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Effects of Almix® Herbicide on Oxidative Stress Parameters in Three Freshwater Teleostean Fishes in Natural Condition

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Abstract

Background: Aquatic pollution by pesticidal application, in recent times, has gained much attention throughout the world, as they ultimately reach to the aquatic bodies through agricultural runoff or by aerial spraying and finally, impair the health status of fish. The aim of the present study was to evaluate the toxicological responses of Almix® herbicide on oxidative stress parameters in three Indian freshwater teleosts namely, *Anabas testudineus, Heteropneustes fossilis* and *Oreochromis niloticus* in natural condition.

Methods: Almix® herbicide was applied at field concentration (8 g/acre) used for rice cultivation to evaluate the oxidative stress responses in freshwater teleostean fishes for a period of 30 days. Special type of cage was installed in pond for culturing the fish species.

Results: Acetylcholinesterase (AChE) activity was increased significantly in all fish tissues ($p<0.05$) and highest enhancement was observed in spinal cord of *A. testudineus*, but minimum activity was observed in spinal cord of *H. fossilis*. Significant increased ($p<0.05$) lipid peroxidation (LPO) level in all tissues was observed after Almix® exposure; highest in muscle of *O. niloticus* and lowest in brain of *H. fossilis*. Catalase (CAT) activity also showed significant enhancement ($p<0.05$), and was maximum in gill of *O. niloticus* and minimum in liver of *O. niloticus*, while glutathione-S-transferase (GST) activity was reduced significantly in liver ($p<0.05$), and in particular, highest reduction was observed in case of *H. fossilis*. Protein content also showed significant reduction ($p<0.05$) after Almix® exposure in all fish tissues.

Conclusion: Long-term exposure of Almix® herbicide even at environment friendly concentration caused significant induction on oxidative stress parameters and these responses could be considered as useful tools for monitoring herbicidal contamination in freshwater ecosystem.

Keywords: Almix®; Antioxidant; Oxidative stress; Rice field; Teleostean fish

Introduction

Integrated paddy-cum-fish-culture system is one of the most common culture systems practised in most of the South-East Asian countries. Sometimes, this diversified agricultural system implies close proximity with crop fields and fish ponds. Nowadays, use of herbicides in crop fields is increased several folds for protection of crop from weed infestation and to improve the productivity. In addition, caused threats to the non-target aquatic organisms especially fishes [1]. Almix®, fourth generation herbicide, is widely used in paddy fields, especially in paddy-cum-fish-culture systems to destroy the weeds such as *Cyperus iria, Cyperus defformis, Frimbristylis* sp., *Eclipta alba, Ludwigia parviflora, Cyanotis axillaris, Monochoria vaginalis, Marsilea quadrifoliata,* etc., both through contact and systematic pathway. It is a selective, both pre-emergent and post-emergent herbicide of sulfonylurea group. Almix® works at a very low use rate i.e., 8 g per acre. It is a mixture of 10% metsulfuron methyl ($C_{14}H_{15}N_5O_6S$), 10% chlorimuron ethyl ($C_{15}H_{15}ClN_4O_6S$) and 80% adjuvants [2]. Almix® did not show any volatilization property, therefore do not harm adjacent crops such as mustards, cotton, vegetables, fruit crops, castor *etc.,* unless they are sprayed onto them [2].

Fishes are considered as sentinel organism for ecotoxicological studies and are continuously exposed to wide variety of environmental contaminants [3]. In this context, the use of fishes in evaluating the risk in aquatic ecosystem is of sensitive and reliable approach [4,5]. Fish itself have self-defensive mechanism to withstand the impact of reactive oxygen species (ROS). ROS are generally produced during metabolism and results from an imbalance between pro-oxidants and antioxidants ratio [6-8]. ROS reacts with biological macromolecules to develop oxidative stress via the malfunction of a series of enzymes which ultimately leading to DNA damage and even cell death [9]. Among the oxidative stress parameters, acetylcholinesterase (AChE), which is mainly found in basal lamina of synapses and neuromuscular junction, is considered as one of the sensitive and reliable tool for evaluating the sublethal effects of pollutants in fish by different authors and is responsible for the termination of cholinergic impulse by the hydrolysis of acetylcholine (ACh) to choline and acetic acid [10-13]. Catalase, an enzymatic antioxidant, catalyzes H_2O_2, which is produced by dismutation of superoxide anions into less toxic water and oxygen molecules [14,15]. Glutathione-S-transferases, a phase II biotransformation enzyme, catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for detoxification [16]. Lipid peroxidation (LPO) is a secondary TBARS product,

