www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(12): 189-192

www.thepharmajournal.com
Received: 10-10-2019
Accepted: 12-11-2019

Nandini Pal
Post Graduate Department of Zoology, Bidhannagar College, EB-2, Sector-1, Salt Lake City, Kolkata, West Bengal, India

Suman Bej
Post Graduate Department of Zoology, Bidhannagar College, EB-2, Sector-1, Salt Lake City, Kolkata, West Bengal, India

Corresponding Author:
Nandini Pal
Post Graduate Department of Zoology, Bidhannagar College, EB-2, Sector-1, Salt Lake City, Kolkata, West Bengal, India

Study of Ecotoxicological impacts of alpha cypermethrin 10 EC on fresh water snail *Bellamya bengalensis* Lamark, 1822 (Gastropoda: Viviparidae) in West Bengal, India

Nandini Pal and Suman Bej

Abstract
Bellamya bengalensis is one of the most important edible protein sources having great economic importance. Here the experiment was conducted to study the impact of Alpha Cypermethrin (10 EC) emphasizing on different physiological aspects on *Bellamya bengalensis*. It has been found that exposure of the test animal in different concentrations of Alpha Cypermethrin lead to various physiological changes like mucous secretion, floating behavior, avoidance reaction. Considering the chronic toxicity, exposed to 0.0015ppm of Alpha Cypermethrin for 7 days expressed an increase in glass surface adhesion of haemocytes and maximum percentage of haemocyte aggregation was recorded in 0.0015ppm of pesticide formulation for 7 and 10 days of exposure.

Keywords: *Bellamya bengalensis*, alpha cypermethrin, acute toxicity, chronic toxicity, lethal concentration

Introduction

Molluscs are the group of organisms which constitute the second largest invertebrates and most successful group next to insects (Abbot, 1989; Rao, 1989; Bouchet, 1991) [1, 8]. Extensive study throughout the world has been carried out against the Mollusc and it is estimated that number of described species varies from 80,000 to 135,000 and it has been found that among the described species about 5000 species are belong to fresh water ecosystem (Abbott, 1989; Seddon, 2000) [1, 10]. Though viviparids prefer stagnant water bodies for their long time sustainability, these are also found in the irrigated paddy fields and rarely running water (Saha *et al*., 2017). Most of the snails specially focusing on freshwater habitat served as an intermediate hosts for various parasitic worms of man and his domestic animals. The predilection of snails for fungal foods increases the attractiveness of diseased plant and possibility of spreading of the disease by these snails (Ahirrao, 2002) [2]. The fresh water snail *Bellamya bengalensis* is considered as an important economical species with a high demand of edible protein (17.2%), carbohydrate (13.12%) and lipid (4.83%) and they play an important role in aquatic ecosystem as Bioindicator (Khalua and Tripathya, 2014) [6]. After green revolution the modern agriculture is solely dependent upon the application of pesticides which are widely used to combat agricultural pest and their application has greatly contributed to boost up the agricultural production. But none of these pesticides employed are specific and due to their indiscriminate and wide spread use, several non-target organisms like snails, fishes, crabs etc. of the ecosystem are adversely affected (Magare, 1993). [7] Alpha Cypermethrin is the fourth generation synthetic pyrethroid insecticide used extensively in agricultural field to suppress the pest population due to its quick knock-down property but bioacuumulation in the living aquatic organisms is another important related issue of this insecticide due to its high lipophilic property and low water solubility which leads various physiological hazards to these aquatic fauna (Bacci *et al*., 1987) [3]. Henceforth considering from all possible angles the present study has been conducted to investigate the physiological change of freshwater snail, *Bellamya bengalensis* exposed to various concentration of Alpha Cypermethrin.

Materials and Methods

Determination of Acute Toxicity

Collection of test animals and chemicals

Adult healthy *Bellamya bengalensis* of same size (5.5-7.5 gm weight and 3-4 cm. shell height) were manually collected from the nearby water body (Mathura Beel) of North 24 Parganas, District of West Bengal. Animals were taken into the laboratory by keeping them in round plastic container of 3 liter volume of 10 individuals per container in a moist condition. Commercial grade Alpha Cypermethrin (10 EC) was collected from the local market for the experiment.

Laboratory maintenance of test animals

Prior to starting the above experiment, test animals were acclimatized for 5 days in the laboratory ambient condition. During this period *B. bengalensis* was maintained with aquaria with fresh supply of pond water having temperature of 29 °C ± 3 °C. During the course of acclimatization and experiment, the animals were fed with chopped *Hydrilla* sp. and common aquatic weeds.

