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Research Article

Acute exposure of Pyrazosulfuron Ethyl induced Haematological and Blood Biochemical changes in the Freshwater Teleost fish *Oreochromis mossambicus*

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Abstract

Buildup of agriculture pesticides in the aquatic habitat through natural run-off is a major problem faced by developing and developed countries. The present study was designed to evaluate the 96 hr LC_{50} of herbicide, Pyrazosulfuron-Ethyl which belongs to Sulfonylurea group. Using static bioassay with continuous aeration under laboratory conditions, acute toxicity of the herbicide was determined for fresh water fish *Oreochromis mossambicus*. Hematological and biochemical parameters are used as health indicators to detect the functional status of fish under acute exposure. Hence, biochemical parameters like plasma glucose, protein, albumin and globulin were also studied to evaluate the toxic potential of the herbicide. The herbicide led to significant changes in the hematological parameters such as RBC count, Hb, PCV, MCH, MCHC, MCV and WBC count and biochemical parameters. These alterations can be used as non specific biomarkers in herbicide contaminated aquatic ecosystem.

Keywords: Pyrazonsulfuron-ethyl (PE), *Oreochromis mossambicus*, Haemetology, Blood biochemistry.

Introduction

Pesticides are essential for stable and proficient agricultural crop production; however, pesticides used on arable lands can be transported to waterways. Pesticide which are applied on agricultural land, upto 90% of this never reach the intended targets (Sparling *et al*., 2001) as a result, many other non-target organisms sharing the same environment are in disguise unintentionally poisoned. Water bodies became illegally the end point of the discharge of pesticides. Public concern about the adverse effects of pesticides on aquatic organisms, and bioaccumulation in fish and other aquatic invertebrate is increasing; therefore, there have been many monitoring surveys and research on pesticides in freshwater system (Iwafune *et al*., 2011). One of the non-target biological groups mostly affected by pesticides is fishes (Velmurugan, 2007; Majumdar and Gupta, 2009). Contamination of water by pesticides either directly or indirectly can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable toxicant in fresh water edible fish tissue which can affect the health of humans consuming these fishes (Adedeji *et al*., 2009.).

Sulfonylurea herbicides are an important class of herbicides used worldwide for controlling weeds in all major agronomic crops. Among sulfonylurea products, PE herbicide is widely used for selective post-emergence control of annual, perennial grasses and broad-leaved weeds in cereals, and is currently recommended for use on some relevant crops in over 30 countries (Singh *et al.*, 2012; Giovanni *et al*., 2011). Due its widespread use, it has become a potential water pollutant and presents environmental risk, especially for aquatic organisms, owing to its fairly high water solubility which result in its high mobility. It has been detected in surface and groundwater (Battaglin *et al*., 2000). Phytotoxicity of chlorsulfuron, sulfometuron-methyl and metsulfuron-methyl has been reported for higher plants (Sabater and Carrasco, 1997). Toxicity of triasulfuron on aquatic organisms has been reported earlier (Baghfalaki *et al.*, 2012). However, the toxic potential of PE on fresh water teleost *O.mossambicus* is lacking.

Acute toxicity test usually provide estimates at the exposure concentration causing 50% mortality (LC_{50}) to test organisms during a specified period of the time. The application at LC_{50} has gained acceptance among toxicologists and is generally the most highly rated test for assessing potential adverse effects of chemical contaminats to aquatic life. The exposure of fish to chemical agents induce changes in several hematological variables (Heath, 1995), and are recurrently used to evaluate fish health (Martinez and Souza, 2002). The study of blood parameters in fishes has been extensively used for the detection of physiological alterations in different conditions of stress (Pathak *et al*., 2013 and Parikh *et al*., 2014) Hematological parameters such as hematocrit, hemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Sancho *et al*., 1997; Adedeji *et al*., 2000). Moreover, haematological and biochemical parameters are used as health indicators to detect the structural and functional status of fish under stress condition (Ramesh and Saravanan, 2010). In recent years, biochemical variables are used more to determine the effects of external stressors and toxic substances. Therefore, the biochemical evaluations are gradually becoming a routine practice for determining the health status in fish (Padma *et al*., 2012). Hence in the present study an attempt is made to have an insight to the toxicity deviations on the hematological as well as biochemical alteration on *O.mossambicus* on acute exposure of the herbicide.