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(thiobarbituric acid reacting substances) produced during oxidative degradation of lipids and regarded as an indicator of biochemical toxicity of environmental contaminants [17]. A large number of studies regarding physiological and biochemical parameters in aquatic organisms mainly fish exposed to xenobiotic compounds are available [18,19] but very little work on Almix® toxicity particularly on oxidative stress parameters in fish species were reported [20-26]. Therefore, the aim of the present study is to evaluate the toxicological responses of Almix® herbicide on acetylcholinesterase activity, oxidative stress parameter and antioxidant profile in three freshwater teleostean fishes namely *Anabas testudineus, Heteropneustes fossilis* and *Oreochromis niloticus*. These fish species were selected as ecotoxicological aliquot because of wide distribution in freshwater environment, ease availability throughout the year, easy acclimatization to laboratory conditions and commercial importance.

Materials and Methods

Chemicals

Almix® herbicide, manufactured by DuPont India Pvt. Ltd., India, was purchased from the local market. Bovine serum albumin (BSA), hydrogen peroxide (H_2O_2), 1-chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH) 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), 2-thiobarbituric acid (TBA) and sodium dodecyl sulfate (SDS) of AR grade were purchased from HiMedia Laboratories Pvt. Ltd. All other chemicals were purchased from Merck Specialities Private Limited.

Experimental design

Indian freshwater teleosts namely *Anabas testudineus* (Bloch), *Heteropneustes fossilis* (Bloch) and *Oreochromis niloticus* (Linnaeus) were procured from local market and were acclimatized for 15 days separately in the pond situated at Crop Research Farm premises of the University of Burdwan. The average weight and length taken after acclimatization of these fish species were 30.43 ± 5.14 g, 55.33 ± 4.82 g and 73.10 ± 14.70 g respectively and 11.46 ± 0.70 cm, 21.92 ± 0.84 cm and 17.44 ± 1.06 cm respectively. Fish were fed daily with commercial fish pellets (Tokyu, 32% crude protein) during acclimatization and experimentation. After acclimatization, fish were segregated into two different groups (control and Almix®-treated) in eighteen cages, containing 10 fish in each cage (triplicate). During the experimentation period, fish care and handling was performed in accordance with the guidelines of the University of Burdwan and was also approved by the Ethical Committee. A special type of cage (2.5 m × 1.22 m × 1.83 m) was prepared with nylon net and it was embraced by two PVC nets: the inner and outer having mesh sizes of 1.0 × 1.0 mm^2 and 3.0 × 3.0 mm^2 respectively. During experimentation Almix® was applied at rice cultivation dose of 8 g/acre in nine groups (Almix®-treated) [22,23,26] and control sets were subjected to maintain in separate adjacent crop field pond with same environmental condition and fish were fed with commercial feed daily (@ 1% of the total body weight) in each plot. The desired dose of the herbicide was dissolved in water and applied with the help of a sprayer on first day of the experiment.

Sampling

Water quality of the pond water was assessed as per APHA [27] during the experimentation period. After completion of the experiment i.e., 30th day fishes were sacrificed after anesthetising with tricaine methanesulphonate (MS 222 @ 100 mg/L) and gill, liver, brain, muscle and spinal cord were rapidly taken out, then washed in 0.75% saline solution and soaked with filter paper, then placed in Teflon tubes and finally, stored at -80°C for biochemical analysis.

