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Toxic impact of deltamethrin pesticides on the nutritional physiology of the insect model organism silkworm, (*Bombyx mori.* L)

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Abstract
Pesticides are typically used in agriculture or public health programmes to protect plants from pests, weeds, and diseases, as well as to protect humans from vector-borne diseases such as malaria, dengue fever, and schistosomiasis. Residues of pesticides can be found in a variety of common foods and beverages, including prepared meals, water, wine, fruit juices, snacks, and animal feeds. It's also worth noting that washing and peeling won't totally eliminate the residues. Deltamethrin belongs to the pyrethroid family of insecticides. Pyrethroids are synthetic counterparts of pyrethrins, which are naturally occurring insecticides found in chrysanthemum flowers. Deltamethrin assaults the CNS (central nervous system) of any animal upon contact with even for a short period of time. Model organism silkworm is used for studying the toxicity effect of pesticides *in-vivo*. Silkworms are a promising model animal for health safety and environmental pollution assessment due to their sensitivity to chemical compounds such as pesticides, drugs, and heavy metals, as well as their low cost, body characteristics, and complete genome sequencing.

Keywords: pesticides, pyrethrins, insecticides schistosomiasis, deltamethrin, toxicity

Introduction
Pyrethroids are a chemical class that includes deltamethrin (DELTA). Pyrethroids are chemical compounds inspired by the pyrethrin components of pyrethrum. Deltamethrin, unlike other pyrethroids, is a single pure chemical. Deltamethrin belongs to the pyrethroid family of insecticides Deltamethrin, can induce allergic reactions and asthma in certain persons. Deltamethrin assaults the CNS (central nervous system) of any animal upon contact with even for a short period of time. Skin contact can cause tingling or reddening in the area where the application is made. Facial paraesthesia, which can feel like burning, partial numbness, "pins and needles," skin crawling, and other odd sensations if taken in through the eyes or lips, is the most common symptom. One case report describes pyrethroid insecticide overdose that leads to a state that is clinically comparable to motor neuron disease (Rehman *et al*., 2014) [9]. There are no antidotes, thus therapy must be symptomatic and doctor-approved. Silkworm must be a model organism for the study of longevity because it is an economically important insect for the production of silk, wherein longevity plays an important role in metabolic activities during its life cycle and understanding of its durability to reduce still shorter is the need of the hour for the benefit of rearer, breeder, physiologist, biochemist, geneticist, and farmers, among others. With the completion of the silkworm genome project and the establishment of the silkworm genomic database and the silkworm protein database, the silkworm emerges as a model for scientific research. Environmental issues such as heavy metal pollution and pesticide residues have emerged as a result of rapid economic growth. Finding a model animal that can be used for environmental monitoring is essential for assessing the safety of an ecological environment. The silkworm is sensitive to environmental pollution, particularly pesticides, heavy metals, and other toxic chemicals (Sekimura 2005, Hamamoto *et al*. 2009) [10].

Materials and Methods
Mulberry silkworms that were free from any sorts of silkworm diseases were obtained from the Tirupati farm of Jagadonna Sericulture. The silkworms were grown in the SPMVV's Seri-biotech laboratory at 75±5% relative humidity, 25–27ºC room temperature, and 12 hours: 12 hours light and dark photoperiod under the recommended conditions. *Morus alba* leaves from the SPMVV's garden was used as the feed for the silkworms.

Chemicals
A commercial formulation of (S)—cyano-3-phenoxybenzyl deltamethrin (1R, 3R) -3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate was purchased from A fertiliser house in Tirupati, Andhra Pradesh. By dissolving 0.05 ml of Decis- 20 EC in 99.95 ml of acetone, a stock solution containing 100 mg/L was created. For the determination of toxicity, further dilutions were made with distilled water. The concentrations were

calculated on the basis of the pesticide's active ingredients. As acetone is known to be less toxic to silkworms than other solvents and is frequently used in pesticide assays (Burchfield *et al*., 1952; Reddy *et al*., 1991; Nath, 2002) [3, 8, 6] it was utilised in the present study. All other chemicals utilised in this investigation were of analytical quality.