Materials and Methods

Experimental design

The freshwater fish, *O. mossambiccus* of similar size in length (12 ± 2 cm) and weight (25 ± 1.9 g) were brought from a local pond of Baroda district and were acclimatized at laboratory conditions for 10 days in well aerated test aquaria containing de-chlorinated water. They were fed with commercial fish pallets. 30% water was renewed every day to provide freshwater, rich in oxygen. To evaluate the acute toxicity of the PE herbicide 96-hour LC_{50} values were determined. The day before and during the tests the fish were not fed. For each concentration, 10 fish were tested and the experiment was repeated thrice. Probit analysis (Finney, 1971) was followed to calculate the LC_{50} values.

Experimental Procedure

The experiments were conducted in a series of glass aquariums filled with 40 liter de-chlorinated tap water. Healthy fishes *O. mossambicus* was selected for the test (n=10) to determine the LC_{50} value of each fish. Based on the pilot experiments, the experiment was conducted to determine the toxicity in different concentrations. The concentrations used included 50 mg/l, 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, 500 mg/l, 600 mg/l, 700 mg/l, 800 mg/l and 900 mg/l and 1000 mg/l with three replicates each. The stock solutions were prepared and the required quantity of PE was drawn from the stock solution to find out the LC_{50} values for 96 h. Group 1 served as control, while Group 2, 3, 4 and 5 were treated with PE with concentration 50 mg/l, 100 mg/l, 200 mg/l and 400 mg/l respectively. Ten acclimatized fishes of uniform size were exposed to each concentration. The control and the exposed fish were aerated frequently to prevent hypoxic condition of the medium. Feeding to fishes was stopped during the experiment. Mortality of the fish was recorded from time to time till 96 hours.

Haematological estimation of fish

After the completion of 96 hr acute toxicity Test, fishes were collected from each aquarium for blood analysis. About 3 - 4ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes. The blood was stored in -4°C prior to estimation of hematology. Haemoglobin estimation (HB), Pack Cell Volume (PCV), blood glucose level and total serum protein were analyzed by NIHON KOHDEN Automated Hematology Analyzer (Celtac alpha, Japan). Red blood cell count (RBC), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) was determined using the formulas given below.

MCHC = HB/PCV*1000 g/dL

MCV =PCV*1000/ RBCs fL

MCH = HB/RBCs pg

Statistical analysis

LC_{50} value was determined by Probit analysis using StatPlus 2009 Professional, 5.8.4.version software. Statistical analysis was performed using Graph pad prism 6 software. The data was analyzed using one-way ANOVA test. Results were presented as mean ± SD. The significance was set as $P<0.05$, $P<0.01$ and $P<0.001$.

Results

Table I shows the relation between concentration of PE and mortality rate of fish. The LC_{50} values according to Probit regression curves was found to be 501.65 mg/l, however the Lower Confidence Limit (LCL) value and Upper confidence limit (ULC) were 407.83 mg/l and 595.47mg/l respectively (Fig. 1 & 2). While the LC_{10}, LC_{16}, LC_{84} and LC_{90} were found to be 95.68mg/l, 184.91 mg/l, 818.38mg/l and 90-907.62mg/l respectively.
A significant increase ($p<0.01$) with 50 and 100mg/L dosage in the values of RBC count, HB, and PCV was obtained in the exposed group compared to control (Table – 2). On the other hand MCV showed an insignificant increase while MCHC showed a significant decrease with insignificant alteration in MCH. There were no significant changes in the parameters at higher doses (Table 2, Fig. 3). WBC count and blood glucose showed a significant ($p<0.05$, $p<0.01$) increase compared to control groups (Table-2, Fig-3 & 4). Serum protein level showed a dose dependant significant increase ($p<0.05$) in the experimental groups compared to the control. There was a significant increase ($p< 0.05$) in the globulin and decrease ($p<0.05$) in albumin values (Table 2, Fig. 4).

