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# Effects of Herbicide, Glyphosate on Haematological Profile of *Clarias batrachus* (Linn.)

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## ABSTRACT

The glyphosate ($C_3H_8NO_5P$) is an herbicide widely used by farmers in agriculture to eradicate weeds. The present study includes the airbreathing fish, Clarias batrachus (Linn.) induced to sublethal concentration (2.46 mg/L) of inorganic herbicide, glyphosate for 30 days showed haematological profile alterations. In the treated group significant changes were observed in the haematological profile. The parameters Hb, RBC and WBC were decreases while in DLC, Neutrophil, Monocytes and Eosinophil an increases were observed. The present study concluded that Clarias batrachus is susceptible to herbicide, glyphosate at very low concentration. So, it is suggested that water contamination with glyphosate concentration of less than 2.46 mg/L in fish culture pond may be suitable for optimum growth performance.

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*Keywords: Clarias batrachus; herbicide; glyphosate; haematological profile.*

## 1. INTRODUCTION

"Water pollution by agriculture waste discharge has become one of the most considerable problems in the world" [1]. "Pesticide is one of the major agriculture waste toxicant of aquatic environment such problem considered by researchers worldwide" [2]. "Pesticides are becoming threats to aquatic organisms especially fish. Fish are more susceptible to these pollutants" [3].

Glyphosate Glyphosate (N-phosphonomethyl-glycine) ($C_3H_8NO_5P$) is an herbicide widely used to eradicate weeds from agricultural fields [4]. "It acts on plant by attached with phosphoenolpyruvate, the substrate of EPSP synthase, and subsequently inhibiting synthesis of aromatic amino acid via the shikimate pathway" [5,6]. It is an organo phosphorus based post emergence herbicide and used for the control of broad-spectrum of plants. It can easily ionize as anions in water and can be adsorbed to sediments of pH > 3.5 [7].

*Clarias batrachus* (Linn.), locally known as "Mangur"*,* is a highly nutritious easily digestible prescribed to pregnant women, anaemic patient and children diet [8]. It is an airbreathing fish cultured in stagnant water and recognized as integral part of paddy field culture of the country [9].

The present investigation undertaken was to understand the chronic toxicity impact on the haematological profile in freshwater fish, *Clarias batrachus* exposed to herbicide, glyphosate*.*

## 2. MATERIALS AND METHODS

The air-breathing fish, *Clarias batrachus* has average length (10–12 cm) and weight (30–34 g) was procured live from the local fish market, Darbhanga. It was washed with 0.1% $KMnO_4$ solution to remove dermal infection if any and acclimated for 15 days to laboratory conditions. Fish was fed commercial feed (containing 28% crude protein), ration rate 3% of body weight in the morning (10.00 AM) and follows the methods of APHA [10].

The $LC_{50}$ values of glyphosate were determined according to static acute bioassays and were calculated by the Finney method [11]. The mortality was recorded after 24, 48, 72 and 96 h, the $LC_{50}$ values for the above periods with concentration of glyphosate obtained 34.7 mg, 31.9 mg, 27.4 mg and 24.6 mg respectively. The sublethal concentration was obtained by 1/10th value of the $LC_{50}$ value for 96 hr by method follow Sprague, [12]. A treated group of twenty fish was obtained after exposure of sub-lethal concentration (2.4 mg) of glyphosate for 30 days. Simultaneously the control group of twenty fish was maintained for same period of time. On 30$^{th}$ day the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. At the end of exposure period blood samples were extracted from the caudal vien of the test fish and estimation of haemoglobin, RBC, WBC, Lymphocytes, neutrophil, monocytes, basophil, eosinophil and determination of PCV (packed cell volume), MCV, MCH and MCHC levels were carried out by method Akela et al*.* 1996 and Shrivastav, [13] For the MCV, MCH and MCHC calculation standard formulae of Dacie and Lewis, [14] was followed: MCV (fl) = [PCV (%) x 10] / [RBC count in millions/mm$^3$], MCH (pg) = [HB (g/dl) x 10] / [RBC count in millions/mm$^3$] and MCHC (g/dl) = [HB (g/dl) x 100] / [PCV (%)]

