INFLUENCE OF λ- CYHALOTHRIN (5% EC) ON ENZYME ACTIVITIES IN FRESHWATER FISH *CTENOPHARYNGODON IDELLA*

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ABSTRACT

Rapid industrialization and green revolution have introduced a large variety of agricultural chemicals like pesticides. Pesticides were found to pollute every source of water. The present study toxicity effect of synthetic pyrethroid pesticide λ-cyhalothrin on certain enzymes were evaluated in gill, liver, kidney, muscle and brain of freshwater fish *Ctenopharyngodon idella* during sublethal exposures of 1, 4, 8 and 12 days. A dose of 0.26mg/l was taken as the sublethal($1/10^{th}$ of lethal) concentration. Various organs were taken from control fish exposed after the end of exposure periods and used for estimation of AAT and ALAT activities. All the organs showed the significant difference between control and exposed groups in all the estimated parameters on all days of exposure. In the present study, there is a significant elevation was observed in AAT and ALAT activities. These enzymatic studies act as important biomarkers in determining the level of toxicity caused by the pesticide λ-cyhalothrin.

Keywords: λ-cyhalothrin, Sublethal concentrations, *Ctenopharyngodon idella*, AAT and ALAT.

INTRODUCTION

Generally pesticides are used very extensively in agriculture, forestry, public health and veterinary practices and are gaining immense importance due to their ability to control weeds, insects, aquatic weeds etc, Abu-Darwish *et al.*,(2011). The major chemical groups of pesticides that are usually applied in fields are organophosphate, organochlorine, carbamates and pyrethroids, Sarba and Mehana, (2015). Although they has credited with economic potential to enhance production of food and fibres, they have been found to be highly toxic not only for fish but also to the human beings which pass though the food chain. Long-term exposure of pesticides induces physiological disturbance, behavioural changes, histopathological damages, haematological alterations, biochemical changes, immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer, Pandey *et al.*, (2014); Ullah *et al.*, (2014); Ullah and Zorriehzahra, (2015). Hence, biochemical parameters are the best physiological indicators of the fish health. Therefore it is important to be focused while studying the toxic effects of various pesticides and pollutants on fish.

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OBJECTIVES OF THE PRESENT STUDY

In the present study λ-cyhalothrin pesticide was selected to evaluate the impact of sublethal exposures for 1, 4, 8 and 12 days in freshwater fish *Ctenopharyngodon idella*. Certain enzymes like Aspartate Aminotransferase (AAT) and Alanine Aminotransferase (ALAT) are being extensively used as potential biomarkers for measurement of tissue and organ damage due to pesticidal toxicity.

MATERIALS AND METHODS:

The freshwater fish *Ctenopharyngodon idella*, an exotic fish commonly called grass carp were collected from ponds in Kuchipudi, Guntur District, brought to the laboratory, then acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28± 2^0C prior to experimentation. 96h LC_{50} Sublethal (0.26mg/l) concentration for 1, 4, 8 and 12 days was found out by using probit method, Finney, (1971). For biochemical studies fishes were reared in sublethal concentration for a period of 1, 4, 8 and 12 days. The activities of AAT and ALAT were estimated by the method Reitman and Frankel (1957).

Estimation of Aspartate amino transferase (AAT) activity

The reaction mixture of 1.5 ml contains 1 ml phosphate buffer (pH 7.4), 0.1 ml of Aspartate (L-Aspartic acid), 0.1 ml of α-Ketoglutaric acid and 0.3 ml of supernatant as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by the adding 1 ml of 2,4-Dinitrophenyl hydrazine(DNPH) solution prepared in 0.1N HCl and was allowed to stand for 20 minutes at room temperature. The rest of the details were the same as for alanine aminotransferase. The enzyme activity was expressed as μ moles of pyruvate formed/mg protein/hr.

Estimation of Alanine Aminotrasferase (ALAT) activity

The reaction mixture of 1.5 ml contains 0.1 ml phosphate buffer (pH 7.4), 0.1 ml of L-Alanine 0.1 ml of α-Ketoglutarate and 0.3 ml of supernatant as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by addition of 1 ml of 2,-4 dinitrophenyl hydrazine solution.After 20 minutes, 10ml of 0.4N sodium hydroxide was added and the colour developed was read at 545nm in a spectrophotometer (ELICO Model SL 207) against a reagent blank. The enzyme activity was expressed as μ moles of pyruvate formed/mg protein/hr.