Acetylcholinesterase assay

Acetylcholinesterase activity was analysed based on Ellman et al. [28]. Tissue samples (brain, muscle and spinal cord) were homogenized in Teflon homogenizer with chilled phosphate buffer (0.1 M, pH 8) and centrifuged at 14,000 rpm for 5 minutes at 4°C. Briefly, supernatant (400 µl) was taken in cuvette, then 2.6 ml phosphate buffer (0.1 M and pH 7.0) and 100 µl of dithiobisnitrobenzoic acid (DTNB) were added to it. After that 20 µl of acetylthiocholine iodide was added and reading was taken at 410 nm for 3 minutes against blank and expressed as micromol of acetylthiocholine (AcSCh) hydrolyzed/min/mg protein.

Lipid peroxidation assay

Lipid peroxidation was analysed according to the method described by Ohkawa et al. [29]. Tissue samples (10%) were homogenized in Teflon homogenizer with 1.15% KCl (pH 7.4) and centrifuged at 4,500 rpm for 10 minutes at 4°C. Homogenate (200 µl) were added to 3.8 ml TBA mixture solution (0.2 ml SDS, 1.5 ml acetic acid, 1.5 ml TBA and 0.6 ml distilled water) and was placed in water bath for 1 hour at 95°C and again centrifuged at 4,500 rpm for 10 minutes at 4°C. The LPO level was evaluated by measuring the production of thiobarbituric acid reactive substances (TBARS) spectrophotometrically at 532 nm.

Catalase assay

Catalase (CAT) activity was measured spectrophotometrically based on the method described by Aebi [30]. Tissue samples were homogenized in mortar driven Teflon homogenizer with chilled phosphate buffer (50 mM and pH 7.8) and centrifuged at 4,500 rpm for 10 minutes at 4°C. CAT activity was measured based on the decrease in absorbance at 240 nm due to H_2O_2 consumption (ε^{mM} = 0.0436). The reaction volume contained 20 µL of the supernatant, 1.98 mL of 50 mM phosphate buffer (pH 7.0), and 1 mL of 30 mM H_2O_2.

Glutathione-S-transferase assay

Glutathione-S-transferase (GST) activity was analysed as described by Habig et al. [31]. Tissue sample was homogenized with 20 mM phosphate buffer (pH 6.5) using motor drive Teflon homogenizer. Homogenates were then centrifuged at 10,000 rpm for 10 minutes at 4°C and the supernatants were used for further analysis. The reaction volume consisted of 50 µL of the supernatant, 2.5 mL 20 mM phosphate buffer (pH 6.5), 0.3 mL reduced glutathione (10 mM), and 0.15 mL CDNB solution (1.5 mM). GST activity toward 1-chloro-2,4-dinitrobenzene (CDNB) was determined spectrophotometrically at 340 nm (ε^{mM} = 9.6).

Protein estimation

Protein content of all tissue samples was determined spectrophotometrically based on Lowry et al. [32] using BSA as standard, and enzyme activity was recorded as units per mg protein ($U.mg^{-1}$).

Statistical analysis

Factorial ANOVA was conducted in SPSS program (version 16.0) to analyze biochemical changes. Tukey's a post-hock comparisons were used to test the differences between tissues at $p<0.05$. All the data were expressed as mean ± standard error of mean (n = 9).

Results and Discussion

Present study is the maiden attempt to report the Almix® toxicity in regard to oxidative stress parameters namely AChE, LPO, CAT and GST in Indian freshwater teleosts namely *A. testudineus*, *H. fossilis*,

and *O. niloticus* in natural condition, although very little work on biochemical parameters such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glucose-6-phosphatase in different fish species were reported under the laboratory condition [33,20-26].

Physicochemical characteristics of the test water

Physicochemical properties of the ponds water were measured during experimentation. Water temperature was ranged from 14.0°C to 14.4°C, pH was varied from 7.82 to 7.92, electrical conductivity (EC) ranged from 386.0-393.0 µS/cm, total dissolved solids (TDS) varied from 274.0-279.0 mg/l, dissolved oxygen (DO) varied from 7.3-7.6 (mg/l), total alkalinity ranged from 100.0-102.0 mg/l as $CaCO_3$, total hardness varied from 148.0-156.0 mg/l as $CaCO_3$, orthophosphate varied from 0.11-0.14 mg/l, nitrate-nitrogen varied from 0.55-0.60 mg/l, ammoniacal-nitrogen ranged from 5.10-7.81 mg/l, sodium varied from 20.0-21.0 mg/l and potassium ranged from 2.67-3.00 mg/l. The results showed that physicochemical parameters of test water did not show any significant variation during the Almix® intoxication.

