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Effect of Pesticide's Residue on the Physiological Changes in the Silkworm (*Bombyx mori* L.) Haemolymph

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ABSTRACT: The organic constituents of haemolymph (enzymes) play an important role in biochemical processes underlying growth and development of insects. The study on haemolymph of silkworm (*Bombyx mori* L.) during the fifth instar revealed that pesticide exposure affects haemolymph enzyme activities, crucial for growth and development. Amylase activity in control groups (water spray, absolute control) increased until day 5, then declined, while pesticide treatments caused an early peak and subsequent sharp decline. Acetylcholinesterase (AChE) activity rose steadily in the carbofuron 3G treatment but was dampened with novaluron 10EC exposure. Peroxidase (POX) activity spiked initially under pesticides but dropped significantly by day 6. Novaluron 10EC and chlorfenapyr 10SC were notably toxic, affecting enzyme activity even after a waiting period.

Keywords: Silkworm, peroxidase, amylase, acetylcholinesterase, haemolymph, pesticides.

INTRODUCTION

Enzymes which are highly specialized proteins play a vital role in the metabolism of dietary food in the body of an organism since they catalyze various reactions of any biological system (Campbell *et al*., 2002). Amylase is a hydrolytic enzyme found in micro-organisms, plants and animals which is involved in the digestion and carbohydrate metabolism in insects including the carbohydrates available in the form of starch in mulberry leaves (Horie and Watanabe 1980; Chatterjee *et al*., 1989). Further, the ability to digest more food influences the growth, development and resistance to diseases, stresses and better survival under different environmental conditions (Manjula *et al*., 2010). Acetylcholinesterases (AChEs) are enzymes that hydrolyze the neurotransmitter acetylcholine (ACh) to acetate and choline. AChE is known to be the target of many insecticides which cause modifications of the active site of the AChE enzyme leading to the inhibition of AChE activity and block the hydrolysis of acetylcholine. Thus, AChE activity is one of the main resistance mechanisms in various insect species against many insecticide-resistant insects. Changes in AChE activity may result from exposure to certain insecticides, which act as cholinesterase inhibitors. Inhibitors of AChE are also used to treat certain conditions such as dementia. The inactivation of cholinesterase by cholinesterase inhibitor, pesticides allows the accumulation of large amounts of acetylcholine (Derisbach, 1987). The antioxidant and detoxifying enzymes in insects play an important role in helping insects to cope with the exogenous stress caused by ingestion of plant allelochemicals. These enzymes prevent damage to biological molecules by detoxifying toxic compounds and rendering them non-toxic and hastening their excretion. Cells contain a large number of antioxidants to prevent or repair the damage caused by ROS and to regulate redox sensitive signaling pathways. Catalases and peroxidises (POX) help in removal of hydrogen peroxide in non-specific and specific ways in insect systems, respectively and thereby stabilize the internal balance of toxic free radicals. Like any other insects, silkworms also exhibit enzymatic and non-enzymatic antioxidative defense mechanisms to counter the oxidative damage. The most imperative antioxidant enzyme includes peroxidases (POX) in silkworm; POX transforms hydrogen peroxide to water and oxygen molecules (Heckel, 2018).

MATERIALS AND METHOD

A. Experimental details

Crop	: Mulberry
Variety	: Victory-1 (V-1)
Silkworm breed	: PM×CSR2
No. of treatment	: 9
No. of replication	: 3
Design	: RCBD

B. Collection and storage of haemolymph

The haemolymph was collected from the first day to sixth day of fifth instar in each treatment. From each replication ten larvae were randomly selected. For extracting the haemolymph in fifth instar, third abdominal legs were amputated with sterilized blade and the haemolymph thus bled was immediately drawn into pre-cooled eppendorf tubes containing phenyl

thiourea at 1mg/tube. Phenyl thiourea was used to avoid the activity of prophenol oxidase that causes melanization of the haemolymph samples (Etebari *et al*., 2006). To ensure complete extraction of haemolymph, the larvae were gently pressed from anterior and posterior ends simultaneously until no more haemolymph oozed out of the wound. The samples were centrifuged at 3000 rpm for 15 minutes to separate out the phenylthiourea crystals and haemocytes. The supernatant was used for the estimation after proper dilution (Mahesha *et al*., 2000). The samples were labelled and then preserved in deep freezer at - 20°C till further use.

