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Larval exposure to lambda cyhalothrin affects nutritional physiology in silkworm model (*Bombyx mori.* L)

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Abstract
The synthetic pyrethroids, which are derived from pyrethrins, a natural compound isolated from Chrysanthemum species, constitute the world's most important insecticide market. Lambda cyalothrin, permethrin, deltamethrin fenvalerate, tetramethrin, cypermethrin, and resmethrin are included in this group. These pesticides are widely used in agriculture to combat pest infestation. The pyrethroid insecticides and their introduction metabolite increase the release of neurotransmitters GABA, dopamine, and noradrenaline (and disrupt the endocrine equilibrium). Some pesticides can harm or kill non-target organisms, including humans, but the harmful effects caused by poisoning or injury can be prevented by limiting exposure time. An animal's internal organs and other systems are harmed by pesticide poisoning. The primary entry points for pesticides are oral exposure, inhalation, ocular exposure, and dermal exposure. Even very low levels of developmental exposure can have adverse health effects. The silkworm model has been utilised successfully in numerous aspects of life science research and has significantly aided the advancement of science in this field. In the near future, silkworm may replace mammals as a model for toxicology research. It serves as a supplement and complement.

Keywords: pyrethroids, chrysanthemum, lambda cyhalothrin, insecticides, neurotransmitters, toxicology

Introduction
Lambda-cyhalothrin is a pesticide, belonging to the pyrethroid class, which is used in agricultural, household pest management, food protection, and disease vector control all over the world. The goal of this study was to see if Vitamin C could reduce the propensity of lambda- cyhalothrin (LAMB) induced changes in biochemical markers, oxidative stress, and mammalian kidney enzyme activity. Under normal circumstances, lambda-cyhalothrin is unlikely to cause persistent consequences in humans. Skin irritation, burning facial feeling, headache, respiratory tract irritation, vomiting, nausea, salivation, anorexia, exhaustion, convulsions, and fluid in the lungs are all symptoms of chemical poisoning. Face tingling and burning sensations occurred within 30 minutes after exposure and continued for 6 hours to 2 days, according to laboratory workers. Skin rush was reported by four field workers out of a total of 38. (de Castro 2020) [3] Pyrethroids, such as lambda-cyhalothrin, disturb an organism& nervous system& regular functioning.
Lambda-cyhalothrin may induce paralysis or de bbath in insects by affecting their neural systems. Temperature has an effect on insect paralysis and lambda-cyhalothrin toxicity when insects ingest or touch lambda-cyhalothrin, it affects a wide range of indoor and outdoor insects. Lambda-cyhalothrin contains insect-repelling characteristics. Face tingling and burning feelings were observed by laboratory workers who worked with lambda-cyhalothrin. Symptoms appeared 30 minutes after exposure and continued for 6 to 2 days (de Castro 2020) [3]. All of the occurrences involved humans handling lambda-cyhalothrin that was relatively pure or concentrated. 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

[(R)-cyano-(3-phenoxyphenyl)methyl] is a commercial formulation of Lambda Cyhalothrin. (1S,3S) -3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl] -2,2-dimethylcyclopropane-1-carboxylate) active ingredient 20 g per 100 ml) Karate -20 EC was acquired from A fertiliser house in Tirupati, Andhra Pradesh. By dissolving 0.05 ml of Decis-20 EC in 99.95 ml of hexane, a 100 mg/L stock solution was produced. 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 hexane 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) [2, 8, 6] it was utilised in the present study. All other substances utilised in this investigation were of analytical grade.

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 of lambda cyhalothrin (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 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) [5], and two sublethal doses of lambda cyhalothrin were selected for further study. All the experiments were performed three times in triplicates (50 larvae per replication) (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. In addition, control was prepared by adding the same volume of 0.1 M sodium acetate (Blakemore *et al*., 1995) [1]. (Blakemore *et al*., 1995) [1].

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) [13].

Amylase Assay

Amylase enzyme activity was determined utilising a standard protocol (Bernfeld, 1955; Weidlich *et al*., 2013) [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

For the calculation of the LC50 value (n = 50), a Probit analysis was conducted using the Biostat (2009) software suite three times in triplicate. All other experiments were conducted three times in triplicate (n = 10), whereas this experiment was conducted five times in triplicate (n = 5) and the results are reported as the mean standard deviation. For statistical analysis, a one-way analysis of variance (ANOVA) was conducted with P 0.05, P 0.01

and P 0.001, which were considered significant and highly significant, respectively. Additionally, means were compared using Tukey's post hoc test. Version 6.1 of Microcal Origin was used to generate the graphical displays.