Acetylcholinesterase activity induction

Acetylcholinesterase is considered as one of the fine tool for evaluating the impacts of environmental contaminants. Acetylcholinesterase activity, in the present study, was increased significantly in all fish tissues ($p<0.05$) after Almix® intoxication compared with control value (Table 1). AChE activity showed the usual trend of enhancement as compared to control value after almix intoxication. Muscle tissue showed highest AChE elevation in *O. niloticus* (209.49%) followed by *A. testudineus* (194.51%) and lowest in *H. fossilis* (186.81%). Brain showed highest AChE elevation in *A. testudineus* (265.59%), while lowest AChE activity was observed in *H. fossilis* (191.32%). Similar pattern as observed in brain was also observed in spinal cord. Results clearly showed that AChE activity was raised in the order of muscle>brain>spinal cord for both *H. fossilis* and *O. niloticus*, while in *A. testudineus* pattern of spinal cord>brain>muscle was observed. The results clearly demonstrates that *A. testudineus* was more sensitive to AChE induction. Increased AChE activity observed in the present might be due to acethylcholine accumulation in concerned tissues and finally caused overstimulation of the receptors to herbicide exposure. Similar results of enhanced AChE activity as observed under natural condition can also be corroborated with the findings of laboratory study [24]. Higher AChE activity in brain compared with muscle and spinal cord, observed under present investigation was also described by Glusczak et al. [34] in piava (*L. obtusidens*) after glyphosate exposure and by Miron et al. [35] and Crestani et al. [36] in silver catfish (*Rhamdia quelen*) after clomazone exposure. On the other hand, Bretaudt et al. [37] reported higher AChE activity in skeletal muscle than brain in goldfish (*Carassius auratus*) after carbofuran, diuron and nicosulfuron exposure. On contrary, reduced AChE activity in brain and muscle tissue after herbicide exposure were also reported by several authors [34,38-40]. Therefore, this induced AChE activity may adversely affect cholinergic neurotransmission process which ultimately leads to toxicity in fish, and finally the responses displayed by these fish species to the Almix® exposure could be considered as biomarker of herbicide toxicity.

Lipid peroxidation level induction

Lipid peroxidation is one the most important indicator of oxidative damage in cells and tissues [41]. Significant enhanced ($p<0.05$) LPO level was observed in the present study in all fish tissues (Table 2). In the present study, LPO level in liver was raised 184.05% in *A. testudineus*, 149.01% in *H. fossilis* and 144.07% in *O. niloticus*. Muscle and brain tissue showed highest elevation of 278.79% and 270.68%, respectively in *O. niloticus*, while lowest activity was observed in *H. fossilis* and it was 125.76% and 108.57%, respectively. Gill tissue showed maximum LPO level in *A. testudineus* (179.28%) and minimum in *H. fossilis* (121.77%). Considering all the tissues, muscle of *O. niloticus* (278.79%) showed highest LPO level and brain of *H. fossilis* (108.57%) showed lowest LPO level after Almix® exposure. The results showed that enhanced LPO level was in the tune of liver>gill>muscle>brain in *A. testudineus*, but it was liver>muscle>gill>brain in *H. fossilis* and *O. niloticus* showed the pattern of muscle>brain>gill>liver. Results clearly showed species and tissue dependent and *O. niloticus* was more sensitive to herbicide exposure compared with *A. testudineus* and *H. fossilis*. Significant enhanced LPO level in liver, muscle, gill and brain at field concentration observed under present study indicated development of oxidative stress in these tissues as compensatory response against herbicidal toxicity. Similar results of enhanced LPO level as observed under present study can also be corroborated with the laboratory study findings [24]. Highest LPO level in muscle and liver, observed under present study was also reported by Atli et al. [42] and this may be due to site specificity of oxidative reactions and subsequently maximum generation of free radicals. Different LPO level among different fish species may probably be due to feeding behaviour of fishes; type, duration and concentration of stressors. In addition, different LPO level could reflect different antioxidant response of fish species to Almix® toxicity. Similar trend of increased LPO level in liver, muscle, gill and brain of *Leporinus obtusidens* was reported by Miron

