I J R B A T, Issue (IX), Vol. III, Sept 2021: 67-74
A Double-Blind Peer Reviewed & Refereed Journal

OPEN ACCESS

e-ISSN 2347 – 517X
Original Article

INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY

 www.ijrbat.in

CHANGES IN HAEMATOLOGICAL PARAMETERS OF *CLARIAS GARIEPINUS* EXPOSED TO SUB-LETHAL CONCENTRATIONS OF CYPERMETHRIN

***Dahegaonkar, J. S., Gajbhiye, A.B. and Mohite, A. S.**
P. G. Department of Zoology & Research Academy, Sevadal college for Women, Nagpur-440024, India
*Corresponding author: arnavjd1610@gmail.com

Communicated: 20.06.21	Revision : 23.07.21 & 27.08.2021 Accepted: 02.09.2021	Published: 30.09.2021

ABSTRACT:
The effect of synthetic pyrethroid, Cypermethrin on the haematological parameters of *Clarias gariepinus* was assessed with three exposures of sub-lethal concentrations and control. The fishes were exposed to 0.2µl/L, 0.3µl/L and 0.4µl/L cypermethrin concentrations for 96h. Red blood cell (RBC) count, White blood cell (WBC) count, Haemoglobin (Hb) content, Packed cell volume (PCV) showed significant ($P \leq 0.05$) increase. Mean corpuscular haemoglobin (MCH) values at 0.3µl/L and 0.4µl/L cypermethrin concentrations were not significantly different. Mean corpuscular haemoglobin count (MCHC) and Mean corpuscular volume (MCV) also exhibited significantly different values with that of control. Alterations in the haematological parameters led to the conclusion that cypermethrin has toxic effects on *Clarias gariepinus*.
Key words: - Cypermethrin, *Clarias gariepinus*, haematological parameters.

INTRODUCTION:

Pyrethroids are synthetic analogs of pyrethrins belonging to non-systemic chemical group of insecticides (Burr and Ray, 2004). Approximately 40% of pyrethroid insecticides are being used for the control of insect pests (Masud and Singh, 2013). Cypermethrin, a synthetic pyrethroid has become one of the most important insecticide widely used in agricultural areas all over the world in order to increase the food grains and other agricultural products (Usmani and Knowles, 2001). There is increased risk of food being contaminated with the pesticides, which may harm humans and domesticated animals. Pesticides, which frequently enter the aquatic ecosystem through the agricultural run-off and spraying operations adversely affect non-target animals (Murphy, 1996 and Singh et al., 2003). Synthetic pyrethroids are preferably used over organochlorine, organophosphorus and carbamate pesticides due to their high effectiveness, low toxicity in warm blooded animals and easy biodegradability (Kale et al., 1999).

The assessment of the ecotoxicological risks caused by pesticides to ecosystem is based on toxicity data and the effects of pesticide preparations on non-target organisms (Masud and Singh, 2013). Pesticides represent a relevant stressor for many aquatic and terrestrial species (Leiss et al., 2005). Fish are among the group of non-target aquatic organisms. Haematological parameters are considered pathological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari et al., 2004). It has also been reported that haematological parameters would readily respond to incidental factor such as physical

stress and environmental stress due to pesticide contamination of water (Bhatnagar and Bana, 1992; Ojutiku et al., 2013; Masud and Singh, 2013; Neelima et al., 2015; Thirumavalavan and Ganesan, 2016). Haematological parameters such as haematocrit, haemoglobin, number of RBC and WBC are indicators of toxicity (Neelima et al., 2015). Therefore, haematology has been widely used as potent bioindicator in aquatic toxicology (Sancho et al., 2000; Barcellos et al., 2003). Most of the previous studies have reported negative effects of pyrethroids on blood parameters of exposed fish species (Masud and Singh, 2013; Narra, 2016; Gabriel and Ugbomeh, 2016; Thirumavalavan and Ganesan , 2016). However, in some of the studies were reportedly less affected and the fish recovered soon after they were returned to non-polluted water (Adhikari et al., 2004; Ramesh et al., 2015).

