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Integrative assessment of biomarker responses in teleostean fishes exposed to glyphosate-based herbicide (Excel Mera 71)

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A B S T R A C T

Present study deals with the effects of glyphosate-based herbicide, Excel Mera 71 on *Anabas testudineus*, *Heteropnestes fossilis* and *Oreochromis niloticus* in field conditions (1.85 kg/ha) based on anti-oxidative, metabolic and digestive responses. For this study following biomarkers *viz.*, acetylcholinesterase (AChE), lipid peroxidation (LPO), catalase (CAT), glutathione-S-transferase (GST), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, lipase and protease were investigated in gill, stomach, intestine, liver, kidney, brain, muscle and spinal cord of the concerned fish species. Enzyme activities were significantly altered by glyphosate exposure after 30 days, these activities were tissue as well as species specific. The results suggested that these biomarkers could be used to assess the ecological risks of glyphosate on fish. Bioaccumulation factor (BAF) studied in different aquatic natural macrophytes showed order of *Alternanthera philoxeroides* > *Azolla pinnata* > *Lemna* sp. (Minor) > *Lemna* sp. (Major) > *Pistia stratiotes*, while transfer factor (TF) showed the order of *Pistia stratiotes* > *Alternanthera philoxeroides* > *Lemna* sp. Bioconcentration factor (BCF) study showed maximum accumulation of glyphosate in liver, kidney or intestine, and minimum either in bone or stomach irrespective of fish species. An integrated biomarker response (IBR), which uses a battery of biomarkers to calculate the standardized scores for each biomarker responses ranging from physiological to biochemical/molecular responses, was evaluated by combining the multiple biomarkers into a single value to evaluate quantitatively the toxicological effects of glyphosate. In general, the multiple indices exhibited variations and *A. testudineus* was more affected than other fish species; maximum IBR value was observed for LPO and minimum in case of ALT. The order of integrated biomarkers caused by glyphosate treatment was recorded as follows: LPO > Amylase > CAT > AST > Protease > Lipase > ALP > GST > AChE > ALT for *A. testudineus*, LPO > AChE > AST > Protease > CAT > Amylase > Lipase > GST > ALP > ALT for *H. fossilis* and AChE > CAT > LPO > AST > Amylase > GST > Protease > ALP > Lipase > ALT for *O. niloticus*. Finally, IBR analysis is able to distinguish the variations between different parameters and might be a useful tool for the quantification of integrated responses induced by glyphosate toward fish.

1. Introduction

Integrated paddy-cum-fish-culture system is one of the most common culture systems practised in most of the South-East Asian countries, Southern America and Europe. This diversified agricultural system implies close proximity of crop field areas and fish culture ponds. Nowadays, substantial augmentation in agriculture

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is based on use of herbicide in crop fields for protection of crop from weed infestation and to improve the productivity in terms of biomass, but simultaneously posing a great threat to non-target aquatic organisms especially fin-fishes and shell-fishes [1]. The response of an aquatic organism to these pollutants induces damage at cellular and biochemical levels, which ultimately leading to changes in function of the cells, tissues as well as physiology and behavior of the organism [2].

Excel Mera 71, a new 71% WDG (water dispersible granule) formulation of ammonium salt of glyphosate is non-selective, post-emergent herbicide and is used for controlling the weeds in agriculture, forestry, urban areas and even aquatic bodies [3]. The half-life of glyphosate in soil ranges between 2 and 197 days [4]. Soil and climatic conditions also affect the glyphosate's persistence in soil. The median half-life of glyphosate in water varies from 4 to 91 days [5]. Generally, soils having higher pH and organic matter in cooler climates have shown the most re-cropping problem. Symptoms of injury include an immediate cessation of growth, shortened internodes, chlorotic yellowing, and a gradual necrosis of leaf and stem tissue [6]. A number of studies have been done to determine the bioaccumulation of glyphosate herbicides in macrophytes and fish tissues and subsequent biochemical response in some freshwater fishes in field condition [7,8]. Toxicity study of the glyphosate herbicide in fish including metabolic, oxidative, and haematological parameters were also reported by several authors [3,5,9–11]. Moreover, a number of studies have also been demonstrated the toxicity of glyphosate on other invertebrates as amphibians, rat and mice [12–14] *etc.*