	Concentration		Type of fishes	
Tissues	**(g/acre)**	***A. testudineus***	***H. fossilis***	***O. niloticus***
Muscle	00	0.043 ± 0.005^{a1A}	0.038 ± 0.001^{a1A}	0.045 ± 0.003^{a1A}
	8	0.084 ± 0.008^{a2A}	0.071 ± 0.011^{a2A}	0.093 ± 0.006^{a2A}
Brain	00	0.040 ± 0.008^{a1B}	0.046 ± 0.004^{a1B}	0.051 ± 0.003^{a1B}
	8	0.107 ± 0.014^{a2B}	0.089 ± 0.007^{a2B}	0.100 ± 0.005^{a2B}
Spinal cord	00	0.030 ± 0.001^{a1C}	0.036 ± 0.001^{a1C}	0.039 ± 0.002^{a1C}
	8	0.086 ± 0.009^{a2C}	0.066 ± 0.006^{a1C}	0.075 ± 0.007^{a1C}

Note: Data are presented as mean ± SEM (n = 9). 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.

Table 1: Acetylcholinesterase activity (unit/mg protein/min) in test fish species exposed to commercial herbicide Almix (8 g/acre) for 30 days in field condition.

	Concentration		Type of fishes	
Tissues	**(mg/l)**	***A. testudineus***	***H. fossilis***	***O. niloticus***
Liver	00	2.33 ± 0.139^{a1A}	3.39 ± 0.966^{a1A}	2.10 ± 0.163^{a1A}
	8	4.29 ± 0.112^{a1A}	5.04 ± 0.868^{a1A}	3.03 ± 0.103^{a1A}
Muscle	00	0.92 ± 0.027^{a1B}	3.94 ± 0.082^{b1B}	0.48 ± 0.068^{a1B}
	8	1.17 ± 0.055^{a1B}	4.95 ± 0.272^{b1B}	1.34 ± 0.088^{a1B}
Gill	00	2.06 ± 0.158^{a1C}	5.71 ± 0.674^{b1C}	2.28 ± 0.116^{a1C}
	8	3.69 ± 0.154^{a1C}	6.95 ± 0.820^{b1C}	3.43 ± 0.091^{a1C}
Brain	00	2.46 ± 0.146^{a1D}	9.33 ± 0.860^{b1D}	0.95 ± 0.057^{a1D}
	8	3.02 ± 0.080^{a1D}	10.13 ± 1.080^{b1D}	2.57 ± 0.223^{a1D}

Note: Data are presented as mean ± SEM (n = 9). 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.

Table 2: Lipid peroxidation level (unit/mg protein/min) in test fish species exposed to commercial herbicide Almix (8 g/acre) for 30 days in field condition.

et al. [39] after clomazone exposure. Several authors also reported enhanced LPO level in different fish tissues after pesticide exposure [43-47]. On contrary, Lushchak et al. [48] reported reduced LPO level in brain and liver of goldfish *Crassius auratus* after Roundup exposure. Therefore, the present investigation clearly indicated that Almix® at rice cultivation concentration significantly induced TBARS production in the concerned fish species and could be used as biomarker.

