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ORIGINAL ARTICLE

Lethal and Sub-lethal Toxic effects of a Pyrethroid Insecticide, λ-Cyhalothrin on activities of Acetylcholinesterase, Glutamic Oxaloacetic Transaminase, Glutamic Pyruvic Transaminase and Catalase in the Post-larvae of the Prawn *Macrobrachium rosenbergii*

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ABSTRACT
In order to see the toxic effects of a pyrethroid insecticide, λ-cyhalothrin on one of the non-target organisms, the economically important giant freshwater prawn, Macrobrachium rosenbergii was subjected to 96 h bio-assay, its lethal and sub-lethal toxic effects on the activities of neurotransmitter enzyme, acetylcholinesterase (AChE), metabolic enzymes, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), and antioxidant enzyme, catalase (CAT) were assayed. The 96 h LC_{50} value of λ-cyhalothrin for the post-larvae (PL) of M. rosenbergii was found to be 0.066 µg/L. The PL was exposed to one lethal (0.066 µg/L) and two sub lethal concentrations (0.033 and 0.0165 µg/L) of λ-cyhalothrin for 12 days and sampling was done on the whole PL on 4, 8 and 12 days of exposure. Activity of AChE was significantly (P<0.05) inhibited on all sampling days irrespective of the concentration of λ-cyhalothrin. This indicates the fact that λ-cyhalothrin affects neurotransmission in M. rosenbergii PL. The activities of GOT and GPT were found to be significantly (P<0.05) elevated on all sampling days irrespective of the concentration of λ-cyhalothrin. This suggests that λ-cyhalothrin alter hepatopancreas functions to coop-up with toxic stress as an adaptive mechanism. Activity of catalase (CAT) was found to be significantly (P<0.05) decreased in the PL exposed to 0.066 and 0.033 µg/L of λ-cyhalothrin. This indicates the fact that λ-cyhalothrin affects antioxidant enzyme status in M. rosenbergii PL. In the case of 0.0165 µg/L of λ-cyhalothrin, the activity of catalase (CAT) was found to be significantly (P<0.05) elevated. This suggests that the stress coop-up mechanism was in operation at this and below this toxic level. At the outset λ-cyhalothrin has the potential to affect the neurotransmission, hepatopancreas function and antioxidant capacity of M. rosenbergii PL.
Keywords: *λ-cyhalothrin; Macrobrachium rosenbergii; AChE; GOT; GPT; Catalase.*

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INTRODUCTION

Synthetic pyrethroid insecticides are used worldwide in agriculture and vector control programmes, because of their high toxicity to a wide range of insects and low toxicity to mammals and birds, and rapid biodegradability [1]. Pyrethroids act primarily on the nervous system. The pyrethroids are broadly categorised into two types, (i) pyrethroids have a basic cyclopropane carboxylic ester structure, which generally affects the peripheral nerves and are best suitable to small-scale personal applications, and, (ii) pyrethroids with the cyano group, which affects the entire nervous system and are preferred for large scale uses due to their higher potency [2, 3].

λ-cyhalothrin ($C_{23}H_{19}ClF_3NO_3$) belongs to the second category, it is used for the control of agriculture and household pests. It is commonly mixed with buprofezin, pirimicarb, dimethoate, and tetramethrin. It is marketed as Charge, Excaliber, Grenade, Hallmark, Icon, Karate, Matador, OMS 0321, PP321, Saber,

Samurai, and Sentinel. It is used to control a wide range of insect pests in a variety of crops, including cotton and vegetable production [4]. It is also used as indoor residual spray, space spray for treatment of mosquito control and pests of public health importance [1, 5].

Residue of λ-cyhalothrin may cause environmental impact. It would adversely affect the aquatic biota, animal and human health [6-13]. Human exposure to pyrethroids is reported to occur mainly occupational during application or through pyrethroid residues, which have been detected in milk, blood of dairy cows and cattle meat as well as vegetables and fruits [14, 15]. Moreover, pyrethroid metabolites have been detected in urine of adults, children and pregnant women [10].

