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RESEARCH ARTICLE

EFFECT OF PRE-EMERGENCE AND POST-EMERGENCE HERBICIDE ON FEW BIOCHEMICAL PARAMETERS TO FRESHWATER FISH *LABEO ROHITA*

Rajini. A*[1]., Gopi R.A[1] and Bhuvana .V[2]

[1]Department of Ecotoxicology
[2]Formerly as Biochemist in Department of Biochemistry International Institute of Biotechnology and Toxicology (IIBAT), Kancheepuram Dist, Padappai- 601 301, Tamil Nadu

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ABSTRACT

Impact of pre-emergence herbicide Butachlor 50% EC and post-emergence herbicide 2, 4-D 80% WP was evaluated to freshwater fish, *Labeo rohita*. Chronically fish were exposed for 21 days to 0.066 mg/L Butachlor 50% EC, 100 mg/L 2,4-D 80% WP and in combination of the exposed concentration. Significant increase in total protein was observed in the gill, liver, kidney and muscle tissues exposed to the combination of herbicides and similar change was observed in the gill, liver, kidney and muscle in groups of fish exposed to Butachlor 50% EC. Whereas, in 2,4-D 80% WP significant increase was observed in liver tissue. Increase in aspartate transaminase and alanine transaminase in gill, liver, kidney and muscle tissues were observed in the fish exposed to the 2, 4-D 80% WP + Butachlor 50% EC. Elevation and inhibition in enzymes might be due to stress, disruption of enzyme systems and tissue damage.

INTRODUCTION

Aquatic ecosystems are the ultimate descend of natural and anthropogenic inputs of contaminants into the environment. Many of the environmental pollutant especially pesticides enter into aquatic ecosystem through agricultural run off and ultimately affects the different non target aquatic animals like bivalves, crustaceans, molluscs, prawn and fish, which are of great economics important to humans (Parikh *et al*., 2014). The effects of toxic chemicals on biological organisms, especially at the population, community, ecosystem level are monitored and studied in the ecotoxicology. Ecotoxicology is the science to study the effect of toxic substances on living organisms, encompassing all levels of biological organization from single organisms to ecosystems (Fontanetti *et al.,* 2010). Herbicides are used extensively in the agricultural fields, forests and gardens to kill the plant or inhibit its normal growth. The advantage of the herbicides is the easy application and effective control of weeds and in a particular formulation and application it can have selective or nonselective effect. In agriculture, selective herbicides are often used for tillage, or in combination with tillage and other agronomic practices, to control weeds without damaging crops. Herbicides either drifts or leaches from the agricultural area to the nearby natural resources and the contamination may have toxic effect to the aquatic organisms.

Many herbicides are short-lived and do not accumulate in the environment. However, some are highly toxic to aquatic animals and should be avoided or used with extreme caution near aquatic environments. The length of time which the herbicide remains in the water column determines the length of exposure. The fate of an aquatic herbicide determines whether aquatic life could be exposed at a later time (Eric A Paul *et al.,* 1994). In aquatic ecosystems, fish are regarded as bioindicator of overall system health. Fish can be affected directly or indirectly. The direct effects are initiated at the lower level of biological organization such as molecular level. Indirect affects are where the effect is on the food chain and the behavior of the organism (Alaa *et al.,* 2010). Butachlor and 2, 4 dichlorophenoxyacetic acid are stable indefinitely. 2, 4 dichlorophenoxyacetic acid (2, 4-D) is a widely used herbicide in Southern Brazil due to its low cost and good selectivity (Anusuya *et al.,* 2014). It is available as granular or liquid formulation (Michael *et al.,* 2001). LC_{50} values for fish vary considerably and its variation is partly due to differences in species sensitivity, chemical structure (esters salts, or free acid), and formulation of the herbicide. The no-observed-effect-level (NOEL) varies with the species and the formulation (WHO, Environmental Health Criteria, 1989).

Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may

Corresponding author:* **Rajini. A
Department of Ecotoxicology

lead to toxicity. The biochemical processes represent the most sensitive and relatively early events of pollutant damage (Ghousia Begum, 2004). Indiscriminate usage of herbicides to kill weeds can have impact on the aquatic fauna. Further, these herbicides can have sublethal effects which can affect the survival of the fish. Small "sublethal" concentration of some pesticides can lead to changes in behavior, weight loss, impaired reproduction, inability to avoid predators, and lowered tolerance to extreme temperatures. The overall consequences of sublethal doses of pesticides can be reduced adult survival and lowered population abundance (Louis *et al.,* 1996). Many of the clinical tools used to evaluate mammalian health are not developed for use in fishes. As the aquaculture industry expands, there is an increasing need for improved diagnostic methods (Terry *et al.,* 2000). Fish species exhibit a characteristic response to pesticide stress and it can be measured through variation in enzyme activities.

The pre-emergence and post emergence herbicide Butachlor 50% EC and 2,4-dichlorophenoxyacetic acid (2,4-D) 80% WP are widely and frequently used herbicides in rice ecosystem. The repeated use of these herbicides may lead to the contamination of nearby ponds and freshwater ways by drift and leaching. In the present study an attempt has been made to evaluate the effect of these herbicides on the freshwater fish *Labeo rohita* and its impact on few biochemical parameters, since not much biochemical data in fish tissues are available due to the effect of these herbicides.

MATERIALS AND METHODS

Hatchery bred fish *Labeo rohita*, 18-20g and length 12-14 cm was procured from commercial fish farm and was quarantined for a period of one month in cement tanks in the laboratory. The fish were fed with groundnut oil cake during the quarantine. The fish were acclimatized in glass aquaria for 10 days to the laboratory condition and were fed with groundnut oil cake. Feed was withdrawn 24 h prior to exposure. Physico-chemical parameter such as temperature, pH, dissolved oxygen and hardness were analysed during the conduct of experiment. Semi-static acute toxicity exposure based on OECD guideline 203 was conducted for a period of 96 hour with ten fish/concentration for Butachlor 50% EC and 2,4-dichlorophenoxyacetic acid (2,4-D) 80% WP to evalvuate the LC_{50}. Based on the acute LC_{50} sub lethal concentration of 0.066mg/L ($1/10^{th}$ of LC_{50}) of Butachlor 50% EC and a maximum concentration of 100 mg/l as per OECD guideline (Limit test) for 2,4-dichlorophenoxyacetic acid 80% WP and its combination of Butachlor 50% EC (0.066 mg/l) + 100mg/l of 2,4-D 80% WP were maintained chronically for 21 days with 10 fish in each group along with control group of fish.

On completion of the exposure, five fish were removed from the exposure medium and killed by stunning. The fish were dissected and tissues such as, gill, liver, kidney and muscle was collected in ice-cold phosphate buffer, blotted and weighed. Tissues were pooled organ wise and group wise and the samples were homogenated in 5 ml of ice-cold phosphate buffer and centrifuged at 3000 rpm for 10 minutes and the supernatant was analysed for total protein, alanine transaminase, aspartate transaminase and alkaline phosphatase. The biochemical determinations were done using Erba reagent kits on a semi-autoanalyzer (Erba chem-5 plus, Transasia, India). The values were compared using student's't' test (NCSS, 2000).

RESULTS

Acute 96h LC_{50} was determined as 0.66mg/l for Butachlor 50% EC using Finney probit analysis (NCSS 2000). Limit test concentration of 100 mg/L was used for the exposure of 2, 4-Dichlorophenoxyacetic acid 80% WP. Behavior abnormalities such as loss of equilibrium, rapid opercular movement and surfacing movement were observed in fish exposed to Butachlor 50% EC. No toxicity or behavioral abnormalities was observed in fish exposed to 100 mg/l 2, 4-dichlorophenoxyacetic acid 80% WP. The water was checked for there quality conditions of pH (7.3-7.6), dissolved oxygen (7.4-7.8 mg/l), hardness (285 mg/l) (APHA *et al.,* 1998.) and temperature (22 ± 2°C).