Results

Cellulase Assay

A. OD value at 540nm

Table 1

S. No	Concentration of the Test sample (µg/ml)	OD Value calculated at 540 nm absorbance (in triplicates)		
1.	Control	3.123	3.124	3.102
2.	50 ppm	4	4	4
3.	100 ppm	3.75	3.77	3.619
4.	500 ppm	3.45	3.63	3.74
5.	1000 ppm	3.18	3.25	3.24
6.	2000 ppm	3.07	3.06	3.19

B. Percentage of inhibition

Table 2

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	6.3	6.3	6.9	6.7
4.	500 ppm	5.66	6.41	6.95	6.34
5.	1000 ppm	4.54	4.83	4.79	4.72
6.	2000 ppm	4.08	4.08	4.58	4.24

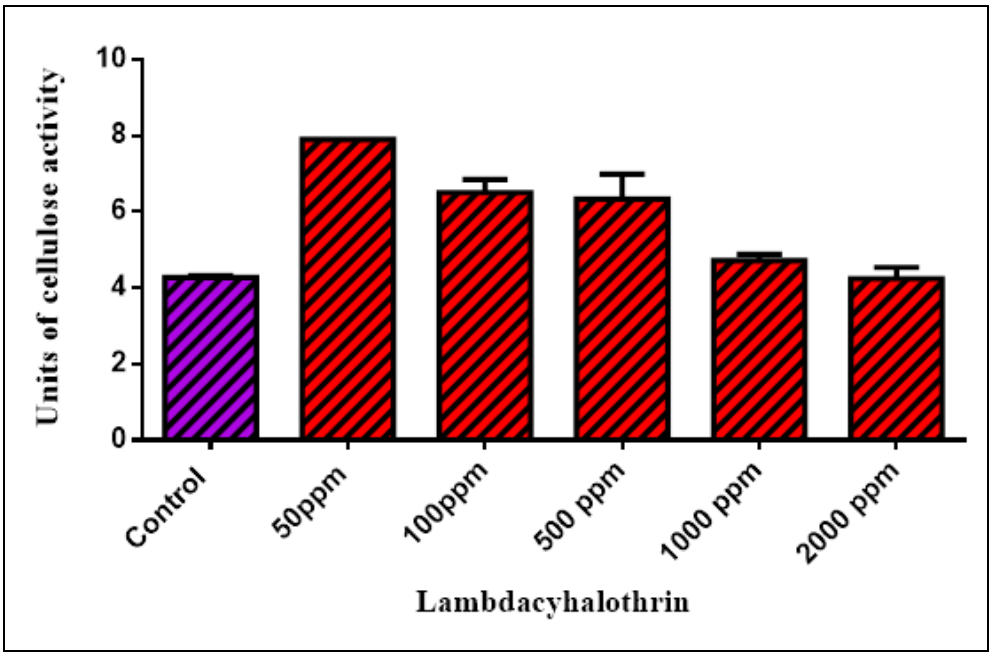

Fig 1

Amylase Assay

A. Results OD Value at 540 nm

Table 3

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	3.77
3.	100 ppm	3.645	3.71	3.642

4.	500 ppm	3.457	3.337	3.365
5.	1000 ppm	3.357	3.331	3.324
6.	2000 ppm	3.218	3.217	3.298