Catalase activity induction

Catalase, in this cascade, is the prime antioxidant enzyme and provides first line of defence against the production of ROS. Significant enhanced ($p<0.05$) catalase activity was observed in all fish tissues after Almix® exposure (Table 3). Catalase activity, in liver, showed maximum elevation in *H. fossilis* (121.10%) followed by 116.82% in *A. testudineus* and lowest in *O. niloticus* (108.91%). Muscle tissue showed highest increased CAT activity in *A. testudineus* (133.46%) and lowest in *H. fossilis* (123.69%). In case of gill and brain, highest elevation of 150.53% and 118.08%, respectively was observed in *O. niloticus*, but lowest CAT activity of 115.56% and 115.48%, respectively was observed in *H. fossilis*. Based on different tissues, the results demonstrated that highest CAT activity was observed in gill of *O. niloticus* (150.53%) and lowest in liver of *O. niloticus* (108.91%). Although CAT activity was increased in all concerned fish tissues, the trend of increment was different for different fish species and in case of *A. testudineus* and *O. niloticus* it was gill>muscle>brain>liver, while *H. fossilis* showed the trend of muscle>liver>gill>brain. Enhanced CAT activity in different fish species may probably be due to production of ROS in liver. Increased liver CAT activity in the present study can also be explained with enhanced TRABS level [49]. Similar results of enhanced CAT activity as observed under present study can also be correlated with the laboratory study findings [24]. The results are also supported by several authors [13,50-52]. On contrary, Crestani et al. [36] reported reduced liver CAT activity in silver catfish after clomazone exposure and by Sayeed et al. [53] in *C. punctatus* after deltamethin exposure. Varied catalase activity observed among different fish species indicated development of oxidative stress condition which ultimately impair the antioxidant defence system due to production of ROS species. Therefore, present study reflects two things: Oxidative change in the tissue system due to increased CAT activity and subsequent elimination of the herbicide from the interior.

Glutathione-S-transferase activity induction

Glutathione-S-transferase is an enzymatic antioxidant, and plays a pivotal role to protect the cells against xenobiotic compounds by catalyzing electrophilic substrates [54]. Significant reduction ($p<0.05$) in liver GST activity was observed in the present investigation compared with control value (Table 4). Liver of *H. fossilis* showed highest reduction in GST activity i.e., 71.63%, moderate in *A. testudineus* (19.09%) and lowest in *O. niloticus* (15.49%). Similar results of reduced GST activity as observed under present study can also be resembled with the laboratory study findings [24]. The results clearly showed that *H. fossilis* was much more sensitive than other two fish species to GST responses. Reduced GST activity in liver indicated failure of detoxification process and oxidative stress development. Similar result was also reported by Ballesteros et al. [55] in *Jenynsia multidentata* after endosulfan exposure and by Menezes et al. [56] in *R. quelen* after Roundup exposure. Higher GST activity compared with LPO level observed under present study indicated higher scavenging of ROS substances. Reduced glutathione-S-transferase activity here indicated that Almix® caused damage to the antioxidant defence system as well as the detoxification process of teleostean fish species. So, it could be used as biomarker of Almix® toxicity.

Protein content

Proteins play an important role in the architecture and physiology of the cell by catabolising different amino acids in fish [57]. Protein content showed significant decline ($p<0.05$) in all fish tissues after Almix® intoxication (Table 5). The results clearly demonstrated that brain of *A. testudineus* (94.85%) showed maximum reduction after Almix® exposure. Reduced protein levels in all fish tissues at rice cultivation concentration may be due to protein catabolism for meeting the high energy requirement to overcome the imposed stress and indicated loss of compensatory mechanism and oxidative stress

	Concentration		Type of fishes	
Tissues	**(mg/l)**	***A. testudineus***	***H. fossilis***	***O. niloticus***
Liver	00	48.51 ± 3.84^{a1A}	62.84 ± 1.47^{a1A}	50.08 ± 4.98^{a1A}
	8	56.67 ± 4.78^{a1A}	76.10 ± 1.90^{b1A}	54.54 ± 3.64^{a1A}
Muscle	00	61.99 ± 1.73^{a1B}	78.54 ± 1.48^{a1B}	64.19 ± 3.13^{a1B}
	8	82.73 ± 6.27^{a2B}	97.15 ± 1.97^{a2B}	85.56 ± 4.00^{a2B}
Gill	00	86.77 ± 4.38^{a1C}	50.51 ± 1.36^{b1C}	50.19 ± 2.64^{cb1C}
	8	118.19 ± 4.26^{a2C}	58.37 ± 1.03^{b1C}	75.55 ± 5.85^{cb2C}
Brain	00	77.88 ± 1.42^{a1D}	128.95 ± 1.15^{b1D}	69.29 ± 2.28^{a1D}
	8	91.85 ± 2.82^{a1D}	148.91 ± 3.09^{b2D}	81.82 ± 1.93^{a1D}

Note: Data are presented as mean ± SEM (n = 9). 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.