Clarias gariepinus is an economically important freshwater fish. In the present investigation, an attempt has been made to evaluate the acute toxicity of cypermethrin *Clarius gariepinus* measured by its mortality. The effects of sub-lethal concentration of cypermethrin was measured by registering the changes in haemoglobin concentration, haematocrit values, RBC and WBC counts in a static system.

MATERIALS AND METHODS:

Live and healthy specimens of catfish, *Clarias gariepinus*, were procured from the fish market, situated at Sakkardara, Nagpur. The fish weight and length ranges from 110-140 g and 30-34 cm, respectively were taken. The fish specimens were kept in glass aquaria to get acclimatized to laboratory conditions for two weeks. The fish were fed with locally manufactured food to satiety twice daily for two weeks. The aquarium water conditions like pH, dissolved oxygen, temperature, hardness and alkalinity were examined weekly. Water quality characteristics in the experiment was recorded according to APHA (1980).

When the fish behaviours interact that it is normal, indicating the acclimatization time, five fishes were kept in each aquarium filled with 30 liters of water. The known volume of stock solution of cypermethrin was added to three different aquarium for obtaining the test concentrations (0.2µl, 0.3µl, 0.4µl) for 96hrs. A control set was also run without toxicant having the same number of fish in same volume of water. At a time, three sets of experimental and control group were run. Aeration was provided through the electrical air pumps in the aquaria. During the exposure period of toxicant, the feeding was stopped. The number of dead fish were noted and removed immediately from the aquaria. Fresh concentration of cypermethrin was prepared daily and the water was also changed. The food was supplied twice in a day to satiety. The experimental fish was removed from each aquarium of five replicates. Blood was collected from the caudal peduncle of the fish after excision, allowed to flow through the dorsal aorta and collected in heparinized vials. Two or more fishes were excised to pool enough blood for the analysis of different indices. The blood was collected in eppendorf tubes. The clotted blood samples were avoided.

Test specified pesticide: Cypermethrin, an agriculture grade trade named as Challenger 25(25%EC with 100%purity) was used in the experiment. Micropipette to measure the doses of cypermethrin was used for test exposure.

Experimental design for static bioassay: Fish aquarium of 50 litres capacity were used for the experimentation with twenty number of *Clarias gariepinus*, divided into four groups of five fishes in each.

Fish exposure to cypermethrin: Three groups were exposed to dose of 0.2µl,0.3µl and 0.4µl concentrations of cypermethrin for 96 hours, while the fourth group served as control. Feeding

and adding of fresh dose of cypermethrin were done after changing water on alternate days, during the experimentation. Fish were observed thrice daily for mortality and their behavioural study.

Haematological study: A set/group of five fishes kept for the experimentation were sacrificed after 96hrs. at the end of the experiment. During each sampling, blood samples were obtained making excision in caudal peduncle and collected in eppendorf tubes containing EDTA anticoagulant (Mgbenka et al., 2003) for determination of RBC, WBC, haemoglobin content. Physico-chemical characteristics of water like pH, temperature, dissolved oxygen, alkalinity and hardness were also recorded (APHA,1980).

Total count of RBC: Neubauer haemocytometer was used for counting total red blood cells (RBCs). Dilution of blood 1:200 was done with Hayem's fluid (Mishra et al., 1977). Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as 10^6 mm^3 (Wintrobe, 1967).

Total count of WBC: Total white blood cells (WBC) were counted using Neubauer haemocytometer (Mgbenka et al., 2003). Blood was diluted 1: 20 proportion with WBC diluting fluid and placed in haemocytometer. 4 large (1sq mm) corner squares of the haemocytometer were counted under the microscope. The total number of WBC was calculated in mm^3 $x10^3$ (Wintrobe, 1967).

