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Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of vitamin C

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ABSTRACT

The present study was carried out to investigate the sub-lethal toxicity of technical grade deltamethrin (a synthetic pyrethroid) of concentration 1.61 μg/L (1/3rd of 96 h LC_{50}) on hematological and biochemical parameters of catla (*Catla catla*) fingerlings and its amelioration through dietary vitamin C. The deltamethrin exposed fishes were fed with different levels of supplemented vitamin C such as 50, 250, 500 and 1000 mg/kg diet to see its ameliorating effect by assaying hematological parameters *viz.* total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin content (Hb), total serum protein, albumin, globulin, albumin–globulin ratio and biochemical parameters such as lactate dehydrogenase (LDH), acetylcholine esterase (AChE), alanine amino transferase (ALT), aspartate amino transferase (AST), total adenosine triphosphatase (ATPase), magnesium adenosine triphosphatase (Mg^{2+}-ATPase) and sodium potassium adenosine triphosphatase (Na^+, K^+-ATPase) activities. The finding of this study showed that deltamethrin had negative effect on the hematological and biochemical parameters of *Catla catla*. The experimental group, which was exposed to deltamethrin and fed with normal diet showed significantly lower values ($P \leqslant 0.05$) of all parameters studied except ALT activity. This might be due to possible disruption of hematopoiesis and proteosynthesis. However, the fish fed with varied concentration of vitamin C in diets neutralized the toxic effect of deltamethrin, as evidenced by significantly lowered hematological and biochemical response. Vitamin C @ 1000 mg/kg diet was the most effective in amelioration of harmful effect of deltamethrin on hematological and biochemical parameters of catla fingerlings. The result suggests that vitamin C can be effectively used to neutralize the toxic effect of deltamethrin on catla.

1. Introduction

The synthetic pyrethroids is a diverse class of more than 1000 powerful broad spectrum insecticides that are environmentally compatible by virtue of their moderate persistence, low volatility and poor aqueous mobility in soil [1]. Due to their high efficacy, easy biodegradability and low toxicity in birds and mammal [2], synthetic pyrethroids are chosen over organochlorine, organophosphorus and carbamate insecticides [3].

Deltamethrin, a synthetic pyrethroid is used to control the pests of various agricultural crops, used in public health programme and protection of stored crops [4]. The extensive use of deltamethrin on land may be washed into surface water and kill or at least adversely influence the life of aquatic organisms and other higher animals. Aquatic organisms, particularly fish, are highly sensitive to deltamethrin [5–7]. The toxicity may be lethal to fish or may lead to stress which in turn causes immunosuppression and susceptibility to secondary infection.

The effects of insecticide pollution on non-target organisms in the environment can be studied by detecting changes in organisms at the physiological, biochemical or molecular levels, providing "early warning" tools in monitoring environment quality [8,9]. These sensitive early warning biomarkers can measure interaction between environmental xenobiotics and biological effects. Inhibition and induction of these biomarkers is a good approach to measure potential impacts of pollutants on environmental organisms [10]. The analysis of hematological and biochemical parameters

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in fish can contribute to the assessment of the animal's health and also the habitat conditions [11].

Most fish species cannot synthesize vitamin C, and have to depend on external sources to meet their needs [12]. The vitamin C requirement for normal growth and survival is quite low [13], however, a higher level is required to improve the stress resistance of fish [14]. The dietary supplementation of ascorbic acid at higher doses could counter the stress of the pesticide deltamethrin in *Clarias gariepinus* [15]. Similar results were also reported by other authors [16,17].

Several studies have been carried out on the effect of different pesticides on hematological and biochemical aspects of fish and other aquatic animals, but very few on its amelioration through dietary vitamin C. Especially, there is lack of data on this topic in relation to *Catla catla* species. Considering the commercial value of this species, it is necessary to obtain scientific information about the effect of deltamethrin on *Catla catla* to improve risk-assessment studies and its attenuation with the help of dietary Vitamin C. The main objective of this study was to assess the effect of single sub-lethal concentration of deltamethrin over a long exposure period on hematological and biochemical parameters of *Catla catla* and its amelioration through a gradient of dietary Vitamin C.

2. Materials and methods

2.1. Fish and husbandry

Fingerlings of catla weighing 14 ± 0.2 g were brought from a commercial fish farm at Palghar, Mumbai, India and were used in the experimental study. Fishes were acclimatized for 2 weeks prior to experimentation. Chlorine free tap water was used throughout the course of the experiment. The physico-chemical characteristics of the test water were as follows: temperature 27 ± 2.0 °C; pH 7.4; hardness 80 mg l^{-1} (as $CaCO_3$); alkalinity 88 mg l^{-1} (as $CaCO_3$) and dissolved oxygen concentration 5.6 ± 0.2 mg l^{-1}.