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RESULTS AND DISCUSSION:

Aspartate Aminotransferase (AAT) Activity

Present study the AAT was elevated in all the tissues of test fish *Ctenopharyngodon idella* exposed to λ-cyhalothrin in sublethal concentrations for 1st, 4th, 8th and 12 days. The calculated values for AAT activity along with percent change over control and standard deviations were given in Table 1, and Fig. 1. In the tissues of control fish, *Ctenopharyngodon idella* AAT activity with reference to lyotrophic gradation series was in the order of: Liver > Kidney > Brain > Muscle > Gill.

Under sublethal exposure to λ-cyhalothrin for day 1, maximum percentage of elevation was in kidney (181.95%) followed by muscle (123.80%), liver (46.27%), brain (28.49%) and minimum elevation was in gill (24.2%); whereas day 4 sublethal exposure, maximum elevation was in kidney (363.90%) followed by muscle (161.90%), liver (121.54%), brain (111.82%) and minimum elevation in gill (72.61%); for day 8 sublethal exposure, maximum elevation was in kidney (599.51%) followed by muscle (200%), brain (180.10%), liver (175%) and minimum elevation in gill (114%) and for day 12 sublethal exposure, the maximum percentage of elevation was in kidney (768.29%) followed by liver (236.7%), brain (227.95%), muscle (221.42%) and minimum elevation in gill (147.13%) were observed in test fish *Ctenopharyngodon idella.*

Table 1: Change in the Specific activity levels of AAT (μ moles of pyruvate formed/mg/h) and % change over the control in different tissues of *Ctenopharyngodon idella* on exposure to sublethal concentrations of λ-cyhalothrin (5% EC)

Tissue	Control	Exposure period (days)							
		1		4		8		12	
		Sublethal	% Change	Sublethal	% Change	Sublethal	% Change	Sublethal	% Change
Gill	1.57±0.01	1.95±0.1	+24.2	2.71±0.07	+72.61	3.36±0.06	+114	3.88±0.07	+147.13
Muscle	1.68±0.01	3.76±0.11	+123.80	4.4±0.03	+161.90	5.04±0.03	+200	5.4±0.06	+221.42
Liver	3.76±0.03	5.5±0.1	+46.27	8.33±0.1	+121.54	10.34±0.06	+175	12.66± 0.1	+236.7
Kidney	2.05±0.01	5.78±0.04	+181.95	9.51±0.21	+363.90	14.34±0.02	+599.51	17.80±0.05	+768.29

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Brain	1.86±0.02	2.39±0.04	+28.49	3.94±0.05	+111.82	5.21±0.06	+180.10	6.1±0.09	+227.95

Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. Values are significant (P<0.05)

Fig. 1: Change in AAT (µ moles of pyruvate formed/mg/h) in different tissues of *Ctenopharyngodon idella* on exposure to sublethal concentration of λ-cyhalothrin (5% EC)