The acute and chronic toxic effects of pesticides, such as endosulfan, carbaryl, carbendazim, carbofuran, chlorpyrifos, dichlorvos, quinalphos, dimethoate, lindane, methomyl, monocrotophos and profenofos have been reported on aquatic animals, particularly freshwater prawns [16-31]. However, no report is available pertaining to λ-cyhalothrin induced changes on the biochemistry and physiology of aquatic organism in general, crustacean in particular and *Macrobrachium* in very particular. Therefore, in the present study, investigation was made on λ-cyhalothrin induced changes on the activities of the neurotransmitter, acetylcholinesterase (AChE), the metabolic enzymes, glutamic oxaloacetic transaminase (GOT) also known as aspartate aminotransferase (ASAT) and glutamic pyruvic transaminase (GPT) also known as alanine aminotransferase (ALAT), and antioxidant enzyme, catalase (CAT) in the post-larvae of the commercially important freshwater prawn *Macrobrachium rosenbergii*, commonly called the scampi.

MATERIALS AND METHODS

Maintenance of PL

The post-larvae of *M. rosenbergii* were purchased from Happy Bay Aqua Nova Hatchery, Mugaiyur, Marakanam Taluk, Kancheepuram District, Tamil Nadu, India. They were safely brought to the laboratory in polythene bags filled with hatchery water and well-oxygenated. They were stocked in large cement tank (6' × 4' × 3') and acclimatized for 2 weeks in ground water. During which they were fed with boiled egg albumin, *Artemia* nauplii and commercially available scampi crumble feed alternatively thrice a day *ad libitum*. The excreta, unfed feed and exuvia if any were removed, three fourth of the water was renewed daily and adequately aerated.

Determination of LC_{50} value

The pyrethroid insecticide, λ-cyhalothrin 2.5% EC (CONSTANT) was purchased from local agro service centre. Ten concentrations of λ-cyhalothrin (0.0125-0.1250 µg/L) were prepared by mixing in double distilled water afresh on every day. Post larvae (PL) of *M.rosenbergii* (1.8 ±0.4 cm and 0.14 ± 0.02 g) were transferred to plastic aquaria of 10 L capacity (each with 10 PL) with ground water for 2 days. Out of eleven groups, one PL group was served as control and others were exposed to ten different known concentrations (0.0125-0.1250 µg/L) of λ-cyhalothrin for 96 h to assess their LC_{50} value as per the guidelines prescribed by ASTM [32]. The experiment was conducted in triplicates. The toxic water medium was renewed daily by siphoning method causing minimum disturbance to the prawns and freshly prepared concentrations of λ-cyhalothrin was added separately to maintain the toxic level in a steady state. During the experiment the prawns were neither fed nor aerated. The concentrations and their respective mortality percentage were subjected to computation for calculation of the median lethal concentration. The 96 h LC_{50} value with 95% confidence limits was assessed using computerized program of Finney [33] method of probit analysis and it was found to be 0.066 µg/L.

Lethal and sub-lethal toxicity

The 96 h LC_{50} concentration (0.066 µg/L) and two sub-lethal concentrations derived from it (0.033 µg/L and 0.0165 µg/L) were chosen and the PL were exposed to these concentrations for a period of 12 days. A common control was also maintained. Each group comprised 5 aquaria (15 L capacity) and each aquarium housed 20 PL. The PL were fed with scampi crumble feed *ad libitum* and the medium was not aerated. The toxic water medium was renewed daily by siphoning method causing minimum disturbance to the prawns and freshly prepared concentrations of λ-cyhalothrin was added. The dead PL was removed during the experiment. The mortality was higher in 0.066 µg/L followed by 0.033 µg/L and 0.0165 µg/L. The remaining PL was sampled on day 4, 8 and 12 of exposure for enzyme assays, by excluding the exoskeleton, carapace, uropod and telson.

Enzyme assays

Acetylcholinesterase

The activity of acetylcholinesterase (AChE) was determined according to the method of Ellman et al. [34]. 100 mg of test PL tissues was homogenized (10% w/v) in ice-cold 50 mM Tris buffer (pH 7.4), centrifuged at 9300 g for 20 min at 4 ºC and the supernatant was used to assay the enzyme activity. Based on

degradation of acetylthiocholine iodide by AChE into a product which binds to 5,5-dithiobis-2-nitrobezoic acid (DTNB) and the formation of yellow colour was measured at 412 nm. One unit of AChE activity was expressed as µmoles acetylcholine hydrolyzed/min/mg protein under experimental conditions. Soluble protein concentration was determined by the method of Lowry et al. [35].

Metabolic enzymes

The metabolic enzymes such as glutamic oxaloacetate transaminase (GOT) and glutamic pyruvic transaminase (GPT) were analyzed by the method of Reitman and Frankel [36] using a med source kit (Medsource Ozone Biomedicals Pvt. Ltd. Haryana, India). 100 mg of PL tissues were homogenized in 0.25 M sucrose and centrifuged at 3300 g for 20 min in a high speed cooling centrifuge at 4 ºC. The supernatant was used as the enzyme source.

