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Changes in Cytochrome P450 activity in 4th and 5th instar larvae of *Samiaricini* on exposure to the two organophosphorous pesticides, malathion and dimethoate- A comparative study

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ABSTRACT: Cytochrome p450 dependent monooxygenases are an extremely important enzyme of metabolic system involved in the metabolism of xenobiotics and endogenous compounds. In the present investigation the activity of cytochrome p450 in 4th and 5th instar larvae of eri silkworm on treatment with the two sub lethal doses (0.2 ppm and 0.4 ppm)of malathion and dimethoate has been assessed. Exposure of eri silkworm to the sub lethal doses of both the pesticides causes highly increased cytochrome p450 activity. Highest deviation is observed in the midgut tissues of 5th instar larvae exposed to 0.4 ppm sub lethal dose of dimethoate at 72nd hour.

KEYWORDS: Eri silkworm larvae, midgut, fat body, cytochrome P450, malathion, dimethoate

I. INTRODUCION

Pesticides and toxicants always exert a systemic effect on both target and non target organisms, which could be identified by studying the mobilization of different key substrates and by following the activity of important enzymes. Chemical control of insect pests is the most dominant approach at present. It is responsible for many health hazards among animals. Variation in cellular or biochemical components or process, structures or functions i.e., measurable in a biological system or samples provide information on the amplitude of response of an organism in relation to the magnitude of chemical insult and on the relation between biological effects and environmental contamination. The often used bio-marker to evaluate xenobiotic toxicity is the mixed function monooxygenases (MFO), indicators of chemical stress due to different pesticides (Fossi and Leonzio, 1994). The MFO system plays a determinant role in the initial stage of detoxification of xenobiotic lipophilic compounds. One of the basic features of this system is its substrate inducibility. De Matteis (1988) reported that the xenobiotic compounds actively stimulate the synthesis of new functional proteins. Thus induction is a quantitative or semi quantitative signal of the presence of xenobiotic substances. The inductive response is substrate specific, i.e., a given class of xenobiotics can specifically induce a single class of enzymes because of its substrate specificity-inducibility. The MFO system is one of the most specific biomarkers enabling the identification of various families of liposoluble chemicals responsible for induction. This system contains a number of different components with cytochrome P-450 occupying a key position. The reaction catalysed by this system involves oxidations, usually from carbon atoms to the alcohol level. However, a number of different kinds of oxidations are catalysed and the term mixed function oxygenase (MFO) is frequently used. The oxidising agent is molecular oxygen but only one atom of oxygen molecule is used, so this is frequently also referred to as monooxygenase.

The P-450 monooxygenases are ubiquitous enzymes, found from bacteria to mammals. In insects these activities are essential for the synthesis and the degradation of the steroid moulting hormones and juvenile hormones and also in the

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metabolism of pheromones. The P-450 enzymes are also important for the adaptative mechanisms of insects to the toxic chemicals synthesized by their host plants (Gould, 1984). Frank and Fogleman (1992) and Berenbaum*et al*. (1990) reported that this adaptation is notable for the fact that the biosynthesis of these enzymes can be induced by the presence of the toxins in the food. P-450 monooxygenase activities can be involved in the metabolism of virtually all insecticides, leading to an activation of the molecule or, more generally, to a detoxification (Agosin, 1985). Taylor and Feyereisen in 1996 observed that for some insects, this detoxification is so active that the insecticide does not reach its molecular target before being metabolized and degraded by these enzymes: such individuals are resistant to insecticides.

Feyereisen (1999) reported that the cytochrome P450s (monooxygenases) compose a large enzyme superfamily involved in the detoxification of many xenobiotics and several studies have shown that increased cytochrome P450s catalysed metabolism is an important mechanism of pyrethroid resistance in many insect species (Maitraet al., 1996).

