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Effect of Cypermethrin on Protease activity in Laevicaulis alte.

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Research Paper - Zoology

Abstract

The effect of cypermethrin on protease activity in gastropod *Laevicaulis alte* were estimated slug were exposed to lethal concentration of cypermethrin (L_{C50}) for 96 hours after exposure due to cypermethrin increase in protease activity 42.50% , during pre-productive 17.86% during reproductive 13.99% in post-reproductive period was observed from 24 to 96 hrs.

Keywords

Pesticides, Enzymes, Cypermethrin, Toxicity.

Introduction

To meet the increasing food demands, several types of pesticides are used for controlling various types of agricultural pests. As such many useful non-target organisms also have the toxic effect of pesticide. Most of the pesticides interfere with the enzyme action and produce many physiological and biochemical changes in the bodies of non-target organisms.

Gastropods exposed to toxicants for even a short span of time may produce considerable destruction of the internal organs especially their enzymatic architecture. Majority of enzymes are functional in various metabolic pathways and changed pattern of enzyme activity induced by pesticide, is the surest indicator of functional disorder, enzyme assays and estimation of metabolites have been proposed as valid biochemical means of monitoring toxicity. Pollutants act at the biochemical level, at a number of sites but an organism may be able to adapt by normal homeostatic mechanisms, so that enzyme inhibition may reduce the overall fitness of an organism. Enzyme bio-assays remain, however, useful techniques in

looking for sub lethal effects of toxic pollution.

A number of studies have been carried out on the pesticide toxicity Ramana Rao and Ramamurthi, 1978; Muley and Mane,1989; Magare, 1991;Chaudhari and Lomte, 1992, Jadhav *et. al.*,1995; Lomte and Waykar, 2000, Ahirrao *et.al.*,2004; Ahirrrao and Kulkarni, 2005; Ahirrao and Khedkar, 2012; Ahirrao and Borale, 2013And Borale and Ahirrao, 2013) Molluscs show a variety of digestion patterns as they have learnt to feed in different ways due to their ability to adapt themselves to life in so many different types of habitats. The comparative physiology of digestion in molluscs has been reviewed by Vanwheel (1961). The correlation between digestive enzymes and diet has been established but specific characterization of different enzymes of different animals presents many interesting and puzzling question (Prosser, 1973).

Mackee and Wolf (1963) state that the important poisoning of an enzyme system depends on its capacity to react with ligand. It has been also argued that some of the cytoplasmic enzymes are leaked out of the tissues due to damaged cells. They are often released in fluids resulting in the decreased enzyme activities in tissues and corresponding increase in the fluids. The pollutants may cause injury to organism and the damaged tissues show dysfunction which results in quantitative altered enzyme activity.

The molluscan digestive gland has been investigated for molluscan physiological studies. However, enough attention has not been paid to the effect of pollutants on the digestive enzymes of gastropods. In gastropods, hepatopancreas is the principle site of detoxification. All the toxic substances while passing through it cause significant histopathological and enzymatic changes. It is the digestive gland, which combines in itself the enzyme secretary functions of pancreas, small intestine and liver of the higher animal. Due to accumulation of pesticides in tissues produces many physiological and biochemical changes. (Ghanbahadur *et al.*, 2015). Several workers have reported the effect of pesticides and heavy metals on enzyme activity in molluscs, which was shown by either depletion of enzymes (Jackim *et al.*,1970; Hinton and Koehing, 1975) or elevation of enzymes (Banerjee *et al.*, 1978; Verma and Prasad, 1972).

The effect of heavy metal salts on the digestive enzymes, alpha-amylase and protease have been studied on crab (Tonapi and Verghese, 1985). Jadhav and Lomte (1983) studied influence of pH and temperature on the digestive enzyme, amylase, lipase and protease in the mid gut gland of the fresh water bivalve, *Lamellidens corrianus*.

Lomte and Godhamgaonkar (1984) have recorded the specific activity of protease in

the digestive gland of *Thiara tuberculata*.