C. Estimation of amylase activity

The quantitative estimation of amylase activity in the haemolymph of silkworm was done as per the procedure given by Tanaka and Kusano (1980). In this assay, starch was the substrate and maltose was the end product.

(i) Principle. Since the maltose is colourless, the end product should be coloured by using some chemical reaction, so that the concentration of the end product will be measured calorimetrically to quantify the activity of the enzyme. Addition of 3-5 dinitro salicylic acid (DNS) solution would not only stop the reaction of amylase (enzymes) over the starch (substrate), but it also gave reddish orange colour to the maltose (end product).

(ii) Reagents

1. Preparation of 0.2 per cent starch solution. Soluble starch of 200 mg was dissolved in 100 mL of phosphate buffer (0.01 M Sodium dihydrogen orthophosphate + 0.001 M Disodium dihydrogen orthophosphate). Then NaCl of 0.05 M was added and pH was adjusted to 6.8 using acid or alkali (*i.e*., HCl or NaOH). The solution was shaken well on hot water both to dissolve the starch completely and filtered.

2. Preparation of 3-5 dinitro salicyclic acid. One gram of 3-5 dinitro salicylic acid powder was dissolved in 2 mL of 2N sodium hydroxide solution and 50 mL of distilled water. Then, 30 mg of sodium potassium tartarate was added. The mixture was shaken well on magnetie stirrer to dissolve the solutes completely. Then the volume was made upto 100 mL and finally the solution was filtered and preserved under air tight container in order to protect DNS solution from carbon dioxide.

(iii) Procedure

1. For each treatment, three test tubes were taken and 2 mL of 0.2 per cent starch and 200 µL of samples were added to each test tube.
2. The test tubes were incubated at 37°C for 30 minutes on hot water bath.
3. Then 2 ml of 3-5 DNS was added to each test tube to stop the reaction and boiled on water bath at 50 to 60°C for 5 minutes.
4. The test tubes were cooled immediately under running water and the optical density (OD) values of the end products was measured separately, treatmentwise and replicationwise using colorimeter at 525 nm.

(iv) Conversion of OD values to the concentration of maltose. By using the standard curve of maltose, the OD values were converted into concentration of maltose which was released during the chemical reaction. For preparing the standard curve 100 mg of maltose was dissolved in 100 mL of phosphate buffer of 6.8 pH. This served as stock solution. From stock solution, serial dilutions of maltose were prepared from 0.1mL to 1 mL (which had 0.1mg to 1mg of maltose). Thus, the total volume of all the serial dilutions was exactly made upto 2 ml using phosphate buffer. For each test tube 2ml of DNS was added and boiled for 5 minutes on water bath. After cooling, the OD values of the serially diluted maltose solutions were recorded at 525 nm. The standard graph was prepared by plotting the OD values against the serially diluted maltose. Finally the amylase activity was estimated by adopting the following formula:

$$\text{Amylase activity} = \frac{\text{Standard graph value} \times \text{No.of dilutions} \times 60}{\text{Amount of sample used} \times \text{Incubation time}}$$

The amylase activity was expressed as mg of maltose released per mL of sample per hour (*i.e*., mg/mL/min) at 37°C.

D. Estimation of Acetylcholinesterase activity

The Acetylcholinesterase activity assay kit provides a simple and direct procedure for measurement of AChE levels in a haemolymph sample as estimated by Ellman *et al*. (1961).

(i) Principle. This assay is an optimized version of the Ellman's method in which thiocholine produced by AChE, reacts with 5, 5'-dithiobis (2-nitrobenzoic acid) to form a colorimetric (412 nm) product, proportional to the AChE activity present.

(ii) Components. The kit is sufficient for 100 assays in 96 well plates.

- Assay buffer, pH 7.5 – 30 mL
- Calibrator, equivalent to 200 U/L – 4 mL
- Reagent – 240 mg

(ii) Preparation of reagents

Sample preparation:

(a) Haemolymph samples were diluted 10 folds in assay buffer.

