Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 205-208

Received: 24-05-2020
Accepted: 25-08-2020

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Effect of Cypermethrin on hepatopancreatic α-amylase Activity and on Ribonucleic Acid content of Hepatopancreas of *Colisa fasciatus*

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Abstract
Insecticides are the widely used chemicals in the agricultural, economic, human, and environmental sectors. Cypermethrin is a highly toxic organochlorine pesticide for aquatic species that often leads to death by impairing the metabolism. During the present study effect of cypermethrin on α-amylase activity at 24h, 48 h LC_{50} doses was determined. Effect was also observed at sub-lethal concentration along with the determination of bio-concentration factor. Reversal of the changes along with changes in RNA content was also determined. Hepatopancreatic activity was observed and results showed that the boiled enzyme (in a water bath) showed no in-vitro enzyme activity where the enzyme activity in the ice bucket was observed as significant. It was noted, from the that the LC_{50} doses of Cypermethrin inhibited the ∝-amylase activity by 32.1%, 64.7% and 58.8% at 24h, 48h and 72h (LC_{50} doses) respectively. It was found that during 24h, 48h and 72 h of exposure of fish to LC_{50} endosulfan concentration there was 52.17%, 64.7% and 58.8% loss of enzyme activity.

Keywords: Cypermethrin, organochlorine pesticide, enzyme and Hepatopancreas

Introduction
Insecticides are the chemicals that are commonly used in the agricultural, economic, human and environmental sectors. Areas of animal-health. Insecticidal exposure has been related to several dangerous effects including antioxidant metabolism. Endosulfan is an extremely toxic organochlorine pesticide to aquatic organisms which might be hampering fish health through impairment of metabolism sometimes leading to death. Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, including moth pests of cotton, fruit and vegetable crops [1]. It is also used for crack, crevice and spot treatment for control of insect pests in stores, warehouses, industrial buildings, houses, apartment buildings, greenhouses, laboratories and on ships, rail, buses, trucks and aircraft. It may also be used in non-food areas in schools, nursing, homes, hospitals, restaurants, hotels, and in food processing plants and as a barrier treatment insect repellent for horses. Cypermethrin is available in emulsifiable concentrate, and wettable powder formulations [1]. Technical cypermethrin is a mixture of eight different isomers, each of which may have its own chemical and biological properties. Singh and Srivastava (1999) [2] exposed freshwater fish *Channa striatus* (Bloch) to permethrin at a dose of 2.0 mg/l (24-h LC_{50}) and found significant reduction in the activity of lactate dehydrogenase and cytochrome oxidase and enhancement in succinate dehydrogenase activity in the tissue. They concluded that the mechanism of action was blocking of aerobic as well as anaerobic metabolism in the exposed fishes.
The present study deals with the effect of cypermethrin on α-amylase activity at 24h, 48 h LC_{50} doses. Effect was also observed at sub-lethal concentration along with the determination of bio-concentration factor. Reversal of the changes along with changes in RNA content was determined.

Materials and Methods
Adults and healthy *Colisa fasciatus* were regularly obtained from the local market pond and acclimatised in the glass aquaria for seven days (*Colisa fasciatus* were not easily available some generally it was brought from ponds north of Ganges in Bihar around Gandak). During the acclimatization of fishes normal level of dissolved O (d) was maintained. For the determination of lethal concentration and toxicity of the pesticides the fishes were exposed in

each aquarium in batches of ten specimens. The test concentrations of the pesticides were selected from a decilog series and are expressed here in terms of part active ingredient per million parts of water.

Preparation of Enzyme: Control and treated fishes were sacrificed and hepatopancreas was dissected out and homogenized separately in a potter Elevehem homogenizer with 0.02M Na-phosphate buffer. The crude homogenate (0.5%) were centrifuged separately at 1000 g and the supernatant was use as enzyme solution of control and treated.

∝-Amylase assay: ∝-amylase activity was determined following the method of Bernfeld (1955) [3] with--- slight modifications. 0.5 ml of aliquot of the enzyme solution was used for enzyme assay. 1 ml. Of 1% buffered starch solution for 10 min at 37^0 C in atmosphere of air. The incubation mixture was then cooled to room temperature and the enzyme reaction was interrupted by adding 2ml of 3, 5 dinitrasalicylic acid. The tube containing the mixture was then boiled in water bath for about 5 min to stop the reaction completely. It was then cooled to room temperature and added 20ml of distilled water. A blank was run simultaneously where 1 ml of distilled water added instead of enzyme. The optical density of the solution was determined at 540 nm.