Cytochrome P-450 is the site where both the substrate and oxygen bind. The most characteristic feature of the reaction cycle catalysed by cytochrome P-450 is the ability of the heme iron to undergo cyclic oxidation-reduction reactions in relation to substrate binding and oxygen activity. The reactions of a particular xenobiotic is determined by the structure of the molecule. The cytochrome P-450 (CYP) dependent monooxygenases represent the first line of defense against toxic lipophilic chemicals because they catalyse reactions involving incorporation of an atom of molecular oxygen into the substrate (Guengerich*et al*., 1994). The resulting increase in hydrophilicity facilitates further metabolic processing and excretion. Xenobiotics enhance the expression of P-450 gene through receptor and non receptor mediated mechanisms. The differences in detoxifying enzymes are important in determining the differential toxicity of organophosphorous compounds in different organisms.

II. MATERIALS AND METHODS

The Eri silkworm (*Samiaricini*) 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 (*Ricinuscommunis*). 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. 4^{th} and 5^{th} instar larvae were used for experimental purpose.

After the emergence of 3^{rd} 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 3^{rd} instar larvae cultured separately, from which required number of 4^{th} and 5^{th} instar larvae were sacrificed on the fixed dates along with the experimental groups.

The experimental Group (Group II) is 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 cytochrome P450 activity was estimated in both normal control group and pesticide treated experimental group from the day of treatment at three different time intervals (24^{th} hour, 48^{th} hour and 72^{nd} hour) in two different larval instars (4^{th} and 5^{th} instars). A Pilot experiment was done to find out LD_{50}values (Finney, 1971) of the pesticides for both 4^{th} and 5^{th} instar larvae of eri silkworm. The LD_{50} of Malathion for 4^{th} and 5^{th} instar larvae were found as 1.32 ppm and 1.66 ppm respectively. The LD_{50} of Dimethoate for 4^{th} and 5^{th} 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. The selected doses of both the pesticides had been administered and periodically monitored by taking hourly changes.

4^{th} and 5^{th} instar larvae of Eri silkworms were dissected alive for collection of tissues i.e. midgut tissues and fat bodies. 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 24^{th} hour, 48^{th} hour and 72^{nd} hour of treatment for both 4^{th} and 5^{th} instar larvae.

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Activities of cytochrome P-450 in the different tissues of eri silkworm were estimated by following the method of Omura and Sato (1964).

III. RESULTS

Changes in Cytochrome P450 activity (nmol/mg protein) in different tissues of 4th and 5th instar larvae of *S. ricini*during malathion and dimethoate treatments are discussed below-

A. In fat bodies

The mean, SEM, SD, CV% values and percentage deviation from normal control mean values of Cytochrome P450 activity (nmol/mg protein) in fat bodies of 4th and 5th instar larvae of *S. ricini* in different experimental groups are presented in Table 1. The comparison of mean values with significance of variance are presented in Table 2.

The mean values (mean±SEM) of Cytochrome P450 activity in fat bodies of normal control group of *S. ricini*at 24th, 48th and 72nd hours in 4th instar larvae were recorded as 0.14±0.01, 0.15±0.01 and 0.16±0.01 nmol/mg protein respectively.The observed mean values (mean±SEM) of Cytochrome P450 activity in the 4th instar larvae treated with different doses of both the pesticides showed a gradual increasing trend with the increasing doses and time period. Maximum activity was observed at 48th and 72nd hours of exposure with the mean value of 0.21±0.01 nmol/mg protein and in 5th instar larvae maximum activity was observed at 72nd hour of exposure with the mean value (mean±SEM) of 0.24±0.01 nmol/mg protein when treated with 0.4 ppm dimethoate.

B. In Midgut

The mean, SEM, SD, CV% values and percentage deviation from normal control mean values of Cytochrome P450 activity (nmol/mg protein) in midgut of 4th and 5th instar larvae of *S. ricini* in different experimental groups are presented in Table 3. The comparison of mean values with significance of variance are presented in Table 4.