GOT analysis, the substrate solution, L-aspartic acid (500 µL; pH, 7.4) was added to a 100 µL sample and incubated at 37 ºC for 1 h. Further, 500 µL of 2, 4-dinitrophenyl hydrazine was added and allowed to stand for 20 min at room temperature. Then 3 mL of freshly prepared 4 N sodium hydroxide solution was added to the above solution. The color development was read at 505 nm using spectrophotometer within 15 min. Sodium pyruvate (160 U/L) was used as a calibrator. The activity of GOT was expressed as U/L.

GPT analysis, buffered L-alanine, 2-oxoglutarate substrate (500 µL; pH, 7.4) was added to a 100 µL sample and incubated at 37 ºC for 20 min. With this, 500 µL of 2, 4-dinitrophenyl hydrazine was added and allowed to stand at room temperature for 30 min followed by the addition of 3 mL of freshly prepared 4 N sodium hydroxide solution. The color development was read at 505 nm using a spectrophotometer within 15 min. Sodium pyruvate (170 U/L) was used as a calibrator. The activity of GPT was expressed as U/L.

Catalase

Catalase (CAT) activity was assayed by the method Sinha et al. [37]. Briefly, 0.1 mL of 5% tissue homogenate was incubated with 0.5 mL of H_2O_2 (0.2 M) at 37 ºC for 90 sec in the presence of 0.01 M phosphate buffer (pH 7.4). Reaction was stopped by adding 5% dichromate solution. Further, samples were incubated at 100 ºC for 15 min in boiling water bath. Amount of H_2O_2 consumed was determined by recording absorbance at 570 nm. CAT activity was calculated in terms of µmol H_2O_2 consumed/min/mg protein.

Statistical analysis

The differences between control and experimental groups were analyzed by adopting student-'t' test using SPSS software (version 20.0). All measurements were performed in triplicates and the results are expressed as mean ± SD of three individual observations. $P<0.05$ was fixed to assess the statistical significance.

RESULTS AND DISCUSSION

Bio-assay

In the present study, the 96 h LC_{50} of λ-cyhalothrin for *M. rosenbergii* PL was assessed to be 0.066 µg/L; 95% confidence limits: upper, 0.074 µg/L; lower, 0.058 µg/L (Table 1). The death of PL was faster and mortality was higher in response to higher concentrations of λ-cyhalothrin. The reported 96 h LC_{50} value of λ-cyhalothrin for *Macrobrachium nipponense* was 0.04 µg/L [38]. These values are very similar to that of the value evaluated in the present study. Therefore, λ-cyhalothrin is a potent toxicant even at lower concentration, and thus, demanding a proper attention. It has been reported that the 96 h LC_{50} of 5% EC λ-cyhalothrin for the fish, *Cyprinus carpio* as 0.50 µg/L [39]. Similarly, the 96 h LC_{50} values of λ-cyhalothrin for *Oncorhynchus mykiss* Rich (rainbow trout) and *Lepomis macrochirus* (bluegill sunfish) were 0.24 and 0.21 µg/L respectively [40]. These values are much higher than that of the value evaluated in the present study. Therefore, it is valid to mention here that toxicity of a chemical is attributed to many factors, such as water temperature, purity of the toxin, life stage of an organism, evolutionary hierarchy of the organism etc. Moreover, the reported 96 h LC_{50} value of gamma-cyhalothrin for *M. nippoense* was 0.28 µg/L [38].

Behavioural abnormalities

During the bio-assay, the test PL was exhibited behavioural abnormalities, such as fast jerking, frequent jumping, erratic swimming, spiraling, convulsions and tendency to escape from the aquaria. Following this state of hyper excitability, the test PL become inactive and showed muscular spasms and loss of orientation. There was loss of equilibrium and paralysis, which ultimately caused death. Moreover, the test PL showed mucus secretion over the gill chamber. Actually, mucus secretion is an adaptive mechanism to reduce the vulnerable surface area of the gills in order to prevent absorption of toxicant, which led to inhibition of oxygen consumption due to 'coagulation film anoxia' which in turn led to 'histotoxic anoxia' and ultimately caused death of test PL. These behavioural changes were severe in

higher acute concentrations of λ-cyhalothrin to which the PL was exposed (> 0.05 µg/L). Similar behavioural abnormalities have also been reported in the juveniles of the freshwater prawn, *Macrobrachium malcolmsonii* exposed to acute concentrations of endosulfan and carbaryl [26, 27].