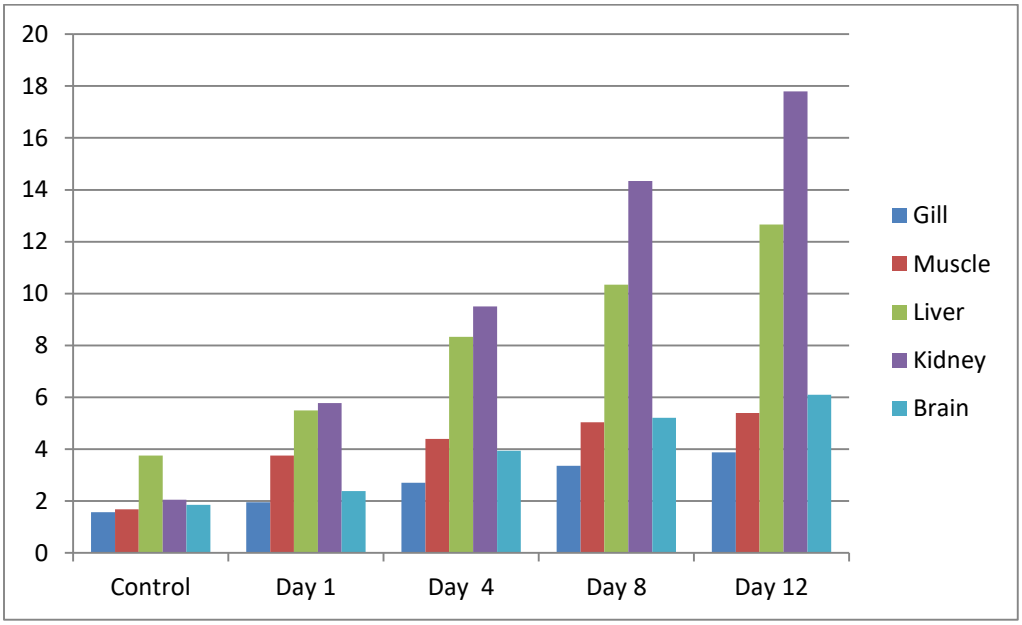

Table 2: Change in the Specific activity levels of ALAT (µ moles of pyruvate formed/mg/h) and % change over the control in different tissues of *Ctenopharyngodon idella* on exposure to sub lethal concentrations of λ-cyhalothrin (5% EC)

Tissue	Control	Exposure period (days)							
		1		4		8		12	
		Sublethal	% Change	Sublethal	% Change	Sublethal	% Change	Sublethal	% Change
Gill	2.28±0.14	2.6±0.02	+14.03	2.67±0.02	+17.10	2.80±0.07	+22.80	3.09±0.01	+35.52
Muscle	2.27±0.01	2.37±0.01	+4.4	3.28±0.01	+44.49	3.88±0.03	+70.92	4.16±0.01	+83.25
Liver	11.73±0.09	12.6±0.18	+7.41	13.55±0.09	+15.51	14.82±0.04	+26.34	15.56±0.21	+32.65
Kidney	2.11±0.08	2.60±0.07	+23.22	2.99±0.08	+41.70	3.73±0.06	+76.77	4.73±0.02	+124.17

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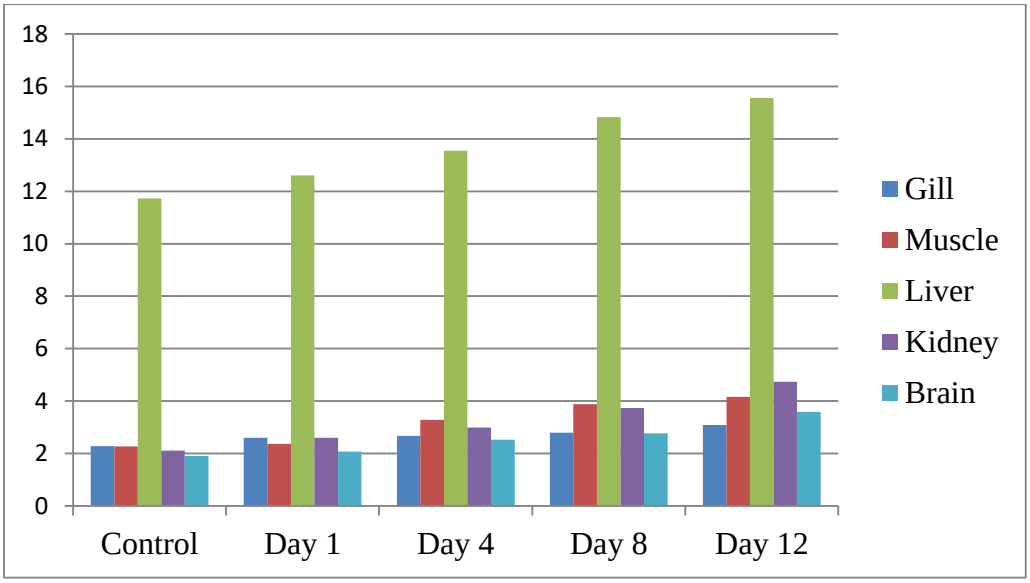
Brain	1.90±0.08	2.08±0.15	+9.47	2.53±0.05	+33.15	2.77±0.02	+45.78	3.59±0.07	+88.94

Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. Values are significant P<0.05.