## 3. RESULTS AND DISCUSSION

The Table 1 contains the result of present experiment revealed the haematological profile alteration. The fish, *Clarias batrachus* induced to glyphosate (2.4 mg/l) sublethal concentration for 30 days showed alteration in haemoglobin. The control fish group Hb value was 10.85 ±0.05 gm/dl and treated group had 6.72±0.10 gm/dl. A highly significant (P<0.001) decreases was observed in haemoglobin (Hb) in treated fish group. The haematological parameters has found distinguishable variable as decreases was observed in haemoglobin (Hb) (6.72±0.10 gm/dl) in present study (Fig. 1) was conformity with findings of Jerald and Saradhamani, [15] have observed decrease value in the Hb of fish, *C. catla* exposed to glyphosate. Arjun et al*.* [9] have observed similar decrease in the level of haemoglobin and showed highly significant (P<0.001) in the chromium exposed fish, *Clarias batrachus*. Similar haematological alterations results were observed by earlier workers with various toxicants treated fish; Hb decline was reported by Revathi et al*.* [16], Shipra et al*.* [17], Bruska et al*.* [18], Anwar and Choudhary [19]. Roy and Nath, [20] also reported similar haematological changes in case of Thiamethoxam treated *Oreochromis niloticus.*

In the control fish group RBC count was 6.32±0.05 x $10^6$ cell/$mm^3$ and treated fish group had RBC 5.1±0.05 x $10^6$ cell/$mm^3$of blood. A highly significant value (P<0.001) decreases was observed in treated group. The present investigation finding supported by Raizada and Gupta, [21] have found the decrease in RBC count and haemoglobin in the *Trichogaster fasciatus* induced to the fungicide RH-216. Haematological profiles have been recognized as indicator of stress induced by pesticides and infection of parasites, and variation in RBCs count and haemoglobin concentration was due to deleterious effect of toxicants on the erythropoietic tissue of *Mystus vittatus* [22]. Mishra and Srivastava, [23] have observed that a decrease in RBC count from 6,400,000 to 3,460,000/cm in *Heteropneustes fossilis* induced to malathion 7.6 mg/l in 96 hr. Muthalagi [24] has reported a decrease in RBC count in IMC, *C. mrigala* exposed to sewage treatment. The present study showed also conformity with Arjun et al. [9] reported that a decrease in RBC count in airbreathing fish, *C. batrachus* exposed to chromium. A decrease in RBC in the *H. fossilis* (Bloch) induced to mercury chloride was reported by Pratibha and Kumar [25].

In the control fish group Neutrophil was 5.85±2.05 x$10^3$ cell/$mm^3$ and treated group had 12.82±0.05x$10^3$ cell/$mm^3$. A highly significant value (P< 0.001) increase was observed in treated fish group of present study.

In the control fish group Monocytes was 4.2±0.05 x$10^3$ cell/$mm^3$ and treated group had 7.6±0.05 x$10^3$ cell/$mm^3$. A non significant value (P<0.05) increase was observed in treated group.

The value of Eosinophil in control group was 2.3±0.05 x$10^3$ cell/$mm^3$ and treated group had 3.6±0.05 x$10^3$ cell/$mm^3$ showed significant value (P < 0.01).

In the control group Basophil value was 1.8 ±0.02 x$10^3$ cell/$mm^3$. A decrease value was exhibited by Basophil 1.5 ±0.02 x$10^3$ cell/$mm^3$ in treated fish group has non significant value (P < 0.05). Lymphocytes, Basophil value decreases in present investigation and Neutrophil, Monocytes and Esnophil increase in *Clarias batrachus* exposed to glyphosate (Fig. 2, Fig. 3) such changes in haematological parameters are in close conformity with earlier works, Muthalagi [24], Arjun [26]; Pratibha and Kumar [25]; Sasikala et al. [27] under various exposure of sewage, chromium and mercury chloride to the fishes. Gomulka et al. [28] have reported a significant decrease in the counts of lymphocytes, neutrophils and monocytes in European whitefish exposed to propofol. Neutropaemia and Lymphopaemia were observed in fish under in 30 days exposure to glyphosate (Table 1).

