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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

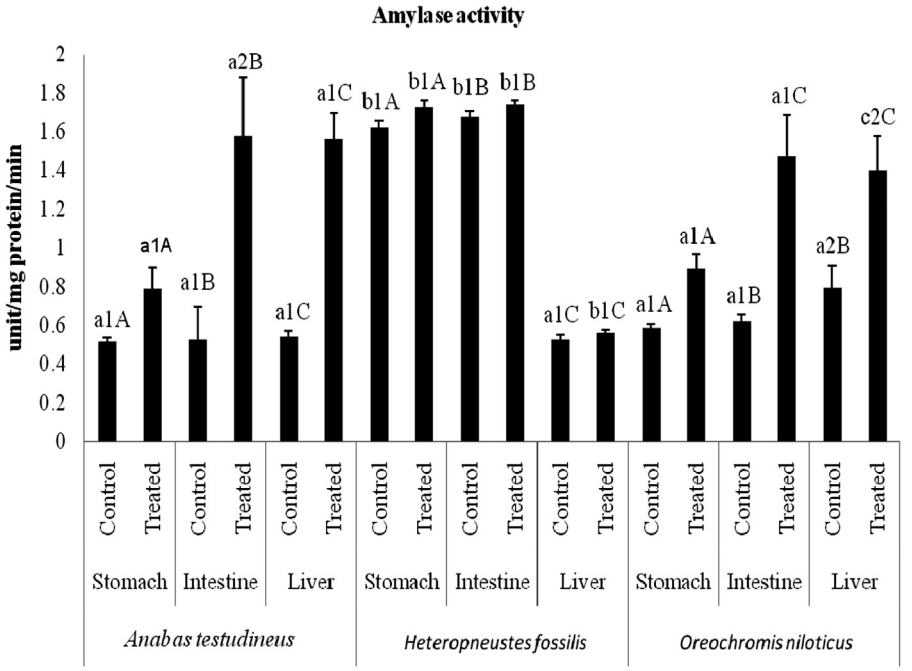

Fig. 1. Amylase activity in different tissues of three test fish species. Data expressed as mean ± SEM ($n = 9$) in μg of maltose liberated/mg protein/min. Values with different lowercase superscripts (alphabet) differ significantly ($p < 0.05$) between fishes within tissue and concentration. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within tissue and fishes. Values with different uppercase superscripts (alphabet) differ significantly ($p < 0.05$) between tissues within fishes and concentration.

expressed as μg of maltose liberated/mg protein/min at 37 °C.

2.3.2. Lipase

Measurement of lipase activity was followed by the method of Cherry and Crandall [19]. Activity of the enzyme was expressed as μg of fatty acid liberated/mg protein/min at 37 °C.

2.3.3. Protease

Protease activity was measured as per Snell and Snell [20]. Activity of the enzyme was expressed as μg of tyrosine liberated/mg protein/min at 37 °C.

2.3.4. Total protein

Total protein content in the tissue samples was determined spectrophotometrically based on the method described by Lowry et al. [21] using BSA as standard.

2.4. Statistical analysis

The data were statistically analyzed using SPSS package (version 16.0). Two-way analysis of variance (ANOVA) was applied to compare the mean values of different parameters among different tissues, concentrations and fishes. Means were compared by Tukey's test. The values of all biochemical parameters were expressed as mean ± SE ($n = 9$). A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Amylase activity induction

Amylase activity after almix exposure in the field condition was significantly increased ($p < 0.05$) compared to control value in all the investigated tissues of test fish species (Fig. 1). Stomach showed highest elevation of amylase activity in *A. testudineus* (153.50%) followed by *O. niloticus* (152.44%) and lowest in *H. fossilis* (106.41%). In the intestine and liver, maximum increase in amylase activity was observed in *A. testudineus* (300.76% and 288.89% respectively), while it was minimum in case of *H. fossilis*, 103.89% and 113.83% respectively. It was revealed that among all the tissues, highest amylase activity was observed in intestine (300.76%) of *A. testudineus* and lowest activity in intestine of *H. fossilis* (103.89%). The increment of amylase activity in the tissues of almix exposed fishes was in the pattern of intestine > liver > stomach in case of *A. testudineus* and *O. niloticus*, while in *H. fossilis* was liver > stomach > intestine. The pattern of elevation of amylase activity, in the present study, was species specific where *A. testudineus* was more sensitive than other two fish species.