Discussion

PE, a new rice herbicide belonging to the sulfonyl urea group has recently been registered in India for weed control in rice crops (Singh *et al*., 2012).Several studies indicate that these group of herbicides on leaching enters ground water and tend to persist (Battaglin *et al*., 2000). However once it enters surface waters it may affect other organisms such as fish as a non-target organism in natural conditions (Aktar *et al*., 2009).From the LC_{50} value detected in the present study PE can be categorized into least toxic compound.

Blood is a pathophysiological reflector of the whole body and therefore, blood parameters are important in diagnosing altered physiological status of fish exposed to toxicants (Adhikari *et al*., 2004). PE exposure resulted into a significant increase in the hematological parameters: RBC count, haematocrit (PCV), and HB compared to control. Maximum alteration was noticed at 50 mg/l, followed by a decrease. The initial increase could be due to increased hypoxia (Rifkind *et al*., 1980 and wepener *et al*., 1992). Liver through activating erythopoiesis is probably preventing the physiological hypoxic

Table 1. The relation between concentration of PE and mortality rate of *O. mossambicus*

Concentration	Actualpercent%	Log10 conc.	Total no.	No. dead	Probit
Control	-----		10	0	-
50	0.025	1.699	10	0	3.0396
100	0.1	2.0	10	1	3.3183
200	0.2	2.301	10	2	4.1585
300	0.3	2.4771	10	3	4.476
400	0.4	2.6021	10	4	4.7471
500	0.5	2.699	10	5	5.0
600	0.6	2.7782	10	6	5.2529
700	0.7	2.8451	10	7	5.524
800	0.8	2.9031	10	8	5.8415
900	0.9	2.9542	10	9	6.2817
1000	0.975	3.0	10	10	6.9604

Table 2. Haemetological Parameter, Blood Glucose and Total Protein in *O.mossambicus* affected by acute exposure of PE

Parameters	Concentration mg/l of PY (Mean ± SD)				
	Control	50mg/l	100mg/l	200mg/l	400mg/l
RBCs 10^6/μL	1.04± 0.025	1.90±0.027	1.69 ±0.023	1.40±0.028	1.28±0.022
HB g/dL	5.3±0.154	9.7±0.159	8.6±0.152	6±0.157	5.80±0.162
PCV(Htc)%	14.6±0.555	29.6±0.551	32.6±0.558	23.4±0.557	19±0.554
MCV fL	140.38±3.52	155.79±3.56	192.9± 3.54	144.44±3.57	142.42±3.51
MCHC g/dL	36.3±1.03	32.77±1.03	26.38±1.03	34.62±1.03	31.3±1.03
MCH pg	50.96± 1.66	51.96± 1.68	50.89± 1.64	50± 1.65	53.54± 1.69
TotalWBC10^3/μL	45,000± 655	151,600±653	109,500±653.64	79,600±657.89	76,800±652.23
Glucose	138±3.162	215± 3.113	322±3.15	133±3.19	222±3.14
Protein	10.3± 0.462	11.9± 0.471	12.7± 0.469	14.3± 0.465	16.5±0.473
Albumin	5.64± 0.48	3.4± 0.49	2.40±0.48	4.80±0.47	5.70±0.48
Globulin	6.3±0.354	6.9±0.355	5.50±0.357	8.00±0.360	10.8±0.359

Figure 1. Plot of adjusted probits and predicted regression line of PE to *O.mossambicus*