Materials and Methods:

In the month of sept-2016 early in the morning 6.00 a.m. Medium sized terrestrial snail *Laevicaulis alte* (8 to 10 cm in length and 2 to 3 cm in width) used in the present study were collected from Kalwan Taluka area. Freshly collected slugs were immediately brought to the laboratory and kept in large glass containers. The slugs were cleaned to remove fouling biomass and mud. They were acclimatized to the laboratory conditions for four to five days. The air temperature was 21.25° + 2.2173°. Since the animals are micro feeders, no special food was supplied during the experiment.

To study the effect of pesticides cypermethrin on the enzyme activity of gastropods, L. alte were exposed to lethal concentration (L_{C50} ppm of 96 hrs) for acute treatment. The active and acclimatized medium sized animals were divided into two group, one was maintained as control and other group was exposed for acute treatment of pesticides cypermethrin up to 96 hours during pre-reproductive, reproductive and post-reproductive periods.

For digestive enzymes such as protease the animals were dissected and the digestive gland was taken out, cleaned and homogenized in ice cold distilled water.

Protease:

The protease activity was determined by following Sorenson's formaldehyde titration method as modified by Prosser and Vanwheel (1958). The reaction mixture contained 3.0 ml gelatin (3%), 1.0 ml phosphate buffer (pH 7.5) and 1.0 ml tissue homogenate (10% w/v). The reaction mixture was incubated for 60 minutes at 37°C. The enzyme activity was terminated by keeping it in boiling water bath for 5 minutes, and then equal amount of neutral formaldehyde was added and titrated against 0.1 N KOH solutions by using alcoholic phenolphthalein (0.5%) as an indicator. The difference between boiled and unboiled tissue homogenate showed protease activity. The amount of amino acid liberated in terms of ml of KOH (0.1 N) solution was taken as an index of enzyme activity.

Result and Discussion:

Protease

In the present investigation on toxicity evaluation of *Lauvicaulis alte* our findings are almost similar to that of Borole (2002) and Ahirrao (2002). Considerable amount of work has been done on the determination of toxicity of various pesticides to invertebrates, fishes and vertebrates, indicating that the toxicity and the susceptibility vary with the type of pesticide and

the test species. The increase in protease activity in the digestive gland of the slug was different in different pesticidal stresses. The fluctuation in the elevation of activity was observed. Due to Cypermethrin the increase in protease activity was observed from 24 to 96 h exposure. The percent increase was varies from 75 % ($P < 0.01$) to 42.50 ($P < 0.001$) in pre-reproductive period, 12.3660 % ($P < 0.001$) to 17.8600 % during reproductive period and from 9.5672 % to 13.9939 % ($P < 0.001$) in post-reproductive period.

Fig.1: Changes in the protease activity of *Laevicaulis alte* after acute pesticidal stress during Pre-reproductive period.

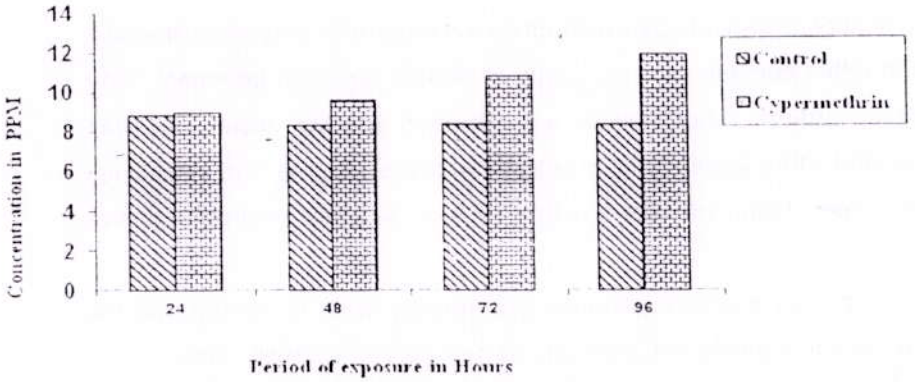


Fig. 2: Changes in the protease activity of *Laevicaulis alte* after acute pesticidal stress during reproductive period.