3.2. Lipase activity induction

Lipase activity in the stomach, intestine and liver of the test fishes was presented in Fig. 2. Lipase activity was significantly elevated ($p < 0.05$) in all the tissues of all the test fishes from control value. In stomach, highest lipase activity was observed in *O. niloticus* (203.27%), moderate in

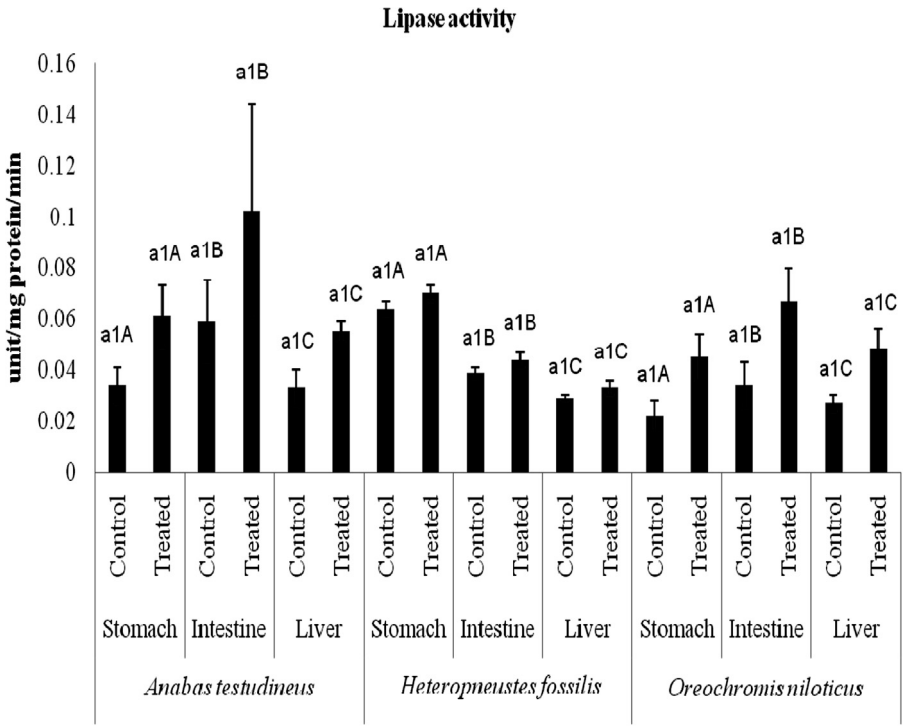

Fig. 2. Lipase activity in different tissues of three test fish species. Data expressed as mean ± SEM ($n = 9$) in μg of fatty acid liberated/mg protein/min. Values with different lowercase superscripts (alphabet) differ significantly ($p < 0.05$) between fishes within tissue and concentration. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within tissue and fishes. Values with different uppercase superscripts (alphabet) differ significantly ($p < 0.05$) between tissues within fishes and concentration.

A. testudineus (177.91%) and lowest in *H. fossilis* (109.65%). Intestine showed highest increased activity of lipase (194.17%) in *O. niloticus* and lowest activity (112.70%) in *H. fossilis*. Likewise, liver showed similar trend of enhanced activity of lipase as observed in intestine, but it was 179.89% and 115.19% in *O. niloticus* and *H. fossilis* respectively. Here, the increment of lipase activity in different tissues of *A. testudineus* and *O. niloticus* was in the order of stomach > intestine > liver, while *H. fossilis* showed a different trend of liver > intestine > stomach. So, the present study showed that elevation pattern of enzyme activity was different for different fish species as well as tissues, and *A. testudineus* and *O. niloticus* were more sensitive than *H. fossilis*.