(b) Samples were prepared by briefly sonicating or by homogenizing in 0.1 M phosphate buffer, pH 7.5, followed by centrifugation at 14,000 rpm for 5 minutes. Cleared supernatants were used for the assay.

(c) Best results are obtained when samples are freshly prepared. If this is not feasible, samples should be stored at 2–8 °C and used within 24 hours.

Assay reaction:

(a) The working reagent was prepared fresh and used within 30 minutes. Each reaction required 2 mg of reagent. To prepare working reagent, the amount of reagent needed in total was weighed into a centrifuge tube. Added 200 µL of assay buffer per 2 mg of reaction and vortexed to dissolve.

(b) Transfered 200 µL of water (assay blank) and 200 µL of calibrator into separate wells of a 96 well plate. Added 10 µL of samples into separate wells of the 96-well plate.

(c) Transfered 190 µL of the freshly prepared working reagent to all sample wells and taped the plate briefly to mix.

(d) Incubated the samples at room temperature. After 2 minutes, the initial absorbance was measured at 412 ηm [(A_{412}) initial].
(e) Continued to incubate the plate at room temperature. At 10 minutes, took final measurement at 412 ηm [(A_{412}) final].

(iv) Calculations

$$\text{AChE Activity (units/L)} = \frac{A_{412}\ (\text{final}) - A_{412}\ (\text{initial})}{A_{412}\ (\text{calibrator}) - A_{412}\ (\text{blank})} \times\ n \times 200$$

Where,
200 = equivalent activity (units/L) of the calibrator when assayed is read at 2 minutes and 10 minutes
n = dilution factor (n = 10 for whole haemolymph)
A = Absorbance at 412 nm
A (calibrator) = Absorbance of the calibrator at 10 minutes
A (blank) = Absorbance of the blank at 10 minutes
One unit of AChE is the amount of enzyme that catalyzes the production of 1.0 µmole of thiocholine per minute at room temperature at pH 7.5. This kit has a linear range of 10–600 units/L of AChE activity.

E. Estimation of Peroxidase activity in haemolymph

The method proposed by Castillo *et al*. (1984) was adopted with slight modifications for assaying the activity of peroxidase in the protein extract using Guaiacol as substrate. The reaction catalyzed by peroxidase is given below:

$RH_2 + H_2O_2 \rightarrow 2H_2O + R$

(i) Priniciple. The resulting oxidized (dehydrogenated) guaiacol is probably more than one compound and depends on the reaction conditions. The rate of formation of guaiacol dehydrogenation product is a measure of the POX activity and can be assayed spectrophotometrically at 436ηm.

(ii) Reagents

(a) 0.1M Phosphate buffer (pH 6.1)
(b) Guaiacol solution 96mM: Dilute 2.169 mL guaiacol in 200 mL distilled water. It can be stored frozen for many months
(c) Hydrogen peroxide solution (12mM): Dilute 124 µL of 30% H_2O_2 to 100ml of distilled water. The extinction of this solution should be 0.485 at 240ηm. Prepare freshly.
(d) Sample: Centrifuge the haemolymph sample at 18000g at 5°C for 15 min. Store on ice till the assay is carried out.

(iii) Procedure

(a) Pipette out 900µL of distilled water, sample (varies based on the protein concentration), 1mL of phosphate buffer, 0.5 mL Guaiacol solution and 0.5 mL hydrogen peroxide solution to the cuvette. (Buffer solution was adjusted to 25°C before assay).
(b) Mixed well and placed the cuvette in the spectrophotometer.
(c) Absorbance due to the formation of tetra-guaiacol was measured at a time interval of 30 sec up to 1 min at 436ηm.

Enzyme unit: One unit of peroxidase is defined as the amount of enzyme that oxidizes 1µmole of guaiacol per min per gram fresh weight or per mg protein under standard assay condition.

(iv) Calculations. Since the extinction co-efficient of guaiacol dehydrogenation product at 436ηm under the conditions specified is 6.39 per micromole, the enzyme activity per litre of sample is calculated as below

$$\text{Enzyme activity units/litre} = \frac{\text{Absorbance} \times \text{Total assay volume}}{\Delta t \times E \times I \times \text{enzyme sample volume}}$$

Where, Δt = Incubation time, E = Extinction co-efficient, I = Cuvette diameter

RESULTS AND DISCUSSION

The results and discussion on the research topic entitled "Effect of pesticide's residue on the physiological changes in the silkworm (*Bombyx mori* L.) haemolymph" conducted during 2021-2022 at College of Sericulture, Chintamani and biochemical studies were conducted in Advanced Centre for Plant Biotechnology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bangalore, presented below and discussed in the light of earlier reports published.