Amount of maltose produced was determined from a calibration curve of maltose prepared from standard maltose solution.

Estimation of protein: Protein was measured by the method of Lowry *et al*., (1951) [4] taking bovine serum albumin as standard.

Results

Hepatopancreatic ∝-amylase activity was observed and findings have shown that the boiled (in a water bath) did not show any in-vitro enzyme activity where as significant the enzyme activity was observed in the enzyme kept in ice-bucket. Observation of hepatopancreatic ∝-enzyme activity was done by sacrificing a group of fish and then hepatopancreas was excised out then homogenized in a potter Elevehgem homogenizer. The homogenate was then centrifuged at 1000g and supernatant was used as enzyme. The extract thus prepared showed significant ∝-amylase activity (Table 1) when the same was boiled in a water bath (100 ^{0}C) for 20min.

Table 1: Demonstration of fish ∝-amylase activity in the hepatopancreas of *Colisa fasciatus*.

System	Mg of maltose liberated/ Mg of enzymes protein
Hepatopancreatic homogenate	0.310
Boiled homogenate	0.002

Addition were same as mentioned in the "Methods"

The studies on variation of pH was observed with Na-phosphate buffer of different pH value taking liver supernatant as enzyme. Optimum pH for ∝-amylase activity was found to be at 6.5.

Effect of Cypermethrin of ∝-amylase activity

To observe the effect of pesticide on fish hepatopancreatic ∝-amylase activity, a group of Colisa fasciatus with the Cypermethrin pesticide for 24hr, LC_{50}, 48h LC_{50} and 72h LC_{50} concentrations and were sacrificed, the enzyme was prepared in similar manner as mentioned in the Method. There was marked inhibition of ∝-amylase activity due to pesticide treatment.

Table 2: Effect of Cypermethrin on fish hepatopancreas ∝-amylase activity

System	mg of maltose liberated/Mg of protein	Inhibition %
Control	0.31	-----
Boiled control	0.013	-----
Treated (24h LC_{50})	0.130	52.1
Treated (48h LC_{50})	0.09	64.7
(Treated (72h LC_{50})	0.130	58.8

Table 3: Effect of Cypermethrin of fish hapatopancreas ∝-amylase activity

Control	Boiled Control	Treated(24h LC_{50})	Treated(48h LC_{50})	Treated(72h LC_{50})
0.31	0.013	0.23	0.20	0.19

Additions were same as mentioned in the 'Method'

The fishes were exposed to sub-lethal concentrations (1/3 of LC_{50}) for a period of 8 days. A control group was maintained in the identical environment. The fishes were sacrificed from both experimental and control batches on 1st, 4th and 8th day of exposure. For recovery studies fishes were transferred to pollutant free water and ∝-amylase activity was observed on 1st, 4th and 8th day of post-exposure period (Table 4).

Table 4: Effect of Cypermethrin on ∝-amylase activity at sub-lethal concentration.

Concentration of Cypermethrin	Exposure Period			Recovery Period		
	1st	4th	8th	1st	4th	8th
0	0.31±0.03	0.31±0.03	0.45±0.02	0.34±0.02	0.034±0.01	0.34±0.02
Sub Lethal at	0.20±0.03	0.22±0.02	0.30±0.02	0.30±0.03	0.040±0.02	0.33±0.02
1/3 of 24h, LC_{50}						
Sub Lethal at	0.19±0.02	0.26±0.02	0.30±0.03	0.31±0.03	0.040±0.04	0.32±0.02
1/3 of 48h, LC_{50}						
Sub Lethal at	0.17±0.01	0.20±0.02	0.20±0.02	0.30±0.02	0.031±0.02	0.31±0.02
1/3 of 72h, LC_{50}						

The rate of enzyme activity by increasing concentration of enzyme proteins was altered when the enzyme was extracted and fractionated from the hepatopancreas treated (24h LC_{50}, 48h LC_{50} and 72h LC_{50}) fish. There was a significant inhibition of pesticide polluted enzyme when compared to the rate of activity of the control enzyme.

The effect of increasing concentration of the substrate (starch) on the control enzyme increases the activity linearly up to 0.70 mg/ incubation mixture and then it was levelled off. In this connection it was observed that the inhibition of ∝-amylase activity by was found to be non-competitive in nature as the rate of inhibition could not be changed by increasing the concentration off the substrate.

