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Research Article

STUDIES ON ACTIVITY LEVELS OF ASPARTATE AMINO TRANSFERASE (ASAT) AND ALANINE AMINO TRANSFERASE (ALAT) IN THE TISSUES OF *Cyprinus carpio* (LINN.) EXPOSED TO CYPERMETHRIN (25% EC)

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ABSTRACT

Aspartate and Alanine transaminases (ASAT&ALAT) are widely distributed in the cells of all animals and serve to link the carbohydrate and protein metabolism by interconverting dynamic substances. Freshwater fish, *Cyprinus carpio* were exposed to sublethal concentrations (5, 10, 15 and 20% of 96hLC_{50}) of synthetic pyrethroid, cypermethrin (25% EC) for 2, 4, 6 and 8 days and analysed the activity of ASAT and ALAT in gill, muscle and liver. The activities of ASAT and ALAT were increased in all the tissues and dose as well as exposure period depended and statistically significant (*P>0.05) alterations were observed. Enhanced activities of these enzymes during the toxic stress of cypermethrin resulting in incorporation of amino acids into TCA cycle for energy production.

Keywords: Cypermethrin, *Cyprinus carpio*, ASAT, ALAT, Gill, Muscle, Liver.

INTRODUCTION

Aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides [1]. Pesticides are one of the most potentially harmful chemicals introduced into the environment. Though they have contributed considerably to human welfare, their harmful effects on non-target organisms are significant. Pesticides by their very nature create some risk or harm to biota or the environment because they are designed to kill or otherwise adversely affect living things. Synthetic pyrethroid pesticides are derived from natural compounds isolated from the Chrysanthemum genus of plants [2]. They possess relatively low mammalian toxicity, potent insecticidal action and phosphostability with low volatility and persistence. They are broad spectrum insecticides with tremendous ability to kill insect pests. They do not bioaccumulate and excert small effects on mammals but are very toxic to fish.

Cypermethrin is a synthetic pyrethroid broadly used for residential, commercial, industrial and agricultural pest control originally associated with cotton crops [3]. In recent years, it has also been adopted for pest control in soya bean culture [4]. It is well known that cypermethrin is extremely toxic to fish and aquatic arthropods under laboratory conditions [5] and it is also recognized that its toxicity is reduced under field conditions in water bodies with abundant particulate material [6].

Fishes are sensitive to water contamination and pollutants may significantly damage certain biochemical processes when they enter organs of these animals. Fish is a good indicator of aquatic contamination because its biochemical responses are quite similar to those found in mammals [7]. Fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds [8].

Changes in plasma enzyme activity are used as indicators of tissues injury, environmental stress, or a diseased condition. The rate of increase of plasma enzyme activity depends on the concentration of an enzyme in cells, the rate of leakage caused by injury and the rate of clearance of the enzyme from plasma [9]. Enzymatic malfunctioning can cause a rapid breakdown of cell components that are otherwise quite stable leading to death of an organism. Chemical induced alterations in enzyme activities can be used to monitor the functional alterations in the intoxicated tissue of an organism [10] [11]. In the present investigation an attempt was made to study activity levels of transaminases (ASAT&ALAT) in *Cyprinus carpio* exposed to sublethal concentrations of cypermethrin (25%EC), a synthetic pyrethroid.

MATERIALS AND METHODS

Freshwater fish, *Cyprinus carpio* weighing average of 6.2±2g and 7.5±0.5cm in length were procured from local fish farm in Tenali, Guntur District Andhra Pradesh, India and acclimated to laboratory conditions for 20 days. They were stored in large glass aquaria and water in the tanks was changed daily. The average temperature of water was 24-26°C. They were fed with rice bran, oil cake and soya bean in the ratio of 2:2:1 daily. The physico-chemical parameters of water are given in Table 1. Commercial grade cypermethrin (25% EC) of liquid formulations manufactured by Agro Ltd., India was used in the present study. After the normal process of acclimatization, a group of ten fish each were transferred to plastic tubs (15L capacity) containing 10L of water. Fish were exposed to different concentrations of cypermethrin to determine median lethal concentration (LC_{50}). From this, 4 sublethal concentrations i.e. 5, 10, 15 and 20% of 96hLC_{50} were selected and fish were exposed for a period of 2, 4, 6 and 8 days along with the control. Control and exposed fishes were sacrificed at the end of each day. Gill, liver and muscle were isolated and immediately transferred to deep freezer prior to analysis. Aspartate Amino Transferase (ASAT) and Alanine Amino Transferase (ALAT) activities were assayed by colorimetric method [12]. 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	814 micro ohms/cm
3	pH at 28°C	7.8
4	Alkalinity	
	1. Phenolphthalein	Nil
	2. Methyl orange	470
5	Total hardness as ($CaCO_3$)	256mg/l
6	Calcium hardness (as N)	74mg/l
7	Sulphate (as SO_4)	Trace
8	Chloride (as Cl)	36mg/l
9	Fluoride (as F)	1.6mg/l
10	Iron (as Fe)	Nil
11	Dissolved Oxygen	8.5 – 10ppm
12	Temperature	24 - 26°C

RESULTS

The activity of ASAT and ALAT was observed to increase in gill, muscle and liver of *Cyprinus carpio* exposed to cypermethrin over control.

