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RESEARCH ARTICLE

EFFECT OF HERBICIDE 2, 4-D ON HEMATOLOGICAL PARAMETERS OF *CLARIAS BATRACHUS*

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ABSTRACT

The objective of this study was to observe the effect of 2-4 dichlorophenoxy acetic acid on the haematology of Clarias batrachus fish. The haematological parameters studies such as Red Blood Cell (RBC), White Blood Cell (WBC), differential lucocytes counts (neutrophils, lymphocytes, eosinophils and monocytes), Hb %, platelets, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Packed Cell Volume (PCV). The results revealed a significant decrease in Red Blood Cell (RBC), Hb %, platelets, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Packed Cell Volume (PCV) levels but White blood cells (WBCs) count and neutrophils, eosinophils, basophils, monocytes were increased and fluctuated significantly in experimental group compare with control group. Thus on the basis of obtain result in the present investigation it can be concluded that 96 hrs. exposure of 34.64 ppm of 2-4 D aqueous solution has toxic effect and suggests that exposure of 2-4 D could cause some level of stress as indicated by changes in the haematological indices of the fish under consideration.

Key words: Haematology, 2-4 dichlorophenoxy acetic acid, Clarias batrachus, Herbicide, Toxic, Blood.

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INTRODUCTION

2,4-D (2,4-dichlorophenoxyacetic acid) is a systemic herbicide and is used to control many types of broadleaf weeds, Agricultural uses include pastureland, wheat, corn, soybeans, barley, rice, oats, and sugar cane. The toxicity of 2,4-D to fish is variable, with the ester form of 2,4-D expressing greater toxicity than other forms. 2,4-D has also been demonstrated to bio-accumulate in fish (Wang *et al*., 1994). Though agriculture products have been greatly depended on using of herbicides to control unwanted plant and weeds, however they may endanger other organisms specially when they rich to aquatic environment (Sarikaya, and Yılmaz, 2003). Floods and run off could dissolve applied herbicides and shift them into the rivers or seas. Aquatic animals may uptake herbicide from surrounding medium by different routs as nontarget organisms. Herbicides make up about 40% of the production of pesticides in the world. They have been shown to cause deleterious effects on fish health (Banhawy *et al*., 1996). Synthetic herbicides are commonly used by farmers to control weeds and nuisance aquatic vegetations around rivers, lakes and reserviours. However, these pesticides ultimately find their way to these water bodies, inducing adverse impacts on fishes living therein (Tsuda *et al*., 1997). The widespread use of herbicides has resulted in a steady increase in water pollution, evoking considerable damage of phytoplankton and zooplankton, thus depleting essential sources of the food chain for fish (Montanes *et al*., 1995). According to Musa and Omoregie, (1999) fish are intimately associated with the aqueous environment, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish. The use of haematological technique in fish culture is growing in importance for toxicological research, environmental monitoring and fish health conditions. Many works has been conducted on haematological changes of pesticides in the fish such as Das and Mukherjee (2000) Adebayo *et al*. Non biodegradable compounds even can enter food chains and affect people who consume contaminated foods. Fish in close association with their aquatic environment and any changes in this environment would be reflected in alterations in their haematological studies (Huges *et al*., 1979, Suzana Golemi, *et al*., 2012). Hematological parameters are important for reflecting the pathophysiological status of a fish. These parameters have been widely used as indicators of disease or stress due to pollutants Blood is an important component for studying the effects of toxicants as it is highly susceptible to environmental fluctuations (Pandey and Pandey, 2001). Blood is a pathophysiological indicator of the body as it is highly susceptible to internal and external environmental fluctuations in stress conditions. It is affected by the toxic pollutants that

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have gained momentum in recent years and are in fact important diagnosing tools to investigate disease or stress in fish (Seth and Saxena, 2003). The current investigation was undertaken to investigate Effect of herbicide 2, 4-D on hematological parameters of *clarias batrachus*

MATERIAL AND METHOD

Experimental animal: Healthy *Clarias batrachus* were used as a experimental animal and it is was collected from local fish market & acclimatized to the laboratory for one week during which they were regularly feed with prawn powder & soya meal.

Test chemical: 2-4 D was used as a test chemical. Test fishes were exposed to sub-lethal doses (34.64 ul/l) for maximum 96 hrs.

Experimental design: In the present investigation experimental fishes were divided into two groups.

- **Control group:** In this group 10 fishes were kept and exposed to normal water.
- **Experimental group:** In this group 40 fishes were exposed to 34.64 µl concentration of 2-4 D solution.

Experimental duration: In both control and experimental group fishes were exposed to maximum 96 hrs.

Autopsy: Fishes of control and experimental groups were sacrificed at 0 hrs. 24 hrs, 48hrs, 72 hrs and 96 hrs. Blood collected by cardiac puncture of *Clarias batrachus* then processed for various haematological tests.

Haematological analysis

RBC & WBC Counting: RBC &WBC counting were done by Manual method (Sharma and Singh, 2000).