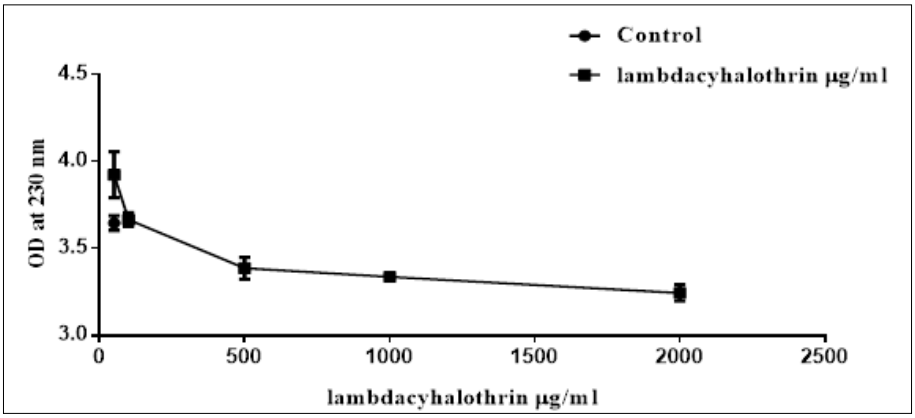

Fig 2

B. **Percentage of inhibition**

Table 4

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	4.52	5.426
3.	100 ppm	3.76	4.17	3.76	3.896
4.	500 ppm	2.46	1.94	2.117	2.172
5.	1000 ppm	2.05	1.94	1.94	1.976
6.	2000 ppm	1.23	1.23	1.7	1.387

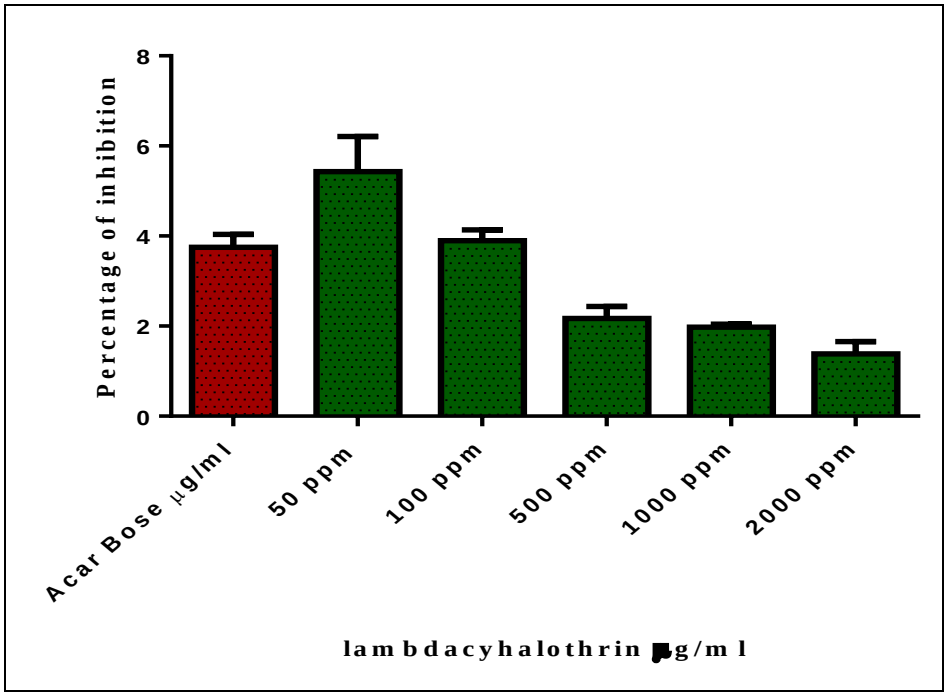

Fig 3

Catalase Assay

A. OD Value at 240 nm

Table 5

S. No	Concentration of the Test Sample (ppm/ml)	OD value		
1.	control	0.832	0.751	0.762
2.	50ppm	0.369	0.303	0.462

3.	100ppm	0.493	0.612	0.442
4.	500ppm	0.814	0.514	0.859
5.	1000ppm	0.761	0.91	0.772
6.	2000ppm	0.725	0.61	0.672

B. Units of Enzyme Activity

Table 6

S. No	Concentration of the Test Sample (ppm/ml)	Units of Catalase Activity (in triplicates)			Mean value
1.	control	0.49	0.43	0.44	0.453
2.	50ppm	1.00	0.84	0.93	0.923
3.	100ppm	0.65	0.82	0.69	0.72
4.	500ppm	0.65	0.41	0.68	0.58
5.	1000ppm	0.38	0.47	0.37	0.407
6.	2000ppm	0.2	0.22	0.34	0.253

