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Effect of Pesticide on Fish Enzyme Activity

Abstract

Pesticides significantly affect the fish enzyme activity and fish physiology. The present studies were conducted on α-amylase activity of hepatopancreas of the fish due to pesticide at sub-lethal and LC_{50} concentration.

There is little evidence of pollution affecting the health of fish all over the world. There is disrupt that pollution can affect the all aquatic organism. All evidence is show mostly disease in fish in open water.

Experiments were conducted to find out the nature of inhibition and the probable dose of the two pesticides responsible for changes in α-amylase activity. Mechanism of reaction phenomenon and reversal of the changes enzyme activity was also observed.

Keywords: Pesticide, Enzyme, α-amylase, Organochlorine, Organophosphates.

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Introduction

We have been gifted by aquatic resources such as ponds, lakes, rivers and ocean nature which provides employment opportunities to millions of Indians. But directly and indirectly these valuable fishes which give us direct economic and physical benefits, human activities are affecting the quality of these aquatic organisms. Due to the increasing use of pesticides, a large group of toxic compounds are having a profound effect on aquatic animals and water quality.Due to which the physical structure of the fish and the activities of the enzyme have been greatly affected.

Now in the present studies effect caused by pesticides at sub-lethal and LC_{50} concentration where observed. Due to insecticides in fish, hepatopancraticα-amylase activities are being affected. Due to which the physical activities of the fish and the actions of enzymes have been greatly affected. Here have studied the activities and its effects of two of the main pesticides responsible for this organophosphate and organochlorine. For which specific experiment has been prescribed. Because fish is an important animal worldwide.

Pesticides are chemical compounds that are used to kill pests, including insects, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture, to kill pests that damage crops.

Pesticides include herbicides for destroying weeds and other unwanted vegetation, insecticides for controlling a wide variety of insects, fungicides used to prevent the growth of molds and mildew, disinfectants' for preventing the spread of bacteria, and compounds used to control fishes.

There are various types of pesticide found Example that Organochlorine, Organophosphate, Organosulfur, Organotin etc.

1st Organochlorine (Aldrin, Chlordane, Chlordecone, DDT, etc.) Vijverberg et al.(1982)

2nd Organophosphates and Carbamates, it is a synthetic insecticide, act on the insect's nervous system, and interferes with ACHE and other cholinesterases, disrupting nerve impulses, killing or disabling the insect. Organophosphate insecticides such as Sarin, Tabun, Soman and VX (are chemical warfare nerve agent), have an accumulative toxic effect to wildlife. Carbamates have shorter duration, less toxic and similar to the others. Other examples of Organophosphates insecticides are Acephate, Azinphos-methyl, Bensulide, Chlorethoxyfos, chlorpyrifos, Chlorpyriphos-methyl and Diazinon, Palmer et al. (2007)

Aim of the Study

The present studies aim to observed the impact of pesticide on none target species.

Disturbances of biochemical and physiological processes processes may take miniutes to hours, but on a higher organizational scale such as tissues or for example, behavior of organisms, these organisms, these organisms may not induce changes or , if they do, only after the disturbance follows another temporal or spatial regimen.

Ecotoxicology as a science can be defined from different points of view:

1. One may consider it as the toxicology of chemical compounds which are emitted voluntarily or accidentally in to the environment. Studies concerning these problems can be performed in the laboratory. In the recent years ecotoxicology is mainly dominated by the physiologists and they measure different responses of individuals, e.g. changes of metabolic rates or behavior.
2. From an ecologist point of view the changes due to toxic effects not only modify the property of individuals but also influence the whole ecosystem. Seitz and Ratte (1991) investigated the casual chain from effects on individuals (primary effect) to populations, communities and ecosystem (secondary effects).

Materials and Methods

Materials

Soluble starch and 3, 5-dinitraslicylic acids were purchased from E.Merk. Specimens of *Clariasbatrachus* were collected from local pond and market and were maintained in the laboratory.

METHODS

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 (05%) were centrifuged separately at 1000 g and the supernatant was use as enzyme solution of control and treated.

