



## STUDY THE MALATHION ACTIVITY ON MORTALITY AND BIOCHEMICAL CHANGES OF HETEROPNEUSTUS FOSSILIS

### Zoology

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### ABSTRACT

Malathion was used in vivo on fresh water fish *Heteropneustus fossilis* to study its toxicity. The acute toxicity tests were conducted during certain intervals in various concentrations (5, 10, 15, and 20 mg/L) of malathion. The physical and chemical analyses of water were carried out by following APHA methods. While treating with malathion, the percentage of fish mortality was assessed during 24, 48, and 72 hours. The lethal and sub-lethal concentration of malathion were found to be LC100 (25 mg/L) and LC0 (5 mg/L), respectively. The antioxidant enzyme activity in the liver, muscle and gill, respectively increased during the accumulation of malathion, whereas it decreased during depuration period. Hence, the pesticide intoxication has made defective consequences in the normal metabolic pathways which led increasing the rate of mortality in fish population.

### KEYWORDS:

*Heteropneustus fossilis*, Malathion, and Antioxidant enzymes

### INTRODUCTION

Deliberate or accidental contamination of ponds by widely utilized organophosphorous (OP) insecticides such as malathion is a potential problem for aquaculture in tropical countries. The pesticide, on reaching to aquatic systems, greatly influences the non target organisms such as fish and birds. The toxic effects of the chemicals may be physiological, biochemical and pathological in nature. The changes produced by these agents may be complex, damaging different organs, tissues or cells. Examinations of tissues from organisms after death may possibly reveal the causative agent. Histological studies on fish have revealed that various toxicants have produced pathological changes in the tissues such as macrobiotic changes in the liver, tubular damage of kidneys, gill and lamellar abnormalities (Ramalingam, 2000). Due to growth of agriculture in and around fresh water bodies the pesticides are used abundantly during the cultivation season and found their way into water bodies. The degree of toxicity produced by the poisonous substance is dose independent upon environmental conditions such as temperature, pH, oxygen content and presence of residue molecules (Capkin et al., 2006; Singh and Mishra, 2009). Many authors have reported the changes in acid and alkaline proteases in fish exposed to sub lethal levels of pesticides. The present study has been made to investigate the biochemical changes followed by mortality in the fresh water fish *Heteropneustus fossilis* induced by sub lethal dosages of the pesticide malathion.

### MATERIALS AND METHODS

Fish (*Heteropneustus fossilis*) were collected and kept in aquarium. They were fed daily and acclimatized in laboratory for 30 days. The physical and chemical analyses of the water were carried out (APHA, 2005). Fish were divided into seven groups (each containing 10 fish) where six were experimental and one group as control. Acute toxicity study was carried out using the standard guidelines (EPA/ROC, 1998) to determine the lethal (LC100), median (LC50) and safe sub lethal (LC0) levels of malathion in various concentrations (5, 10, 15, and 20mg/L). The mortality of fish (%) was assessed during the interval of 24, 48 and 72 hours. 1/3rd of median lethal concentration (5 mg/L) was taken to study the effect of malathion on the biochemical constituents and detoxifying ability of fish. Catalase activity assay was performed according to Beaumont et al., 1990.

### RESULTS

The percentage of mortality of *Heteropneustus fossilis* exposed to malathion in 5, 10, 15, and 20mg/L for 24h, 48h and 72h was assessed. The median lethal concentration was observed as 15mg/L since it caused 50% mortality in 72 h using the "Maximum likelihood method" (Finney, 1971). 1/3rd of median lethal concentration (5 mg/L) was taken to study the effect of malathion on the biochemical constituents and detoxifying ability of fish. The physico-chemical parameters were assessed in water and water containing different malathion concentration.

The activity of antioxidant enzymes in the liver, muscle and gill of *Heteropneustus fossilis* exposed to LC0 concentration of 5 mg/L malathion during accumulation were observed (Catalase  $43.1 \pm 2.3$ ,  $16.5 \pm 0.57$ ,  $23.9 \pm 0.17$   $\mu$  moles of phenol liberated/min/100mg protein and Glutathion-S-transferase  $270.5 \pm 0.16$ ,  $143.2 \pm 1.03$ ,  $215.5 \pm 0.72$   $\mu$  moles of phenol liberated/min/100mg protein) in the liver, muscle and gill, respectively increased during the accumulation of malathion whereas, it decreased (Catalase  $17 \pm 1.44$ ,  $7.9 \pm 0.23$ ,  $10.7 \pm 0.69$   $\mu$  moles of phenol liberated/min/100mg protein and GST  $219.5 \pm 1.12$ ,  $108.1 \pm 0.34$ ,  $160.2 \pm 0.46$   $\mu$  moles of phenol liberated/min/100mg protein) during depuration period. The concentration of acid phosphatase and alkaline phosphatase in tissue of *Heteropneustus fossilis* exposed to malathion were estimated ( $0.420 \pm 0.01$ ,  $2.249 \pm 0.00$ ,  $0.424 \pm 0.01$   $\mu$ m/mg protein/h and  $0.850 \pm 0.02$ ,  $1.025 \pm 0.01$ ,  $1.825 \pm 0.01$   $\mu$ m/mg protein/h, respectively). The sublethal effects of malathion on nucleic acid content in different tissues of *Heteropneustus fossilis* during accumulation and depuration periods were fluctuated. Depletion on biochemical parameters like Protein, Glycogen and Free amino acid were evaluated during various periods of exposure.

