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Behavioural responses and acute toxicity of *Anabas testudineus* to pesticide methyl parathion

ARTI KUMARI AND RISHIKESH KUMAR

Department of Zoology, MLSM College, Darbhanga 846004, India. E-mail: chaturvedi.manju85@gmail.com

ABSTRACT

Toxic components are important for assessing the impact of aquatic ecosystems. In aquatic toxicity tests, groups of selected organisms are exposed to test materials under defined conditions to determine potential adverse effects. Bioassay tests were conducted on freshwater air breathing fish *Anbabas testudineus* to evaluate the acute toxicity of methyl parathion insecticide to deter mine their LC_{50} after 24, 48, 72 and 96 h exposure. The safe concentration of this biocide was calculated on the basis of LC_{50} for 96 h which was investigated earlier by Arti and Rishikesh, 2015.

Behavioural characteristics are sensitive indicators of toxicant effect. Many chemical contaminants target specific physiological systems and exert their effects on behaviour. Fish in toxic media exhibited irregular, erratic swimming movements, hyper excitability, loss of equilibrium spiraling, loss of balance, rapid respiration, lightdiscolouration, surfacing and gulping air. The present investigation was designed to elucidate the acute toxic effect of pesticide, methyl parathion on the toxicity and behaviour of air breathing fish *Anabas testudineus*.

Keywords: *Methyl parathion, LC_{50}, Behaviour, Anabas testudineus, Toxicity*

INTRODUCTION

Parathion belongs to the most commonly used pesticides worldwide. Their massive expansion is a threat to the natural environment including the aquatic medium. Although they are rapidly degraded in soil and plants, they are extremely toxic to fish because of high sensitivity to them. There are synthetic analogues of the natural pyrethrins, extracts of the ornamental *Chrysanthemum cinerariae folium* and its related species. The derived compounds tetramethrin, resmethrin, fenvalerate, permethrin, cypermethrin, ②-cyhalothrin, and deltamethrin are used extensively in agriculture. They exhibit low mammalian toxicity insecticidal action and photostability with low volatility and persistence. They are broad-spectrum insecticides with tremendous effect to kill insect. Although they were acutely toxic to fish, very few accidental poisoning occurred because they aer not registered for aquatic use and they seldom had enough persistence to reach water from normal application (Di Giulio RT, Hinton DE, 2008). However, the significant increase of chemical emissions in water resources has led to deleterious effects for aquatic organisms (Livingstone, 2001 and Matsumoto *et al.*, 2006)

MATERIALS AND METHODS

Freshwater air-breathing *Anabas testudineus* were procured from local Donar fish market of District Darbhanga. This species was selected for bioassay because of some ecotoxicological characteristics such as wide distribution, availability throughout the year, easy maintenance under laboratory conditions and of commercial importance.

Survival studies on the fish of 2.5±0.5g were carried out. The feeding was stopped 24 h prior to the exposure period. Fish specimens were subjected to prophylactic treatment by batting twice in 0.05% potassium permanganate ($KMnO_4$) for two minutes to avoid any dermal infections. The fishes were then acclimatized for one week under laboratory conditions in semi statis systems. The test solutions were replaced at every 24 h. Dead animals was removed immediately from the medium. For each bioassay test, a control was used. Experiments were conducted using triplicates.

Acute Toxicity: The median tolerance limit of any pollutant is meant as an elementary guide in the field of toxicology (Ward and Parrish, 1982). Without reference to the medium tolerance limit, no information on sub-lethal effects can be deduced (Patin, 1982).

RESULTS AND DISCUSSION

From the results of mortality readings the 96h LC_{50} value and 95% confidence limits for ②-cyhalothrin based on probit analysis was found to be 0.09 ③g1^{-1}. The lower and upper lethal confidence limits indicated a range between 0.038 ③g1^{-1}to 0.134 ③g1^{-1}. The LC_{50} values and exposure period showed a direct relationship. The LC_{50} values obtained for ②-cyhalothrin were 0.40 ③g1^{-1}, 0.44 ③g1^{-1}, 0.12 ③g1^{1}, and 0.095 ③g1^{-1}for24, 48, 72 and 96 h respectively. From the above results it was observed that the LC_{50} values of 96 h were found lowest among all the exposure periods. The 96 h LC_{50} values provide a useful means of comparing the relative acute lethal toxicity of specific toxicants to organisms under specific conditions (Table 1).

Table 1
LC_{50} values of Anabas testudineus exposed to methyl parathion acute toxicity test

Hours	LC_{50} Value (③g1^{-1})
24	0.34
48	0.24
72	0.12
96	0.095

All pesticides induce toxic stress in form of behavioural changes in the fish. The control fish behaved in a natural manner i.e. displayed uncoordinated behaviour. At the initial exposure, fish were alert, stopped swimming and remained static in position in response to sudden changes in the surrounding environment. They were alert to the slightest disturbance, but in the toxic environment fishes tried to avoid the toxic water with fast swimming and jumping. Observations from the bioassay revealed that fishes exhibited increase in stress as evidenced by slow and uncoordinated movement. Faster opercular activity was observed as surfacing and gulping of air.