Determination of LC_{50} and behavioral abnormality

Static replacement bioassays with the *B. bengalensis* was conducted in 3 liter Borosil glass jar each containing 2 liter pond water (Temperature 28.7 ± 0.4 °C, pH 7.1 ± 0.2, Free CO_2 9.9 ± 0.5 mg/ liter, Dissolved Oxygen (DO) 5.5 ± 0.3 mg/ liter, Total alkalinity 181 ± 8.6 mg/ liter as CaCO3, Hardness 119 ± 6.2 mg/ liter as CaCO3). The experiment was set by using four jars with the test animal exposed to same concentration (10 EC) of Alpha Cypermethrin with four replications along with control. Stock solution of the test chemical and its dilutions were
made following the method of American Public Health Association (2012). Initially, rough range finding tests were conducted for the test organism to determine the dose range at which mortality occurs. The selected test concentrations of Alpha Cypermethrin were finally used for the determination of LC_{50} values for the *B. bengalensis*. Ten test organisms (7.7±0.5 gm weight and 4.2±0.8 cm shell height) were used in each replicate. The number of dead organisms was counted at every 24h during the experiment. Water chemical analysis and the bio assays were done following the methods outlined in American Public Health Association (2012).

Determination of Chronic Toxicity

For determining the chronic toxicity, *B. bengalensis* were exposed to 10 and 20% sub lethal doses of Alpha Cypermethrin for 20 days to take the different observations viz. cell adhesion, aggregation and morphology of haemocytes on 7th, 10th and 20th day of exposure.

Collection of haemocyte

Shells of the control and post treated animals were cleaned by gentle brushing under running tap water to remove adhered plant species and clay particles followed by sterilization with ethanol and were bled aseptically and the haemolymph was collected from foot muscle at a volume not exceeding 1ml bleed per day. The bleeding and collection procedure was carried out at 4 ºC to prevent cell aggregation.

Adhesion assay of haemocytes

Viability of haemocytes was checked by Trypan Blue staining following the principle of dye exclusion and uniform cell density was adjusted by sterile snail saline (SSS). Viable cells (> 95%) of uniform density were carefully plated over grease free sterile glass slide kept in sterile humid chamber. Haemocytes of control and treated *B. bengalensis* were incubated in humid chamber for 150 minutes over glass surface for complete adhesion. Gentle jetting of SSS was applied to remove non-adherent haemocyte population settled over slide due to gravity and was enumerated by Neubauer hemocytometer. Adherent and non adherent cell populations were examined microscopically after cytofixation and staining with Giemsa's stain.

Aggregation assay

Aggregation assay (Percent of aggregation) was determined using following formula:

$$\text{The percentage of cells Remaining free} = \frac{\text{Total free cell numbers in test treatment}}{\text{Total free cell numbers in fixed fresh haemolymph}} \times 100$$

Fixation and staining

The fixed monolayer of cells was further fixed with methanol and stained with Giemsa's stain on slide for examination under microscope.

Result and Discussion

Acute toxicity

The lethal concentration of Alpha Cypermethrin to *Bellamya bengalensis* is summarized in (Table 1). No mortality of test animal was recorded in control during the experiment. In the present study LC_{50} value of Alpha Cypermethrin to *B. bengalensis* is estimated at 0.015 mg/ liter. None of the literature was found on the ecotoxicological impact on *Bellamya bengalensis*. But based on other perusal available literature on other gastropods it has been estimated that the findings of the present experiment follow the similar trends with other findings. Bej *et al.*, 2015 [5] reported that 96h LC_{50} value of Alpha Cypermethrin to *Branchiura sowerbyi* was 0.013 mg / liter. Another experiment was conducted by Yordanova *et al*., 2009 [11] and they reported that the 48h EC_{50} value of Alpha Cypermethrin for *Daphnia magna* was 0.0008 mg / liter and for *Gammarus pulex* 24h LC_{50} value was 0.0003 mg/ liter. There is no report on the LC_{50} value of alpha cypermethrin on *Bellamya* or other molluscs, but in comparison to the data of fish and other aquatic invertebrates it can conclude that the pesticide Alpha Cypermethrin is highly toxic to the test animal *Bellamya bengalensis.*

Table 1: Lethal concentrations (LC Values) along with 95% confidence limits of alpha cypermethrin to the *Bellamya bengalensis* at different hours of exposure