A. Haemolymph amylase activity (mg/ml/h)

The day wise haemolymph amylase activity was measured in terms of mg of maltose released per ml of haemolymph per hour. The data on the activity of heamolymph amylase during fifth instar are presented (Table and Fig. 1). There was no significant difference among the treatments with respect to the haemolymph amylase activity during first day of the fifth instar but its activity significantly increased from second day to third day of fifth instar in treatments carbofuron 3G (T_1), dimethoate 30EC (T_2), novluron 10EC (T_3), azadirechtin 0.03EC (T_4), fenazaquin 10EC (T_5), dinotefuron 20 SG (T_6) and chlorfenapyr 10EC (T_7) and thereafter it exhibited significantly decreased activity from third day to sixth day of fifth instar. Whereas, in treatments water spray (T_8) and absolute control (T_9) there was increased trend from first day to fifth day of fifth instar but sudden decreased activity was exhibited on sixth day of fifth instar.

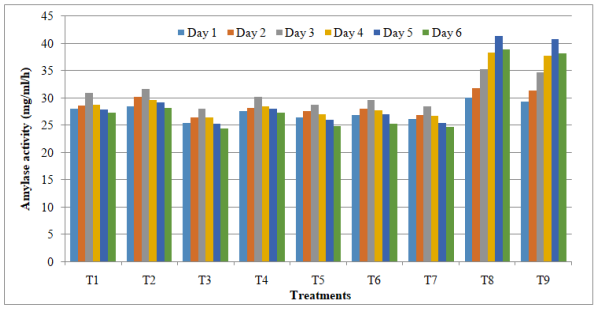

Fig. 1. Amylase activity (mg/mL/h) in the haemolymph of fifth instar silkworm as influenced by feeding pesticide treated mulberry leaves.

Table 1: Amylase activity (mg/mL/h) in the haemolymph of fifth instar silkworm (*B.mori* L.) as influenced by feeding pesticide treated mulberry leaves.

Treatments	Amylase activity (mg/ml/h) in 5th instar					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T1 – Carbofuron 3G	27.96	28.61	30.92	28.77	27.81	27.29
T2 – Dimethoate 30EC	28.48	30.23	31.60	29.59	29.12	28.20
T3 – Novluron 10EC	25.43	26.36	28.06	26.36	25.22	24.39
T4 – Azadirechtin 0.03EC	27.51	28.23	30.22	28.42	28.06	27.30
T5 – Fenazaquin 10EC	26.36	27.65	28.81	27.07	25.98	24.85
T6 – Dinotofuron 20SG	26.90	28.08	29.58	27.70	26.93	25.26
T7 – Chlorfenapyr 10EC	26.15	26.81	28.40	26.67	25.38	24.62
T8 – Water spray	29.98	31.74	35.27	38.26	41.42	38.87
T9 – Absolute control	29.32	31.29	34.74	37.70	40.75	38.22
F - test	NS	*	*	*	*	*
SEm ±	-	1.17	1.63	1.59	1.70	1.58
CD @ 5%	-	3.47	4.84	4.73	5.04	4.70
C.V (%)	-	7.03	9.15	9.16	9.77	9.53

In present study, it was found that lowest haemolymph amylase activity of 24.39 mg/ml/h was recorded in novluron 10EC (T_3), followed by chlorfenapyr 10EC (T_7) recorded 24.62 mg/mL/H and fenazaquin 10EC (T_5) recorded 24.85 mg/mL/h which was statistically lower than rest of the treatments on sixth day of fifth instar. The results are supported by the findings of Amarnatha (2004), who mentioned that haemolymph amylase activity increased from first day to fifth day of fifth instar in all the breeds and thereafter it fluctuated, exhibiting decreased activity on sixth day of fifth instar. In the present study, it was also found that highest peak of haemolymph amylase activity of 41.42 mg/mL/h in water spray (T_8), followed by 40.75 mg/mL/h in absolute control (T_9) was recorded on fifth day of fifth instar, which was higher than rest of days.