In DLC (Differential leucocytes count) the values of Lymphocytes in control group was 52.43±2.40. A decrease was found in treatment groups 32.4±0.02, the Lymphocytes showed significant value (P < 0.01). The present findings a decrease in WBC are close conformity with reports of earlier workers, Revathi et al. [16], Shipra et al. [17], Das, et al. [29], Anwar and Choudhary [9] under the exposure of various toxicants like fertilizers, pesticides, alkaloids to fishes or mammals. In fishes Muthalagi [24] has reported a decrease in WBC count in IMC, *C. mrigala* exposed to sewage treatment. Arjun [26] has also explained similar decrease of WBC in *Clarias batrachus* exposed to chromium. Pratibha [25] have explained a fair decrease in WBC in the fish *H. fossilis* induced to mercury chloride. The WBC plays a very important role in the defense mechanism of body. So, may be fish become prone to other diseases due to loss of WBC. The leucopaemia term used here for decrease WBC count in fish exposed to certain toxicants. Another observation support the present work, Vasait and Patil [30] found decreasing lymphocyte count in *Nemacheilus botia* fish induced to organophosphorous insecticide.

PCV (Packed Cell Volume) value in control fish group was 35.1±0.06. Similar decrease was observed in treated fish group as 12.65±0.03. Its value showed significant (P < 0.01), (Table 1).

The present study (Table 1) is conformity with Muthalagi [24], Arjun [26], Kreutz, et al. [31], Pratibha [25] and Narayna [32] in fish when fishes exposed to various pollutants such as sewage, chromium as well as cadmium chloride. Revathi et al. [16] have reported a decrease in PC, MCV, MCH and MCHC in fish induced with increasing concentration of tannery effluent. The cellular blood iron decline reflects the reduction in haemoglobin (Hb) and increase haemolysis of RBC count has been found in present study. Increase in MCH and MCHC levels of the haematology profile of fish induced to herbicide, glyphosate. Arjun [26] has reported similar alteration in haematology profile in *Clarias batrachus* induced to chromium. Pratibha [25] have explained to same decrease of PCV, MCV,

MCH and MCHC in fish *H. fossilis* (Bloch) induced to mercuric chloride. Olojo and Ladeji [33] found an increase in MCV, MCH and MCHC levels of *C. gariepinus* induced to manganese exposure. Ahmed et al*.,* [34] have found haematological changes in *Cyprinus carpio* exposed to diazinon. Sharma and Langer [35] also reported an increase in lymphocyte, eosinophils and monocytes with a decrease in neutrophils and basophils concentration in *Garra gotyla gotyla* exposed to various concentrations of manganese.

**Table 1. Haematological profile changes in glyphosate induced *Clarias batrachus* (mean ± SE of 5)**

| Variable | | Glyphosate (30 days) exposure |
|---|---|---|
| **Parameter** | **Control** | **2.46 mg/l** |
| Blood Hb (gm/dl) | 10.85 ±0.05 | 6.72±0.10 *** |
| TEC(RBC) ($10^6$ cell/$mm^3$) | 6.32±0.05 | 5.1±0.05 *** |
| WBC ($10^4$ cell/$mm^3$) | 5.52±0.05% | 3.65±0.05% *** |
| Neutrophil ($10^3$ cell/$mm^3$) | 5.85±2.05 | 12.82±0.05 *** |
| Lymphocytes ($10^3$ cell/$mm^3$) | 52.43±2.40 | 32.4±0.02 ** |
| Monocytes ($10^3$ cell/$mm^3$) | 4.2±0.05 | 7.6±0.05 * |
| Eosinophil ($10^3$ cell/$mm^3$) | 2.3±0.05 | 3.6±0.05 ** |
| Basophil ($10^3$ cell/$mm^3$) | 1.8 ±0.02 | 1.5 ±0.02 * |
| PVC (%values) | 35.1±0.06 | 12.65±0.03 ** |
| MCV(fl/cell) | 150.25±0.85 | 158.00±1.35 |
| MCH(pg) | 43.55±1.02 | 48.21±1.45 |
| MCHC(g/dl) | 24.15±1.05 | 29.58±1.05 |

*Values are mean ± SE of 5 individual observations:- * P<0.5 Non Significant, ** P<0.01 Significant, *** P<0.001 Highly Significant*