Test organisms	Concentration (mg/ liter)				
	LC Values	24 hours	48 hours	72 hours	96 hours
Bellamya bengalensis	LC_{1}	0.004 (0.001-0.008)	0.003 (0.001-0.006)	0.002 (0.001-0.005)	0.002 (0.000-0.004)
	LC_{5}	0.008 (0.003-0.012)	0.006 (0.002-0.009)	0.004 (0.001-0.007)	0.003 (0.001-0.006)
	LC_{10}	0.010 (0.005-0.015)	0.008 (0.003-0.012)	0.006 (0.002-0.009)	0.005 (0.001-0.008)
	LC_{15}	0.013 (0.006-0.017)	0.010 (0.004-0.014)	0.008 (0.003-0.011)	0.006 (0.002-0.009)
	LC_{50}	0.030 (0.024-0.038)	0.024 (0.018-0.031)	0.019 (0.013-0.024)	0.015 (0.010-0.020)
	LC_{85}	0.071 (0.051-0.139)	0.062 (0.045-0.115)	0.046 (0.035-0.075)	0.040 (0.030-0.075)
	LC_{90}	0.087 (0.060-0.195)	0.077 (0.054-0.163)	0.058 (0.042-0.104)	0.051 (0.035-0.110)
	LC_{95}	0.118 (0.075-0.324)	0.116 (0.069-0.277)	0.079 (0.054-0.170)	0.071 (0.046-0.196)
	LC_{99}	0.208 (0.114-0.843)	0.196 (0.108-0.758)	0.144 (0.086-0.437)	0.134 (0.073-0.589)

Behavioral responses shown by the test animals during the bio-assay were also observed and tabulated in the second table (Table 2). Different types of behavioral responses showed by the *Bellamya bengalensis* were accounted as Mucous Secretion (MS) (Fig 1), Avoidance Reaction (AR) and Floating Behavior (FB) (Fig 2). The test animals showed maximum mucous secretion in the following concentrations 0.025, 0.03, 0.035, 0.04 and 0.045 mg/ liter in 24 hours and 48 hours and gradually decrease in 72 and 96 hours respectively. The maximum avoidance reaction was recorded in 72h in following doses from 0.02, 0.025, 0.03 mg/ liter. Floating behavior was shown maximum by the test animals in 24 hours and 72hours exposure time. In 48 hours and 96 hours the floating behavior became gradually decrease.

Table 2: Impact of Alpha Cypermethrin on behavioral response of *Bellamya bengalensis* exposed to various concentrations during different hours of exposure (MS: mucus secretion; AR: avoidance reaction; FB: floating behavior; –: none; +: mild; ++: moderate; +++: strong)

DOSE (mg/ liter)		24h			48h			72h			96h		
		MS	AR	FB	MS	AR	FB	MS	AR	FB	MS	AR	FB
Sl. No.	Conc.												
1	00	-	-	-	-	-	-	-	-	-	-	-	-
2	0.005	+	-	-	+	-	-	+	-	-	+	-	-
3	0.01	+	-	-	+	-	-	+	+	-	+	++	-
4	0.015	+	-	+	+	+	+	+	++	+	+	++	+
5	0.02	++	++	+	++	++	++	+	+++	+	+	+++	+
6	0.025	++	++	++	+++	++	++	+++	+++	+	+	++	+
7	0.03	+++	++	++	+++	+++	++	++	+++	++	+	+	++
8	0.035	+++	+++	+++	+++	+++	+++	++	++	++	-	+	+
9	0.04	+++	++	+++	+++	++	++	++	+	+	-	-	-
10	0.045	+++	+	++	+++	+	+	+	-	-	-	-	-
11	0.05	+++	+	++	++	-	+	-	-	-			
12	0.055	+++	-	+	+	-	-	-	-	-			

Fig 1: Mucous secretion

Fig 2: Floating behavior

Chronic toxicity

Adhesion behavior of Haemocytes

After isolation of haemocytes from haemocoel, the clumps or aggregates were formed within 10-15 minutes and subsequently when cells or aggregates were allowed to settle down on glass surface, they got adhered flattened and dispersed. During subsequent incubation periods cells were wetted at the surface of the clump and in contact with the substratum got adhered to the substratum. Haemocytes of *Bellamya bengalensis* exposed to 0.0015ppm of Alpha Cypermethrin for 7 days expressed an increase in glass surface adhesion in comparison to control. Followed by

depression in adhesion response. A treatment of 0.003ppm Alpha Cypermethrin for 10 and 20 days exhibit a depression in glass surface adherence compared to control.

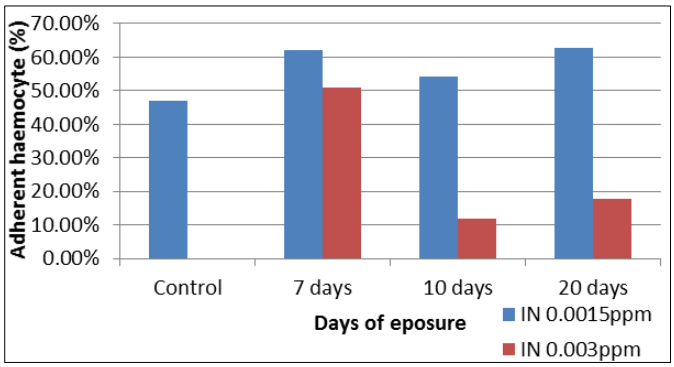

Fig 3: Graphical presentation of adherent response of haemocyte of *Bellamya bengalensis* exposed to sub lethal concentration of Alpha Cypermethrin