α- Amylase assay

α-amylase activity was determined following the method of Bernfeld (1955) with slight modifications. 0.5ml of alquot of the enzyme solution was used of enzyme assay. 1ml. of 1% buffered starch solution for 10min at 37^0C in atmosphere of air. The incubation mixuture was then cooled to room temperature and the enzyme reaction was interrupte by adding 2ml of 3, 5dinitrasalicylic acid. The tube containing the mixture was then cooled in water bath for about 5 min to stop the reaction completely. It was then cooled to temperature and added 20ml of distilled water. A black was run simultaneously where 1 ml of distilled water was added instead of enzyme. The optical density of the solution was determined at 540nm. 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) taking bovine serum albumin as standard.

Results & Observations

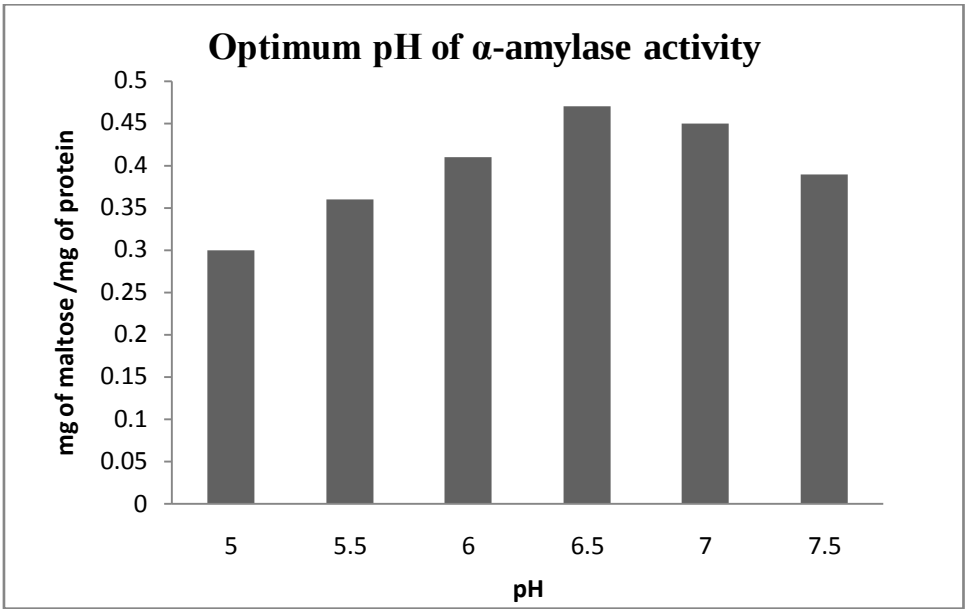
Hepatopancreatic α-amylase activity was observed and findings have been shown that the boiled (in a water bath) did not show any in-vitro enzyme activity where as significant the enzyme activity was observed in the enzyme kept in ice-bucket. Observation of hepatopancreatic α-enzyme activity was done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

Table :- Demonstration of fish *Clariasbatrachus*hepatopancreas α-amylase activity.

System	mg of maltose liberated/ mg of enzymes protein
Liver homogenate	0.450
Boiled homogenate	0.002

Additions 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 of α-amylase activity was found to be at 6.5 (Fig)



Effect of and organophosphate pesticide on α-amylase activity

To observe the effect of pesticide on fish hepatopancreatice α-amylase activity, a group of *C. batrachus* treated with the organochlorine and organophophate pesticides for 24hr LC_{50}, 48h LC_{50} and 72h LC_{50} concentration and were sacrificed, the enzyme was prepared in similar manner as mentioned in the 'Method'. There was marked inhibition of α-amylase activity due to pesticide treatment.

Table: - Effect of organophosphate on fish hepatopancreasα-amylase activity

System	mg of maltose liberated/ mg of protein	inhihition %
Control	0.45	---
Boiled control	0.013	---
Sub-lethal concentrations:-		
Treated (24h)	0.23	48.8
Treated (48h)	0.20	55.5
Treated (72h)	0.19	57.7

Additions were same as mentioned in the 'Method'

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

Conclusion

The pesticide inhibition mechanisum of α-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 inhibition its activity or pesticides may hamper the availability of NA^+ ion which is an activator of hepatopancreatic α-amylase.

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