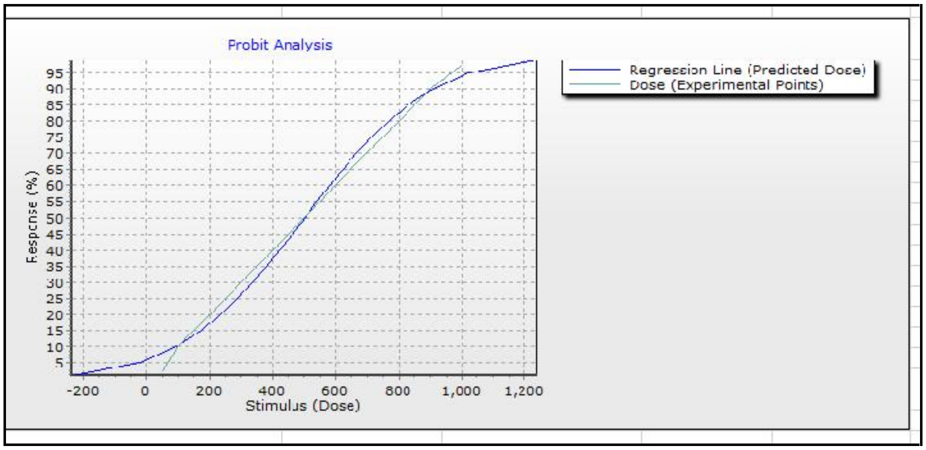


Figure 2. Plot of adjusted probits and predicted regression line of PE to *O.mossambicus* in log10base