Acute Toxicity Bioassay

According to protocol, the acute toxicity of a substance to silkworm was determined using the semistatic method under laboratory conditions (Zhang *et al*., 2008) [14]. The experiment was conducted with concentrations ranging from 0.5 to 5.0 mg/L Deltamethrin (0.5, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, and 5.0 mg/L) and was repeated three times with nine replicates for each concentration. Fifty grammes of fresh mulberry leaves were sprayed with each freshly prepared working concentration of deltamethrin and then fed to pre-starved (5th instar) healthy larvae for eight hours. A control group was also maintained from the same stock in the same environment and fed mulberry leaves sprayed with distilled water (Nwani *et al*., 2010) [7]. After pesticide exposure, alterations in feeding, movement, and any abnormal behaviour were observed and recorded at regular intervals. The LC50 value that would kill 50 percent of silkworms at different time intervals, i.e., 24–96 h, was determined using the probit analysis method (Finney, 1971), and then two sublethal doses of deltamethrin were chosen for further research. Every experiment was conducted three times in triplicate (50 larvae per replication).

Enzyme Assay

For enzyme assays, 4th instar larvae were acclimatised to sublethal concentrations of pesticide (1.5 and 2.0 mg/L) and then exposed to sublethal concentrations of pesticide (1.5 mg/L). All enzyme assay experiments were conducted three times in triplicate (n = 10). After 24 hours of exposure to deltamethrin, the entire gut of mature 5th instar larvae was isolated, rinsed three times in 1X phosphate buffer saline (pH 7.4), and cut into foregut, midgut, and hindgut. For crude enzyme preparation, various gut sections were homogenised in 500 l of sodium acetate buffer (0.1 M, pH 5.6) and centrifuged at 15,000 rpm for 15 minutes at 4 degrees Celsius. Moreover, the control was made by adding the same volume of 0.1 M sodium acetate (Blakemore *et al*., 1995) [2].

Cellulase Assay

For cellulase assay, 200 l of sodium acetate buffer (0.1M, pH 5.6) was mixed with the substrate (20 l of 0.1% sodium carboxymethyl cellulose), then 50 l of crude enzyme was added, and the mixture was incubated in a water bath at 37 degrees Celsius for 30 minutes. The mixture was then boiled for 15 minutes after 90l of dinitro salicylic acid solution (1.6 g sodium hydroxide, 30 g sodium potassium tartarate, and 1 g 3, 5– dinitrosalicylic acid per 100 ml H2O) was added. At 540 nm, glucose production was detected by measuring absorbance with a multimode reader. Using the glucose standard curve, the activity was measured as g glucose release per 30 min (Bernfeld, 1955; Weidlich *et al*., 2013) [1, 13].

Amylase Assay

Amylase enzyme activity was determined utilising a standard protocol (Bernfeld, 1955; Weidlich *et al*., 2013) [1, 13]. Twenty microliters of 1% glucose was combined with 200 microliters of sodium acetate buffer (0.1M, pH 5.6) before 50 microliters of crude enzyme was added. The mixture was incubated for 30 minutes at 37°C. Following incubation, 90 l of dinitro salicylic acid solution was added, and the mixture was boiled for 15 minutes before being cooled to room temperature. Using a multi-mode reader to measure absorbance at 620 nm, maltose production was detected. The maltose standard curve was created, and the activity was measured as g maltose per 30 minutes.

Catalase Assay

Catalase enzyme activity in the haemolymph of silkworm was estimated by following the method of Aebi (1974). 2 ml of 50 mM potassium phosphate buffer (pH 7.0) was taken in a test tube and then add 450 µl of 30 mM hydrogen peroxide was added, to 25 µl of haemolymph. Immediately the decrease in absorbance was recorded after every 15 seconds up to 60 seconds at 240 nm by using a spectrophotometer. Enzyme activity was expressed as µ moles of H2O2 consumed/min/mg protein

Statistical Analysis

Probit analysis was performed with Biostat (2009) software package with three times in triplicates for LC50 value calculation (n = 50). All the other experiments were performed three times in triplicates (n = 10) which was performed five times in triplicates (n = 5) and results are considered as mean ± standard deviation. One way analysis of variance (ANOVA) was carried out for statistical analysis with $P < 0.05$, $P < 0.01$, and $P < 0.001$, which was considered significant and highly significant respectively. Further, means were compared by post hoc Tukey's test. The graphical representations were performed using Microcal Origin version 6.1.