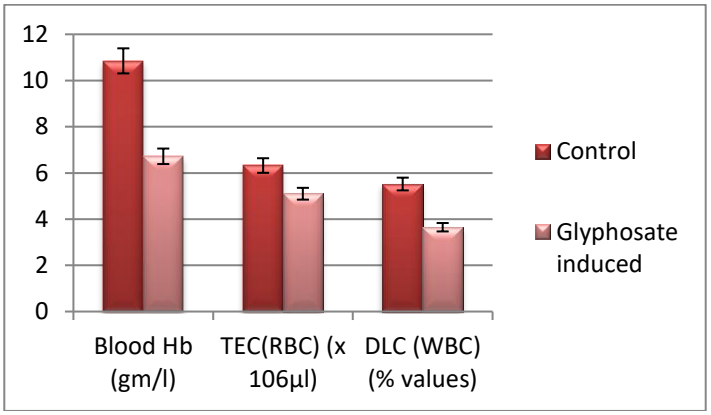


**Fig. 1. Bar graph showing Haematological parameters**

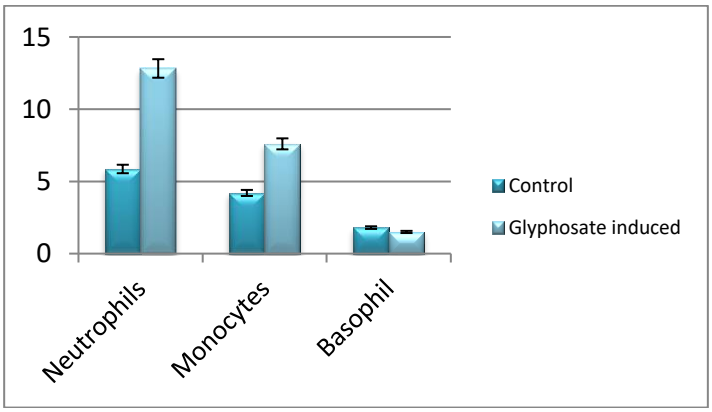


**Fig. 2. Effect of glyphosate on neutrophil, monocytes and basophil**

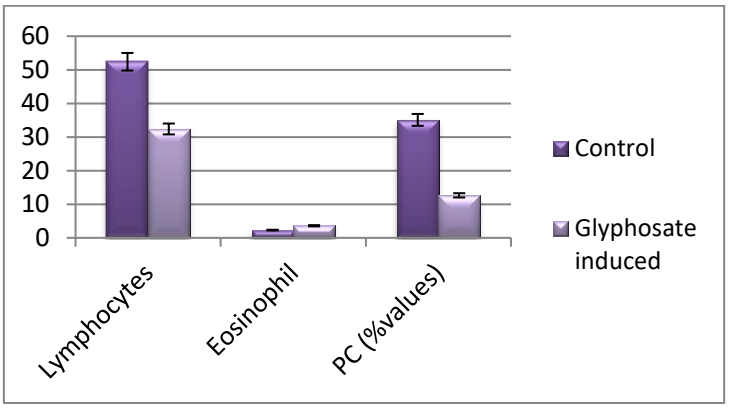


**Fig. 3. Effect of glyphosate on lymphocytes and eosinophil**

"The haematological profile was assessed on various parameters, such as Hb, RBC, WBC decreases while in DLC, Neutrophil, Monocytes and Eosinophil values increases while Lymphocytes and PCV values decrease. The increase or decrease value analyzed and appeared in three ways as significant, highly significant or non-significant" [36,37]. Such physiological changes causes various diseases in herbicide glyphosate induced fishes like Erythropoesis, anaemia, Leucocytopaemia, Neutropaemia, Lymphopaemia, Eosinophilia and Erythropaemia.

Germysz-Kathowaska [38] have found "a reduction in HB, HCT and RBC count by an organophosphorous insecticide in japanese quail. The reduction of HB might be attributed to the blood coagulation. The reduction can be related to decrease RBC number which indicates haemolysis, haemorrahage and reduced erythropoisis in fishes on exposure to herbicide. Decrease in MCHC reveals that loss of HB is comparatively at higher rate than that of the PVC. The decreased MCV, MCH, and MCHC clearly indicate hypochronic microlytic anemia". The above findings are supported by Ramalingam, et al. [39]. Decrease PVC shows the magnitude of shrinking of cell size [40].

