

PROTECTIVE EFFECT OF ASCORBIC ACID ON CYPERMETHRIN INDUCED ALTERNATION IN THE R.B.C. OF THE FRESHWATER FISH, *PUNTIUS SOPHORE*.

B.R. Shinde, V.N. Khandare, V.R. Borane.

Department of zoology, Jijamata Art's, Science & Commerce College, Nandurbar (MS).

Abstract: Fresh water fishes, *Puntius sophore* were exposed to chronic dose of cypermethrin without & with Ascorbic acid. Decrease in R.B.C. was observed in cypermethrin exposed fishes. Fishes were exposed to pesticides with Ascorbic acid shows less present variation in the R.B.Cs. Pre exposed fishes to pesticides showed fast recovery with Ascorbic acid as compared to those cured naturally.

Keywords: Cypermethrin, Ascorbic acid, R.B.Cs, *Puntius sophore*.

INTRODUCTION

The indiscriminate use of agrochemicals including pesticides, fertilizers, weedicides and growth regulators are being used now-a-days in developed and developing countries to boost crop yield. But their extensive use poses a constant threat to aquatic life of non-target animals like fishes to affect on biological system. The blood carries heavy metals and pesticides to different organs or systems of an animal, hence blood components are directly affected. Decrease in hematological parameters in Malathion exposed freshwater fish, *Cyprinus carpio* (Ramesh and Mahavalaramanujam 1992).

Alteration of hematological parameters of fish has been associated with their physiological state and may be induced directly by genotoxic compounds. Clinical chemistry analyses are faster and cheaper than analytical chemistry. Since the variations of these parameters have been demonstrated to be sensitive to sublethal concentration of different toxic agents, they can be used for detecting pollutants exposure in the environment (National Research Council, 1989). Pyrethroid insecticides are commonly used to control insect pests and represent about 30% (Amdur et al., 1991) of world insecticide consumption. Their mean life in water is two weeks but they get rapidly absorbed by aquatic organisms with a significant toxicity (Phillip and Rajasree, 1996). Also, some investigations suggest that they can be absorbed by fish gills even at very low concentration in water Clark et al., (1985). Saxena and Seth (2002) showed a significant change in the hematology of the common fresh water fish, *Channa punctatus* on exposure. The information suggests that hematological parameters could be used as potential biomarkers of pyrethroid insecticides. Cypermethrin is one of the synthetic pyrethroid uses in normal practice. Cypermethrin is readily absorbed by gills and mouth even from very low concentration in water. The symptoms of poisoning in insects and animals are hypersensitivity, hyperactivity with violent burst of convulsions and finally complete prostration with convulsive movement, disturbance in the ganglia of the central nervous system rather than in peripheral nerves.

Ascorbic acid plays an important role in distribution and excretion of toxic components. Ascorbic acid has reversed dysfunction of cells lining blood vessels. The normalization of functioning of these cells may be link to prevention of heart diseases (Chambers, 1999). It has been realized that antioxidants can play significant role in the treatment of toxic components induced oxidative stress. Some antioxidants behave as efficient chelators (Gurer and Eracel, 2000). The SH group of protein is mainly responsible for metal interaction or binding L-ascorbic acid is antioxidant and may extent in protective effects by chelating the

metal and removing them from the system, Tajmir Riahi (1991). During toxicosis ascorbic acid indicate positive role in detoxification. It is necessary for the synthesis of collagen, growth and maintenance of epithelial tissue. It can acts as a hydrogen carrier, it may have an essential role in the metabolism of carbohydrate or protein or both. It appears to function in maintaining strength in blood vessels.

MATERIALS AND METHODS

Medium sized fresh water fishes *Puntius sophore* were collected from Shivan river area Nandurbar. The physico-chemical parameters of the water used for the maintenance of the fishes were analyzed as per the methods given in APHA and AWWA (2005). The fishes were divided into three groups A, B, and C. Group A fishes were maintained as a control. The group B fishes were exposed to LC 50/10 dose of Cypermethrin (0.6912 ppm) for 30 days, while group C fishes were exposed to respective chronic concentration of pesticide with 50mg/l of ascorbic acid for 30 days. Fishes from B group were divided into two groups after 30 days exposure to Cypermethrin into D and E groups. Fishes of D group were allowed to cure naturally while those of E group were exposed to Ascorbic acid (50mg/l). RBC count were recorded from A, B and C group fishes after 15 and 30 days of exposure and from D and E group after 35th and 40th days of recovery. Blood was obtained by cutting the caudal peduncle dissection method (Reichenbach-Klinke, 1982, Roberts, 1978), using heparin as anticoagulant. First few drops were discarded and only the first 2ml of blood was taken since the entry of lymph into the blood is reported (Schreck, 1975) to affect haematocrit value. RBC was counted by using Naubauer's haemocytometer.