Haemoglobin content /percentage (Hb): Haemoglobin estimation was done by using Sahli's (1962) method. Graduated haemoglobin tube was filled with N/10 HCl upto mark 10, then blood was sucked in haemoglobin pipette upto mark 20 cu.mm. The blood was poured into haemoglobin tube already containing N/10 HCl. Allow it to settle for 10mins. The mixture was diluted with N/10 HCl, stirring continuously with a glass rod, till the colour matches with that of the standard brown glass rod. The reading was taken on haemoglobin tube showing percentage of haemoglobin.

Statistical analysis: Haematological changes were tested by using one way ANOVA (analysis of variance). Tukey's test was used for calculation of significant differences.

$P \leq 0.05$ values were regarded as significant.

Determination of packed cell volume (PCV) or haematocrit value: Packed cell volume was determined by micro haematocrit method of Schalm et al., (1975). The heparinised blood was filled up to the mark 100 of the haematocrit tube with the help of Pasteur pipette and centrifuged at 3000rpm for 30mins. The relative volume of the height of the RBC's packed at the bottom of the haematocrit tube was recorded as packed cell volume in terms of percentage of total blood column taken in the haematocrit tube.

Determination of mean corpuscular volume (MCV): MCV indicates the average size of the red blood cell in a given sample of blood. MCV was calculated by the following formula and expressed as femtoliter (fL).

Determination of mean corpuscular haemoglobin (MCH): MCH represents the average content of the Hb in each red blood cell. MCH is influenced by the Hb concentration and the number of RBC. MCH was calculated by the following formula and expressed in pictogram (pg).

Mean corpuscular haemoglobin concentration (MCHC): MCHC reflects the average concentration of the haemoglobin in the red blood cells in a given volume of the blood. MCHC was obtained by the following formula and expressed in terms of gram percent (g %).

RESULT & DISCUSSION:

Abnormal behaviours like gulping of air, high opercular movement, erratic and agitated swimming, restlessness, hanging in the water column, burst swimming have been recorded (Table. 2) during the present study. Similar observations were reported by Aguigwo (2002);

Ayoola and Ajani (2008) and Ikele et al.(2011). Table -3 have been observed during the present study.

Table-2. Behavioural study of the *Clarias gaiepinus* under the influence of the chemical pesticide cypermethrin.

The results obtained on the haematology parameters of *Clarias gariepinus* exposed to sublethal concentrations of cypermethrin for a 96h period have been presented in Table-3.

Exposure to cypermethrin at 0.2µl/ L, 0.3µl/L and 0.4 µl/L concentrations results in disturbances in haematology. Sub-lethal concentrations of cypermethrin at 0.2µl/L, 0.3µl/L and 0.4µl/L significantly ($p \leq 0.05$) increase the red blood corpuscle (RBC) count with mean values of (1.35±0.03), (1.67±0.10) and(2.16 ±0.05) 10^6/µl respectively, with in 96h of exposure. The control fish had (1.02±0.02) 10^6/µl of RBC. Similar results had been reported by Kannan et al., (2014) and Al-Otaibi et al., (2019) while investigating the effects of cypermethrin (10%EC) in the concentration of 0.0006ml/L on *Catla catla* for 24h and effects of acute concentration of diazinon on *Clarias gariepinus,* respectively. The results of white blood corpuscle (WBC) count revealed that the blood of the control fish showed a mean value of 48.41±0.46 10^3/ µl. The fishes exposed to sub-lethal concentrations of cypermethrin showed significant increase ($p \leq 0.05$) in the mean values of WBC to be 64.28 ±0.43, 75.46±0.53 and 101.40±0.16 10^3/µl after 96h of exposure. The increase in WBC is considered as an adaptive mechanism. This may be due to the direct stimulation of the immunological defence mechanism against stress (Henry et al., 1978; Labelo et al., 2001; Hassen, 2002; Das and Mukherjee, 2006). Such a significant increase in the WBC can be correlated with an increase in antibody production which helps in survival and recovery of fish exposed to pesticides (Joshi et al., 2000; Masud and Singh. 2013). Adhikari et al., (2004) have suggested increase in the number of WBC in *Clarias gariepinus* exposed to cypermethrin may be due to haemoconcentration.

