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Effects of almix herbicide on profile of digestive enzymes of three freshwater teleostean fishes in rice field condition

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ABSTRACT

The present investigation was carried out to compare the alterations of digestive enzymes like amylase, lipase, and protease activities in three teleostean fishes *viz*., *Anabas testudineus*, *Heteropneustes fossilis* and *Oreochromis niloticus* after application of almix herbicide for 30 days at rice field concentration *i.e.*, 8 g/acre. Highest amylase activity was observed in intestine of *A. testudineus* (300.76%) and lowest in intestine of *H. fossilis* (103.89%), while maximum lipase activity was found in stomach of *O. niloticus* (203.27%) and lowest in stomach of *H. fossilis* (109.65%). Protease activity was also highest in liver of *O. niloticus* (270.47%) but lowest in stomach of *H. fossilis* (114.04%). Changes in the enzymes' activity were different in respect to fishes and their tissues. According to this analysis, *A. testudineus* and *O. niloticus* were more sensitive. So, it can be inferred that long-term exposure of almix even at environment-friendly concentration may cause alterations in the digestive functions.

1. Introduction

Aquatic inhabitants particularly fishes, the secondary level of consumers, are frequently exposed to water contaminants like pesticides, herbicides *etc*., and their derivatives through agricultural runoff or rain. Much emphasis is given in those aquatic bodies which are in close proximity with paddy crop fields where a large amount of herbicides are used throughout the

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year for protection and production of crop [1]. Now-a-days, the use of herbicides increased several folds to kill or control the unwanted weeds especially in paddy fields to increase the productivity. Almix, a very effective third generation herbicide, is widely used to control the broad leaf weeds and sedges in the paddy fields. The chemical composition of almix is 10% metsulfuron methyl, ($C_{14}H_{15}N_5O_6S$) [methyl 2-(4-methoxy-6-methyl-1, 3, 5-triazin-2-ylcarbamoyl-sulfamoyl) benzoate], 10% chlorimuron ethyl, ($C_{15}H_{15}ClN_4O_6S$) [ethyl 2-(4-chloro-6-methoxypyrimidin 2 ylcarbamoyl-sulfamoyl) benzoate] and 80% adjuvants [2]. Study on alterations in physiological and biochemical activities of aquatic organisms such as fish induced by xenobiotic compounds are available [3,4] but very little work particularly on almix toxicity in fishes were reported [5–7].

Study on digestive physiology in the aquatic organisms is very essential, as the enzyme profile are indicative to digestive processes [8]. Although, the array of digestive enzymes in bony fishes is the same as that of other vertebrates [9]. Among these digestive enzymes, amylase starts digestive processes as a catalytic enzyme and plays a vital role in the breakdown of starch into sugar. Amylase estimation is essential to know the rate of carbohydrate digestion because carbohydrate becomes the potential source during the stress condition. Lipolytic enzymes play an important role in the break down and turnover of lipids which are considered as energy storage molecules and are involved in cell signalling in biological systems [10]. Different authors measured the amylase, lipase and protease activity in the different tissues exposed to different pesticides (Gupta et al. [11] in *Channa striatus* exposed to diazinon and endosulfan; Shoba Rani et al. [12] in *Clarias batrachus* exposed to trichlorfon; Venkateshwarlu et al. [13] in *C. batrachus* (Linn) exposed to endosulfan; and Ganesh et al. [14] in *C. batrachus* exposed to profenophos). Measurement of these enzymes in the tissues helps to understand the mechanism of digestive processes and subsequent adaptation to the changes due to intoxication, and therefore can be used as a method of evaluation for fish health status.

Fishes are considered as more susceptible organisms to environmental contamination; therefore, their enzymatic evaluation can be the promising indicator of chemical intoxication [15]. Three Indian freshwater food teleosts of different trophic levels *viz., Anabas testudineus, Heteropneustes fossilis* and *Oreochromis niloticus* in the present study, were selected as model organisms due to its greater sensitivity to the xenobiotics. In this context, aim of the present study is to evaluate the effects of almix herbicide at rice field concentration on digestive enzymes of *A. testudineus*, *H. fossilis* and *O. niloticus*.

2. Materials and methods

2.1. Experimental design

Freshwater teleosts, *A. testudineus* (Bloch), *H. fossilis* (Bloch), and *O. niloticus* (Linnaeus) of both sexes with an average weight of 30.43 ± 5.14 g, 55.33 ± 4.82 g, and 73.10 ± 14.70 g respectively and total length of 11.46 ± 0.70 cm, 21.92 ± 0.84 cm, and 17.44 ± 1.06 cm respectively procured from the local market and were acclimatized in the control pond for 15 days. After acclimatization fishes were divided into two groups (control and almix-treated) and maintained in twelve experimental plots, containing 10 fishes in each cage installed separately at paddy field of Burdwan University Crop Research Farm, the University of Burdwan: three for *A. testudineus*, three for *H. fossilis*, three for *O. niloticus*, and three controls in separate field (one for *A. testudineus*, one for *H. fossilis* and one for *O. niloticus*). The cages were prepared in the field for the culture of the experimental fish species as per Chattopadhyay et al. [16] with some modifications. All the cages were square in shape having an area of $2.5\,m \times 1.22\,m$ and height of the cage was 1.83 m (submerged height was 1 m). The cages were framed by light strong bamboo. The four-sided wall, floor of the cage and top of the cage cover was fabricated with nylon net and was embraced by two PVC nets: the inner and outer bearing mesh sizes of $1.0 \times 1.0\,mm^2$ and $3.0 \times 3.0\,mm^2$ respectively. The desired dose of 8 g/acre was dissolved in water and applied once (considering the mode of use of the farmers). It was sprayed on first day of the experiment on the surface of each cage of almix-treated plots. During experimentation for a period of 30 days both the groups, almix-treated and control were subjected to same environmental conditions and fishes were fed daily with commercial feed (42% crude protein) at a rate of 1% of the total body weight of fish in each plot. During the experimentation period in rice field, the average value of water parameters were as follows: temperature $15.67 \pm 0.145\,^{\circ}C$, pH 7.89 ± 0.033, electrical conductivity $390.33 \pm 2.19\,\mu S/cm$, total dissolved solids 276.33 ± 1.45 mg/l, dissolved oxygen 7.47 ± 0.088 mg/l, total alkalinity 101.33 ± 0.67 mg/l as $CaCO_3$, total hardness 152.00 ± 2.31 mg/l as $CaCO_3$, sodium 20.56 ± 0.294 mg/l, potassium 2.89 ± 0.111 mg/l, orthophosphate 0.12 ± 0.007 mg/l, ammoniacal-nitrogen 6.06 ± 0.875 mg/l, nitrate-nitrogen 0.58 ± 0.016 mg/l.

2.2. Tissue sampling

During experimentation period the quality of the rice field water was assessed as per APHA [17]. Desired tissues of stomach, intestine and liver from almix-treated and control fishes were collected at the end of the experiment *i.e.*, after 30 days. The tissues were removed, washed in 0.75% saline solution, blotted with tissue paper, and homogenized in 2 ml of 0.2 M, pH 7.4 phosphate buffer by using the mortar and pestle and finally centrifuged at 8000 rpm for 25 min at $4\,^{\circ}C$ and the supernatants were taken in Teflon tubes and finally stored at $-80\,^{\circ}C$ for biochemical analysis of amylase, lipase, protease and total protein content.

2.3. Measurement of enzyme activity

2.3.1. Amylase

Amylase activity was measured according to Bernfeld [18] using starch as substrate. Activity of the enzyme was