Acetylcholinesterase

In the present study, activity of AChE was found to be significantly (P<0.05) decreased on all the sampling days in both lethal and sub-lethal concentrations of λ-cyhalothrin when compared with control (Table 2). However, maximum inhibition was seen on 12th day in 0.066 µg/L followed by 0.033 µg/L and 0.0165 µg/L concentrations of λ-cyhalothrin. This indicates the fact that the toxicity of λ-cyhalothrin causes accumulation of ACh at neuronal and neuromuscular junctions, which led to impairment in hydrolysis of ACh, suggesting disruption of synaptic transmission in the cholinergic system of *M. rosenbergii* PL. The recorded inhibition of AChE activity can be well correlated with observed behavioural changes in this study. Furthermore, this might have led to reduce locomotion, feeding, ventilation of gills etc., in *M. rosenbergii*. Similar inhibition of AChE activity has been reported in fishes exposed to specific type of pyrethroids [41, 42] and chlorpyrifos [43]. Inhibition in AChE activity has been reported in the grass shrimp, *Palaemonetes pugio* embryos exposed to OP pesticides, chlorpyrifos and malathion [44], in *M. malcolmsonii* exposed to dichlorvos, endosulfan, and carbaryl [19, 20, 24], in the freshwater shrimp *Paratya australiensis* exposed to dimethoate [45], in the clam, *Ruditapes decussatus* exposed to malathion [46], in the riceland prawn, *Macrobrachium lanchesteri* on exposure to chlorpyrifos [47], in freshwater fairy shrimp, *Streptocephalus dichotomus* exposed to malathion and glyphosate [48], and in *M. rosenbergii* exposed to heavy metals, $CdCl_2$, CrO_3 and $Pb(NO_3)_2$, and OP insecticides, quinalphos and dimethoate [30, 49].

Fulton and Key [50] reported that inhibition of cholinesterase (ChE) activity has been widely used as a specific biomarker for pesticide contamination in aquatic animals. AChE plays a key role in regulation of cholinergic nervous transmission. Indeed, AChE is responsible for the hydrolytic degradation of acetylcholine, which is the primary neurotransmitter in the sensory and neuromuscular systems. AChE inhibition leads to over stimulation of the central and peripheral nervous systems, resulting in deleterious neurotoxic effects in organisms, which can lead to death.

Metabolic enzymes

GOT and GPT are known to play a key role in mobilizing L-amino acids for gluconeogenesis, and they function as links between carbohydrate and protein metabolism under altered physiological, pathological, and induced environmental stress conditions [51]. In the present study, activities of GOT and GPT were found to be significantly (P<0.05) elevated in test PL on all sampling days irrespective of concentrations of λ-cyhalothrin when compared with control (Table 2). However, maximum elevation in these enzymes activities was seen on 12th day in 0.066 µg/L followed by 0.033 µg/L and 0.0165 µg/L concentration of λ-cyhalothrin. The increase in GOT and GPT activities suggest the extent of liver damage in aquatic organisms [52]. Elevation in activities of GOT and GPT have also been reported in the marine shrimp, *Litopenaeus vannamei* exposed to DDT, lorsban, diazinon, folidal, guzation and lindane [53], in the green mussel, *Perna viridis* exposed to copper, lead and zinc [54] and in *M. rosenbergii* exposed to heavy metals, $CdCl_2$, CrO_3 and $Pb(NO_3)_2$, and OP insecticides, quinalphos and dimethoate [30, 49].

Increase/ decrease in GOT and GPT activity suggested tissue/ organ damage/ dysfunction [55, 56]. In the present study, the increase recorded in GOT activity suggests that an important reaction ofthe molecular rearrangement involving amino acids linked to the citric acid cycle at two points (oxaloacetic and ketoglutaric acids) was altered. Similarly, the increasein GPT indicates the fact that the test PL terribly required intensive glycogenesis to coop-up the energy crisis occurred due to λ-cyhalothrin toxicity.