The mean values (mean±SEM) of Cytochrome P450 activity in the midgut of normal control group of *S .ricini*at 24th, 48th and 72nd hours of 4th instar larvae were observed as 0.11±0.01, 0.13±0.01 and 0.13±0.01 nmol/mg protein respectively. Maximum activity is observed from the results at 72nd hour of exposure with the mean value of 0.19±0.01 nmol/mg protein treated with 0.4 ppm dimethoate but in 5th instar larvae this value rises to 0.21±0.01 nmol/mg protein which was highly significant. However, minimum activity was recorded on 0.2 ppm malathion exposure at 24th hour.

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Table 1 : Showing the mean values of Cytochrome P-450 activity (nmol/mg protein) in fat bodies of 4th and 5th instar larvae of *Samiaricini* in different experimental groups.

Groups		Mean,SEM,SD, CV%, % deviation	Hours of treatment (4th Instar)			Hours of treatment (5th Instar)		
			24thhr	48thhr	72ndhr	24thhr	48thhr	72ndhr
Normal		Mean	0.14			0.17		
		±SEM	0.01			0.01		
		±SD	0.04			0.02		
		CV %	28.57			11.76		
Normal control (Group I)		Mean	0.14	0.15	0.16	0.17	0.17	0.17
		±SEM	0.01	0.01	0.01	0.01	0.01	0.01
		±SD	0.04	0.03	0.03	0.02	0.02	0.02
		CV %	28.57	20.00	18.75	11.76	11.76	11.76
		% deviation from Normal	0	7.15	14.29	0	0	0
Malathion treated	0.2ppm Group II (Ai)	Mean	0.15	0.16	0.15	0.17	0.17	0.17
		±SEM	0.01	0.01	0.01	0.01	0.01	0.01
		±SD	0.02	0.01	0.02	0.02	0.02	0.02
		CV %	13.33	6.25	13.33	11.76	11.76	11.76
		% deviation from Normal control	7.14	6.67	-6.25	0	0	0
	0.4ppm Group II (Aii)	Mean	0.15	0.16	0.17	0.18	0.20	0.21
		±SEM	0.01	0.01	0.01	0.01	0.01	0.001
		±SD	0.02	0.02	0.02	0.02	0.02	0.004
		CV %	13.33	12.50	11.76	11.11	10	19.05
		% deviation from Normal control	7.14	6.67	6.25	5.88	17.65	23.53
Dimethoate Treated	0.2ppm Group II (Bi)	Mean	0.15	0.15	0.17	0.16	0.18	0.19
		±SEM	0.01	0.01	0.01	0.01	0.01	0.01
		±SD	0.03	0.02	0.04	0.03	0.03	0.02
		CV %	20.00	13.33	23.53	18.75	16.67	10.53
		% deviation from Normal control	7.14	0	6.25	23.59	5.88	11.76
	0.4ppm Group II(Bii)	Mean	0.20	0.21	0.21	0.18	0.21	0.24
		±SEM	0.01	0.01	0.01	0.01	0.01	0.01
		±SD	0.05	0.04	0.02	0.04	0.03	0.04
		CV %	25	19.05	9.52	22.22	14.28	16.67
		% deviation from Normal control	42.86	40.00	31.25	5.88	23.53	41.18

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Table 2 : **Showing the comparison of mean values of Cytochrome P450 activity (nmol/mg protein) in Fat bodies 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)		
		24thhr	48thhr	72ndhr	24thhr	48thhr	72ndhr
Between Normal and Group I	T	0	0.71	1.41	0	0	0
	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	0.71	0.71	0.71	0	0	0
	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	0.71	0.71	0.71	0.71	2.12	2.83
	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	0.71	0.71	0.71	0.71	0.71	1.41
	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 IIB (ii)	T	4.24	4.24	3.54	0.71	2.83	4.95
	P	<0.01	<0.01	<0.01	>0.05	<0.05	<0.01
	df	18	18	18	18	18	18
Between Group IIA (i) and Group IIA (ii)	T	0	1.64	18.22	0	0.71	2.83
	P	>0.05	>0.05	<0.01	>0.05	>0.05	<0.05
	df	18	18	18	18	18	18
Between Group IIB (i) and Group IIB (ii)	T	3.54	4.24	2.83	1.41	2.12	3.54
	P	<0.01	<0.01	<0.05	>0.05	<0.05	<0.05
	df	18	18	18	18	18	18
Between Group IIA (i)and Group IIB (i)	T	0	0.71	1.41	0.71	0.71	1.41
	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	3.54	2.83	0	0.71	2.12
	P	>0.05	<0.01	<0.05	>0.05	>0.05	<0.05
	df	18	18	18	18	18	18