No mortality or toxicity signs was observed during the 21 days chronic exposure. Exposure to concentration of chemicals may be the most prevalent threat to which fish may confront in an aquatic environment. Though behavioral abnormalities may not be observed, there are possibilities to cause biochemical alterations. All metabolic processes are governed by enzymes and it activates, controls the metabolism within the cell. Proteins are important constituents of cells and tissues and are polymers of amino acids that are linked covalently through peptide bonds, it has numerous biological functions. The most common changes in protein concentrations results due to the acute-phase reaction. Significant changes in total protein was observed in gill, liver, kidney and muscle tissues of the fish exposed to 2, 4 D 80% WP and butachlor 50% EC combination. Similarly, fish exposed to butachlor 50% EC showed significant changes in gill, liver, kidney and muscle tissues. In fish exposed to 2, 4 D 80% WP significant change in the total protein of liver tissue was observed (**Table 1**). Remarkable protein loss at lethal as well as sub lethal concentration in fish *Clarias batrachus* exposed to butachlor and concluded that the agricultural use of butachlor must be checked especially near the water bodies (Vishal *et al*., 2012).

Table 1

	Total Protein (g/wet wt. of tissue)			
Fish Tissues	**Control**	**2,4 D 80% WP**	**Butachlor 50% EC**	**2,4 D 80% WP + Butachlor 50% EC**
Gill	26.573±0.093	28.447±1.030	22.413*±0.053	33.086*±1.045
Liver	11.135±0.425	25.441*±1.275	16.179*±0.718	28.582*±0.295
Kidney	12.721± 0.109	12.485± 0.351	19.579*± 1.209	33.497*± 0.306
Muscle	5.707±0.096	4.396±0.460	3.857*±0.366	4.395*±0.151

Values are Mean ± Standard error of 3 observations. Values marked * are statistically significant (P<0.05) when compared with control.

Alkaline Phosphatase is present in all tissues of the body and the toxicant effect on tissues may show either inhibition or elevated levels of alkaline phosphatase. Alkaline phosphatase is involved in transphorylation reactions, secretion activities and also mediates membrane transport. Acid and alkaline phosphatase activity has been studied in the liver and intestine of *Labeo rohita* (Deshpande *et al*., 1999). Significant change in the alkaline phosphatase was observed in gill, liver and muscle tissue of the fish exposed to the combination of 2, 4 D 80% WP and butachlor 50% EC. In fish exposed to 2, 4 D 80% WP and butachlor 50% EC significant changes were observed in gill, liver and kidney when compared with control (**Table 2**).

DISCUSSION

The exposure to the concentrations of the herbicides revealed a significant change in the tissue biochemical parameters of freshwater fish, *Labeo rohita*. Alterations in Protein, Alanine and Aspartate transaminase in the liver and muscle tissues had also been observed in the fish, *Clarais batrachus* exposed to insecticide carbofuran (Ghousia Begum 2004). Farombi *et al*., (2008) has reported both mutagenicity and carcinogenicity demonstrated the severe risk butachlor possesses. Suggests, further restrictions on use should be considered and that actual environmental stress from this compound is investigated.

Table 2

Alkaline phosphatase (IU/l)				
Fish Tissues	**Control**	**2,4 D 80% WP**	**Butachlor 50% EC**	**2,4 D 80% WP + Butachlor 50% EC**
Gill	8.437±0.043	17.416*±0.264	19.982*±0.140	16.854*±0.098
Liver	3.827±0.015	7.188*±0.098	5.989*±0.094	4.451*±0.065
Kidney	4.862± 0.139	8.397*± 0.191	12.151*± 0.343	6.549± 0.410
Muscle	0.841±0.067	0.551±0.043	0.510±0.021	0.421*±0.013

Values are Mean ± Standard error of 3 observations. Values marked * are statistically significant (P<0.05) when compared with control.

Table 3

Aspartate transaminase (IU/l)				
Fish Tissues	**Control**	**2,4 D 80% WP**	**Butachlor 50% EC**	**2,4 D 80% WP + Butachlor 50% EC**
Gill	2.958±0.031	3.645*±0.023	5.429*±0.010	4.616*±0.014
Liver	2.855±0.022	5.901*±0.033	5.542*±0.029	5.222*±0.052
Kidney	3.181±0.029	2.828*±0.033	7.938*±0.082	9.478*±0.051
Muscle	1.594±0.014	1.310*±0.010	1.180*±0.010	0.614*±0.006

Values are Mean ± Standard error of 3 observations. Values marked * are statistically significant (P<0.05) when compared with control.