This increase was observed to be directly proportional to sublethal concentrations of cypermethrin and exposure period (Figs 1&2). The results were statistically significant at (*P>0.05). The activity of ASAT in gill of control fish was observed to be 59.42±0.02µm oxaloacetate/mg protein/h and in sublethal concentrations of cypermethrin, the activity of ASAT was observed to increase over control irrespective of concentrations and exposure period (Fig. 1). This increase was observed to be 62.03±0.03, 63.48±0.09, 66.71±0.06 and 76.54±0.08µm oxaloacetate/mg protein/h equal to 4.39, 6.83, 12.26 and 28.81% respectively for 5, 10, 15 and 20%96hLC$_{50}$ after 2 days of exposure period. This increase was much intensified after maximum period of 8 days as the sublethal concentrations increase from 5% to 20%96hLC$_{50}$. Maximum increase of 81.00±0.02µm oxaloacetate/mg protein/h is equal to 35.40% at highest sublethal concentration of 96hLC$_{50}$ and maximum exposure period of 8 days. Similarly activity of ASAT in muscle and liver of *Cyprinus carpio* was also observed to increase as sublethal concentrations and exposure periods increases. The muscle ASAT activity was found to increase to 70.01±0.06 from the control value of 52.38±0.04µm oxaloacetate/mg protein/h equal to 33.65% over control fish. Similarly liver showed an increase of 16.36% activity over control after 20%96hLC$_{50}$ and 8 days of exposure period.