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Table 3 : Showing the mean values of Cytochrome P-450 activity (nmol/mg protein) in midgut of 4th and 5th instar larvae of *Samiaricini* in different experimental groups

Groups		Mean,SEM,SD, CV%, % deviation	Hours of treatment (4th Instar)			Hours of treatment (5th Instar)		
			24thhr	48thhr	72ndhr	24thhr	48thhr	72ndhr
Normal		Mean	0.11			0.13		
		±SEM	0.01			0.01		
		±SD	0.03			0.02		
		CV %	27.27			15.38		
Normal control (Group I)		Mean	0.11	0.13	0.13	0.13	0.14	0.14
		±SEM	0.01	0.01	0.01	0.01	0.01	0.01
		±SD	0.03	0.03	0.03	0.02	0.02	0.04
		CV %	27.27	23.08	23.08	15.38	14.29	28.57
		% deviation from Normal	0	1.18	18.18	0	7.69	7.69
Malathion treated	0.2ppm Group II (Ai)	Mean	0.11	0.12	0.13	0.14	0.15	0.15
		±SEM	0.01	0.01	0.01	0.01	0.01	0.01
		±SD	0.01	0.01	0.01	0.01	0.01	0.01
		CV %	9.09	8.83	7.69	7.14	6.67	6.67
		% deviation from Normal control	0	-7.69	0	7.69	7.14	7.14
	0.4ppm Group II (Aii)	Mean	0.12	0.13	0.18	0.15	0.16	0.17
		±SEM	0.02	0.03	0.02	0.03	0.01	0.01
		±SD	0.05	0.05	0.03	0.04	0.01	0.01
		CV %	41.66	38.46	16.67	26.67	6.25	5.88
		% deviation from Normal control	9.09	0	38.46	15.38	14.29	21.43
Dimethoate Treated	0.2ppm Group II (Bi)	Mean	0.12	0.13	0.13	0.14	0.15	0.15
		±SEM	0.01	0.01	0.07	0.01	0.02	0.04
		±SD	0.03	0.05	0.02	0.03	0.06	0.02
		CV %	25	38.46	15.38	21.43	40	13.33
		% deviation from Normal control	9.09	0	0	7.69	7.14	7.14
	0.4ppm Group II(Bii)	Mean	0.12	0.15	0.19	0.17	0.18	0.21
		±SEM	0.04	0.01	0.01	0.01	0.01	0.01
		±SD	0.01	0.03	0.05	0.03	0.03	0.03
		CV %	8.33	20.00	26.32	17.65	16.67	14.29
		% deviation from Normal control	9.09	15.38	46.15	30.77	28.57	50.00

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Table 4 : **Showing the comparison of mean values of Cytochrome P450 activity (nmol/mg protein) in midgut 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)		
		24thhr	48thhr	72ndhr	24thhr	48thhr	72ndhr
Between Normal and Group I	t	0	1.41	1.41	0	0.71	0.71
	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	0.09	0.71	0	0.71	0.71	0.71
	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	0.45	0.47	2.24	0.63	1.41	2.12
	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	0.71	0.93	0.30	0.71	0.45	0.45
	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	0.24	1.41	4.24	2.83	2.83	4.95
	p	>0.05	>0.05	<0.01	<0.05	<0.05	<0.01
	df	18	18	18	18	18	18
Between Group IIA (i)and Group IIA (ii)	t	0.45	0.32	2.24	0.32	0.71	1.41
	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	0	1.41	0.85	2.12	1.34	1.46
	p	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05
	df	18	18	18	18	18	18
Between Group IIA (i)and Group IIB (i)	t	0.71	0.71	0	0	0	0
	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	0.61	0.63	0.45	0.63	1.41	2.83
	p	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05
	df	18	18	18	18	18	18

