ASCORBATE EFFECT ON CYPERMETHRIN INDUCED ALTERATIONS IN THE PROTEIN LEVELS OF THE FRESHWATER FISH, *GARRA MULLYA*.

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Abstract

Fresh water Fishes, Garra mullya were exposed to chronic dose of cypermethrin with and without ascorbic acid. Total count of Protein content was recorded. Remarkable decreases in Protein content were observed in cypermethrin exposed fishes. Fishes were exposed to cypermethrin with Ascorbic acid showed less present variation in the Protein content . Pre- exposed fishes to pesticides showed fast recovery with ascorbic acid as compared to cure naturally. The role of ascorbic acid on exposure to cypermethrin of an experimental fish, Garra mullya is discussed in the paper.

Keyword : ascorbic acid ; Protein ; Garra mullya ; cypermethrin.

Introduction

Indiscriminate use of different pesticides in agriculture to prevent the crop from pest peril has increased over the years, especially in the developing countries (Santhakumar and Balaji, 2000). These pesticides, even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and alter the physicochemical properties of water (Waykar and Lomte, 2001), which are proved to be highly toxic not only to fishes but also to other organisms (Madhav Prasad *et.al.,* 2002).

The accumulated pesticide interacts with biomolecules and alters the physiology of organisms.The toxic compounds exerts stress to organism and organism responds to it by developing necessary potential to counteract that stress. The chemical changes occuring in the body of organism give first indication of stress (Mayes, 1977). A number of changes in biochemical parameters of aquatic organisms due to pesticide toxicity have been noted by several investigators (Shivaprasad Rao *et. al.* 1981 ; Mule and Lomte, 1992 ;,Muley *et.al*. ; 1996). The indiscriminate use of synthetic pyrethroids; organophosphates; organochlorines; carbamates cause serious pollution problem in aquatic environment. Higher concentrations of toxicants in aquatic environment

cause adverse effect on aquatic organisms at cellular or molecular level and ultimately lead to alterations in the biochemical composition. The pollutant affects the activity of biologically active molecules such as amino acids, co-enzyme and other proteins containing sulphur and phosphorus, and affect physiological processes in tissues (Ghosh and Chatterjee,1985) Biochemical changes induced by pesticide are disturbed metabolism, inhibition of important enzymes, retardation of growth and reduction of fecundity and longivity of organisms (Murty, 1986). Reports are available regarding the toxic effects of pesticides on protein content of some aquatic animals (Mule and Lomte , 1993 ; Kaur and Dhawan 1996; Borah and Yadav, 1995). The effect of pesticides are known to induce biochemical changes in the fishes before the drastic cellular dysfunction (Manohar and Subbiah,1982 and Das and Mukherjee, 2000).

Protein is an important organic constituent, which play important role in metabolism of organisms and metabolic activities. It is an integral part of cell membrane. Harper *et.al.* (1978) reported that, the proteins are among the most abundant biological macromolecules and are extremely versatile in their function .

Ascorbic acid is a powerful antioxidant which plays an important role in intracellular oxidation-reduction system and in binding of free radicals produced endogenously (Laurence et.al., 1997). Ascorbic acid reduced the clastogenic effects generated by certain chemical agents in the in vivo and vitro assays. (Amare et.al., 1996, Khan *et.al.*,1996).Ascorbic acid plays an important role in the distribution and excretion of trace mineral and toxicant Lewin(1974); Hughes(1974) Reported that ascorbic acid is a diffusible biological reducing agent When present in appropriate concentrations. Ascorbic acid acts as an essential factor for normal growth in rainbow trout *salmogairdeneri* reported by (Halver et. al.1969,Tucker and Halver 1986). Chinoy and Seethalakshmi (1977) showed impact of ascorbic acid on steroidogenesis in gastropod. Considering above cited references the effect of pesticides can be detoxified by the ascorbic acid. The present investigation reports the effect of ascorbic acid on cypermethrin induced changes in protein levels of selected tissue of an experimental fresh water fish *Garra mullya.*

Materials and Methods

Healthy, active and medium sized fresh water fishes, *Garra mullya* were collected from Shivan river, near Nandurbar. Physicochemical parameters of water used for experimentation were studied by the methods given in APHA and AWWA (1985) The acclimatized, same sized, active healthy fishes were divided into three groups A, B and C. The group A fishes were maintained as control, group B fishes were exposed to chronic dose ($LC_{50/10}$ dose of cypermethrin (0.4820 ppm) for 30days, while group C fishes were exposed to chronic concentration of pesticide with 50 mg/l of ascorbic acid up to 30 days. After 30 days fishes form B group were divided in to two subgroups D and E , the fishes from group D were allowed to self-cure naturally in the normal water , while fishes form E group were expose to 50 mg/l of ascorbic acid Protein content were

estimated from A,B and C group fishes after 15th and 30th days of exposure and from D and E groups after 35th and 40th days of recovery, by Lowry`s method Lowry's *et.al.,* (1951) using Bovine serum albumin as standard.

