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RESEARCH ARTICLE

IMPACT OF BIOPESTICIDE – *AZADIRACHTA INDICA* ON ALKALINE AND ACID PHOSPHATES OF FRESH WATER CATFISH *HETEROPNEUSTES FOSSILIS*

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ABSTRACT

The side-effect of Azadirachta indica (A. Juss) on certain enzyme activities of a freshwater catfish, Heteropneustes fossilis was studied for a period of 24,48,72,and 96 hr, at sub lethal concentration. During the exposure period the alkaline phosphates and acid phosphates content in the liver, muscle and intestine of treated fish showed decreasing trend. The alterations of these biochemical parameters can be effectively used as nonspecific biomarkers against plant extract toxicity stress and also help safer usage of plant extracts in aquaculturefarms.

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INTRODUCTION

Pesticides are major cause of concern for aquatic environment because of their toxicity, persistency and tendency to concentrate in organisms as they move up the food chain, increase their toxic effects to fauna. These pesticides pose a serious threat to the aquatic organisms bring a change in the cellular functions and affect the vital physiological and biochemical functions of the fishes (Ayotunde *et al* 2005). Ecologically pesticide and herbicides have created two major serious problems, which were not previously anticipated. In the first place many of them have persisted and accumulated in the environment and have harmful effects to numerous animals and secondly many of them have directly or indirectly affected to the human health. Acute toxicity may result in severe damage to the components of nature. Hence it is essential to evaluate the hazard associated with the use of specific pesticide in the aquatic environment (Ayotunde and Ofem 2005). A chemical pesticide is target specific and leaves deleterious impact on the environment (Tilak and Koteswara Rao 2003). To overcome the problems of synthetic chemical hazards, one of the best controls measured is the use of plant origin products *i.e* "Biopesticide".

The popularity of the plant products are increasing day by day because of their biodegradability, least persistence and least toxic to non-target organisms with economic and easy availability (Ayuba and Ofojekwu 2000). *Azadirachta indica* is medicinal plants are part and parcel of human society to combat diseases, form the dawn of civilization and it is one of the most promising natural compounds, where it is less harmful to the environment than the synthetic pesticides. Maximum pesticidal activity is in seed kernel which has been tested for biological effect as repellent, feeding and ovipositon deterrent, growth regulators and sterillant effect. It is also reported to have toxicity and imparts egg. hatchability (Bais and Arasta 1995, Satyanir and Yavad 1999). *Azadirachta indica* (Neem) is one of the most promising natural compounds. It is less harmful to the environment than the synthetic pesticides but shows adverse effects on aquatic animals like fish. Goel *et al*. (1982) reported serum alkaline and acid phosphatases decreased by 15% in *Heteropneustes fossilis*, resulting from the effect of the pesticides, Vorbrodt (1959), has reported that phosphatase is an important enzyme of animal metabolism, which play an important role in the transport of metabolites across the membranes.

Table 1. Effect of *A. indica* on tissue Alkaline Phosphatase of *H. fossilis*

Sr .	Tissue	Control	24 Hr	48 Hr	72 Hr	96 Hr
1	Liver	0.392±0.002	0.336** ± 0.003	0.290*±0.002	0.285**±0.005	0.209**±0.001
2	Muscle	0.359± 0.002	0.281** ±0.004	0.272** ± 0.005	0.249** ±0.0008	0.128**±0.006
3	Intestine	0.327 ±0.003	0.271**±0.003	0.192**±0.003	0.149**±0.004	0.104**±0.006

± SE, * Significant at P< 0.05, ** Significant at P< 0.01 and P< 0.05 both

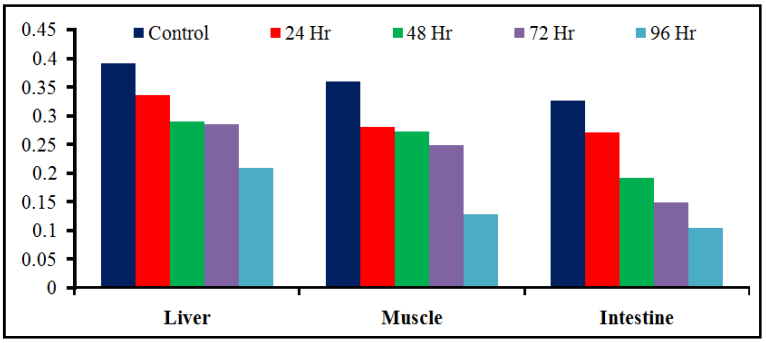

Figure 1. Effect of *A. indica* on tissue Alkaline Phosphatase of *H. fossilis*

Table 2. Effect of *A. indica* on tissue Acid Phosphatase of *H. fossilis*

Sr .	Tissue	Control	24 Hr	48 Hr	72 Hr	96 Hr
1	Liver	0.361+0.001	0.323**+0.002	0.314**+0.001	0.198**+0.0006	0.115**+0.002
2	Muscle	0.358+0.0004	0.317**+0.0006	0.266**+0.001	0.158**+0.002	0.103**+0.003
3	Intestine	0.347+0.0005	0.305**+0.0005	0.257**+0.002	0.151**+0.002	0.096**+0.002

