



RESEARCH ARTICLE

EFFECT OF INSECTICIDE CYPERMETHRIN ON HAEMATOLOGICAL ALTERATIONS IN THE FISH *CATLA CATLA*

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ABSTRACT

Insecticide is common pollutants of freshwater ecosystems where they induce adverse effects on the aquatic biota. Fish, *Catla catla* is an important carp species in Tamil Nadu region having good nutritional values. Fishes living in close association with may accumulate heavy metals. In the present observation, the toxic effects of the pesticide cypermethrin LC₅₀ 0.22 ppm on the total RBC, WBC and Hb in the fish, *Catla catla* were estimated. Sublethal concentrations of insecticide cypermethrin on (10% and 30% sublethal concentrations) showed a decreasing trend in the RBC and Hb compared to controls and the WBC analysis revealed a significant increased compared to control for a period of 5, 10 and 15 days exposures. The results indicated the toxic nature of the insecticide cypermethrin.

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INTRODUCTION

Water pollution is usually caused by various human sources, typically (point and nonpoint) industrial facilities and agrochemicals especially in aquatic ecosystem, has become a serious environmental problem now a days. These agrochemicals and industrial discharges may carried away effectively by rains, winds, rivers and floods into the large water bodies and change their physico-chemical properties with high toxicity. Pesticides represent a relevant stressor for many aquatic and terrestrial species (Leiss *et al.*, 2005b). In aquaculture, organophosphate pesticides are used to control agricultural pests as well as parasites in fish. During rainy season, these pesticides are accumulated to the pool of water bodies and pose the risks of survivability and reproduction capacity. Cypermethrin is widely used against pests all over the world to increase the production of food grains and other agricultural-products (Usmani and Knowles, 2001) and there is increased risk of food being contaminated with the insecticide, which may harm humans and domesticated animals. Insecticides from insecticides-contaminated feed can be transported to young embryos through eggs and thus can cause teratological abnormalities, organ dysfunction and mortality in the young embryos hence affects the growth. Long-term application of toxic chemicals including pesticides in different ecosystems, which due to their high efficacy and easy use have eliminated some biological methods of pest control, was caused by environmental pollution. Pesticides have been applied to fight against pests of plants, animals and humans. However, the introduction of pesticides to the natural environment has also some negative effects, including unintentional intoxication of

useful insects, fish, birds, mammals, and other inhabitants of aquatic and terrestrial biocenoses (Senthil kumar *et al.*, 2001). The reproductive potential of fish is affected, when reared in water containing pesticide residues (Moore and Waring, 2001). Abhilash and Prakasam, (2005) reported alterations in the cellular morphology of pesticide treated fish. Haematological parameters are used as an index of fish health status in a number of species to detect physiological changes following different stress condition like exposure to pollutants, disease, metals hypoxia, etc., (Duthie and Tort, 1985). The exposure of fish to several types of chemical agents may induce changes in several hematological variables (Heath, 1995), which are frequently used to evaluate fish health (Martinez and Souza, 2002). Hematological parameters such as haematocrit, haemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Sancho *et al.*, 2000; Barcellos *et al.*, 2003). Moreover, blood cell profile has been considered as an important indicator of diseases and other toxicants. As pathomorphological changes are indicative of numerous diseases (Yawata, 2003) there is little doubt of the importance of elucidating the mechanism governing erythrocyte shape (Pawlowski *et al.*, 2006). Blood is a pathological indicator of the whole body, and hence haematological parameters are important for analysis of the functional status of an exposed animal to suspected toxicant (Omitoyin, 2006). The exposure of fish to several types of chemical agents may induce changes in several haematological parameters, which are frequently used to evaluate fish health. Haematology has been widely used for the detection of physiopathological alterations following different stress conditions. Therefore, haematological techniques are the most common method to determine the sublethal effects of pollutants (Modesto and

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Martinez, 2010; Kumar *et al.*, 2011). Recent studies indicate that the pesticide toxicity in fish may be related to an increased production of reactive oxygen species (ROS), which induce oxidative damage to biological systems (Yonar and Sakin, 2011).

MATERIALS AND METHODS

Fish, *Catla catla* were collected from Chidambaram area and were brought to the laboratory in large plastic troughs and acclimatized for one week. Healthy, fish having equal size (length 10 to 12 cm) and weight (50 to 100 g) were used for experimentation. Stock solution of insecticide cypermethrin was prepared by dissolving appropriate amount of salt in distilled water. Physico-chemical characteristic of test water have analyzed regularly during the test periods following the standard method describe by APHA (APHA, 1998).