Table 4

Alanine transaminase (IU/l)				
Fish Tissues	**Control**	**2,4 D 80% WP**	**Butachlor 50% EC**	**2,4 D 80% WP + Butachlor 50% EC**
Gill	0.857±0.019	2.509*±0.011	1.377*±0.014	1.641*±0.059
Liver	0.754±0.056	4.825*±0.016	4.396*±0.008	5.431*±0.017
Kidney	0.400±0.004	0.806*±0.017	1.778*±0.040	1.313*±0.035
Muscle	0.327±0.015	0.368±0.007	0.281±0.004	0.132*±0.003

Values are Mean ± Standard error of 3 observations. Values marked * are statistically significant (P<0.05) when compared with control.

The aminotransferases are a group of enzymes that catalyze the interconversion of amino acids to 2-oxo-acids by transfer of amino groups. The level of both aspartate transaminase and alanine transaminase becomes elevated or decreased based on the nature of the damage to the cells.

Alanine transaminase is more liver specific enzyme and its elevations persist longer than the Aspartate transaminase. Measurement of both gives value in assessing the toxicant impact on the organism. Significant changes of Aspartate transaminase was observed in gill, liver, kidney and muscle of the fish exposed to combination (2, 4 D 80% WP + butachlor 50% EC), butachlor 50% EC and 2,4 D 80% WP when compared with the control (Table 3).

Significant change in alanine transaminase was observed in gill, liver, kidney and muscle of the fish exposed to combination of 2, 4 D 80% WP + butachlor 50% EC. Whereas, significant changes were observed only in gill, liver and kidney in fish exposed to butachlor 50% EC and 2,4 D 80% WP when compared with the control (Table 4).

Stress signs in the form of behavioral changes were observed in freshwater fish *Heteropneustes fossilis, Clarias batrachus, Channa punctatus* and mosquito larvae (*Culex pipiens fatigans*) exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-chloro-2,6-diethyl-N-(butoxymethyl) acetanilide (Butachlor) and both types of organisms was recommended as good bioindicator for the risk assessment of aquatic environment (Farah *et al* 2004). Xue *et al*., (2014) has reported butachlor residues below the maximum residue limits set by China (0.5 mg/kg) and Japan (0.1 mg/kg). For 2, 4 D the pesticide residues in food and feed is 0.01 to 400 mg/Kg according to codex alimentarius (FAO/WHO standards, 2013). Groundwater in the lower Central and the lower North eastern region of Thailand was contaminated with pesticides residues, in many cases in concentration above 0.1 µg/l safety limit set by the EU. In the lower Central region during the rainy season in 2001, 68% of the total groundwater samples were contaminated with endosulfan and other insecticides, in concentration ranging from 0.02 to 3.2 µg/l, and paraquat, 2,4-D, butachlor, atrazine and metribuzin herbicide residues ranging from 0.02 to 18.9 µg/l (Tirado *et al*., 2008). Very low levels of residue detected in rice grain below the maximum residue limit of 0.5 mg/kg of butachlor (Rama Lakshmi *et al*., 2012). Pesticide use in India is regulated by the Central Insecticides Board and Registration

Committee (CIBRC) and the Food Safety and Standards Authority of India (FSSAI). The CIBRC registers pesticides for crops while the FSSAI sets the maximum residue limits of pesticides for the crops registered (Chandra Bhushan *et al*., 2013). Though MRL (maximum residue limit) value is 0.01 mg/kg for 2, 4 D, MRL data is not available for butachlor herbicide hence the extensive usage of these herbicides should be made with caution.

CONCLUSION

From the present study it is inferred that sub lethal concentration of the herbicide butachlor 50% EC and 100 mg/L limit test concentration for 2, 4 D 80% WP, and its combination had an adverse effect on the fish enzymes such as total protein, transaminases and alkaline phosphatase. The usage of this pesticide should be done cautiously near the aquatic resources since it will lead to environmental stress to the aquatic species.

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