Catalase

CAT, a sensitive antioxidant biomarker enzyme against oxygen free radicals, the reactive oxygen species generated due to oxidative stress. It facilitates the breakdown of hydrogen peroxide into hydrogen and oxygen, thereby it plays an antioxidant role, its activity increasing in organisms submitted to oxidative stress [57]. In the present study, the activity of CAT was found to be significantly (P<0.05) lower in test PL on all sampling days when treated with λ-cyhalothrin (Table 2). The inhibition of CAT was greater in lethal concentration followed by higher sub-lethal concentration of λ-cyhalothrin. Whereas, the activity of CAT was found to be significantly (P<0.05) elevated in the case of lower sub-lethal concentration of λ-cyhalothrin (0.016 µg/L). This indicates the fact that in the lower sub-lethal concentration of λ-cyhalothrin, the test PL was able to adopt or mitigate the excessive hydrogen peroxide generated by the action of superoxide dismutase on the excessive oxygen free radicals. On the other hand, in the higher sub-lethal and lethal concentrations of λ-cyhalothrin (0.066 µg/L and 0.033 µg/L respectively), excessive hydrogen peroxide or superoxide radical might have produced, which in turn inhibit CAT activity. Therefore, the protective mechanism was hampered in test PL. The decreased activity of CAT has been

reported in fish exposed to deltamethrin [58], chlorpyrifos [43] and malathion [59]. Inhibited catalase activity due to oxidative damage has also been reported in the brackish water prawn *Penaeus monodon* exposed to fenvalerate [60], in the clam, *Ruditapes decussates* [46] and in *M. rosenbergii* exposed to heavy metals, $CdCl_2$, CrO_3 and $Pb(NO_3)_2$, and OP insecticides, quinalphos and dimethoate [30, 49].

Table 1: 96 h LC_{50} evaluation of lambda-cyhalothrin on *M. rosenbergii* PL

Concentrations of λ – cyhalothrin (µg/L)	Observed Mortality			Mortality		LC_{50} (µg/L)	95% confidence limit	
	T1	T2	T3	Mean	%		Upper (µg/L)	Lower (µg/L)
0.0125	0	0	0	0.00	0.00			
0.0250	1	1	1	1.00	10.0			
0.0375	2	3	3	2.66	26.6			
0.0500	3	4	3	33.3	33.3			
0.0625	5	5	5	5.00	50.0	0.066	0.074	0.058
0.0750	6	6	6	6.00	60.0			
0.0875	7	7	7	7.00	70.0			
0.1000	8	8	9	8.33	83.3			
0.1125	10	9	10	9.66	96.6			
0.1250	10	10	10	10.00	100			

T1, T2, and T3 represent triplicates of exposure each with ten numbers of *M. rosenbergii* PL.

Table 2: Activities of AChE (µmol/min/mg protein), GOT (U/L), GPT (U/L) and CAT (µmol H_2O_2 consumed/min/mg protein) in *M. rosenbergii* PL exposed to lethal and sub-lethal concentrations of lambda-cyhalothrin

Enzymes	Days	Control	Lethal and sub-lethal concentration of λ – cyhalothrin		
			Lethal (0.066 µg/L)	Sub-lethal (0.033 µg/L)	Sub-lethal (0.016 µg/L)
AChE	4	3.62±0.05	2.17±0.04	2.25±0.10	2.63±0.11
	8	3.63±0.06	2.10±0.08	2.20±0.09	2.48±0.14
	12	3.64±0.07	2.02±0.05	2.15±0.06	2.28±0.11
GOT	4	26.16±0.40	35.26±0.42	32.54±0.37	30.76±0.29
	8	26.34±0.31	37.61±0.42	33.68±0.33	31.82±0.25
	12	26.43±0.42	38.99±0.33	34.43±0.32	32.12±0.22
GPT	4	22.26±0.29	29.82±0.48	27.52±0.46	25.62±0.31
	8	22.47±0.22	30.52±0.63	29.83±0.51	27.05±0.36
	12	22.70±0.23	32.31±0.41	30.17±0.60	29.30±0.32
CAT	4	37.53±0.51	35.28±0.50	34.53±0.39	39.15±0.45
	8	37.41±0.42	33.17±0.52	32.34±0.36	39.52±0.51
	12	37.22±0.40	32.11±0.43	31.33±0.35	41.12±0.48

Each value is mean ± SD of 3 individual observations.
All the values are significant at $P< 0.05$.
AChE, Acetylcholinesterase; GOT, Glutamic oxaloacetic transaminase;
GPT, Glutamic pyruvic transaminase; CAT, catalase

CONCLUSION

In conclusion, λ-cyhalothrin stress inhibits AChE activity, induces GOT and GPT activities, and induces as well as inhibits CAT activity in *M. rosenbergii* PL. Therefore, the possibility of water pollution by λ-cyhalothrin can be monitored through adopting proper assessment mechanism.

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