+ SE, * Significant at P< 0.05, ** Significant at P< 0.01 and P< 0.05 both

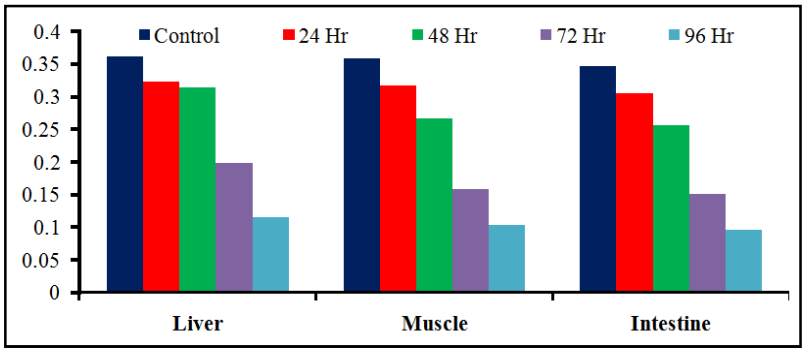

Figure 2. Effect of *A. indica* on tissue Acid Phosphatase of *H. fossilis*

So reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as it plays an important role in protein synthesis (Pilo *et al*. 1972).

MATERIALS AND METHODS

For the experiment fresh water catfish *Heteropneustes fossilis* were selected and divided into two groups with 10 fishes in each aquarium. Each group was exposed to sublethal concentration of the *Azadirachta indica* similar set up was also maintained as control. During sublethal studies, fish were fed by locally available marketed food. The animals were scarifies for optimal concentration of biopesticide for different exposure of 24, 48, 72 and 96 Hrs. For enzymatic studies, fishes were scarified during the exposure period of 24, 48, 72 and 96 Hrs respectively. The toxicant was renewed after fixed period.

Enzymatic Studies: For enzymatic studies, liver, muscle and intestine were dissected out, washed in chilled fish saline, blotted, weighted and homogenized in pestle and mortar. Homogenization was performed in different media as per the requirement of the techniques involved. The biochemical studies were carried out as per standard the methods given below-

Acid Phosphatase by procedure of Tennis wood *et al*, (1976): The tissue was homogenized and centrifuged at 3000 rpm. 0.5 ml of supernatant was taken in a clean test tube and 0.5 ml of the substrate solution (P-nitro phenyl phosphate) and 0.5 ml of 0.1 N. citrate buffers were added. The test tube with above solution was kept in water bath maintained at 37^0C for 30 minutes. After completion of 30 minutes, the reaction was arrested in the extracts by adding 3.8 ml of 0.1N Sodium hydroxide. The colour formed at the end was read at 415 nm in UV-Visible spectrophotometer (Spectronic-20 Bausch and Lamb). Values expressed in µ moles of phenol liberated/ min/100mg protein.

OBSERVATION AND RESULTS

Enzymatic Studies

Alkaline Phosphatase: During the course of experiments, *Heteropneustes fossilis* was exposed to sub lethal concentration of *Azadirachta indica*. The mean (±SE) values mentioned enzyme parameter for selected tissues was observed to be significantly lower for the experimental groups compared with the controlled group.

Acid Phosphatase: During the course of experiments, *Heteropneustes fossilis* was exposed to sub lethal concentration of *Azadirachta indica*. The mean (±SE) values mentioned enzyme parameter for selected tissues was observed to be significantly lower for the experimental groups compared with the controlled group.

DISCUSSION

During the course of experiments, *Heteropneustes fossilis* was exposed to sub lethal concentration of Biopesticide *Azadirachta indica* for 24, 48, 72 and 96 hr. The ALP and ACP showed the decreasing trend in their level according to increase exposure. Alkaline Phosphatase in liver, muscle and intestine tissues were observed to be significantly lower for the experimental groups of fish compared with the controlled group. In biological system, phosphate found as Alkaline and Acidic phosphate. However, phosphates are most commonly found in the form of adenosine phosphates, (AMP, ADP and ATP) and in DNA and RNA and can be released by the hydrolysis of ATP or ADP. Similar reactions exist for the other nucleoside diphosphates and triphosphates. Phosphoanhydride bonds in ADP and ATP, or other nucleoside diphosphates and triphosphates, contain high amounts of energy which give them their vital role in all living organisms. They are generally referred to as high-energy phosphate, as are the phosphagens in muscle tissue. Compounds such as substituted phosphines have uses in organic chemistry but do not seem to have any natural counterparts. The addition and removal of phosphate from proteins in all cells is a pivotal strategy in the regulation of metabolic processes. According to Gnandil *et al.* (2006), the exposure to toxicant leads to depletion in available phosphates that lead to interrupted biochemical process. It suggest that the reduced phosphate level may responsible for alter biochemical level in fish during experiment (Kumar *et al.* 2002)

Conclusion

In the present study results, it is very clear that the fresh water fish *Heteropneustes fossilis* exposed to sub lethal concentration of Biopesticide *Azadirachta indica* showed the reduced level of Alkaline Phosphatase, Acidic Phosphatase So it suggest that may *Azadirachta indica* has several medicinal values as well having utility like biopesticide but its exposure showed adverse effect on the fresh water fish *Heteropneustes fossilis.*

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