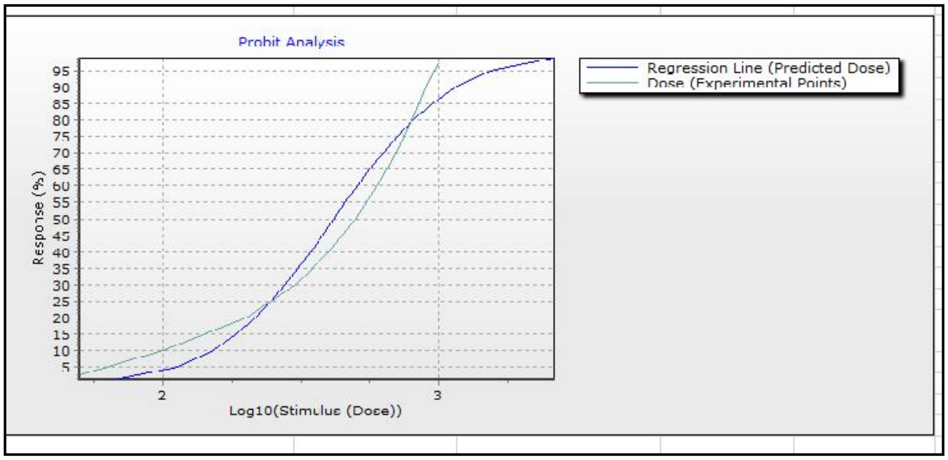
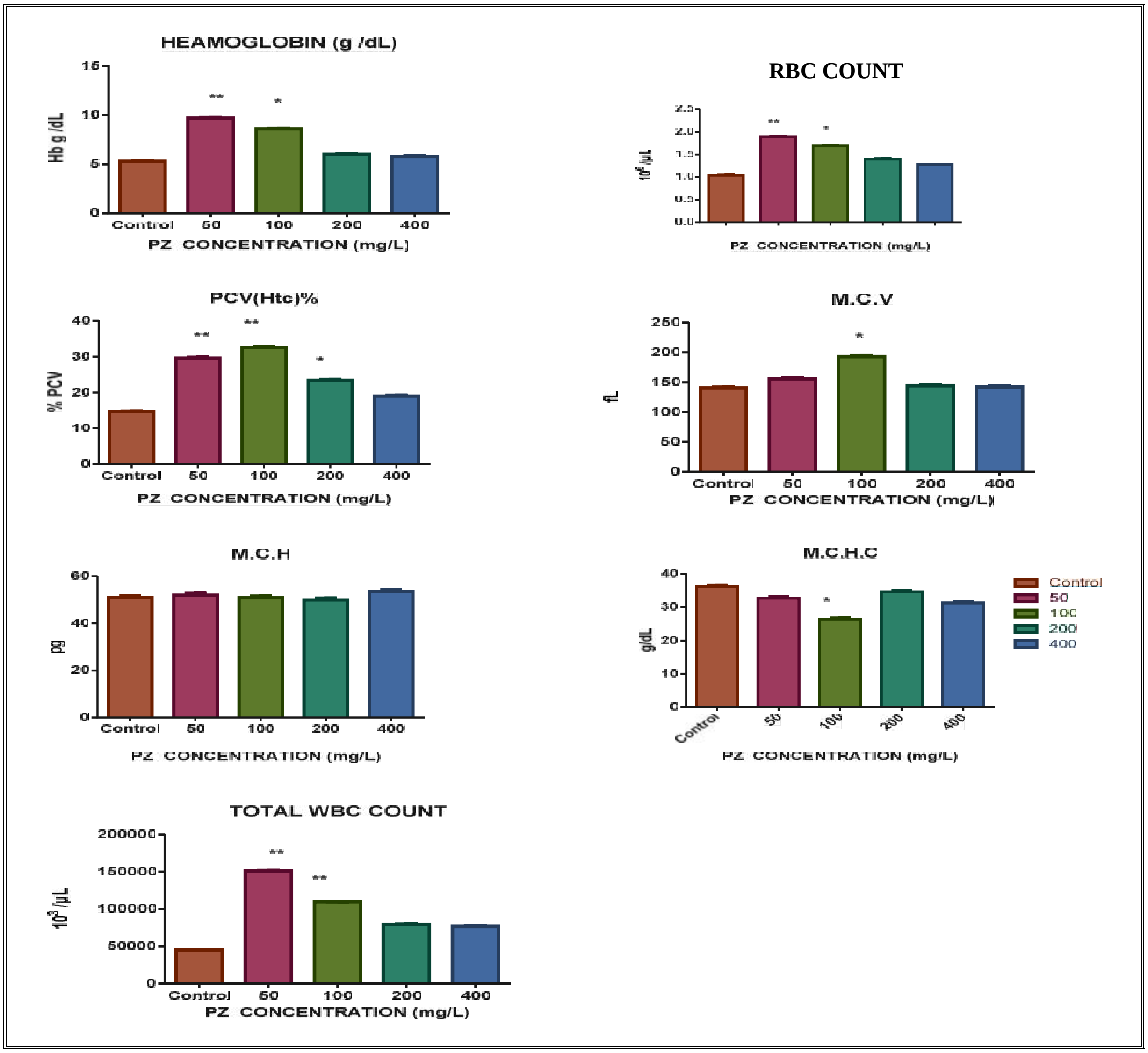
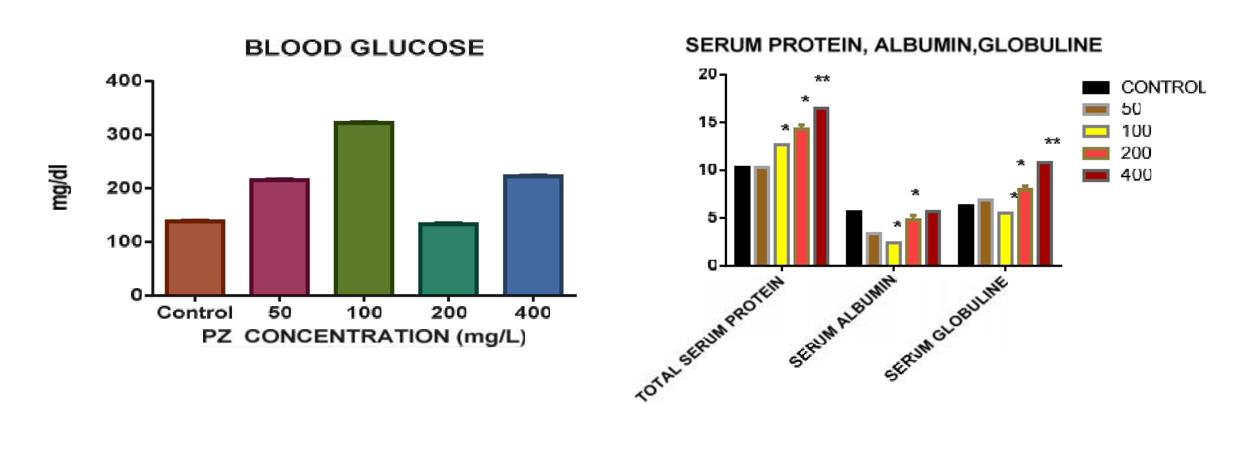


Figure 3. Changes in Haemetological Parameter of PE treated fish *O.mossambicus*.