## 4. CONCLUSION

*Clarias batrachus* with average weight 30.0± 4.0 g has susceptible to herbicides, glyphosate at low concentration as 2.46 mg/l. The chronic toxicity can be detected through haematological profile alteration. So, suggested it may the less than above concentration be suitable for fish culture, optimum growth performance and survival rate than other water conditions. The haematology parameters may be used as a tool to detect the herbicides toxicity to fish culture monitoring.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Urban OJ, Cook NJ. Hazard evaluation division, standard evaluation procedure, ecological risk assessment. EPA540/9-85-001. Final Report, U.S. Environmental protection Agency, Washington, DC; 1986.
2. Ajani F, Awogbade AA. Hematological Changes of the African Catfish *Clarias gariepinus* (Burchell, 1822) Juveniles Induced by Diuron, British Biotechnology Journal. 2012;2(4):247-256.
3. Wekler P. Information Resources in Toxicology 3 Edn. San Diego. Academic Press. 2000;278.
4. Dayan FE, Barker A, Takano H, Bough R, Ortiz M, Duke SO. Herbicide mechanism of action and resistance. In: Moo-Young M,

editor. Comprehensive Biotechnology. 3rd ed. Volume 4. Elsevier; Amsterdam, The Netherlands. 2020;4826.
5. Schonbrunn E, Eschenburg S, Shuttleworth WA, Schloss JV, Amrhein N, Evans JNS, Kabsch W. Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3phosphate synthase in atomic detail. Proc. Natl. Acad. Sci. 2001;98(4):1376−1380.
6. Clair A, Mesnage R, Travert C, Seralini GE. A glyphosate based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, testosterone decrease at lower levels. Toxicol. Vitro. 2012;26(2):269−279.
7. Rzymski P, Klimaszyk P, Kubacki T. The effect of glyphosate– based herbicide on aquatic organisms–a case study. Limnological Review. 2014;13(4):215–220.
8. Debnath S. *Clarias batrachus,* the medicinal fish: An excellent c&idate for aquaculture & employment generation. 2011 International Conference on Asia Agriculture and Animal IPCBEE. 2011;13 (2011) © (2011)IACSIT Press, Singapoore.
9. Arjun Sah, Roy, Nutan DN. Effect of Hb parameters on chromium to *C. batrachus.* J. of Environ & Ecoplaning. 2009;16(1):93-101.
10. APHA. Standard methods for the examination of water and waste water (16th Ed). American Public Health Assoc, Washington D.C.; 1985.
11. Finney DJ. Statistical methods in biological assay. 3rd ed. London UK: Griffin Press. 1978;508.
12. Sprague JB. Measurement of pollution toxicity to fish. III. Sub – lethal effects and 'safe' concentration, Water Res. 1971;5: 245-266.
13. Srivastava AK, Agarwal SJ. Hematological anomalies in a freshwater teleost, *Colisa fasciatus*, on acute exposure to cobalt. Acta. Pharmacol. Toxicol. 1979;44:197-199.
14. Dacie JV, Lewis SM. Practical haematology. 7th edition. Edinburgh: Churchill Livingstone; 1991.
15. Jerald F Felix, Saradhamani N. Impact of the Herbicide Glyphosate Roundup (41%) On The Haematology of the Freshwater Fish, *Catla Catla* (Hamilton). IOSR J. of Env. Sc, Toxicology and Food Technology. 2015;9(4):III, 56-60.
16. Rewathi KM, Yogananda, Kaplarasi K of tannary effluent on the bio-chemical and haematology of wistar albino rats. Indian J. Environ & Ecoplan. 2003;7(3):629-632.
17. Shipra, Shamra, Goyal RP, Geetanjali Chakravaraty, Anjali Sharma. Orange red, a blend of permitted food colour induced haematological changes in Swiss Albino Mice. 2005;24(2):99-103.
18. Bruska-jastrzebska E, Protasowuki M. Effects of cadmium and nickel exposure on hematological parameters of common carp, *Cyprinus carpio*. Actalchthya et Piscatorial. 2005;35(1):29-38.
19. Anwar, Choudhary. Effects of Quinine and Atropine to rat. J. of Natural conservatives Indian. 2009;21(2):325-329.
20. Roy R, Nath S. Some hematological investigations on *Oreocromis niloticus* (Trewavas) following exposure to Thiamethoxam. Acta zool. Lituanica. 2011; 21(4):301-305.
21. Raizada MN, Gupta A. Toxic effect of Rh-2 16 (a systemic fungicide) from total etythro- cyte counts (RBC) and haemoglobin (Hb) content of Trichogasterfasciatus. Comp. Physiol. Ecol. 1982;7(1):29-30.
22. Verma SR, Sarita R, Dable RC. Indicators of stress induced by pesticides in *Mystus vittatus* haematological parameters. Indian J. Environ. Health. 1982;24(1):58-64.
23. Mishra J, Srivastava AK. Malathion-induced haematological and biochemical changes in the Indian catfish, *Heteropneustes fossilis*. Environ. Res. 1983;30:393-398.
24. Muthalgi S. Effect of different concentration of sewage on the haematological parameters of *C. mirgila.* Indian J. Eniron. & Ecoplan. 2006;12(2):409-412.
25. Pratibha K. Haematological & bio-chemical effects of mercuric chloride to *Heteropneustes fossilis.* Ph.D. thesis of L.N.M.U. Darbhanga; 2013.
26. Arjun S. Effects of chromium on haematological and histopathological parameters, *C. batrachus.* Ph. D. thesis, L.N.M.U, Darbhanga; 2010.
27. Sasikala G, Palanisamy P, Mallikaraj D, Bhuvaneshwari N, Natarajan GM. Metasystox Induced Haematological Modulation in the South Indian snake headed *Channa striata.* Int. J. Pharm, Biol. Arch. 2011;2(2):775-777.
28. Gomulka P, Wlasow,T, Szczepkowski M, Misiewicz L, Ziomek E. The effect of propofol anaesthesia on haematological and biochemical blood profile of European