Opercular movements increased initially in all exposure periods but decreased later steadily. The fish exhibited peculiar behaviour of trying to leap out from the pesticide medium, which can be viewed as an escaping phenomenon. A copious amount of thick layer of mucus was found deposited in the buccal cavity and gills. The discolouration of the skin was more pronounced. The fish progressively showed signs of tiredness and lost positive rheotaxis. Fishes spiraled at intervals and engulfed the air through mouth before respiration stopped. Soon they settled at the bottom of the tank, and after some time died with their mouth and operculum wide open.

Toxicity data for a variety of pesticides such as organophosphate, organo chlorine, carbamide and pyrethroid pesticides have been reported for a number of fish species by various authors. Acute toxicity of fish species exposed to type II pyrethroids②-cyhalothrin for 96 h vary among fishes, for example in *Bryconamazonicus* it is 6.5 ③g1^{-1} (F.D. De Moraes *et al.*, 2013) , in *Gambusia affinis* as 1.10 ③g1^{-1} (Guner 2009), in *Brachydanio* *rerio* as 1.94 ③g1^{-1} (Wang *et al.* 2007), in *Clarias batrachus* as 5.0 ③g1^{-1} (Kumar *et al.* 2011).

The differences in the literature LC_{50} values in the sensitivity of different fish species to pesticide exposures seems to be influenced by the age of the fish chosen for the toxicity tests. For example, the 96 h LC_{50} value for zebrafish, *Daniorerio* embryos exposed to ②-cyhalothrin was 0.875 g/L (Xu*et. al.*, 2008) compared with 0.5 g/L of 37-52 days old post-hatch Zebrafish. *Daniorerio*, embryos exposed to ②–cyhalothrin was 0.875 g/L (Xu et al., 2008) compared with 0.5 g/L of 37-52 day old post-hatch Zebrafish fingerling with same exposure times (Wang *et al.*, 2007). Time of exposure, method of determination, chemical purity, age and species of fish when taken into consideration, also determines the toxicity of ②–cyhalothrin.

The present investigation reported lower LC_{50} value of 0.095 ③g1^{-1} for 96 h in the test animal (*Anabas testudineus)* compared to previous researchers. However, the present LC_{50}value was less than that of LC_{50} values for 96 h reported in *Clarias batrachus* 5 ③g/L (Kumar, A., 2011). From all the results and observations, it is inferred that toxicity values were not constgant for any particular group of fishes and it varies from species to species with alteration in physical and chemical factors. Low concentrations of methyl parathion as reported in this study, are usual in water environments and they are close to that reported to fish farms in some countries (Haya, 2005; Marino and Roco, 2005, and Bulluta *et al.*, 2010).

The fish serves as bio-indicator of water quality and this can be easily judged by morphological, physiological and behavioural changes in the altered environment. When the fish were exposed to the lethal concentration of methyl parathion they migrated immediately to the bottom of the tank and independency (spread out) in swimming. The migration of the fish to the bottom of the tank following the addition of methyl parathion clearly in dicates the avoidance behaviour of the fish.Prashanth and Yogesh (2006) have also observed the avoidance nature by *Catla catla* on exposure to sodium cyanide.

The erratic swimming of the treated fish indicates loss of equilibrium. It is proved that the region in the brain associated with the maintenance of equilibrium should have been affected. Santhakumar and Balaji (2000) observed exciting and erratic movements in *Anabas testudineus* exposed to monocrotophos. Similar observations were reported in *Hiteropneustes fossilis* exposed to rogor and endosulfan (Sabita and Yadav, 1995). Loss of positive rheotaxis observed of the fish is a good indication of toxic response. The hyper excitation and abnormal behaviour like fin-flickering observed in the insecticidal exposed fish may be due to the irritating effect of the toxicant. Excess mucus secretion all over the body was another change noticed. Mucus also forms a barrier between the body and the toxic medium, to minimize its irritating effect. Rao (2006) similar observations following RPR-V (a novel phosphorothionate insecticide, 2-butenoic acid-3-3 [diethoxyphosphinothionyl] ethyl ester) exposure to euscharine fish, *Oreochromis mossambicu*. Blood clots and pronounced mucus secretion over the gill were also observed, is related to the uptake of water containing pesticide through gill. Aggressive behaviour such as nudge and nip were increased following exposure to the toxic material. On the other side, caudal bending was noticed in the toxicant exposed fish *Anabas testudineus* and this greatly reduced the normal swimming pattern. Caudal bending may be a sort of paralysis, which might be due to the inhibition of muscular AChE activity resulting in blockage of neural transmissions. The treated fishes also showed fading of their body colour. Fish were also found striking their head against the walls of the experimental containers and body colour was observed to become light. Marked change in colour was observed in the present study which became lighter. It may be due to shifting and degeneration of melanophores and also destruction of mucus cells in the solution was said by various authors. Pai *et al.* (2003) observed the loss of skin coloration in *O. niloticus* fingerlings exposed to different concentrations of textile mill effects. Surfacing phenomenon also seen in the present study might be to gulp maximum possible air to ease the stress.

CONCLUSION

The behavioural changes can be considered as symptoms of stress on account of the toxicological nature of the environment. These behavioural responses can be used as a tool in biomonitoring programme to monitor ecotoxicity risk of methyl parathion to the test species. This study has proved convincingly that toxicants are deleterious to natural population of fish. Even though pesticide pollution may not directly kill aquatic organisms like fishes, the sublethal concentration of pesticide in water can adversely affect several vital behavioural patterns of the fish reducing its overall fitness. Therefore it is suggested to prevent the water animals including fishes from pesticide and insecticide pollution.

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