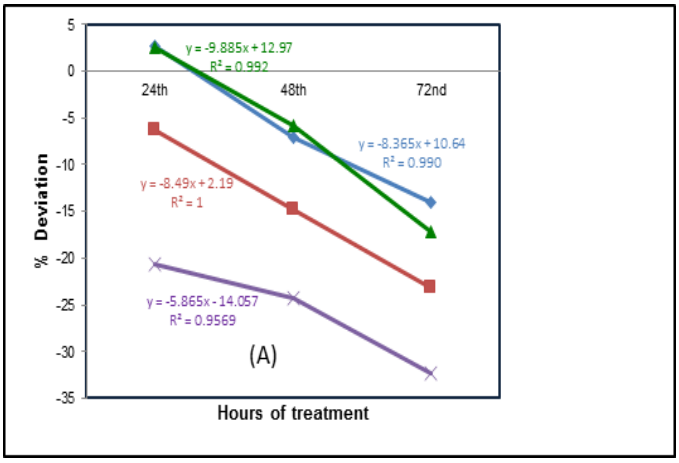

Fig 3

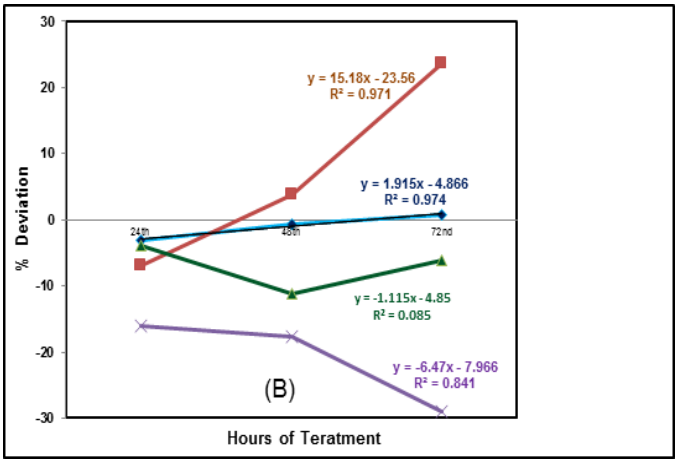

Fig 2: Presenting the % deviation of Acetylcholinesterase activity (U/mg of protein) in the Brain of the experimental groups from the mean values of the normal group. (A) in 4th instar larvae, (B) in 5th instar larvae of *S. ricini*.

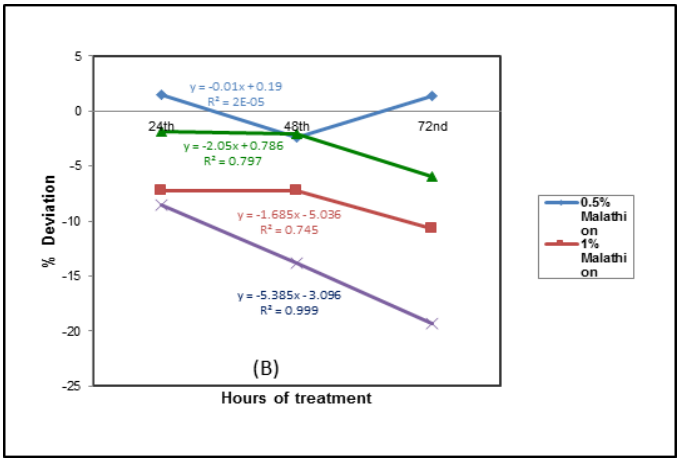

Fig 4: Presenting the % deviation of Acetylcholinesterase activity (U/mg of protein) of the experimental groups from the mean values of the normal group in leg muscles. (A) in 4th instar larvae, (B) in 5th instar larvae of *S. ricini*.

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