Differential Leukocytes counting: DLC Counting was done by Leishmann method (Sharma and Singh, 2000).

Haemoglobin % Analysis: Hb% analysis was done by Sahil's method (Sharma and Singh, 2000).

Platelets, MCV, MCH & PCV counting: Platelets, MCV, MCH & PCV counting were done by Manual method (Sharma and Singh, 2000).

RESULTS

In the present investigation haematological estimation of control and experimental fish were done. The haematological parameter were RBC, WBC, DLC (Neutrophiles, Eosinophiles, Lymphocytes, Basophiles and Monocytes), platelets, PCV, MCV and MCH. In control haematological values (Tables and Figures) were RBC (3.49 million/ml), WBC ($9.70x10^9$cells), Hb (9.50 g/dl), Neutrophiles (30.45%), Eosiniphiles (11.68 %), lymphocytes (52.43%), Basophiles (8.31 %), Monocytes (5.06 %), Platletes (110.0%), PCV (28.35 %) MCV (96.03 fl) and MCH (44.36.00 pg). In the present investigation at the 24 hrs. haematological values were RBC (2.23 million/ml), WBC ($10.60 x10^9$cells), Hb (9.00 g/dl), Neutrophiles (32.67 %), Eosiniphiles (13.01%), lymphocytes (59.21%), Basophiles (9.00%), Monocytes (6.32 %), Platletes (100.06%), PCV (25.00%), MCV (90.45 fl) and MCH (39.63 pg).

Table. Hematological changes in *clarias batrachus* due to 2-4 D herbicide

Peramaters	Control value	Experimental value			
		24 hrs	48 hrs	72 hrs	96 hrs
RBC (million/ml)	3.49	2.23	2.07	1.75	1.48
WBC (cells/cmm)	9.70	10.60	11.70	12.79	13.75
Hb% (g/dl)	9.50	9.00	7.03	5.62	3.68
Neutrophiles (%)	30.45	32.67	31.00	28.13	24.40
Eosinophiles (%)	11.68	13.01	13.09	09.32	8.22
Lymphocytes (%)	52.43	59.21	60.02	50.14	48.23
Basophiles (%)	8.31	9.00	9.20	8.00	7.90
Monocytes (%)	5.06	6.32	6.90	5.01	4.10
Platelets (%)	110.0	100.6	99.02	95.32	89.23
PCV (%)	28.35	25.00	20.53	17.11	13.24
MCV (fl)	96.03	90.45	80.30	73.01	67.40
MCH (gm/l)	44.36	39.63	35.98	29.16	22.96

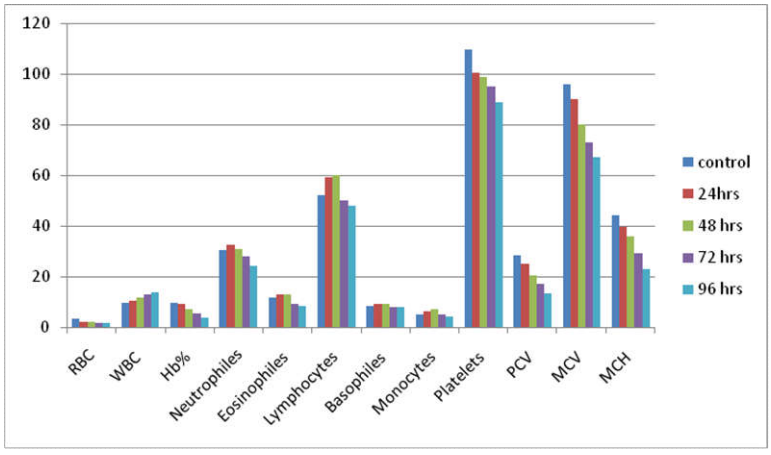

Figure. Hematological changes in clarias batrachus due to 2-4 D herbicide

In the present investigation at the 48 hrs haematological values were RBC (2.07 million/ml), WBC (11.70 x10^9cells), Hb (7.03 g/dl), Neutrophiles (31.00 %), Eosiniphiles (13.09%), lymphocytes (60.02 %), Basophiles (9.20 %), Monocytes (6.90 %), Platletes (99.02%), PCV (20.53%), MCV (80.30 fl) and MCH (35.98 pg). In the present investigation at the 72 hrs haematological values were RBC (1.75 million/ml), WBC (12.79 x10^9cells), Hb (5.62 g/dl), Neutrophiles (28.13 %), Eosiniphiles (9.32 %), lymphocytes (50.14 %), Basophiles (8.00 %), Monocytes (5.01 %), Platletes (95.32%), PCV (17.11%) MCV (73.01 fl) and MCH (29.16 pg). In the present investigation at the 96 hrs haematological values were RBC (1.48 million/ml), WBC (13.75 x10^9cells), Hb (3.68 g/dl), Neutrophiles (24.40 %), Eosiniphiles (8.22 %), lymphocytes (48.23 %), Basophiles (7.90 %), Monocytes (4.10 %), Platletes (89.23%), PCV (13.24 %) MCV (67.46 fl) and MCH (22.96 pg). RBC, Hb, Platelets, PCV, MCV and MCH values were decreased as compared to control value at 24, 48, 72 and 96 hrs. WBC value were increased as compared to control at 24, 48, 72 and 96 hrs. Neutrophil, Basophiles, Eosinophiles, Lymphocytes and Monocytes values increased at 24 and 48 hrs and than decreased at 72 and 96 hrs as compared to control value.