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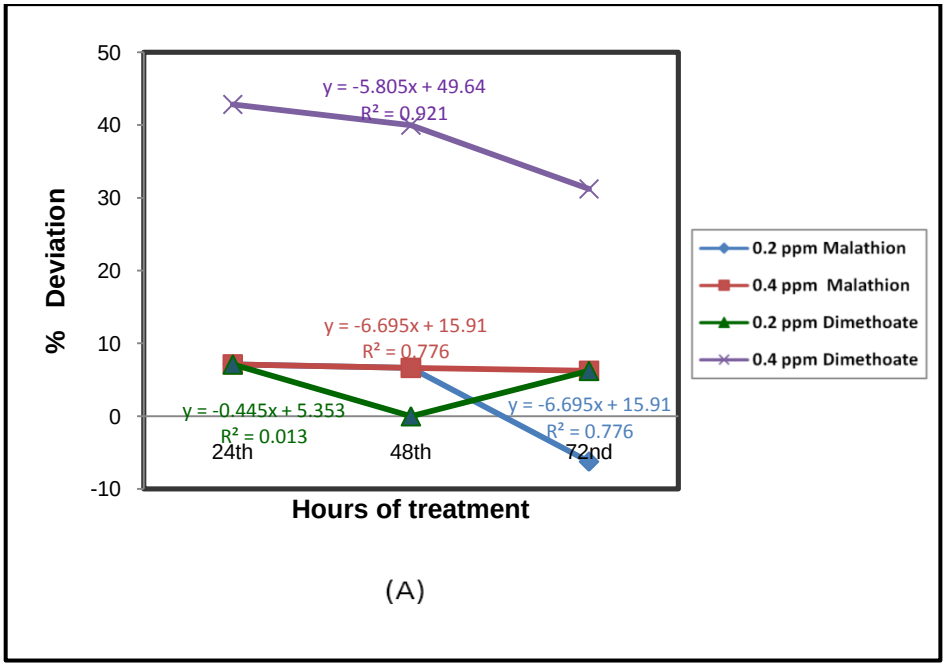

