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Original Research Article

Sublethal Effects of Cypermethrin on Free Amino Acids and Protease Activities in *Cyprinus carpio* (Linn.)

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Abstract

Cyprinus carpio, a freshwater fish was exposed to sublethal concentrations (5, 10, 15 and 20% 96h LC_{50}) of cypermethrin (25% EC) for two, four, six and eight days to evaluate free amino acid levels and protease activity in gill, muscle and liver tissue. Exposure to sublethal doses caused statistically significant ($p \leq 0.05$), time and dose dependant increase in the free amino acid levels and protease activity. The increased levels of free amino acid and protease activity might be due to tissue damage under the toxic stress imposed by cypermethrin. Maximum elevation of free amino acid levels was observed in liver, followed by muscle and gill. Maximum increase in protease activity was observed in liver, followed by gill and muscle.

Keywords

Cypermethrin
Cyprinus carpio
Enzyme activity
Insecticides
Toxicity

Introduction

Pollution of aquatic ecosystems has become a universal problem in the present day world due to rapid industrialization and increase in human population (Bela and Prasad, 2008). Hence, aquatic organisms are often exposed to mixtures of toxicants because it is believed that regardless of where the pollution occurs, it will eventually end up in the aquatic environment (Firat et al., 2011). Fishes are more frequently exposed to the pollutants and may be taken in through gills, skin and contaminated foods (Ling et al., 2011). Pesticides presented in aquatic environments can affect aquatic organisms in different ways (Ventura et al., 2008). Pesticides are not highly selective but are generally toxic to many macrophytes, non-target organisms such as fish (Franklin et al., 2010). Long term exposure to these pollutants causes countless abnormalities and reduces the life span of various fauna (Naz et al., 2011). The contamination affects all groups of organisms in aquatic environment like invertebrates (Castillo et al., 2006) and non target aquatic biota like fish (Singh et al., 2010). Pyrethroids constitute another group of insecticides in addition to organochlorine, organophosphate, carbamate and other new generation compounds. Pyrethroids are preferred above organophosphates,

carbamates and organochlorines as these are highly efficient, low toxic and easily biodegradable (Sharaf et al., 2010). For more than 30 years, pyrethroids are in use for home formulations and agricultural purposes and these insecticides cover nearly 1/4th of the world market (Ahmad et al., 2012).

The biochemical studies are good parameters which help to see the effects of toxicants on metabolism of fish (Kajare et al., 2000). In general, every living component has its own defense mechanism to cope with the negative effects of foreign materials in the body. If toxic elements are encountered in higher concentration, they are bound to bring severe adverse effects. Pesticides produce many physiological and biochemical alterations in the freshwater fauna by influencing enzyme activities and metabolites.

Sublethal effect is in general more subtle and quantitative but it is difficult to monitor sublethal effect at the community level, due to the complexity of an ecosystem and the specificity of the induced effect. Therefore, for the lower toxic concentrations, laboratory studies at organism level are indispensable for the identification of relevant effects. This avoids the complexity of population dynamics and focuses on the study of more specific and mechanistic action (Javed, 2006).

Materials and methods

Freshwater fish, *Cyprinus carpio* of size ranging 6.5±0.5cm in length and 10±0.5gm weight respectively were bought from a local fish farm in Tenali, Andhra Pradesh and acclimatized to laboratory conditions for 15 days and were maintained in large glass aquaria. Water was changed daily fish were fed with groundnut cake twice a day. The physico-chemical parameters of water are given in Table 1.

Commercial grade cypermethrin (25% EC) of liquid formulation was purchased from local agro-chemical store. Fish were exposed to sublethal concentrations of cypermethrin for a period of 2, 4, 6 and 8 days. At the end of the exposure period, fish were sacrificed to death and target organs such as gill, liver and muscle were dissected out and free amino acid levels in the tissues were estimated by the Ninhydrin method described by Moore and Stein (1957). Protease activity in the tissues was estimated using the Ninhydrin method described by Davis and Smith (1955). Statistical analysis was done according to Duncan's multiple range (DMR) test.

Table 1. Physico-chemical parameters of water used for the present experiment.