3.3. Protease activity induction

Protease activity in all three tissues of almix-treated fishes was also significantly increased ($p < 0.05$) in the field condition (Fig. 3). Protease activity in the stomach was highest in *O. niloticus* (183.90%) followed by *A. testudineus* (126.38%) and lowest in *H. fossilis* (114.04%). Similar pattern of increased activity was also observed in case of liver *i.e.*, highest in *O. niloticus* (270.47%) and minimum activity of 117.68% in *H. fossilis* was observed, while the intestine showed maximum enzyme activity in *A. testudineus* (164.58%) and minimum in *H. fossilis* (114.04%) respectively. The result showed that highest protease activity was observed in liver of *O. niloticus* (270.47%) and lowest in stomach of *H. fossilis* (114.04%) in respect to all the tissues. The pattern of increment of protease activity in different tissues of *A. testudineus* was in the order of liver > intestine > stomach, while it was in the tune of intestine > liver > stomach and liver > stomach > intestine in *H. fossilis* and *O. niloticus* respectively. The results clearly depicted that *O. niloticus* was more sensitive to the changes than other two fish species.

4. Discussion

The present study is the maiden attempt to report the toxicity of almix herbicide in regard to digestive enzyme profile namely amylase, lipase and protease in three Indian freshwater teleosts, *A. testudineus*, *H. fossilis* and *O. niloticus*, although some authors worked on some other biochemical parameters such as alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and glucose-6-phosphatase in different fish tissues after exposure to the herbicide, almix in the laboratory condition [5–7].

In teleostean fishes, digestion takes place in the post-oesophageal part of the alimentary canal *i.e.*, stomach followed by intestine. Digestive enzymes in fishes are produced by gastric glands and columnar epithelial cells of stomach and intestine respectively along with pancreatic (hepatopancreas) acinar cells. All the pancreatic enzymes are concentrated in the form of zymogen granules in the pancreatic acinar cells and secreted together. Some of the digestive enzymes are secreted by the anterior part of the intestine and work best at a pH ranging from neutral

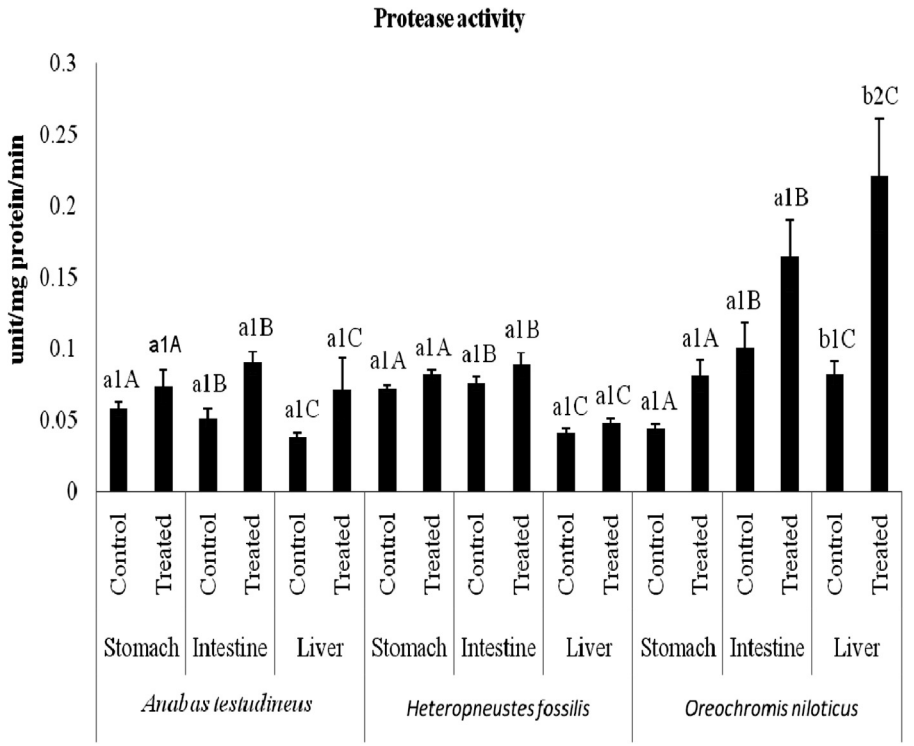

Fig. 3. Protease activity in different tissues of three test fish species. Data expressed as mean ± SEM ($n = 9$) in μg of tyrosine liberated/mg protein/min. Values with different lowercase superscripts (alphabet) differ significantly ($p < 0.05$) between fishes within tissue and concentration. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within tissue and fishes. Values with different uppercase superscripts (alphabet) differ significantly ($p < 0.05$) between tissues within fishes and concentration.