Results

Cellulase Assay

A) Percentage of inhibition

Table 1

S. No	Concentration of the Test sample (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Control	4.3	4.3	4.21	4.27
2.	50 ppm	7.9	7.9	7.9	7.9
3.	100 ppm	6.29	6.29	6.62	6.4
4.	500 ppm	5.37	6.04	6.03	5.813
5.	1000 ppm	4.54	4.5	4.83	4.623
6.	2000 ppm	4.08	4.07	3.7	3.95

B) OD values at 540 nm

Table 2

S. No	Tested sample concentration (µg/ml)	OD Value at 540 nm (in triplicates)		
1.	Control	3.123	3.124	3.102
2.	50 ppm	4	4	4
3.	100 ppm	3.6	3.54	3.68
4.	500 ppm	3.38	3.54	3.52
5.	1000 ppm	3.17	3.19	3.25
6.	2000 ppm	3.06	3.05	2.98

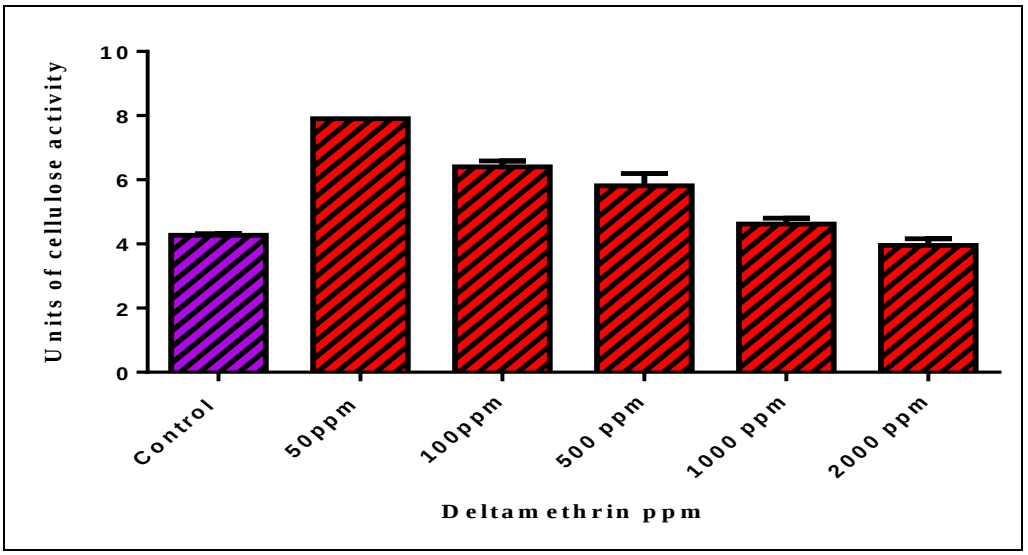

Fig 1

Amylase Assay

A) Percentage of inhibition

Table 3

S. No	Concentration of the Test sample (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Control	3.5	3.7	4.06	3.753
2.	50 ppm	5.88	5.88	5.88	5.88
3.	100 ppm	3.7	2.6	3.11	3.136
4.	500 ppm	1.4	1.9	1.4	1.566
5.	1000 ppm	1.11	1.11	0.5	0.906
6.	2000 ppm	0.52	0.7	0.5	0.555

Table 4

S. No	Concentration of the Test sample (µg/ml)	OD Value calculated at 540 nm absorbance (in triplicates)		
1.	Control	3.609	3.639	3.691
2.	50 ppm	4	4	4
3.	100 ppm	3.662	3.485	3.532
4.	500 ppm	3.248	3.304	3.278
5.	1000 ppm	3.196	3.119	3.103
6.	2000 ppm	3.09	3.12	3.08