Table 3: Catalase activity (unit/mg protein/min) in test fish species exposed to commercial herbicide Almix (8 g/acre) for 30 days in field condition.

	Concentration		Type of fishes	
Tissue	(mg/l)	***A. testudineus***	***H. fossilis***	***O. niloticus***
Liver	00	0.387 ± 0.095^{a1A}	0.157 ± 0.007^{b1A}	0.433 ± 0.052^{a1A}
	8	0.074 ± 0.012^{a1A}	0.113 ± 0.012^{a1A}	0.067 ± 0.005^{a2A}

Note: Data are presented as mean ± SEM (n = 9). 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.

Table 4: GST activity (nmol/mg protein/min) in liver of test fish species exposed to commercial herbicide Almix (8 g/acre) for 30 days in field condition.

	Concentration		Type of fishes	
Tissues	**(mg/l)**	***A. testudineus***	***H. fossilis***	***O. niloticus***
Liver	00	104.63 ± 3.37^{a1A}	65.64 ± 1.00^{b1A}	85.55 ± 1.70^{c1A}
	8	99.04 ± 3.92^{a1A}	57.04 ± 2.22^{b1A}	78.61 ± 2.43^{c1A}
Muscle	00	98.19 ± 2.48^{a1B}	71.57 ± 2.60^{b1B}	75.38 ± 1.56^{cb1B}
	8	92.74 ± 3.23^{a1B}	64.50 ± 2.48^{b1B}	68.32 ± 2.60^{cb1B}
Gill	00	65.19 ± 1.16^{a1C}	72.00 ± 3.99^{a1C}	70.44 ± 0.60^{a1C}
	8	60.85 ± 1.43^{a1C}	67.81 ± 3.97^{a1C}	62.64 ± 2.04^{a1C}
Brain	00	52.61 ± 4.23^{a1D}	49.17 ± 1.21^{a1D}	51.06 ± 0.89^{a1D}
	8	49.90 ± 4.86^{a1D}	43.98 ± 1.02^{a1D}	43.72 ± 1.99^{a1D}
Spinal cord	00	75.57 ± 2.32^{a1F}	49.03 ± 2.12^{b1F}	73.90 ± 2.30^{a1F}
	8	71.34 ± 2.46^{a1F}	45.14 ± 2.59^{b1F}	67.38 ± 2.88^{a1F}

Note: Data are presented as mean ± SEM (n=9). 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.

Table 5: Protein content (mg/g) in test fish species exposed to commercial herbicide Almix (8 g/acre) for 30 days in field condition.

condition in the fish tissues. The present findings were also supported by Sancho et al. [58], David et al. [57] and Fonseca et al. [59]. Similar results of reduced protein content as observed under present study can also be correlated with the laboratory study findings [24]. On contrary, Crestani et al. [36] observed enhanced protein level after clomazone intoxication in liver of *R. quelen*.

Conclusion

Present study demonstrates that commercial herbicide Almix® at rice cultivation concentration caused adverse effects on oxidative stress parameters in three freshwater Indian teleosts namely *A. testudineus*, *H. fossilis*, and *O. niloticus*. Induced activity of AChE, LPO, CAT and GST indicated impairment in the normal biochemical and/or physiological processes by Almix®-induced free radical toxicity, although the alterations are species and tissue dependent. Therefore, the results indicated that the responses displayed by these fish species could be considered as bioindicators of herbicide toxicity in freshwater ecosystem. Finally, from an ecotoxicological view point, the use of this herbicide in agricultural fields and aquatic bodies must be handled carefully and monitored judicially.

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