Batches of 10 healthy fishes were exposed to different concentrations of insecticide cypermethrin to calculate the medium lethal concentration LC₅₀ value (0.22 ppm) using probit analysis Finney method (Finney, 1971). Fishes (Four groups) were exposed to the two sublethal concentrations (1/10th and 1/30th mg/L) of copper for 5, 10 and 15 days respectively. Another group was maintained as control. Fish was collected and gently wiped with a dry cloth to remove water. Caudal peduncle was cut with a sharp blade and the blood was collected in a watch glass containing EDTA, an anticoagulant (6% Ethylene diamine tetra acetic acid). The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the Red Blood Corpuscle Count (RBC), White Blood Corpuscle Count (WBC) and Haemoglobin count (HB).

RESULTS

Median lethal concentration (LC₅₀): Cypermethrin insecticide caused 50% mortality of fish *Labeo rohita* at 96 hours was 0.22 ppm. The LC₅₀ values of cypermethrin for 24, 48, 72 and 96 hours were 0.28, 0.26, 0.24 and 0.22 ppm respectively.

Haematology: Toxic effects of insecticide cypermethrin on the haematological parameter of *Catla catla* were found to be different time dependent. The results of the blood parameters like RBC, WBC and Hb contents after exposing fish *Catla catla* to sub lethal (5, 10 and 15 days) concentrations of cypermethrin.

Red blood corpuscle (RBC): Results of the red blood corpuscle (RBC) are presented in Table.3. In the present study, the fish *Catla catla* exposed to (10% and 30%) different concentrations of cypermethrin indicated a decreasing trend in the RBC values when compared to control fish. The control RBC values were observed 2.71, 2.73 and 2.75 in 5, 10 and 15 days exposure periods. The decrease values 2.57, 2.11 and 1.92 (10⁶/mm³) were recorded in the number of RBC in 10% sub lethal concentration at 5, 10 and 15 days exposure. A marked decrease values 2.38, 2.05 and 1.84 (10⁶/mm³) were recorded in the number of RBC in 30% sublethal concentrations at 5, 10 and 15 days exposure period (Table 1 and Fig. 1).

White Blood Corpuscles (WBC): The blood parameter of white blood corpuscles (WBC) values obtained the (10% and 30%) various concentrations of cypermethrin in 5, 10 and 15 days are showed in Table 1. The WBC counts of *Catla catla* were increased with the increasing trend of cypermethrin for different exposure periods when compared to control fish. The control WBC values were observed 15.73, 15.69 and 15.75 in 5, 10 and 15 days exposure periods. The increase in the number of WBCs was found to be 15.92, 16.27 and 16.68 (10³/mm³) at 5, 10 and 15 days exposure at 10% sub lethal concentration. The increasing trends of WBC were found to be 16.12, 16.35 and 17.21 (10³/mm³) at 5, 10 and 15 days exposure at 30% sub lethal concentrations respectively (Fig. 2).

Haemoglobin (Hb): The changes of blood parameter like haemoglobin (Hb) in the fish *Catla catla* both in control as well as sub lethal concentrations of cypermethrin exposed periods for 5, 10 and 15 days were showed in Table 1. In the present study, the haemoglobin (Hb) level was decreasing trend when compared to control fish. The control fish haemoglobin content was recorded 6.23, 6.16 and 6.22 (g/100 ml) at 5, 10 and 15 days exposure period. The decrease in the amount of haemoglobin was found to be 6.12, 5.78 and 5.44 (g/100 ml) in 10% sub lethal concentration at 5, 10 and 15 days exposure. The 30% sublethal concentration of cypermethrin in *Catla catla* was recorded decreasing trend in 5.95, 5.61 and 5.23 (g/100 ml) at 5, 10 and 15 days exposure period (Fig. 3).