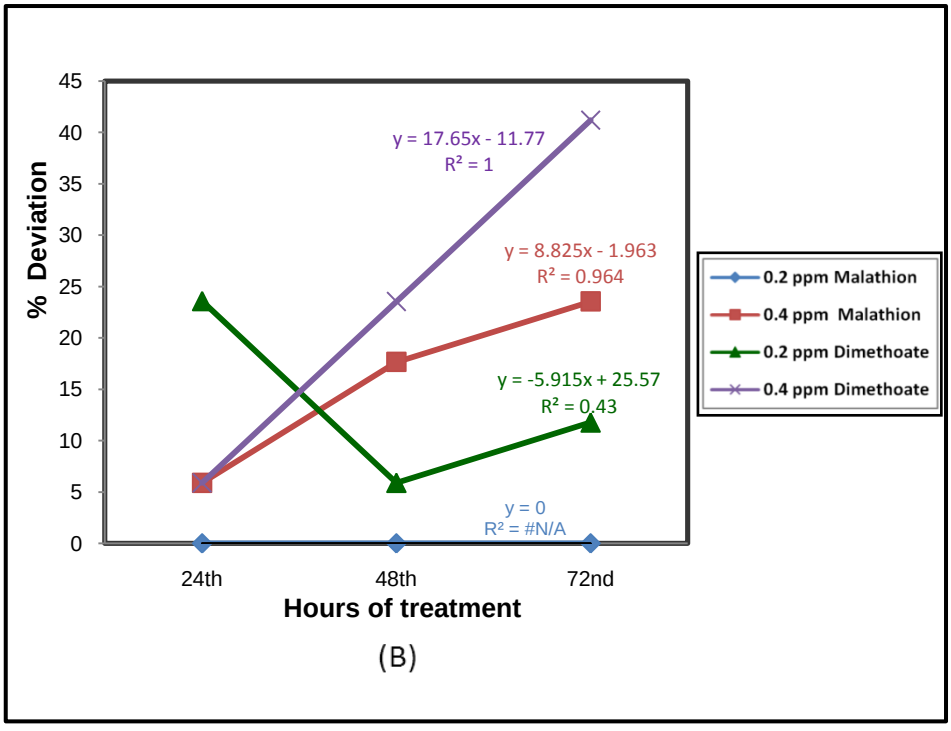

Fig- 1: Presenting the % deviation of Cytochrome P450 activity (nmol/mg of protein) in the Fat Body of the experimental groups from the mean values of the normal group. (A) in 4th instar larvae, (B) in 5th instar larvae of *S. ricini*.

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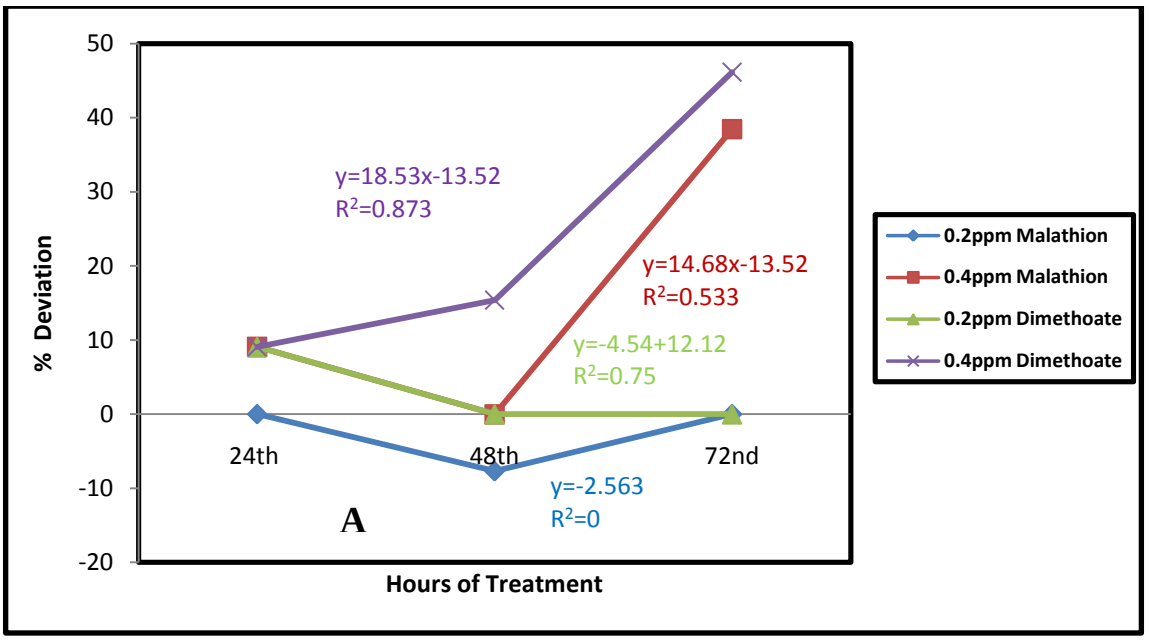