The Haemoglobin concentration (Hb) and Packed cell volume (PCV) showed significant ($p \leq 0.05$) increase as compared to control. Haemoglobin concentration in the blood of fish at 0.2µl/L, 0.3µl/L and 0.4µl/L cypermethrin resulted 6.22±0.30, 7.74±0.20 and 9.29±0.26 g/dl mean values, respectively. Similarly, PCV values of the test fish showed a significant ($p \leq 0.05$) increase as compared to the control (13.56±0.42). The results evidentially showed that increase in the PCV lead to corresponding increase in the haemoglobin content as well. The result obtained in present study has been corroborated by the findings of Olufayo (2009) and Ojutika et al., (2013).

Mean corpuscular haemoglobin (MCH) values at 0.3µl/L and 0.4µl/L cypermethrin concentrations are not significantly different with that of control. Mean value (46.48±0.59 pg/ cell) obtained for 0.2µl/L cypermethrin concentration showed significant ($p \leq 0.05$) increase in MCH. Mean corpuscular haemoglobin count (MCHC) values at different concentrations of cypermethrin are significantly different with that of control. Similarly, the result of mean corpuscular volume (MCV) showed that there was significant increase in the blood of fish exposed at 0.2µl/L as compared to the control mean value 134.8 +- 5.21 FI. Adeyemo et al., (2008) and Ojutika et al., (2013) reported the similar trend for erythrocytes values. The increase in the MCV and MCH suggests the macrocytic normochromic type of anaemia (Gabriel and Ugbomeh, 2016). Increase in MCV may be caused due to endomitosis which results to the haemodilution as suggested by Anand kumar (1994). The toxicity of pollutants depends generally on size and species of fish as well as duration of exposure (Dutta et al.,1995).

REFERENCES:

Adeyemo, O. K.; Ajani F.; Adeddeji, O. B.; Ajiboye, O. O. (2008). Acute toxicity and blood profile of Adult *Clarias gariepinus* exposed to lead nitrate. *Int. J. of Haematology*., Vol. 4 No.2.

Adhikari, S.; Sarkar, B.; Chattterjee, A.; Mahapatra, C. T.; Ayyappan. (2004). Effect of cypermethrin and carbofuran on certain haematological parameters and prediction of recovery in a freshwater teleost, *Labeo rohita* (Hamilton). *Ecotoxicol. Envion. Saf.* 58: 220- 226.

Aguigwo, J. N. (2002). The toxic effect of cymbush pesticide on growth and survival of African catfish, *Clarias gariepinus* (BURCHELL 1822), *J. Aquatic Sci.*, 17(2): 81-84.

Al-Otaibi, A. M.; Al-Balawi, H. F. A.; Ahmad, Z. and Suliman, E. M. (2019). Toxicity bioassay and sub-lethal effects of diazinon on blood profile and histology of liver, gills and kidney of catfish, *Clarias gariepinus. Braz. J. Biol.* (Online).,Vol. 79 (2); PP. 326-336. (http://dx.doi.org/10.1590/1519–6984.185408).

Anand Kumar, A. (1994). Endosulfan induced biochemical and pathophysiological changes in freshwater fish, *Clarias batrachus.* Ph.D. Thesis, Osmania University, Hyderabad, A.P., India.

APHA (1980). Standard Methods for the Examination of Water and Waste water,15th edition, Washington, *D C: American Public Health Association,* PP.1134.

Ayoola, S. O.; Ajani, E. K. (2008). Histopathological effect of cypermethrin on juvenile African catfish (*Clarias gariepinus). World J. Biol. Res.*1(2), PP.1-14.