Table 1: Physico-chemical parameters of water used for experimentation

Temperature	25.1 ± 3.2 ⁰
PH	7.60 ± 0.3
Conductivity	140 ± 15.7 μ mho ^{-cm.}
Free Co2	3.34 ± 1.3 ml ⁻¹ .
Dissolved O2	6.3 ± 1.1 ml ⁻¹ .
Total Hardness	204 ± 12.0 mg ⁻¹ .
Total Alkalinity	585.6 ± 32.8 mg ⁻¹ .
Magnesium	31.67 ± 2.9 mg ⁻¹ .
Calcium	30.46 ± 3.06 mg ⁻¹ .
Chloride	107.92 ± 16.34 mg ⁻¹ .

Table 2: RBC Counts in *Puntius sophore* after chronic exposure to cypermethrin without & with ascorbic acid & during recovery (Values expressed as millions of cells/mm³.)

Group	Treatment	15d	30d	35d	40d
A	Control	4.0 ± 0.081	3.98 ± 0.0082	--	--
B	Cypermethrin (0.6912 ppm)	3.40 ± 0.62** (-15.0)	3.11 ± 0.0081*** (-21.85)	--	--
C	Cypermethrin +AA	3.70 ± 0.0081** (-7.5)	3.58 ± 0.012*** (-10.05)	--	--
D	Recovery in Normal water	--	--	3.18 ± 0.014### [+3.85]	3.21 ± 0.14### [+4.21]
E	Recovery in A.A.	--	--	3.38 ± 0.0129### [+7.07]	3.65 ± 0.005### [+15.11]

AA=Ascorbic acid (50 mg/l.), ± indicates S.D. of three observations. Values in () indicates percent change over respective control. Values in [] indicates percent change over 30 days of respective B. *indicates significance with the respective control. # indicates significance with 30 days of respective B. p<0.05 = * & #, p<0.01 = ** & ##, p<0.001 = *** & ###

RESULTS AND DISCUSSION

Fishes experimentally exposed to cypermethrin for a period of 15 and 30 days in a group B and C showed significant decrease in RBC count in cypermethrin exposed fishes. When dose of cypermethrin along with ascorbic acid was given the depletion in RBCs was 3.65 for 40 days observation. The pre exposed fish to cypermethrin for 40 days showed fast recovery in RBC count and significant improvement with ascorbic acid as compared to those cured naturally in normal water after 5 and 10 days. The decrease in RBCs number and hemoglobin content under pesticide impact was attributed to symptoms leading to hypochronic microlytic anemia, which ascertain the iron deficiency and a consequent reduction in hemoglobin synthesis (Bhai *et.al.*, 1971). Variations in hematological parameters of fish in relation to different pollutant have been noted by Das and Bhattacharya (2002). The decrease in the erythrocyte count can also be correlated with reduction in oxygen carrying capacity, due to damage of gills by zinc toxicity, (Reddy *et.al* 1998). Morgan *et.al.*, (1980) reported damage of bone marrow in the animals exposed to lindane. This essential vitamin C is drastically affected and altered by various environmental pollutants like pesticides, (Bhusari 1983). In fish, the toxic compounds gain access via the gills directly into blood stream, persist there and cause alterations in hematology. A decrease in important blood parameters has been reported during exposure to various pesticides in fishes, (Saxena and Seth, 2002). Changes in hematological parameters might have been brought about by cypermethrin as an anemic condition due to decreased synthesis of RBC number in bone marrow cells. R.B.C. of *Puntius sophore* has been much documented, so this paper would provide an important contribution to the knowledge of the specimen constitution and variation. Finally, hematological alterations may be used for diagnosis in the field to assess pollution related pathophysiological alterations in fish. R.B.C. of the freshwater fish, *Puntius sophore*.

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