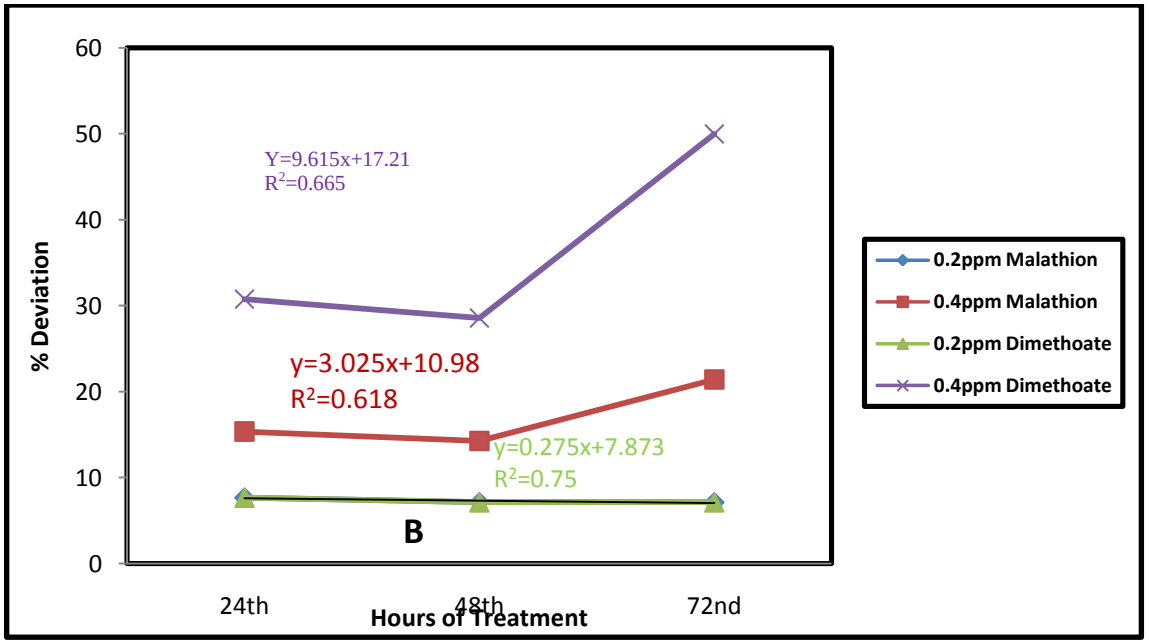

Fig- 2: Presenting the % deviation of Cytochrome P450 activity (nmol/mg of protein) in the midgut of the experimental groups from the mean values of the normal group. (A) in 4th instar larvae, (B) in 5th instar larvae of *S. ricini*.

IV. DISCUSSIONS AND CONCLUSION

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Overall increase in Cytochrome P450 enzyme activity was observed in both fat body and midgut, which was found to be more significant in higher dose of pesticides. Similar trend was observed in treatment with Malathion and Dimethoate but with higher intensity in case of Dimethoate. In case of 4th instar larvae the increase was observed about 10% except in treatment with Dimethoate (0.4ppm) which was 30% above the normal base line (Fig. 1. A) but in 5th instar larvae the enzyme activity increases gradually from above 5% to 25% in all the treated groups (above 40% with 0.4 ppm dimethoate). Fig. 1. B.

In the present experimental setup, it was clearly observed that the midgut of 4thand 5thinstar larvae treated with Malathion and Dimethoate exhibits almost similar trend than that of fat body but the intensity of deviation was higher showing the highest value (50%) at 72nd hour of exposure in 5th instar larvae. (Fig- 2.A, B).

Between the fat body and midgut the highest Cytochrome P450 activity was observed in the midgut on pesticidal exposure. It is noted in the present study that of the two larval instars the enzyme activity in the 5th instar larvae weremore affected than that of the 4th 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 *Samiaricini* than that of Malathion.

The involvement of cytochrome P450s in insecticide resistance and detoxification was suggested by Kulkarni*et al*. (1974). Berge *et al*. (1998) reported that insecticide resistance is associated with an increase in Cytochrome P450 in different insects. Insect P450 monooxygenases are reported to be induced by various insecticides (Terriere, 1984) and herbicides (Kao *et al.*, 1995). The rapid detoxification of insecticide is required to eliminate the toxicants from the body which ultimately increases the enzyme activity involved in this metabolic process. The overall increased activity of Cytochrome P450 also observed in the present study may be due to active involvement of the enzyme in the detoxification process as suggested by earlier workers.

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