Increased trend of amylase activity was recorded in treatments T_8 (Water spray) and T_9 (Absolute control) up to the fifth day (29.98 mg/mL/h to 41.42 mg/mL/h and 29.32 mg/ml/h to 40.75 mg/mL/h, respectively) of the fifth instar and then the enzyme activity was declined on the sixth day (38.87mg/mL/h and 38.22 mg/mL/h, respectively). The results are supported by the findings of Rajitha and Savithri (2014), who reported increased trend of amylase activity was recorded in the haemolymph of healthy larvae up to the 5th day (0.475mg/mL to 0.667 mg/mL) of the instar and then the amylase activity was declined on the 6th day (0.626 mg/mL). Whereas in the present study in treatments (T_1 to T_7) the amylase activity was elevated up to third day and then the activity of the enzyme was declined significantly during the rest of the days. However, compared to water spray and absolute control, the amylase activity was declined significantly in the pesticide treatments which are under stress condition. In contrast to this, Gururaj *et al*. (1999) found that the activity of amylase in the *Bm* NPV infected larva, increased significantly in the haemolymph from 48 h to 144 h, but as disease progressed, it decreased significantly.

The results are also in accordance with Kadam *et al*. (2021), who reported that highest peak of haemolymph amylase activity of 1.43 µmoles was recorded on fifth day of fifth instar which was higher than rest of days. The least haemolymph amylase activity of 1.22 µ moles was recorded on sixth day of fifth instar.

B. Acetylcholinesterase activity in haemolymph (unit/L)

The day wise activity of haemolymph acetylcholinesterase (AChE) was measured in terms of unit/L of haemolymph per minute. The data on the activity of heamolymph AChE during fifth instar are presented (Table 2 and Fig. 2). There was significant difference among the treatments with respect to the haemolymph AChE activity from first day to sixth day of the fifth instar.

Table 2: Acetylcholinesterase activity (units/L) in the haemolymph of fifth instar silkworm (*B.mori* L.) as influenced by feeding pesticide treated mulberry leaves

Treatments	Acetylcholinesterase activity (units/L) in 5th instar					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T1 – Carbofuron 3G	37.49	48.90	53.79	63.62	63.73	66.99
T2 – Dimethoate 30EC	60.31	53.79	65.20	58.72	70.26	73.53
T3 – Novluron 10EC	48.90	48.90	48.90	48.94	49.02	53.92
T4 – Azadirechtin 0.03EC	57.05	53.79	57.05	58.73	63.73	63.73
T5 – Fenazaquin 10EC	65.20	57.05	48.90	58.78	58.82	58.82
T6 – Dinotofuron 20SG	42.38	60.31	47.27	53.88	52.29	58.82
T7 – Chlorfenapyr 10EC	45.64	47.27	58.68	58.78	49.02	63.73
T8 – Water spray	66.83	48.90	35.86	48.98	57.19	55.56
T9 – Absolute control	66.83	53.79	60.31	53.88	68.63	78.43
F - test	*	*	*	*	*	*
SEm ±	1.80	2.49	2.24	2.82	2.83	2.78
CD @ 5%	5.35	7.40	6.65	8.38	8.41	8.25
C.V (%)	5.73	8.21	7.34	8.72	8.28	7.55

In the present study, haemolymph AChE activity was estimated in pesticides treated silkworm, *Bombyx mori* L. and the results revealed that, AChE activity in haemolymph of the fifth instar larvae under novluron 10EC shows an overall dampening effect in fifth instar larvae throughout the experimental period with slight Whereas, in water spray (T_8) and absolute control (T_9) it is found to be high in initial fifth instar and further there was no prominent trend was observed.

The highest haemolymph AChE activity of 78.43 U/L was recorded in water spray (T_8), followed by dimethoate 30 EC (T_2) of sixth day, which was statistically higher than rest of the treatments under study. The least value of haemolymph AChE activity of 35.86 U/L was recorded in water spray (T_8) of third day, followed by carbofuron 3G (T_1) of first day (Table 2).