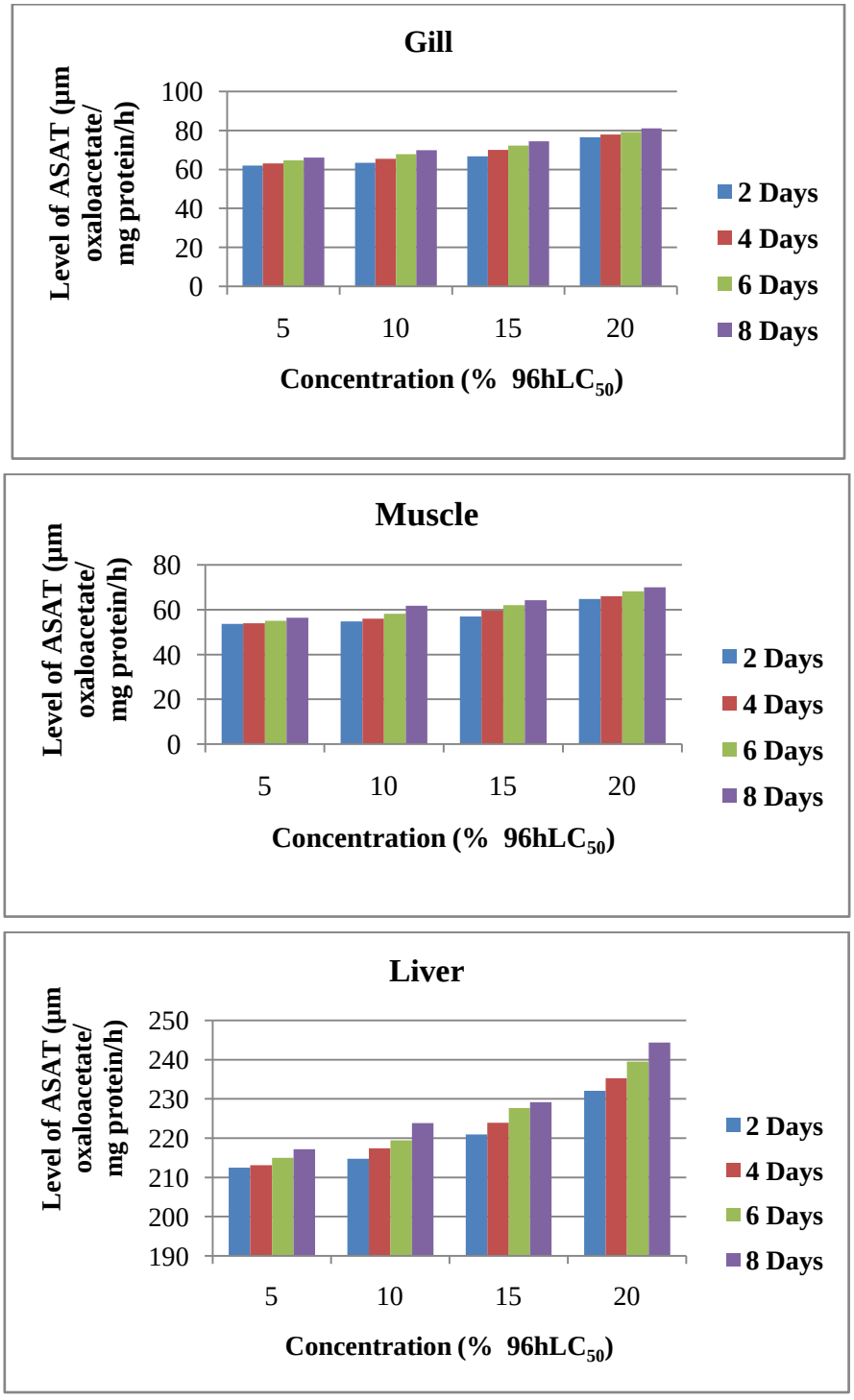

Fig. 1: Activity Levels of Aspartate Amino Transferase in the tissues of *Cyprinus carpio* exposed to sublethal concentrations of cypermethrin (25%EC)

The activity of ALAT was also found to increase in the tissues studied (Fig. 2) under sublethal concentrations of cypermethrin. The gill ALAT of control fish was 54.30±0.05µm oxaloacetate/mg protein/h. This was found to increase to 72.00±0.09µm oxaloacetate/mg protein/h after 2 days at 20%96hLC$_{50}$ (32.59%). This increase was much higher after 8 days at 20%96hLC$_{50}$ and was observed to be 75.74±0.09µm oxaloacetate/mg protein/h equal to 38.11% over control. Similarly muscle and liver ALAT activity was found to increase to 90.16±0.02 and 290.38±0.05 from the control values of 63.89±0.05 and 204.03±0.09µm oxaloacetate/mg protein/h after 20%96hLC$_{50}$ and 8 days respectively. Muscle showed an enhancement of 40.91% and liver showed an enhancement of 42.3% after longest exposure period and highest sublethal concentration.