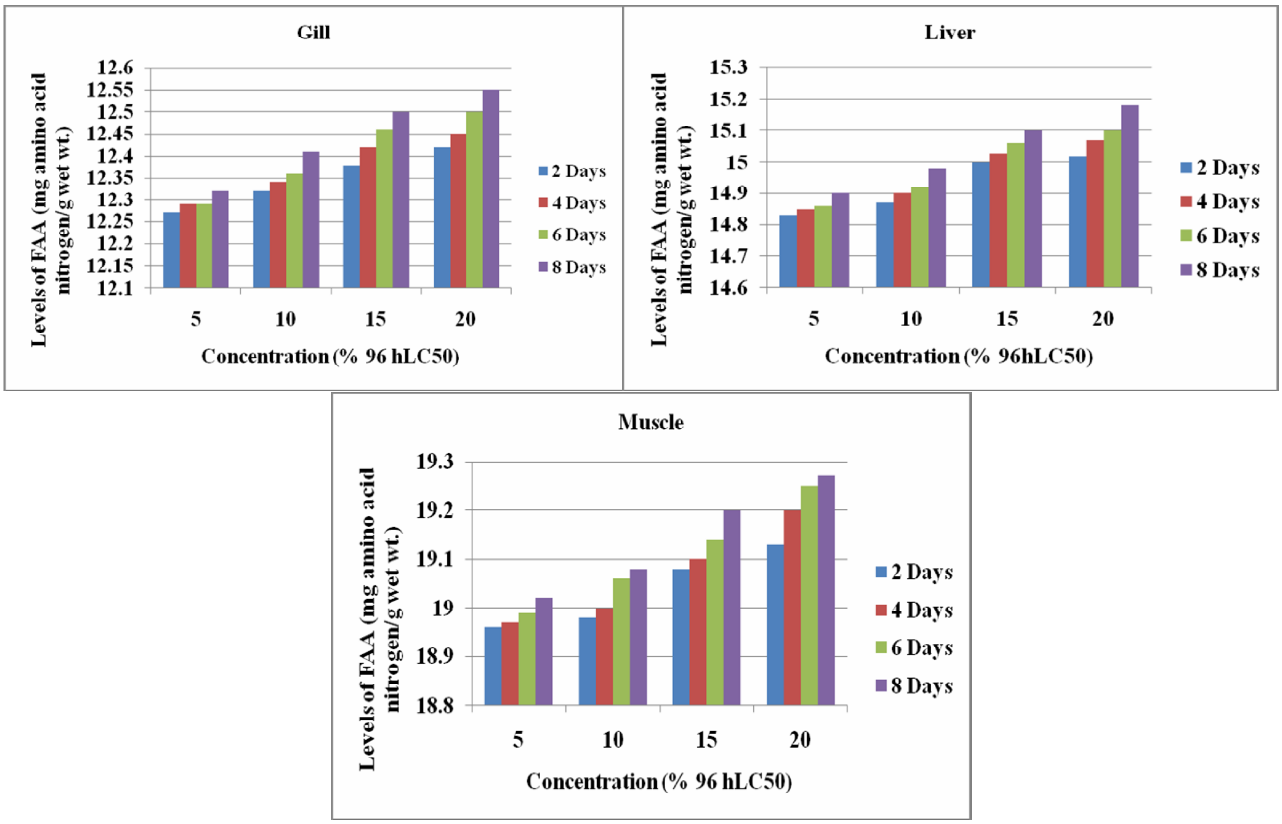
S. No.	Parameter	Value
1	Turbidity	8 Silica units
2	Electrical Conductivity at 28°C	812 micro ohms/cm
3	pH at 28°C	7.7
4	Alkalinity	
	1. Phenolphthalein	Nil
	2. Methyl orange	475
5	Total hardness as ($CaCO_3$)	254mg/l
6	Calcium hardness (as N)	73mg/l
7	Sulphate (as SO_4)	Trace
8	Chloride (as Cl)	37mg/l
9	Fluoride (as F)	1.7mg/l
10	Iron (as Fe)	Nil
11	Dissolved Oxygen	8.6 – 10ppm
12	Temperature	25 – 26°C

Results

Study revealed that there was a gradual increase of free amino acid levels and protease activity in all the tissues irrespective of the concentration and exposure period. The increase in these two parameters was directly proportional to concentration of the pesticide and duration of the exposure period. Alterations in the free amino acid levels in experimental fish *Cyprinus carpio* is given in Fig. 1. Free amino acid levels in the gills of control fish were in the range of 12.24±0.22 -