### DISCUSSION

During the acute toxicity tests, the fish were seen to exhibit several behavioural responses, such as fast jerking, frequently jumping, erratic swimming, spiraling, convulsions and tendency to escape from the aquaria. Following this state of hyper excitability, the fish became inactive and loss of orientation. There was loss of equilibrium and paralysis which ultimately resolved in death of the fish. These altered behavioral abnormalities were observed only at high concentration ranges (values higher than 96 h Lc50). Rao et al., 2005 reported that abnormal changes in behavior in mosquito fish *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos. Acute toxicity of pesticides like Endosulfon, Malathion and Copper sulphate at different concentrations to fresh water prawns *Macrobrachium rosenbergii* were reported (Natarajan et al., 1992). These observations was in support with the study of Chattopadhyay et al., 2006 who reported that certain erratic behavioral patterns of fish was noticed during the exposure period to herbicides.

In the present study, the fish were treated with sublethal concentration of 5mg/L of malathion for a period of 15 days. One set of fish was sacrificed for enzyme analysis. Antioxidant enzymes such as catalase and glutathion S-transferase were analysed in different tissues of liver, muscle and gill of *Heteropneustus fossilis*. Similarly after 15 days exposure of malathion, the fish were released into fresh water for further 15 days (depuration study). At the day 20, the malathion depurated fish were sacrificed and antioxidant enzymes were analysed as it was in accumulation study. During the accumulation study, the catalase activity was more than double fold higher at day 15 in the liver of *Heteropneustus fossilis*, when compared to its control. Similarly glutathion S-transferase activity also increased at day 15 in the liver of fish, when compared to control. fish when compared to control.

Increased responses of both enzyme activities were observed when released into fresh water for another 15 days. At day 30th tissues (Liver, muscle and gill) antioxidant enzymes were monitored. In the depuration study, It has been believed that enhanced antioxidant enzymes in malathion exposed fish would help in the removal of oxyradicals. During this depuration study the antioxidant enzymes were very low at day 16 when compared to control. The liver, muscle and gill tissues showed decreased level of acid phosphatase (ACP) and Alkaline Phosphatase (ALP) activities. Thus the pesticides intoxication has disturbed the normal functioning of cells with the resultant alterations in the fundamental biochemical mechanisms in fish. This would in turn result in the mortality of fish on chronic exposure to the pesticide. In addition, the pesticide might be deposited in fish accidentally or by means of contaminated water bodies with pesticide and it might lead to harmful consequences in human beings on continuous consumption of those fish.

## REFERENCES

1. APHA, (2005). Standard Methods for the examination of water and waste water. 21st Ed. Washington DC.
2. Beaumont, F., Jouve, H-M., Cagnan, J., Gillard, J., Pelment, J., (1990). Purification and properties of a catalase from potato tubers (*Solanum tuberosum*). *Plant Sci.* 72:19-26.
3. Capkin, E., Altinok, I., Karahan, S., (2006). Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. *Chemosphere*, 64:1793-1800.
4. Chattopadhyay, A., Adhikari, S., Adhikary, S.P., Ayyappan, S., (2006). Evaluation of butachlor for control of submerged macrophytes along with its impact on biotic components of freshwater system. *Iran. J. Environ. Health. Sci. Eng.*, 3(2):103-108.
5. EPA/ROC, (1998). Standard guide for conducting acute tests with fishes: static renewal test for common carp. NIEA B904.10B. Environmental Protection Administration of the Republic of China, Taipei, Taiwan (in Chinese).
6. Finney, D.J., (1971). Probit Analysis. 3rd Ed. Cambridge University Press, London, 330 pp.
7. Natarajan, E., Biradar, R.S., George, J.P., (1992). Acute toxicity of pesticides to giant freshwater prawn *Macrobrachium rosenbergii* (De Man). *J. Aquacult. Tropics.*, 7 (2):183-188.
8. Ramalingam, V., Vimaladevi, V., Narmadaraji, R., Prabakaran, P., (2000). Effect of lead on haematological and biochemical parameters in freshwater fish *Cirrhina mrigala*. *Pollu.Res.*, 19:81-84
9. Rao, J.V., Ghousia, B., Pallela, R., Usman, P.K., Nageswara Rao, R., (2005). Changes in behavior and brain acetylcholinesterase activity in mosquito fish, *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos. *Int. J. Environ. Res. Public Health.*, 2(3): 478-483.
10. Singh, S., and Mishra, R.N., (2009). Occurrence of organochlorine pesticides residues in Kuano river of eastern Uttar Pradesh. *J. Environ. Biol.*, 30:467-468.
11. Tennis Wood, M.C., Bind, E., Clark, A.F., (1976). Phosphatases antigen dependent markers of rat prostate. *Can. J. Biochem.*, 54: 340-343.