Fig. 2: Change in ALAT (µ moles of pyruvate formed/mg/h) in different tissues of *Ctenopharyngodon idella* on exposure to sublethal concentration of λ-cyhalothrin (5% EC)



Alanine Aminotransferase (ALAT)Activity

Present study the ALAT was elevated in all the tissues of test fish *Ctenopharyngodon idella* exposed to λ-cyhalothrin in sublethal concentrations for 1st, 4th, 8th and 12 days.The calculated values for ALAT activity along with percent change over control and standard deviations were given in Table 2 and Fig. 2. In the tissues of control fish, *Ctenopharyngodon idella* ALAT activity with reference to lyotrophic gradation series was in the order of: Liver > Gill > Muscle > Kidney > Brain.

Under sublethal exposure to λ-cyhalothrin for day 1, maximum percentage of elevation was in kidney (23.22%) followed by gill (14.02%), brain (9.47%), liver (7.41%) and minimum elevation in muscle (4.4%); whereas day 4 sublethal exposure, maximum elevation was in muscle (44.49%) followed by kidney (41.70%), brain (33.15%), gill (17.10%) and minimum elevation in liver (15.51%); for day 8 sublethal exposure, maximum elevation was in kidney (76.77%) followed by muscle (70.92%), brain

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(45.78%), liver (26.34%) and minimum elevation in gill (22.80%) and for day 12 sublethal exposure, the maximum percentage of elevation was in kidney (124.17%) followed by brain (88.94%), muscle (83.25%), gill (35.52%) and minimum elevation in liver (32.65%) were observed in test fish *Ctenopharyngodon idella.*

AAT and ALAT are located in both mitochondrial and cytosol fractions of the cell and a close relation appear to exist between the mitochondrial integrity and transaminase levels Bonitenko, (1974). Any modification in the organization of mitochondria is bound to alter the enzyme systems associated with it. Aminotransaminases play an important role in the utilization of amino acids for the oxidation and gluconeogenesis, Kumar *et al.*, (2011). AAT and ALAT were frequently used in the diagnosis of damaged tissues such as liver, muscle, and gills exposed to different toxicant De La Torre *et al.,* (2005). The elevation of AAT activity provides the oxaloacetate required forthe gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism, Velumurugan *et al.*, (2007). ALT (Alanine transaminase), AST (Aspartate transaminase) are being extensively used as potential biomarkers for measurement of tissue and organ damage due to pesticidal toxicity, Neelanjana *et al*., (2017).

The present study alteration in the activities of AAT and ALAT is due to the mitochondrial disruption and damage as a result of λ-cyhalothrin induced stress. Similar increase in transaminase enzymes have been reported, David *et al.*, (2004). Pesticide stress was known to induce significant change in protein metabolism, it is likely that the aminotransferases were also considerably affected. Increased activities of AAT and ALAT in different tissues of fish suggest either increased operation of transamination or increased synthesis of amino acids from other sources like glucose or fatty acids during λ-cyhalothrin intoxication. The elevation in AAT and ALAT activities measured in liver tissues of eel during exposure to proponil due to the existence of heavy drain on metabolites during propanil stress to provide intermediates to the Krebs cycle, Sancho *et al*., (2009). The increase in activities of aminotransferases demonstrating under pesticide exposure conditions of fish and enhanced gluconeogenesis.

The reported elevation levels of transaminases ALAT and AAT in the fish *Cnesterodon decemmaculatus* using those enzymes as biomarkers of polluted water, De La Torre *et al.*, (2005). The increased activities of AAT and ALAT might be an active transamination of aminoacids, possibly to provide keto acid in the kreb's cycle and also the synthesis of these enzymes under chronic pesticide

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stress. The measurement of transaminase activities in serum is frequently used to diagnostic tool in human and animals, Barse *et al.*,(2006). The increased enzyme activities could be helpful to the fish *Ctenopharyngodon idella,* the structural reorganization of proteins and incorporation of keto acids into the kreb's cycle serves to favour gluconeogenesis or energy production, it leads to metabolic compensation and allows the animal to adapt the imposed toxic stress, Al-Ghanim, (2014); Prasanth and Neelagund (2008).