Values are mean ± SD of five individual observations, Values are significant at (*) p<0.05, (**) p<0.01

Figure 4. Changes in Blood Glucose and Total Serum Protein of PE treated fish *O.mossambicus*



Values are mean ± SD of five individual observations, Values are significant at (*) $p<0.05$, (**) $p<0.01$

condition as this mechanism work well for the short-term variation in oxygen concentration in blood (Nespolo and Rosenmanm, 2002). Gills are the first organ to come in contact with the toxicant, damaging the gills and impairing the oxygen transport. The increase in PCV is likely to be due to either gill damage or due to increased metabolic demand or both (Varadarajan, 2010). It has been shown that the erythrocyte number and haemoglobin level may vary with oxygen requirements (Hubrec *et al*., 2000; Tavares *et al*., 2004). Similar results were found in reports of acute intoxication by dichlorvos in *Clarias batrachus* (Benarji, 1990), by quinalphos in *O. mossambicus* (Sampath, 1993). Parallel with an increase in the RBCs, WBC also showed a significant increase. Joshi and his co worker (2002) are of the views that increase in WBC count is suggestive of an increase in antibody production for survival and recovery of the fish exposed to pesticides, lindane and Malathion. Thus, the increased WBC counts indicate hypersensitivity of immune cell resulting into immunological reactions to produce antibodies to cope up with the stress induced by PE (Ramesh and Saravanan, 2008).

Biochemical analysis provides valuable information for monitoring the health condition of fishes. Biochemical variations depend on the fish species, age, sexual maturity and health condition. Analysis of glucose concentration in blood is widely used as indication of stress response. Studies have also reported blood glucose to be a sensitive indicator of environmental stress in fish. By and large glucose is continuously required as an energy source by all body cells and therefore must be maintained at adequate levels in the plasma. In the present study the significant increase in glucose may be the manifestation of stress induced by herbicide was seen. The increase of glucose can be interpreted as a consequence of glycogenolytic activity of catecholamines and gluconeogenetic effect of glucocorticoids as in response of an organism to the stress induced by PE. Our results are agreement with the earlier work of Ramesh and Sarvanan (2008).

Proteins are mainly involved in the structural architecture of the cell. During stress conditions fish need more energy to detoxify the toxicant to overcome pesticide trauma. PE exposure resulted into a dose dependent increase in the proteins. Stress increases the physiological activity which in turn will demand mobilization of proteins to meet the energy required. To overcome the stress there is an increase in the protein synthesis (Martinez *et al.*, 2004;Sweety *et al.*, 2008). Furthermore, serum protein mainly contains albumin and globulin. Albumin is thought to have three basic functions in fish: osmotic regulation of blood volume, source of protein reserve and is also involved in transport functions of exogenous chemicals and endogenous metabolites (Andreeva, 1999; Baker, 2002). Hence the significant decrease in the albumin is probably equipping the fish for the removal of the PE; being an exogenous chemical. However there is an overall increase in the proteins which may be due to concomitant increase in the globulin.

Hence, from the present study the mild toxic nature of the herbicide PE is apparent by the significant changes in the hematological and biochemical changes in the blood, and that the fresh water fish *O. mossambicus* are sensitive to herbicide. The alterations of the parameters may provide the early sign for the determination of acute toxic level of herbicide and their effects on aquatic medium. The findings of present study also provide a better understanding of toxicological endpoint of aquatic pollutants and safer level of these herbicides in the aquatic environment and protection of aquatic habitats.

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