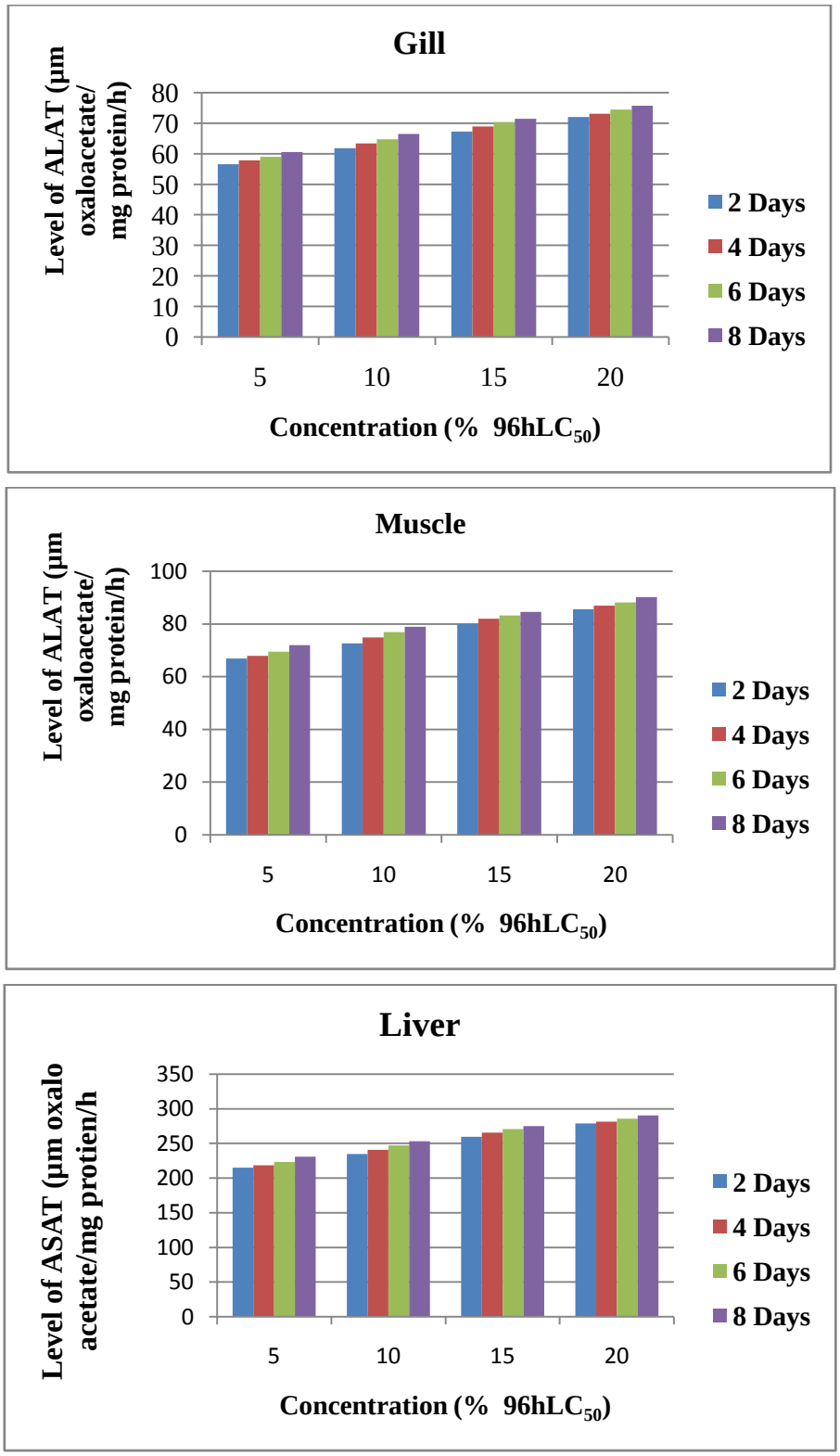

Fig. 2: Activity Levels of Alanine Amino Transferase in the tissues of *Cyprinus carpio* exposed to sublethal concentrations of cypermethrin (25%EC)

DISCUSSION

In the present investigation there is a significant increase in the activity levels of ASAT and ALAT in all the tissues of *Cyprinus carpio* at all the exposure periods of cypermethrin at all the sublethal concentrations. ASAT and ALAT are non plasma specific enzymes that are localized in tissue cells of liver, heart, gills, kidneys, muscles and other organs and their presence in the plasma may give specific information about organ dysfunction [13] [14]. The enhanced activities of transaminases, Aspartate Amino Transferase (ASAT) and Alanine Amino Transferase (ALAT) were observed under toxic stress of cypermethrin in the tissues. During the toxic stress the animal can never take food. So the animal needs additional energy to overcome the toxicity [15] [16]. Hence, the activities of transaminases may be enhanced along with proteolytic activities [17] [18]. The elevated transaminases may indicate that fish can utilize the free amino acids from amino acid pool for energy production. Any malfunction occurs in energy yielding compounds, the cell switches over to the gluconeogenesis process with the help of transaminases [19]. Increase in tissue ASAT and ALAT was the indication of incorporation of amino acids by way of aminotransferase activities of these enzymes into Kreb's cycle to overcome the stress posed by cypermethrin. This may be reason for the increase in the enzyme levels in tissues of exposed fish. During protein metabolism the removal of amino group from different amino acids was observed due to this the elevated levels of the ASAT and ALAT in the exposed fish was noticed, this is because of the breakdown of the proteins.

Alterations in the activities of the aminotransferases would often be reflected in nitrogen metabolism and interdependent biochemical reactions. The increased levels of amino transferases might be attributed to tissue damage under toxic stress [20]. ASAT, a key enzyme of nitrogen metabolism and energy mobilization, is often used as a biochemical indicator of stress [21]. The increased trend in both ASAT and ALAT activities indicates that there is more conversion of amino acids into keto acids than that utilized for energy synthesis [22] [23] [24]. Increased activities of ASAT and ALAT in the present study indicate that there is an active tranamination of aminoacids, possibly to provide keto acid in the TCA cycle. Steady rise in these enzymes may be due to the synthesis of these enzymes under sub acute cypermethrin stress. Increase could be helpful to the fish for structural reorganization of proteins and incorporation of keto acids into the TCA cycle to favour gluconeogenesis or energy production. Increased activities of ASAT and ALAT lead to metabolic compensation and allow the animal to adapt to the imposed cypermethrin stress. These results were in accordance with reports of earlier studies [25] [26] [27].

CONCLUSION

The present work indicates that cypermethrin caused alterations in the activities of Aspartate Amino Transferase (ASAT) and Alanine Amino Transferase (ALAT) of fish *Cyprinus carpio*. Cypermethrin caused an increase in the activity of these transaminases which can be used as relevant stress indicators. Significant increase in the activities of the above mentioned enzymes indicates stress depended tissue impairment. Increased activities of both the transaminases indicated amplified transamination occurs due to amino acid input into the TCA cycle in order to cope with the energy crisis during cypermethrin based stress. We can conclude that cypermethrin is dangerous to aquatic environment, and this should be taken into consideration when this insecticide is used in agriculture or in the control of pests and potential risk from cypermethrin metabolites should be studied to get comprehensive information in terms of its toxic impact.

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