Present study the pesticide stress was known to induce significant change in protein metabolism, it is likely that the amino transferases were also considerably affected. Increased activities of AAT and ALAT in different tissues of fish suggest either increased operation of transamination or increased synthesis of amino acids from other sources like glucose or fatty acids during λ-cyhalothrin intoxication. Begum, (2004), increase in aminotransferase activity in fish *Clarias batrachus*, under carbofuran pesticide stress, enhancement of the activity of the transaminases provided the oxaloacetic acid and pyruvate, α-ketoglutarate and glutaric acid to meet the increased energy demand. Al-Ghanim *et al.*, (2020) the increased activity of SGPT and SGOT was observed at sublethal exposure of fenvalerate in liver and gill tissues of Zebra fish *Danio rerio*. Ozge Cerit and Feride Koc, (2019), the activities of ALT and AST increased significantly in the CP group when compared with the control group of rainbow trout *Oncorhynchus mykiss* on exposure of chrysin(CR) on cypermethrin. This indicates the injury in liver, inflammatory disease of hepatic damage.

Das *et al.*, (2004), elevation of AST activity in serum, brain and gill of *Cirrhinus mrigala* exposed to sublethal concentrations of ammonia. Kori-Siakpere *et al.*, (2007) observed that increased AST and ALT levels in different tissues of African catfish *Clarias gariepinus* exposed to paraquat. Montanha *et al.*,(2014) reported that significant increase in AAT and decrease in ALAT was observed in *Rhamdia quelen* exposed to sublethal concentration of cypermethrin. Similar trend of elevation in AAT and ALAT was observed in fish *Labeo rohita* exposed to Ethion (50% EC), Anitha *et al.*, (2018). Increased ALAT activity was noticed in *Cyprinus carpio* and *Oreochromis niloticus* exposed to cadmium, De Smeth and Blust (2001), Almedia *et al.*, (2002). Increased levels of GOT and GPT in liver and muscle tissues under neem leaf extract exposed to *Cirrhinus mrigala,* Saravanan *et al.*, (2011). Increased activity of aminotransferases due to monocrotophos in *Channa punctatus,* Agrahari *et al.*, (2007), and similar trend was observed in freshwater fish *Labeo rohita* on exposed to phoslone, Manvalaramanujam and Ramesh (1996).

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AAT catalyses reversible transamination of glutamate and oxaloacetate to α-ketoglutarate and aspartate, while ALAT catalyses the reversible transamination of glutamate and pyruvate to α-ketoglutarate and alanine. Thus, the aminotranferases along with GDH contribute some strategic substances such as α-ketoglutarate, pyruvate, oxaloacetate, glutamate etc, to oxidative metabolism, Prashanth and Neelagund (2008). GDH catalyses the reversible deamination of glutamate to α-ketoglutarate and ammonia AAT catalyses reversible transamination of glutamate and oxaloacetate to α-ketoglutarate and aspartate, while ALAT catalyses the reversible transamination of glutamate and pyruvate to α-ketoglutarate and alanine. Thus, the aminotransferases along with GDH contribute some strategic substances such as α-ketoglutarate pyruvate, oxaloacetate, glutamate etc., to oxidative metabolism.

Nagaraju *et al.,* (2013) the activities of aminotransferases demonstrating a constant increase under conditions of enhanced gluconeogenesis. The alterations in the levels of activity of aminotransferases induced by the profenofos and carbosulfan on freshwater fish *Labeo rohita* indicate that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems. Vasantharaja *et al.,* (2014), AAT and ALAT level in blood serum were increased in treated with cypermethrin intoxicating fish *Cirrhinus mrigala*, detoxifying property of *Cardiospermum halicacabum* was observed. Abdul Naveed *et al.,* (2010) enhanced activity of AAT and ALAT during the toxic exposure of triazophos in *Channa punctatus*.

Present study the elevation of AAT activity provides the oxaloacetate required for the gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. Elevation in the levels of AAT and ALAT in different tissues of brain, liver, muscle, gill and kidney of the fish *Ctenopharyngodon idella* can be considered as a response to the stress induced by λ-cyhalothrin to generate ketoacids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and or energy production necessary to meet the excess energy demand under the toxic manifestations. The alterations in the levels of aminotransferases induced by λ-cyhalothrin clearly indicate that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transminase systems.

CONCLUSION

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It is clearly evident from the present study that there is a significant elevation was observed in AAT and ALAT activities. The antioxidant enzymes like AAT and ALAT are being extensively used as potential biomarkers for measurement of tissue and organ damage due to pesticidal toxicity. And also it is concluded that the extensive use of pesticides should be avoided and the applications should be judicious and rationalized.

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