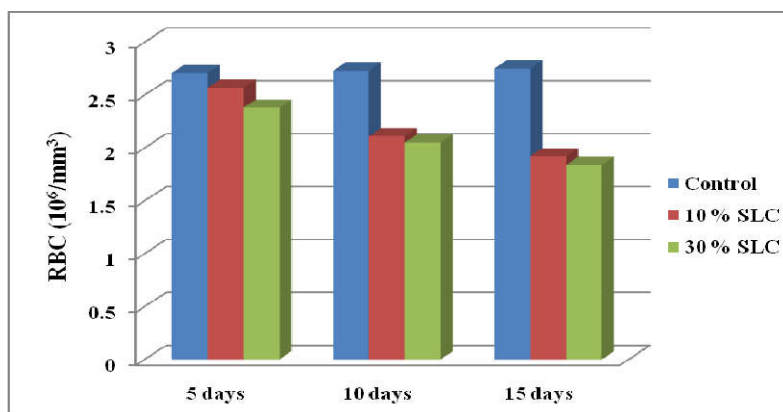
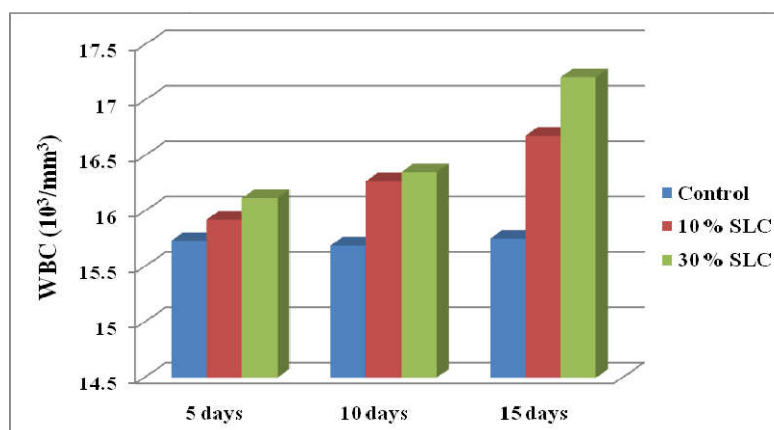
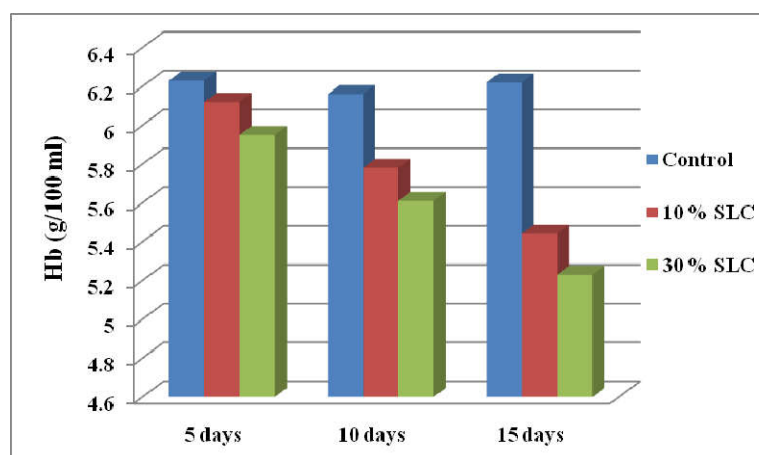
DISCUSSION

In the present study, level of erythrocytes were showed decreasing trend when compared to control fish *Catla catla*. Madhyastha and Nayak, (1979) reported that the progressive reduction in the total number of the erythrocytes in *Rasbora daniconius* (Ham). The response of *Oreochromismossambicus* (peters) subjected to stress by increasing the concentration of pesticides seemed to vary with reference to erythrocytes counts. White a drastic increase has been reported for fish exposed to pesticides, such as aldrin and industrial effluents (Vijayarani, 1983). The hematological parameters in fish can significantly change in response to chemical stressors; however, these alterations were nonspecific to a wide range of substances (Modesto and Martinez, 2010). Ramesh and Saravanan, (2008) also reported that significantly lower values of red blood cells existed in *Cyprinus carpio* that were exposed to cypermethrin. The decrease in the RBC count and the haemoglobin levels may be due to the inhibition of erythropoiesis, haemo synthesis or osmo regulatory dysfunction or due to an increased rate of erythrocyte destruction in the hematopoietic organ (Vani *et al.*, 2011). The reduction of RBC was mainly due to development of hypoxic condition during the treatment which intern leads to increase in destruction of RBC or decrease in the rate of formation of RBC due to non availability of Hb content in cellular medium (Saxena and Seth, 2002).

The damaging of toxicant on erythrocyte may be secondary, as resulting from a primary action of toxicant on erythropoietic tissues on which there exist failures in red cell erythropoietic tissues on which there exist a failure in red cell production and or due to increase in the erythrocyte destruction (Verma *et al.*, 1982). Haematotoxins alter qualitative and quantitative attributes of blood cells to produce toxic sings. When some of these other blood constituents were present or structural anomalies occurring in blood components meddle with normal functioning occur hematotoxicity. Qualitative commutations in blood cell components can consequence in contamination (Moore, 2002). Although adequate in quantity, the quality of these small less colored red blood cells impedes them from covering the normal extent of oxygen (Landis and Yu, 2004). Haematological parameters like RBC could be delicate to sure types of contaminants because of its close relation with the exterior environment and often used to discover the physiological status of fishes (Adhikari *et al.*, 2004). The appraised in the sub lethal toxicity of chemicals, the haematological and biochemical parameters of aquatic organisms may too utilize as possible bio markers (Saravanan *et al.*, 2011). The reduction of total RBC contents in the blood of rainbow trout (*Oncorhynchus mykiss*) when exposed to verapamil, a cardiovascular medicine. Mostly, the reduction in RBC counts in fish may imply the anemic condition of the fish under stress situations was reported by Li *et al.*, (2014). In this work, the haematological parameter of white blood cells level were observed in *Catla catla*, treated with sublethal concentrations of cypermethrin at 5, 10 and 15 days exposure period. The toxicity of cypermethrin on the haematological parameter like WBC values were significantly increasing trend when compared to control.