RESULT AND DISCUSSION

Biochemical changes in the organs of gill of freshwater fish *Garra mullya* exposed to chronic concentration of cypermethrin with and without ascorbic acid in Group A,B and C for 15th and 30th days and from Group D and E during recovery after 5 and 10 days were studied along with control fishes with respect to the percentage of protein in dry tissue powders and are given in the table 1.1 the protein contents in the gill of fish in presence of cypermethrin depleted with the increase in exposure period. The changes in the protein contents of a tissue due to pesticide stress indicate the change in the activity of the organism. In presence of ascorbic acid (50 mg/l) the protein depletion is less as compared to those of cypermethrin intoxicated fishes.

The pre-exposed to cypermethrin showed fast recovery in the protein levels in presence of ascorbic acid than those allowed to cure naturally. Proteins are the most important macromolecules in living being which play vital role in architecture and physiology of the cell and in cellular metabolism. Proteins have a major role in the interactions between intra and extra cellular media. As enzymes,Proteins participate in the intricately balanced subcellular function. After the carbohydrate, the next alternative source of energy is protein. To meet the increased energy demand.Decrease in protein may be due to enhance protein catabolism. Cypermethrin causes deplection of protein. The deplection in tissue protein may be due to impairment or low rate of protein synthesis under or due to utilization in the formation of mucoproteins which are released in the form of mucous.Proteins are essential organic constituents which play a vital role in the cellular metabolism. The increase or decrease in the protein contents due to pesticidal stress may be due to changes in the activity of acid phosphatases, rapidchemical binding of enzymes with pesticides, decrease in the synthesis of enzyme protein or increased metabolism due to stress condition (swami et. al.1983).The decrease in average total protein content in all body tissues after acute and chronic treatment may be due to utilization of amino acids in various metabolic processes (Mule and Lomte,1993), Jha(1988) Supported the idea of consumption of amino acid for metabolic processes as energy source. According to Abel(1974) the depletion of protein may be due to alterations of membrane permeability. Another probability was that pollutant might block protein synthesis. According to Shivaprasad Rao and Ramana Rao(1980) deplection of protein in pollutant treated animal might be due to enhanced proteolytic activity. Increased in protease activity in *Bombax mori* on pesticide treatment also supported depletion of protein content(Srnivas and Purushottam Rao,1987),Waykar(1998) also observed increased protease activity in freshwater

bivalve,*Parreysia cylindrica* after pesticide treatment Gopala Rao et. al.(2006) reported that protein serve as energy.

Table No: 1

Physico - chemical parameters of water used for experimentation.

Sr. No.	Parameter	Value
1	Temperature	$25.1 \pm 3.2\ ^0C$
2	pH	7.61 ± 0.3
3	Conductivity	140 ± 15.7 µ mho /cm
4	Dissolved O_2	6.2 ± 1.1 ml /l
5	Free CO_2	3.34 ± 1.3 ml /l
6	Total Alkalinity	586.6 ± 32.6 mg /l
7	Total Hardness	205 ± 12.0 mg /l
8	Chloride	8107.92 ± 16.34 mg /l
9	Calcium	31.47 ± 3.06 mg /l
10	Magnesium	30.66 ± 2.8 mg /l

Table No. 1.1

Protein contents in the gill of *Garra mullya* after chronic exposure to cypermethrin without and with ascorbic acid and during recovery. (Values represent percentage in dry weight)

Group	Treatment	15 days	30 days	35 days	40 days
A	Control	62.64 ±0.3301	63.47±0.3675	--	--
B	cypermethrin	42.48±0.2432*** (-32.66)	46.86±0.1147*** (-26.70)	--	--
C	cyper +AA	57.66±0.2162*** (-7.81)	54.79±0.2430*** (-10.89)	--	--
D	Recovery in Normal water	--	--	51.43±0.5127### [+10.11]	57.70±0.0129### [+23.12]
E	Recovery in AA	--	--	57.64±0.0141### [+23.51]	61.15± 0.0208 ### [+28.89]

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.

$p<0.05$ = *& $^{\#}$, $p<0.01$ = ** & $^{\#\#}$, $p<0.001$ = *** & $^{\#\#\#}$,

^{NS} = Not significant.

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