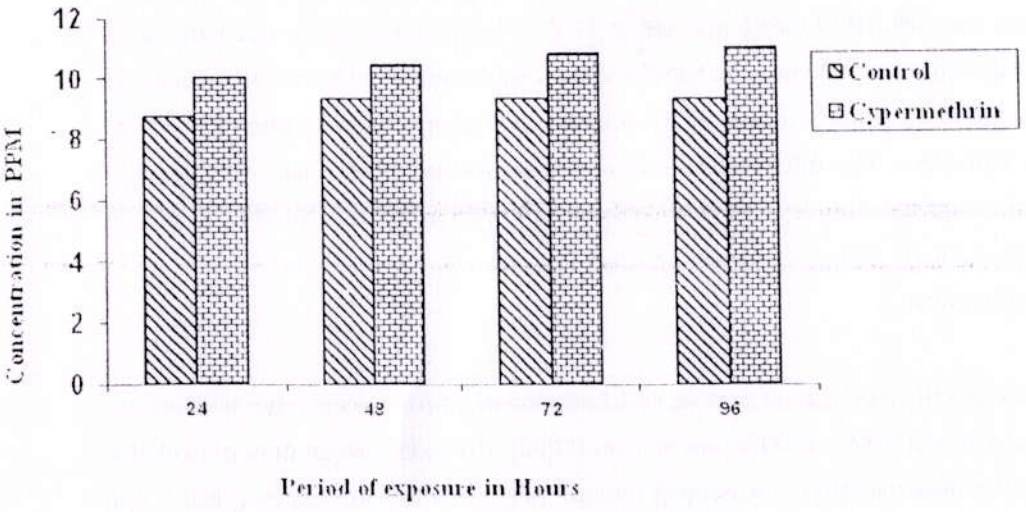
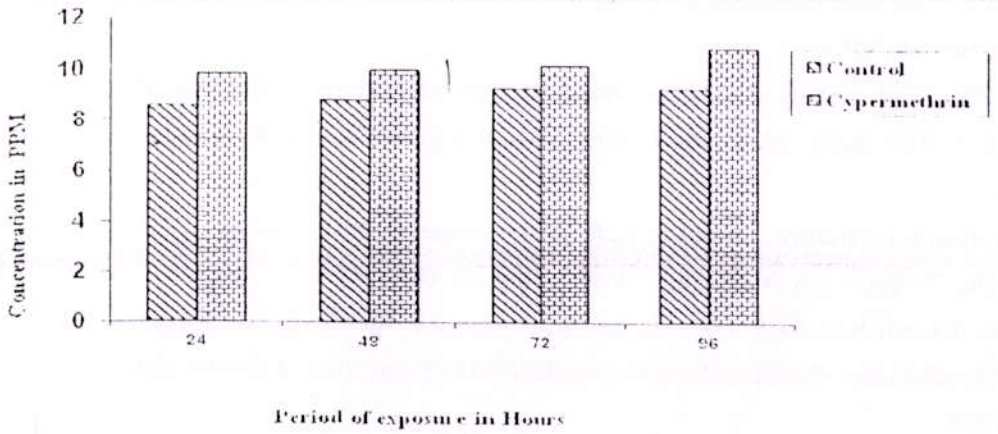


Fig.3: Changes in the protease activity of *Laevicaulis alte* after acute pesticidal stress during Post-reproductive period.



conclusion :

Protease activity increases after cypermethrin treatment on laevicaulis alte. the maximum activity of cypermethrin was recorded in the pre-reproductive period 75% ($P<0.01$) to 42.50% ($P<0.001$)

and less in post-reproductive period 905672% to 13.9939 ($P<0.001$) the activity was recorded during 24 to 96 hrs.

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