Table 1. Haematological parameters of fish *Catla catla* under sublethal concentrations of cypermethrin

Days	Exposure	RBC ($10^6/\text{mm}^3$)	WBC ($10^3/\text{mm}^3$)	Hb (g/100ml)
5 days	Control	2.71	15.73	6.23
	10% SLC	2.57	15.92	6.12
	30% SLC	2.38	16.12	5.95
10 days	Control	2.73	15.69	6.16
	10% SLC	2.11	16.27	5.78
	30% SLC	2.05	16.35	5.61
15 days	Control	2.75	15.75	6.22
	10% SLC	1.92	16.68	5.44
	30% SLC	1.84	17.21	5.23

Fig. 1. Total RBC of *Catla catla* under sublethal concentrations of cypermethrinFig. 2. The total WBC of *Catla catla* under sublethal concentrations of cypermethrinFig. 3. The Hb of *Catla catla* under sublethal concentrations of cypermethrin

Saravanan and Harikrishnan, (1999) have reported that the pollutants generally cause changes in the haematological parameters of the fishes. Haematological parameters are most important parameters for evaluation physiological status of fish in warm blooded animals. These parameters more related the response of the whole organisms. Blood parameters are important in diagnosis the functional status of animals exposed to toxicants. Anemia is one of the most sensitive pathological conditions developed as a result of heavy metal poisoning (Joshi *et al.*, 2002). The earlier reported that the haematological parameter WBC levels were observed in *Tilapia mossambica* when exposed with sub lethal concentrations of arsenic for 21 days. The toxicity of arsenic on the haematological parameter WBC was significantly increased because the arsenic affects the animals and lead to anaemia was reported by Soundararajan and Veeraiyan, (2014). In the present observation, the haemoglobin content were observed decreasing trend when compared to control. A similar finding was reported by (Naveed Abdul *et al.*, 2010). The decrease in the haemoglobin content may be due to the inhibition of erythropoietin, haemo synthesis, osmo regulatory dysfunction (or) due to an increase in the rate of erythrocyte destruction in the haematopoietic organ, which may lead to anaemia in the exposed fish *Cyprinus carpio* was reported by (Jenkins *et al.*, 2003). The haemoglobin level were observed decreasing trend when compared to control (Vutkuru *et al.*, 1996).

Haemoglobin percent were appreciably declined in *Labeo rohita* exposed to chromium reflecting the anemic state of the fish which could be possibly due to iron deficiency and its consequent decreased utilization for haemoglobin synthesis. Similar findings on *labeo rohita*, which also reported hypochromic microlytic anemia under lead chloride stress (Janardhana Reddy *et al.*, 1998). Anemia in fish was early manifestation of acute and chronic intoxication of chromium. Further, a significant decrease in haemoglobin percent and haemtocrit were also reported in *Channa punctatus* exposed to both copper and chromium and that decrease was more pronounced in fishes exposed to chromium suggested that the metal induces acute anemia under toxic conditions (Mahipal Singh, 1995). In the earlier Study, the anemia could be probably due to structural alterations of haeme leading to disturbed hemoglobin synthesis and also the inhibitory effect of chromium on the enzyme system in the synthesis of hemoglobin cannot be ruled out as suggested in earlier studies (Johansson Sjöbeck and Larrsson, 1975). A significant decrease in haemoglobin in the fresh water fish *Channa punctatus* from polluted waters can definitely be related to the pollution due to slaughter house wasters (Iqbal *et al.*, 1997).

Conclusion

Present study revealed that the organophosphorus insecticide cypermethrin is potent to cause toxic responses, even structural alterations, in aquatic organism like fish. The results indicate that the usage of the cypermethrin in the agriculture fields may be a threat to aquatic fauna and flora as well as humans. These reports bring discussions on the deteriorating nature and the lethal effects of the pesticides on ecosystem. Finally, concluded that the assured greater significance due to the increasing emphasis on fish culture and greater awareness of the pollution in aquatic ecosystem. Therefore, the information obtained may be useful for management and monitoring of agricultural insecticide contamination in aquatic ecosystem. It is also recommended that before using cypermethrin in any aquaculture processes, the estimated safe and dischargeable concentrations should be considered important to protect living organisms as well as fish.

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