The significant decrease in AChE activity in the haemolymph of pesticide treated silkworm, in the present study, indicate greater inhibition of the integratory activity of the central nervous system. Acetylcholine accumulation in brain, fat body, haemolymph and silk gland may be due to the uncontrolled release of AChE from the corpus cardiacum or could be due to the degeneration of many biochemical and physiological functions. It is well established that the corpus cardiacum is an important source of neurohormones and a possible site for mediating action of insecticides (Steele, 1980). Present findings are in accordance with the results of Reddy (1984) where the significant inhibition of AChE in the brain tissue of silkworms using carbaryl under *in-vitro* conditions. The earlier work of Watanabe and Kobara (1967) on the esterase system of silkworms under insecticidal stress found that, insecticides interfere with AChE activity, causing inhibition of the enzyme. Further, a marked decline in AChE activity in control larvae from day 1 to 5 clearly indicates that the AChE activity is related to age in silkworms and gradually decreases from the fifth instar to pupation, which is in agreement with the findings of Pant *et al*. (1982). fluctuations. Further, the AChE activity exhibit a different trend and inhibitory effect was not prominent in case of carbofuron 3G (T_1) and azadirechtin 0.03EC (T_4) (Fig. 2). The enzyme activity was found to be increased from first day to sixth day of fifth instar in case of all the pesticides treatments (T_1 to T_7).

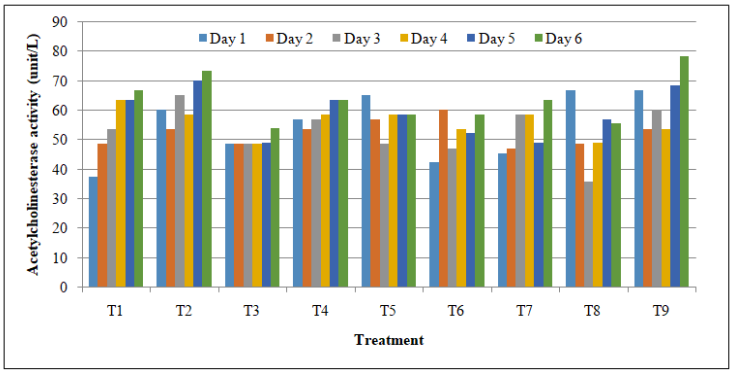

Fig. 2. Acetylcholinesterase activity (units/L) in the haemolymph of fifth instar silkworm as influenced by feeding pesticide treated mulberry leaves.

Surendranath and Kumar (1999), demonstrated that AChE activity was inhibited by exposure to organophosphorus insecticides, followed by a concomitant increase in acetylcholine levels in silkworm, which associated with acceleration in energy metabolism of nerve cells, leading to death of the insects. From the present study, it is seen that pesticide induced physiological disturbances in haemolymph AChE of silkworms can be used as valuable indices for determining the environmental contamination of mulberry fields by widely used pesticides.

C. Peroxidase activity in haemolymph (µg/mL)

The day wise activity of Peroxidase (POX) in haemolymph was measured in terms of µmg/mL of haemolymph. In the present study, POX activity in haemolymph was estimated in silkworm, *Bombyx mori* L. fed with mulberry leaves treated with pesticides. The present study results revealed that, POX activity increased initially from day 1 to 2 then significantly decreased from day 2 to 6 in case of treatments with pesticides. Whereas in water spray (T_8) and absolute control (T_9), decreased trend was noticed from first day itself (Table 3 and Fig. 3).

The highest haemolymph POX activity of 32.45 µg/ml was recorded in novluron 10EC (T_3), followed by chlorfenapyr 10EC (T7) of 31.90 µg/ml of second day, which was statistically higher than rest of the treatments under study. The least value of haemolymph POX activity of 4.16 µg/ml was recorded in absolute control (T_9), followed by water spray (T_8) of 4.45 µg/ml of sixth day (Table 3).

Table 3: Peroxidase activity (µg/mL) in the haemolymph of fifth instar silkworm (*B.mori* L.) as influenced by feeding pesticide treated mulberry leaves.