B) OD values at 540 nm

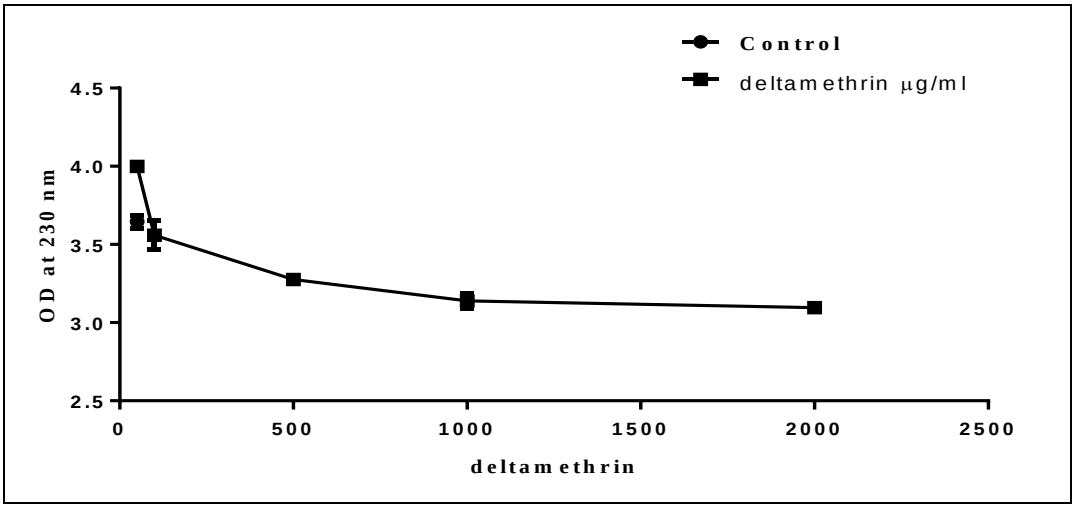

Fig 2

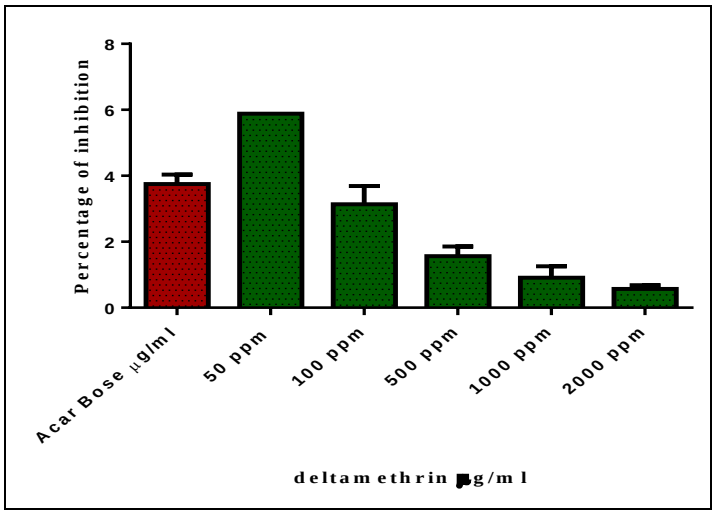

Fig 3

C. IC50 Value of tested sample: 141.6 µg/ml

Table 5

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		2.151
HillSlope		-1.630
IC50		141.6
Std. Error		
LogIC50		0.06519
HillSlope		0.3207
95% Confidence Intervals		

LogIC50		2.010 to 2.292
HillSlope		-2.323 to -0.9375
IC50		102.4 to 195.9
Goodness of Fit		
Degrees of Freedom		13
R square		0.9177
Absolute Sum of Squares		1717
Sy.x		11.49
Number of points		
Analyzed	3	15

Catalase Assay

A) Percentage of inhibition

Table 6

S. No	Concentration of the Test sample (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Control	4.3	4.3	4.21	4.27
2.	50 ppm	7.9	7.9	7.9	7.9
3.	100 ppm	6.29	6.29	6.62	6.4
4.	500 ppm	5.37	6.04	6.03	5.813
5.	1000 ppm	4.54	4.5	4.83	4.623
6.	2000 ppm	4.08	4.07	3.7	3.95