Whitefish. Turkish J. Fish. Aquat. Sci. 2014;14:331-337.
29. Das PC, Ayyappan S, Jena JK. Haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH, Aquaculture. 2006;256: 80–87.
30. Vasait JD, Patil VT. The toxic evaluation of organophosphorous insecticide monocrotophos on the edible fish species *Nemacheilus botia.* Ecol. Env, Cons. 2005; 8(1):95-98.
31. Kreutz LC, Gil Barcellos LJ, de Faria Valle S, de Oliveira Silva Tl, Anziliero D, Davi dos Santos E, et al. Altered hematological and immunological parameters in silver catfish Rhamdia quelen following short term exposure to sublethal concentration of glyphosate. Fish Shellfish Immunol. 2011;30(1):51−57.
32. Narayana Naidu G, Vijay Kumar PPN, Shameem U. Acute and sub-acute toxic effect of Ammonia on Behavioral and Haematological responses of Indian Major Carp *Labeo rohita* Ham, 1822. International Journal of Fisheries and Aquatic Studies. 2017;5(2):332-335.
33. Olojo EAA, Ladeji G. Haematological response of the African catfish, *Clarias gariepinus* (Clariidae) exposed to manganese. Asian J. Biol. Life Sci. 2012;1:126-133.
34. Ahmed Z. Acute toxicity and haematological changes in common carp (*Cyprinus carpio*) caused by diazinon exposure, African Journal of Biotechnology. 2011;10(63):13852-13859.
35. Sharma J, Langer S. Effect of Manganese on hematological parameters of fish, *Garra gotyla gotyla*. J. Entomol. Zool. Stud. 2014;2:77-81.
36. Mala Kumar. Analysis of haematological manifestation of air breathing fish *Clarias batrachus* (Linn.) induced to deltamethrin. International Journal of Fauna and Biological Studies 2023;10(6):20-23
37. Uchenna UB, Uka A, Obiahu OH. The impact of sub-lethal concentrations of glyphosate on growth and haematology of African catfish under aquatic ecological micro-climate. Environmental Chemistry and Ecotoxicology. 2022 Jan 1;4:164-70.
38. Germysz-Kathowaska, Szubartowska KE, Ecezanowaska E. Pheripheral blood the Japanese quail (*Coturnix coturnix* japonica) in acode poisoning by different insecticides, comp. biochem. Physiol. 1985;81c(1):209-212.
39. Ramalingam, Vimaladevi V, Narmadaraji R, Prabakaran P. Effect of lead on the haematological and biochemical changes in the freshwater fish, *Cirrhinus mrigala*, Poll. Res. 2000;19(1):81-84.
40. Shakoori AR, Mughal AL, Iqbal MJ. Effect of sublethal doses of fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver, muscles of a freshwater fish, *Ctenopharyngodon idella*, Bull. Environ. Contam. Toxicol. 1996;57:487-494.



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