Aggregation behavior of haemocyte

Immediately after collection of haemolymph from gastropod, the haemocytes appeared as round in shape. Sometimes aggregation occurred rapidly during the power of bleeding. After initial intercellular contact, cells were found to form aggregate and form clump. Cell aggregation or clump formation is involved in maintaining blood homeostatis and wound healing process in molluscs. An increase of percentage of haemocyte aggregation was recorded in 0.0015ppm of pesticide formulation for 7 and 10 days of exposure followed by decrease in aggregation response for 20 days of exposure. However *Bellamya bengalensis* exposed to 0.03ppm of exposure showed a progressive decrease of aggregation from 7 to 10 days followed by increase in aggregation response in 20 days of exposure.

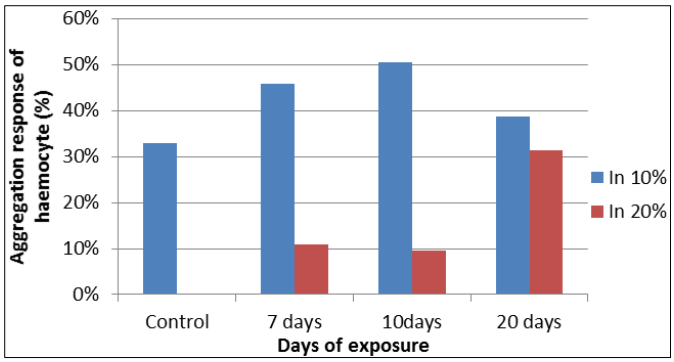

Fig 4: Graphical presentation of aggregation behavior of haemocyte of *Bellamya bengalensis* exposed to sub lethal concentration of Alpha Cypermethrin.

Conclusion

From the present experiment it has been confirmed that the toxic impact of Alpha Cypermethrin is fatal to the *Bellamya bengalensis* and the chronic effects modulate not only the normal behavior such as mucus secretion, avoidance reaction, and floating behavior but also the cell adhesion, cell aggregation and haemocyte morphology. Through this study it can be concluded that indiscriminate use of pesticides in agricultural field should be avoided otherwise it may lead serious hazards or Xenotoxic impact on non-target animals like *Bellamya bengalensis*.

Acknowledgement

The authors are grateful to the Vice Chancellor of West Bengal State University and the Principal of Bidhannagar College for providing the infrastructure and facilities.

References

1. Abbott TR. Compendium of land shells. A full-color guide to more than 2,000 of the World's terrestrial shells. American Malacologists, Inc., Melbourne, FL and Burlington, MA, 1989, 240.
2. Ahirrao DV. Alterations in some physiological processes in a freshwater prosobranch snail, *Bellamya bengalensis* due to Pyrethroids intoxication. Ph.D. Thesis submitted to Dr. Babasaheb Ambedkar Marathawada University, Aurangabad, 2002.
3. Bacci ED, Calamari CG, Vighi M. An approach for the prediction of environmental distribution and fate of cypermethrin. Chemosphere. 1987; 16(7):1373-1380.
4. Bej S, Mukherjee D, Saha N. Acute toxicity of alpha cypermethrin to oligochaete worm, *Branchiura sowerbyi* (Beddard, 1982) along with their behavioural response. International Journal of Scientific Research. 2015; 4(12):325-356.
5. Bouchet P. Extinction and preservation of species in tropical world. What future for Molluscs? American Malacologists. 1991; 20:20-24.
6. Khalua RK, Tripathy S. Seasonal variation of Carbohydrate, Protein and Lipid of common fresh water Edible Gastropod (*Bellamya bengalensis*) of Medinipur District, West Bengal. Research Journal of Biology. 2014; 2:49-52.
7. Magare SR. Effect of the pesticide Hygro in prostate gland of a snail, *Cerastus moussonianus*. Journal of Ecotoxicology and Environmental Monitoring. 1993; 31:59-60.
8. Rao SNV, Dey A. Freshwater Molluscs of India. Zoological Survey of India. Calcutta, 1989, 225-232.
9. Saha BK, Jahan MS, Hossain MA. Ecology and abundance of *Bellamya bengalensis* (Lamarak, 1822) (Gastropoda: Viviparidae) in pond habitats of Rajshahi. Bangladesh Journal of Scientific and Industrial Research. 2017; 52(2):107-114.
10. Seddon BM. Molluscan diversity and impact of large dams. Prepared for thematic review II.1: Dams, ecosystem functions and environmental restoration. IUCN Report. 2000.
11. Yordanova V, Stoyanova T, Traykov I, Boyanovsky B. Toxicological effects of fastac insecticide (alpha-cypermethrin) to *Daphnia magna* and *Grammarus pulex*. Biotechnol. xi Anniversary Scientific Conference, 2009.