Treatments	Peroxidase activity (µg/mL) in 5th instar					
	D1	D2	D3	D4	D5	D6
T1 – Carbofuron 3G	12.14	14.75	12.88	9.95	7.11	6.30
T2 – Dimethoate 30EC	14.59	18.70	17.08	14.95	13.01	11.15
T3 – Novluron 10EC	30.39	32.45	28.93	21.05	18.96	18.00
T4 – Azadirechtin 0.03EC	15.52	17.40	13.78	14.75	11.58	9.82
T5 – Fenazaquin 10EC	18.02	19.80	16.78	15.65	12.86	9.04
T6 – Dinotofuron 20SG	22.07	25.20	21.32	18.80	14.93	13.55
T7 – Chlorfenapyr 10EC	27.77	31.90	29.55	21.55	17.06	16.75
T8 – Water spray	12.77	10.30	9.30	7.30	5.36	4.45
T9 – Absolute control	12.17	10.58	9.45	7.60	5.21	4.16
F - test	*	*	*	*	*	*
SEm ±	0.301	0.512	0.227	0.459	0.310	0.206
CD @ 5%	0.909	1.532	0.688	1.389	0.938	0.617
C.V (%)	2.832	4.404	2.229	5.440	4.392	3.367

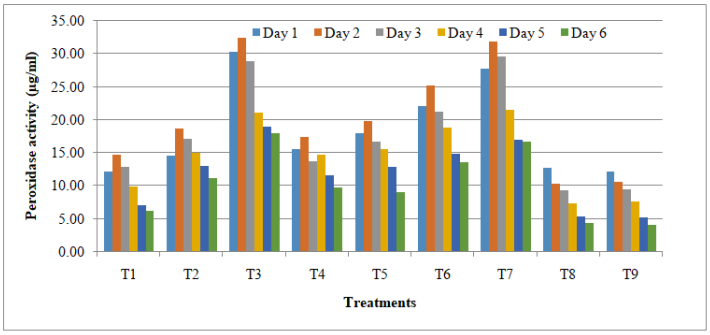

Fig. 3. Peroxidase activity (µg/mL)) in the haemolymph of fifth instar silkworm as influenced by feeding pesticide treated mulberry leaves.

The increased activity of haemolymph POX in treatments with pesticides of our study was in conformity with the findings of Mittler (2002) where the activity of POX significantly increased in larvae of *Chilo suppressalis* exposed to thermal stress and injected with *Beauveria bassiana*, because of ROS production. Jia *et al*. (2016) reported that combined treatment of chlorantraniliprole and *Metarhizium anisopliae* to *Locusta migratoria* (Meyen) (Orthoptera: Acrididae) significantly increased the activities of SOD and POX. Similar to the above mentioned studies, our results indicated the higher activities of POX in haemolymph of silkworm treated with pesticides are noticed, which may be attributed to the higher production of ROS. Karthi *et al*. (2018) opined that, POX activity (0.86 Unit/mg/min) was also increased in *Spodoptera litura* after treatment with conidia (1×10^7 conidia/ml) of *Aspergillus flavus*. Similarly, Butterfield *et al*. (1998) reported that activity of POX in lacewing were all inhibited first and then activated by indoxacarb, emamectin benzoate, imidacloprid and the three sublethal concentrations of lambda-cyhalothrin, this may be due to the stress effect of pesticides on the lacewing, which can destroy some protective enzymes and reduce their activity. However, the present results are in contrary with the findings of Li *et al*. (2012) where, the overproduction of ROS caused by *Bm*NPV infection would break down the balance of the oxidative/antioxidative system in the fifth instar larvae of silkworm, resulting in the lipid peroxidation, which is closely related to the reduction of the antioxidative enzymes like peroxidase.

CONCLUSIONS

The study concludes that pesticide exposure significantly impacts enzyme activities in silkworm haemolymph. Amylase activity in control groups peaked on day 5, while pesticide-treated groups showed an early rise followed by a decline. Acetylcholinesterase (AChE) activity steadily increased with carbofuron 3G but was dampened by novaluron 10EC. Peroxidase (POX) activity initially spiked under pesticides, then decreased sharply. Control treatments showed minimal enzyme fluctuations. Overall, pesticides like novaluron 10EC and chlorfenapyr 10SC proved highly toxic, disrupting enzyme activities.

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