12.26±0.23 mg amino acid nitrogen/g wet wt. when fish exposed to sublethal concentrations. There was a increase in gill free amino acid levels to 12.42±0.21, 12.45±0.19, 12.50±0.21 and 12.55±0.27 mg amino acid nitrogen/g wet wt. after 2, 4, 6 and 8 days of exposure periods respectively. Maximum elevation of 2.36% over control was observed in gill at 20% 96hLC_{50} after 8 days of exposure period followed by 6 days (2.12%), 4 days (1.63%) and 2 days (1.47%). In the same manner, free amino acid levels in muscle and liver were also found to increase as the concentration of the pesticide and exposure period increases. Free amino acid levels of control fish muscle were in the range of 18.94±022 to 18.96±0.24 mg amino acid nitrogen/g wet wt. These levels were found to increase to 19.13±0.19, 19.20±0.18, 19.25±0.22 and 19.27±0.19 mg amino acid nitrogen/g wet wt. equals to 1, 1.37, 1.58 and 1.63% enhancement over control for 2, 4, 6 and 8 days of exposure periods respectively. Liver also showed an increase in free amino acid levels to 15.18±0.29 from the control value of 14.78±0.24 mg amino acid nitrogen/g wet wt. equals to 2.7% enhancement at highest concentration of 20% 96hLC_{50} after longest exposure period of 8 days followed by 2.3% (15.10±0.28), 1.96% (15.07±0.20) and 1.62% (15.02±0.17) for 6, 4 and 2 days respectively. Among the tissues studied maximum percent increase in the free amino acid levels was observed in liver (2.70%) followed by gill (2.36%) and muscle (1.63%).

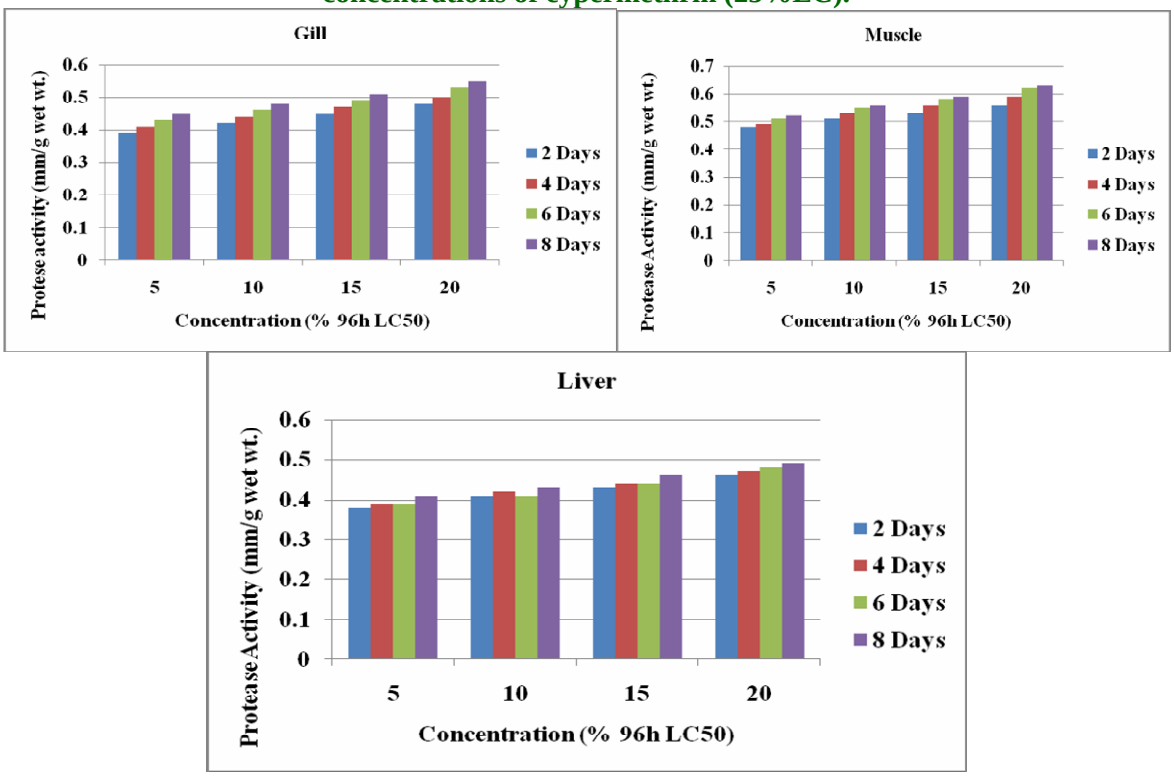
Fig. 1: Free Amino acids levels (mg amino acid nitrogen/gm wet wt.) in the tissues (gill, liver and muscle) of *Cyprinus carpio* on exposure to sub lethal concentrations of cypermethrin (25% EC).