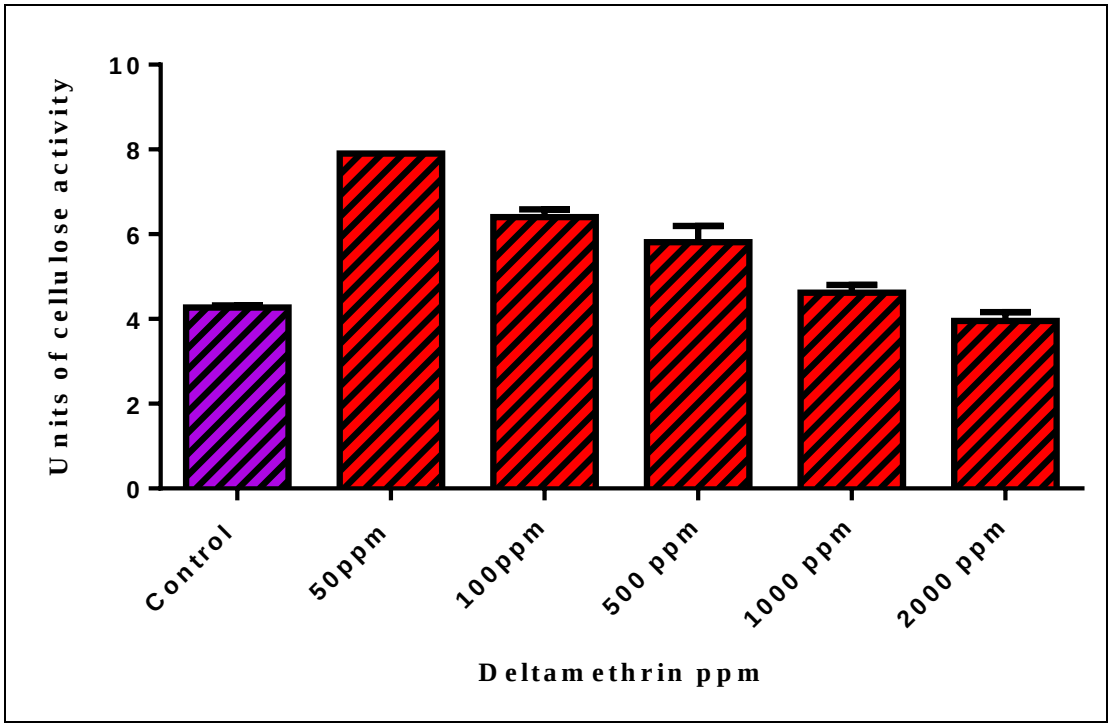

Fig 4

Table 7

S. No	Tested sample concentration (µg/ml)	OD Value at 540 nm (in triplicates)		
1.	Control	3.123	3.124	3.102
2.	50 ppm	4	4	4
3.	100 ppm	3.6	3.54	3.68
4.	500 ppm	3.38	3.54	3.52
5.	1000 ppm	3.17	3.19	3.25
6.	2000 ppm	3.06	3.05	2.98

Discussion

In this study, the acute toxicity of Deltamethrin to silkworm was evaluated in terms of its behavioural and biochemical effects, which play a crucial role in the pesticide resistance mechanisms of organisms. Despite the fact that the lethal concentration of pesticide induced toxicity in organisms, concentrations below the LC50 value

may also have toxicological effects. When larvae were exposed to sublethal concentrations of deltamethrin, a significant reduction in biochemical constituents was observed. The larvae that fed on pesticide-contaminated leaves displayed less feeding behaviour, indicating that their nutritional needs were not met. Etebari *et al*. (2005) suggested that starvation or inadequate feeding could lead to a decrease in a number of biochemical compounds; thus, it could be considered a significant factor in the decline of all biochemical constituents.

Additionally, digestive enzymes play an important role in the development and growth of insects. The process of digestion and the activity of enzymes depend on various factors, including the quantity and quality of food ingested, the larva's age and health, and certain physical factors (Vyjayanthi and Subramanyam, 2002) [12]. Any disruption in enzyme activity prevents insects from obtaining the nutrients necessary for their survival. According to Vyjayanthi and Subramanyam (2002) [12], the decrease in enzyme activity was caused by a lack of substrate due to inadequate dietary intake. Feeding is crucial for stimulating the secretion of digestive enzymes (Shekari *et al*., 2008) [11]. In this study, larvae fed on deltamethrin-treated leaves exhibited less feeding behaviour; consequently, starvation or the inability to form enzyme-substrate complexes may account for the larvae's altered digestive physiology.

Conclusion

Due to the negative impact of sub-lethal concentrations of Deltamethrin on silkworm, there should be grave concern regarding the potential threat posed by this pesticide to society. This study could be useful for the safe and judicious use of pesticides in agricultural practises, so that they do not cause significant harm to non-targeted species, such as mammals, and that their use in the environment is limited. Taken together, our findings gave excellent guidelines for assessing the ecological risk of the insecticides using silkworm as a model organism.

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