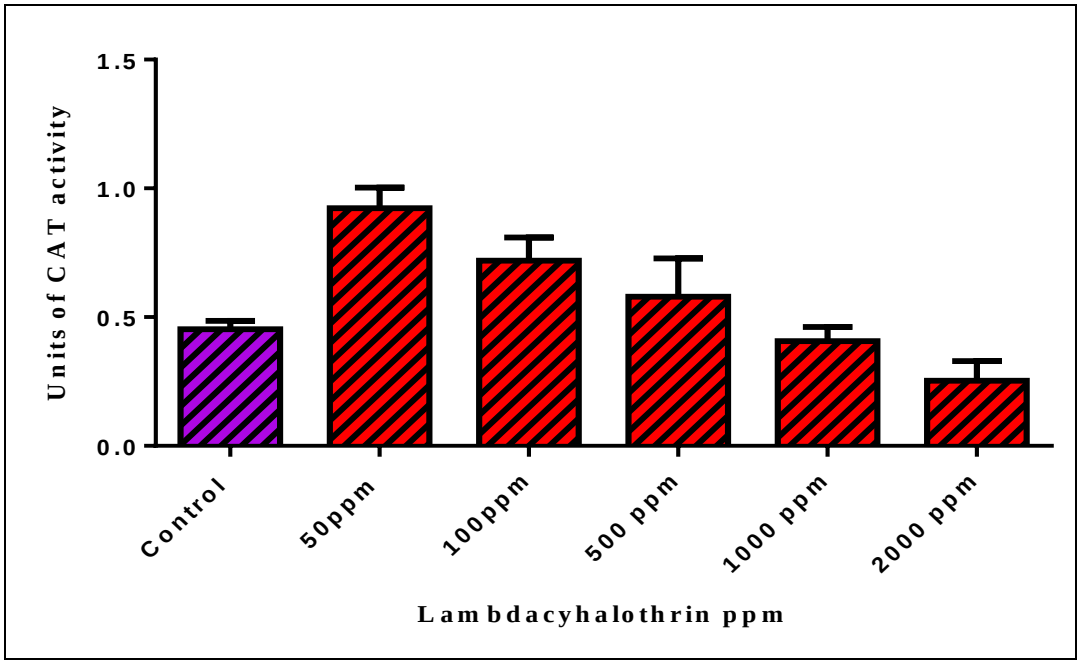

Fig 4

Discussion

The digestive process and enzyme activity depend on a variety of factors, including the quantity and quality of food consumed, the age and health of the insect, and a few physical factors (Vyjayanthi *et al*. 2002) [12]. Any disruption in the activity of digestive enzymes prevents insects from meeting their nutritional needs. In this study, amylase, cellulase, and catalase enzyme activity decreased significantly. According to Vyjayanthi *et al*. (2002) [12], insufficient food intake leads to substrate inaccessibility, which decreases enzyme activity. It was stated previously that exposure to lambda cyhalothrin altered feeding behaviour; therefore, starvation or lack of food could be the cause of the larvae's altered digestive physiology. In addition to the general explanation, the decrease in amylase activity may be due to the cytotoxic effect of sublethal concentrations of lambda cyhalothrin on epithelial cells of the midgut that produce a-amylase. These variations in digestive enzyme activity may affect the food utilisation process and, consequently, the physiological condition of the larava. Simon *et al*. (2008) and Veloso *et al*. (2013) examined the effects of lambda cyhalothrin on the digestive enzyme activity of nontarget organisms, and their findings are consistent with the current findings.

Conclusion

In conclusion, lambda cyhalothrin formulation has a negative effect on silkworm larval hemocytes. In addition, the induced changes in metabolites, nutritional physiology, and immune response were evaluated to better comprehend the possible mechanism of lambda cyhalothrin toxicity in silkworm. Therefore, this study may attribute the severe impact of pyrethroid on silkworms and contribute to the safe use of pesticides for agriculture's sustainable growth. The silkworm model has been utilised successfully in numerous aspects of life science research and has significantly aided the advancement of science in this field. However, there are still numerous obstacles to overcome, and the application of the silkworm model in numerous fields is still in its

infancy, with insufficient animal experiments and clinical trial data. In the near future, silkworms may be used in place of mammals to study the efficacy of drugs. It serves as a supplement and complement. In conclusion, promoting the use of silkworm models in scientific research will provide new insights into traditional problem-solving perspectives, as well as be of great benefit to both science and society.

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