Under sub lethal concentrations of cypermethrin, protease enzyme activity in gill, muscle and liver of *Cyprinus carpio* was observed to increase over the control fish in all the exposure periods and concentrations (Fig. 2). Gill protease activity was found to increase to 0.48±0.02, 0.50±0.01, 0.53±0.01, and 0.55±0.03 over control (0.36±0.02**)** with percent increase of 33.33%, 35.13%, 39.47% and 44.73% for 2, 4, 6 and 8 days respectively at 20% 96h LC_{50}. Protease activity in liver of experimental fish showed an increase from control (0.45±0.01) to 0.46±0.03, 0.47±0.03, 0.48±0.01 and 0.49±0.01 mm/g wet wt. with 31.42, 34.28, 41.17 and 40% increase for 2, 4, 6 and 8 days respectively. Likewise muscle also showed an enhancement of protease activity (36.95%) at highest concentration of 20% 96hLC_{50}

and longest exposure period of 8 days. Maximum enhancement of protease activity was observed at 20% 96hLC_{50} for 8 days in gill (44.73%), followed by liver (40%) and muscle (36.95%).

Fig. 2: Protease activity (mm/g wet wt) in the tissues of *Cyprinus carpio* exposure to sublethal concentrations of cypermethrin (25%EC).



Discussion

Biochemical parameters are sensitive index to alterations caused by pesticide toxicity and can constitute important tools in toxicological investigations. Amino acids regarded as building blocks of proteins and are essential intermediates in protein synthesis and its degradation products appear in the form of different nitrogenous substances. In present investigation, an increase in free amino acids has been observed during sublethal (5, 10, 15 and 20% 96h LC_{50}) toxicity of cypermethrin. The increase in free amino acid level in the present study suggests tissues damage probably due to the increased proteolytic activity under cypermethrin stress.

Increase in the free amino acid levels was the result of protein breakdown for energy and impaired incorporation of amino acids in the protein synthesis (Prashanth and Neelagund, 2007). The increase in free amino acid levels of tissues were the result of stepped up proteases activity and fixation of ammonia into keto acid (Ali, 2003). However, the elevated levels of free amino acid can be utilized for energy production by supplying them as keto acids in to the TCA cycle through aminotransferase reaction to contribute energy needs during toxic stress. The increase in the free amino acid level has functional relevance for meeting energy demands and is involved in osmoregulation as well (Prashanth and Neelagund, 2007).

Enhanced free amino acids may also be due to depletion of reserved glycogen so that the fish can try to yield metabolic energy by gluconeogenesis process (Tripathi et al., 2003, Naveed et al., 2010). Similar findings were observed by several authors (Carlson, 2003; Vijuen et al., 2003; Swetha et al., 2012) in various organisms during various toxic exposures. The lowering of proteins and elevation of free amino acids are apparently inter-related and are indicative of metabolic utilization driving a possible source of energy to meet the energy demand under stress (Prashanth and Neelagund, 2007).

Protease is an enzyme that breaks the peptide bond to produce amino acids and other simpler peptides. In comparison to the control, cypermethrin intoxication induced the highest protease activity. The increased protease activity in liver, gill and muscle tissues was clearly reflected the breakdown of proteins. Under proteolysis, enhanced breakdown dominates over synthesis. While in the case of anabolic process, increased synthesis dominates the protein breakdown (Harper et al., 1979).

Under the increased energy demand associated with cypermethrin induced stress fish may degrade proteins to augment the available energy supply, thus altering the free amino acids pool. Enhanced protease activity has resulted in a marked elevation in the free amino acid content in all the tissue and at all time intervals. Proteolytic enzymes participate in the breakdown of protein molecules into amino acids and these amino acids are in turn oxidized to furnish energy for body function (Saravanan et al., 2010). Induction of proteolysis as a result of elevated protease activity reflecting in the decrease of the protein levels of different tissues of *Cyprinus carpio* exposed to cypermethrin was reported by Prashanth (2006).

Conclusion

It is concluded that synthetic pyrethroids including cypermethrin have attracted farmers and health departments to use them in pest management programmes because of their beneficial aspects. But these compounds are found to be highly toxic to fish and some other aquatic organisms. The analysis of data from the present study clearly evidenced that cypermethrin is highly toxic to *Cyprinus carpio* and had a profound influence on biochemical parameters even at sublethal concentrations. This study also provides an easy diagnosis for cypermethrin contamination and may become useful in evaluating the ecotoxicological effects in aquatic ecosystems close to agricultural fields and possible effects on fish fauna.

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