to alkaline [22]. Disruption of the zymogen granules of gastric glands and pancreatic acinar cells by xenobiotic substances cause alterations in the production of these digestive enzymes [23]. In the present study, amylase activity was increased in all the fish tissues indicating disturbance in the normal digestive process and may be due to break down of the polysaccharides to simple sugar to meet the energy requirement during the herbicidal stress. The results were also supported by Hidalgo et al. [9] with similar findings in the fish tissues. Amylase activity was maximum in the intestinal part and this was possibly because of the starch digestion and glucose absorption that started in the intestinal part. Actually, intestinal enzymes are secreted in an inactive form as zymogens and intestine make them active in digestion [24]. Lundstedt et al. [25] and Uys and Hecht [26] also reported the similar results. Different fish species show different responses to the exogenous herbicidal exposure [27,28].

Lipase plays a vital role in the digestive process, enabling lipid metabolism by the breakdown of these compounds and transferring them to the cells of the organism [29]. Gastric lipase is capable of mild fat-splitting action and little amount of fat is digested in stomach by gastric lipase [22]. In the present study, enhanced activity of lipase in different tissues of *A. testudineus*, *H. fossilis*, and *O. niloticus* exposed to almix herbicide indicated tissue damage as well as development of stress situation and efficient utilization of lipid molecules for digestive purposes to meet the high energy demand during stress condition. Almix caused major destruction of zymogen granules and inhibition of synthesis of zymogen granules in the pancreatic acinar cells which resulted into alteration of the lipase activity. This study also showed the higher lipase activity in the intestine and stomach than liver because they are the prime sites of lipase function to digest lipid molecules along with the digestive tract. The results were in agreement with the observation of Fu et al. [30] who reported increased activity of lipase in the intestine and stomach of *Scophthalmus maximus* and with Wu et al. [31] who found similar results in *Boleophthalmus pectinirostris*.

Chiefly proteins are enzymatically digested in stomach but ends up in the intestine. Gastric glands in the stomach secrete hydrochloric acid and pepsinogen, and are effective in combination to split large protein molecules by the action of HCl and finally the enzyme transforms native protein into proteoses and peptones [24]. Increased protease activity in stomach, intestine and liver of three teleostean fishes in this study indicated the damages mainly due to herbicidal exposure. The increased protease activity indicated the increased degradation of protein compounds to yield the excess energy to meet the energy demand to overcome this herbicidal impact as a compensatory response. The alteration of protease activity in stomach may also be due to alteration of gastric pH caused due to excessive secretion of neutral mucin, and disruption of zymogen granules in gastric glands, which ultimately culminate the production of proteolytic enzymes [22]. Liver showed maximum elevation in enzyme activity in

comparison to stomach and intestine to compensate the toxic effects of almix herbicide and subsequent elimination of their metabolic wastes. The enhanced level of tissue proteolytic activity was also reported by Venkateshwarlu et al. [13], Shoba Rani et al. [12] and Ganesh et al. [14] in different fish species; therefore, evaluation of digestive enzymes in different parts of the alimentary canal of fish species may be considered as a physiological indicator.

5. Conclusion

The present study demonstrated the exposure of almix herbicide at environment friendly rice field concentration in the paddy crop field that caused serious biochemical changes in the digestive enzymes of *A. testudineus*, *H. fossilis*, and *O. niloticus*. The increased activity of the amylase, lipase and protease in different tissues of these fishes indicated tissue damage chiefly in the columnar epithelial cells, pancreatic acinar cells, and gastric glands and it was an adaptive response played by the organism itself to protect them from the almix-induced stress. This ultimately affected the fish health status by disrupting the various physiological and biochemical processes and these changes could be considered as a biomarker of toxicity evaluation in different fish tissues. Finally, from an ecotoxicological point of view, the use of this herbicide in the agricultural fields and aquatic bodies must be carefully evaluated before application.

Conflicts of interest

The authors declare no conflict of interests.

Transparency document

The Transparency document associated with this article can be found in the online version.

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