DISCUSSION

In recent years hematological variables have been used more to determine the sub lethal concentrations of pollutants (Wedemeyer and Yasutake, 1977). The use of immune system parameters to assess alterations in fishes experiencing 2,4-D exposure and interest in defense mechanisms stem from the need to develop healthy management tools support a rapidly growing aquaculture industry (Jones, 2011). In this study significant changes were noticed in blood components of treated fish. These changes included reduction in the red blood cells count, Hb level, Platelets, PCV, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values. On the other hand white blood cells increased and the Differential Leucocytes Count (neutrophils, eosinophiles, lymphocytes, basophiles and monocytes) value showed fluctuations as a result of 2-4 D exposure. The present study shows the high toxic nature of 2, 4-D on fish, the fishes are very sensitive to the presence of even minute quantities of 2, 4-D and are under server metabolic stress.

This study also shows the significance of hematological parameters in assessing the pesticide hazards to fish. In the fish *clarias batrachus* exposed to 2, 4-D hemoglobin percentage decreased significantly. This may be due to a decreased rate of production of Red blood cells or an increased loss of these cells. Gill and Epple (1993) have attribute anemia to: (I) impaired erythroposis due to a direct effect of metal on hematopoietic centers (Kidney/Spleen), (ii) accelerated erythroclasia due to altered membrane permeability and/pr increased due to altered membrane permeability and/pr increased mechanical fragility, and (iii) defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions. The reduction in Hb and several other blood components might be due to the inhibition of RBCs' and haem synthesis, osmoregulatory dysfunction and destruction of RBCs in hematopoietic organs as reported earlier in *Catla catla* (Vani *et al.*, 2011). Kavitha *et al.* (2012) observed a significant reduction in Hb, and RBC levels in fish exposed to *Moringa oleifera* seed extract. White blood cells play a major role in the defense mechanism of the fish and consist of granules. Monocytes, lymphocyte and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies (Ellis *et al.,* 1978; Wedember and McLeay, 1981). Allen, (1994) observed increased WBC (leucocytes) counts in Oreochromis oureus after 2, 4-D exposure. The increase in WBC observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by 2,4-D. Gill and Pant, (1985) have reported that the stimulation of the immune system causes an increase in lymphocyte by an injury or tissue damage. Total WBC count and leucocratic increased in *Tinca tinca* exposed to lethal and sub-lethal treatments with 2, 4-D (shah and altindag, 2005).

The Differential Leucocytes Count (DLC) value showed fluctuations. In this study neutrophils, monocytes and eosinophils increased whereas lymphocytes and basophils decreased in monogenean infected fishes. Similar results were found in helminth infected *Schizothorax* spp. and *Cyprinus* spp. (shah *et al*., 2009). In the light of the present study, the mean value of PCV decreased progressively in the experimented group compared to the control. The result agreed with the work of Akinrotimi *et al.* (2009) in haematological indices of *Tilapia guineensis* subjected to handling stress. The decrease in the PCV indicates the worsening of the condition of the organism and developing of anaemia. Platelets are nucleated cells which are responsible for blood clotting in fish; slight decrease in values observed in this study may signify the effect on platelet (thrombocyte) production.

A significant decrease in PCV value and haemoglobin concentration can be interpreted as a compensatory response that reduces the oxygen carrying capacity to maintain gas exchange in the damaged gill lamellae. Our results are in line with those found by other authors, who assessed the effects of selected pesticides on haematological profile of fish blood (Velisek *et al*., 2009; Velisek *et al*., 2008 ; Ramesh *et al*., 2009 Jee *et al*., 2008). Shamoushaki *et al*. reported decreased RBC, Hb, PCV and MCH in male brood stock (*Rutilus frisii kutum*) after long-term exposure to diazinon. In the present investigation 2-4 D (34.64 ul) exposure for 96 hrs to *Clarias batrachus* was found toxic as it altered rather decreased the red blood cells count, Hb level, Platelets, PCV, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values, flucluated Differential leucocytes count and increased the WBC. Thus the results of present study corroborate with observation of previous authors.

Conclusion: The present study showed that 2,4- D is clearly toxic to cat fish *clarias batrachus*. The exposure of fish to this herbicide resulted in significant reduction in the studied haematological parameters. These alterations may negatively suppress normal growth, reproduction, immunity and even survival of fish in natural environment as well as culture conditions. This should be considered when farmers use 2,4-D to control weeds in their fields. Replacing of 2-4 D with less harmful and more biodegradable pesticide is recommended.

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