Biochemical and physiological responses to the xenobiotic exposure are leading to changes in the structure and function of the cells and/or tissues [15]. Therefore, for biomonitoring and environmental risk assessment in aquatic ecosystem, biochemical and physiological indicators such as enzymes are very useful and are considered as useful biomarkers to identify the environmental contamination in regard to fish health status as well as to develop water quality indices [16]. Evaluation of changes in biochemical enzyme activity as biomarker of toxicant exposure has been used in documenting toxin interaction with biological systems, which ultimately provides the link inbetween the effects of xenobiotic exposure and risk assessment process. In this cascade, biomarkers help to establish a relationship between cause and effect of xenobiotic exposition and ultimately provide an early warning signal of potential damage at cellular and sub-cellular levels in specific organs and/or tissues under contaminated aquatic environment. Although, the use of biomarker-based monitoring approach as a tool for environmental assessment is often critically limited due to lack of integrated statistical analyses. The biomarkers reflect a hierarchy of biological organization and contamination levels measured regardless of the variability in the biomarker [17]. In this regard, in the present study an attempt has been made by using star plots, a simple multivariate graphic method for visual integration of a set of early warning responses measured with biomarkers to determine the global indices of environmental quality that have potentiality to take into account the chemical and biological criteria. This method indicates computation of the star plot area for giving the 'integrated biomarker response' (IBR) described by Beliaeff and Burgeot [18] for the interpretation of biomarker responses which included (1) antioxidant defence mechanism, specifically catalase (CAT); (2) biotransformation enzyme, phase II glutathione-S-transferase (GST); (3) general damage, lipid peroxidation (LPO); (4) acetylcholinesterase (AChE); (5) alkaline phosphatase (ALP); (6) aspartate aminotransferase (AST); (7) alanine aminotransferase (ALT) and (5) physiological and digestive measures, such as amylase, lipase and protease. Studies using an IBR for simplifying the interpretation of biomarker responses have not been verified previously in Indian telostean fishes under field conditions. Moreover, the IBR constitutes a practical and robust tool to assess the susceptibility of glyphosate-based herbicide, Excel Mera 71. Therefore, in the present study, biomarkers selected showed either specific or general responses were acetylcholinesterase, catalase, glutathione-S-transferase, lipid peroxidation, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, amylase, lipase and protease. Additionally, these biomarkers also reflect a straightforward hierarchy relationship between biomarker response to contaminants and biological consequences at cellular or subcellular levels, and were used to develop an integrated biomarker response index (IBR) for interpretation of these biomarker responses.

Use of sentinel organisms for environmental quality monitoring by biological means provide a sensitive and reliable approach to estimate the potential effects of pollutants [19]. Fish, among them, are recognized as an excellent experimental model for toxicological studies because of their importance as protein source [20]. In the present study, *Anabas testudineus* (Bloch), *Heteropnestes fossilis* (Bloch) and *Oreochromis niloticus* (Linnaeus) were selected as test specimen for toxicity study. Several ecotoxicological characteristics of these species such as wide distribution in the freshwater environment, easy availability throughout the year, easy acclimatization to the laboratory conditions and commercial importance make these species as an excellent test species both in the laboratory and field conditions [21–23]. There are some studies on toxicity of glyphosate-based herbicides; therefore, to supplement risk assessment studies, it is very important to obtain information on their toxic effects on these fish species. Therefore, objective of the present study is to use the integrated biomarker responses (IBR) to assess the comprehensive toxic effects of glyphosate-based commercial formulation, Excel Mera 71 particularly on oxidative stress parameters in different freshwater teleostean fishes namely *Anabas testudineus*, *Heteropnestes fossilis* and *Oreochromis niloticus* to explore a wide range of responses. Moreover, herbicide content in water, sediments, macrophytes and concerned fish tissues were also determined.

2. Materials and methods

2.1. Fish

Freshwater teleostean fishes, *Anabas testudineus* (Bloch), *Heteropneustes fossilis* (Bloch) and *Oreochromis niloticus* (Linnaeus) of both the sexes with an average weight of 16.09 ± 1.420 g, 31.77 ± 3.440 g and 38.57 ± 2.47 g respectively and total length of 10.09 ± 0.251 cm, 16.58 ± 0.388 cm and 13.59 ± 0.496 cm respectively, were procured from local market and were acclimatized under congenial pond conditions for 15 days. During the acclimatization period, the average value of water parameters were as follows: temperature 23.37 ± 0.09 °C, pH 6.33 ± 0.03, electrical conductivity 275.00 ± 2.65 μS/cm, total dissolved solids 211.00 ± 2.08 mg/l, dissolved oxygen 6.09 ± 0.10 mg/l, total alkalinity 157.00 ± 2.52 mg/l as $CaCO_3$, total hardness 101.33 ± 1.33 mg/l as $CaCO_3$, sodium 41.67 ± 1.52 mg/l, potassium 9.16 ± 1.47 mg/l, orthophosphate 0.13 ± 0.01 mg/l, ammoniacal-nitrogen 1.69 ± 0.11 mg/l, nitrate-nitrogen 0.45 ± 0.07 mg/l. After acclimatization, fish were transferred to cages immersed in the ponds situated at Crop Research Farm premises of the University of Burdwan. The fish were fed once a day with commercial fish pellets (32% crude protein, Tokyu) during both acclimation and exposure periods.