It was noted, from the results that the LC_{50} doses of Cypermethrin inhibited the ∝-amylase activity by 32.1%, 64.7% and 58.8% at 24h, 48h and 72h (LC_{50} doses) respectively. It was found that during 24h, 48h and 72 h of exposure of fish to LC_{50} endosulfan concentration there was 52.17%, 64.7% and 58.8% loss of enzyme activity. It was however could not be suggested that how much amount of pesticides were accumulated in the hepatopancreas how much amount of pesticides were accumulated in the hepatopancreas to provide the observed percentage of inhibition. An attempt was therefore made to determine the accumulated concentration of pesticide needed for such inhibition by orienting an experiment pattern when pesticides were added in varied concentration in the incubation medium maintaining other factors constant.

It could be seen from Table 5 that RNA content of pesticide treated fish is appreciably deputed in comparison to control. There was about 46.5% inhibition of total RNA content in the liver of cypermethrin treated fish. This decrease after 48 hr, of pesticide treatment may not follow linearly in different time level.

Table 5: Changes in hepatopancreatic RNA Content due to Cypermethrin Treatment.

System	Hepatopancreas (wt. Mg)	Whole (µg)	Cellular RNA (µg/mg wt. Tissue)	% of inhibition
Control	40	346	9.2	48.7
Cypermethrin Treated	40	285	4.9	46.7

Discussion

Bhattacharya *et al*. (1975) [5] have reported that Nation activates ∝-amylase activity possibly not chloride ion. Various concentrations of different salts were used in connection with the present enzyme and showed that both NaCl and NA_2CO_3 activated the fish hepatopancreas ∝-amylase activity. On the other hand, chloride of copper and potassium salt could not activate the ∝-amylase activity, hence supporting the earlier contention. Mishra and Shukla (1997) [6] observed a non-competitive type of inhibition in hepatic enzyme due to organochlorine pesticide. The pesticide inhibition mechanism of ∝-amylase activity is not clearly understood. It may be denaturation of the enzyme protein due to pesticide toxicity or pesticide may bind with the active site of enzyme inhibiting its activity or pesticides may hamper the availability of NA^+ ion which is an activator of hepatopancreatic ∝-amylase.

If the last possibility is considered although this may not be the only cause of ∝-amylase inhibition it was tried to reverse the pesticide pollution by introducing the NA^+ ion exposure after cypermethrin treatment (24h TL_m, 48h TM_m, 72h TL_m). Pesticide treated fishes were removed to aquaria containing fresh water or NA^+ ion and exposed for 48h. Davis and Wedemeyer (1971) [7] studied NA^+, K^+-activated – AT pase inhibition in rainbow trout a site for organochlorine toxicity. Enzyme was prepared in each case according to the method mentioned in the text. Guhathakurata and Bhattacharya (1989) [8] adopted the in vitro technique because studies on biochemical lesions are a fundamental importance in assessing the toxicity of pesticide on enzyme activity. The revival difference of hepatopancreatic ∝-amylase activity between fresh water and Na^+ ion exposed fishes have shown the determined value of reversal effect of Na^+ ion. Therefore, it was evident from table that the loss of hepatopancreatic ∝-amylase activity by Cypermethrin was markedly regained in the presence if Na+ ion. Thus, it was observed here the different concentration of Na+ ion reversing the pesticide effect and 1 ppm (1mg/litre) was found to be suitable concentration for such phenomenon. Thus, it can very easily be explained that Na^+ ion played a very important role to counter act pollution effect in such a physiological condition.

The results of the present studies are similar to the observations done by Kumar and Singh (2000) [9]. They studied the DNA content on the gill and kidney cells of *Clarias batrachus* and effect of fenvalerate on it. They demonstrated that fenvalerate reduced the DNA content in gills, whereas it did not produce any significant effect on kidney whereas RNA and protein content declined substantially in both gills and kidney tissue in response to fenvalerate treatment. In the present study it has been demonstrated that endosulfan and rogor depleted the hepatopancreatic RNA content.

Conclusion

In the present study, it was noted, from the that the LC_{50} doses

of Cypermethrin inhibited the ∝-amylase activity by 32.1%, 64.7% and 58.8% at 24h, 48h and 72h (LC_{50} doses) respectively. It was found that during 24h, 48h and 72 h of exposure of fish to LC_{50} endosulfan concentration there was 52.17%, 64.7% and 58.8% loss of enzyme activity. The pesticide inhibition mechanism of ∝-amylase activity is not clearly understood. It may be denaturation of the enzyme protein due to pesticide toxicity or pesticide may bind with the active site of enzyme inhibiting its activity or pesticides may hamper the availability of NA^+ ion which is an activator of hepatopancreatic ∝-amylase. Thus, it can very easily be explained that Na^+ ion played a very important role to counter act pollution effect in such a physiological condition.

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