2.2. LC_{50} of deltamethrin

Technical grade deltamethrin [S (a) cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropane-carboxylate [from Tagrose Chemical India Ltd., Chennai, India] with active ingredient 98% was used for the experiment. The LC_{50} value of deltamethrin was determined in the laboratory starting with range finding test to acute toxicity trials [18]. Two hundred and sixteen fishes were randomly distributed in eighteen rectangular plastic tanks (100 L) filled with different concentrations of deltamethrin solution (2, 4, 6, 8 and 10 μg l^{-1}) and a control group, water without deltamethrin, in triplicate. The mortality was recorded for 96 h at regular intervals of 12 h and dead fish were counted and immediately removed. The LC50 value of deltamethrin was calculated with the help of probit analysis using SPSS version 12. One-third concentration (1.61 μg l^{-1}) of calculated LC_{50} was selected for sub lethal test trails.

2.3. Experimental diets

In our study, four different vitamin C levels were used for formulation of experimental diets. L-ascorbyl 2-polyphosphate (Sisco Research Laboratories pvt. Ltd., Mumbai, India) was used as the vitamin C source. A basal diet composed of 30% soybean meal, 18% wheat flour, 13% rice polish, 13% fish meal, 10% corn flour, 10% sunflower oilcake, 4% sunflower oil, 1% mineral and vitamin mixture (each 100 g mineral and vitamin mixture contain Vitamin A 200,000 IU; Cholecalciferol 40,000 IU; Vitamin B2 80 mg; Vitamin E 30 units; Vitamin K 40 mg; Calcium pantothenate 100 mg; Nicotinamide 400 mg; Vitamin B12 240 mg; Choline chloride 6 g; Calcium 30 g; Manganese 1.1 g; Iodine 40 mg; Iron 300 mg; zinc 600 mg; copper 80 mg; cobalt 18 mg) and 1% CMC as binder was used. The experimental diets were prepared by incorporating vitamin C in the basal diet at the rate of 50, 250, 500 and 1000 mg/kg of feed. All the feed ingredients were mixed thoroughly, and dough was prepared with required amount of water. Then dough was conditioned for 1 h followed by steaming for 20 min. After cooling, minerals and vitamins mixture and vitamin C were added to the dough and mixed thoroughly. The dough was pressed through a hand pelletizer (0.2 mm). Pellets were dried and stored at 4 °C.

2.4. Experimental design

Fifteen rectangular plastic tanks (100 L) were arranged with continuous aeration. One hundred and eighty fingerlings (14 ± 0.2 g) were randomly distributed equally in five treatment groups with each of three replicates following a complete randomized design. All treatment groups (B, C, D and E) were exposed to sub lethal concentration of LC_{50} of deltamethrin (1.61 μg l^{-1}) except group A. In the diets of treatment groups A and B, a normal required dose of vitamin C (50 mg/kg feed) was incorporated while in the diets of treatment groups C, D and E a different high doses of vitamin C @ 250, 500, and 1000 mg/kg feed were incorporated respectively. The feed was fed twice daily (0700 and 1700 h) to the approximate satiation for 45 days. Every day morning, the test solution (water containing 1.61 μg l^{-1}deltamethrin concentration) of groups B, C, D and E, was completely renewed with fresh one to maintain the required deltamethrin concentration of 1.61 μg l^{-1}. Group A water was also completely changed every day with normal water.

2.5. Hematological studies

Blood was drawn from the caudal peduncle region using a sterile 2 ml syringe rinsed with 2.7% EDTA solution. Blood was then collected in small glass vials coated with 20 μl of 2.7% EDTA solution.

Total erythrocytes count (TEC) and total leukocytes counts (TLC) were estimated using the method of Schaperclaus et al. [19]. The cells were counted using a hemocytometer (Feinoptik, Blakenburg, Germany) and expressed as:

$$\text{Number of RBC/mm}^3 = \text{Nr} \times 10,000$$

$$\text{Number of WBC/mm}^3 = \text{Nw} \times 500$$

where N_r is the total number of red blood cells counted in five squares of the hemocytometer and 10,000 is the factor obtained after taking into consideration the initial dilution factor and N_w denotes the total number of white blood cells counted in four squares of the hemocytometer and the factor obtained after taking into consideration the initial dilution factors was 500. The blood hemoglobin content (Hb) was analyzed following the Cyanmethemoglobin method using Darbkins fluid (Qualigens diagnostics kit, Mumbai, India).

2.6. Serum parameters

Blood was collected from caudal region of fish without rinsing the syringe with anticoagulants and collected in clean and dry Eppendorf tube. The blood was allowed to clot for 45 min in inclined position at room temperature followed by 30 min incubation at 4 °C and then centrifuged at 3000*g* for 10 min at 4 °C.