Barcellos, L. J. G.; Kreutz, L. C.; Rodrigues, L. B.; Fioreze, I.; Quevedo, R. M.; Cericato, L.; Conrad, J.; Soso, A. B.; Fagundes, M.; Lacerda, L. A.; and Terra, S. (2003). Haematological and biochemical characteristics of male jundia (Rhamdia Quelen Quoy and Gaima RDT, Pimelodidoe): changes after acute stress. *Aquacult. Res.*34:1465- 1469.

Bhatnagar, M. C.; Bana, A. L (1992). Respiratory distress to *Clarias batrachus* (Linn.) exposed to endosulfan a histological approach. *J. Environ. Biol.*13: 227-231.

Burr, S. A., Ray, D. E. (2004). Structure activity and interaction affects of 14 different pyrethroids on voltage – gated chloride ion channels. *Toxicol. Sci.* 77: 341-346.

Das, B. K. and Mukherjee, S. C.(2003). Toxicity of cypermethrin in *Labeo rohita* fingerlings biochemical, enzymatic and haematological consequences: *Comparative Biochemistry and Physiology* Part C, 134 :109-121.

Dutta, H. M., Munshi, J. S. D., Dutta, G. R., Singh, N. K., Adhikari, S. and Richmonds, C. R. (1995). Age related differences in the inhibition of brain acetylcholinesterase activity of *Heteropneustes fossilis* (Bloch) by malathion. *Comparative Biochemistry and Physiology*, vol. 111, no. 2, pp. 331-334. http://dx.doi.org/10.1016/0300-9629(94)00166-Q. [Links]

Gabriel, U. U and Ugbomeh, A. P. (2016). Effects of cypermethrin on haematological parameters of African freshwater

catfish, *Clarias gariepinus. J. Aquat. Sci.,* Vol.31 (2B): pp.433-442.

Hassen, F. E. Z. M. (2002). Studies on diseases of fish caused by Henneguya infestation, Ph.D. Thesis, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Henry, E.; Donald, H.; Moroan, P.; Simmons, A. (1978). A pesticide Dieldrin induced immune haemolyticanaemia. *Environ. Res.,*17:55.

Joshi, K. C.; Roychoudhary, N. and Sharma, N. (2000). Microbial pesticides for forest insect control. *In: Recent trends in insect pest control to enhance forest productivity.*

Kale, M. N.; John. S.; Rathore and Bhatnagar. D. (1999). Lipid preoxidative damage on pyrethroid exposure and alterations in antioxidative status in rat erythrocyte a possible involvement of reactive oxygen species. *Toxicol.,* 108: 143-277.

Kannan, M.; Muthusamy, P. and Venkatachalam, U. (2014). Response of Synthetic Pyrethroid Cypermethrin (10% EC) induced Stress in Biochemical and Haematological Parameters of Indian Major Carp, *Catla catla* (Hamilton, 1822). *World J. Pharmaceu.* Res., 3(4) : 1976-1996.

Lebelo, S. L.; Saunders, D. K.; Crawford, T. G. (2001). Observations on blood viscosity in striped bass, *Morone saxtilis (Walbaum)* Associated with fish Hatchery conditions. *Kansa Acad. Sci.*104 : 183-194.

Liess, M.; Brown, C.; Dohmen , P.; Heimback , F.; and Kranger, J. (2005). Effects of pesticides in field – EPIF. Brussels. *SETAC press.*136.

Masud, S. and Singh, I. J. (2013). Effect of cypermethrin on some haematological parameters and prediction of their recovery in a fresh water teleost, *Cyprinus carpio. African J. Environ. Sci. Technol.,* Vol.7 (9): PP. 852–856.

Mgbenka, B. O.; Oluah, N. S.; Umeike, I. (2003). Effect of Gammalin 20 (Linndane) on differential white blood cells count of the African catfish *Clarias albopunctatus. Bull. Environ. Contam. Toxicol.*71 : 248–254.

Mishra, N.; Pandey, P. K.; Datta, H. M.; Munshi, J.S.; Singh, B.R. (1977). Haematological parameter of an air breathing mud eel, *Amphipnous cuchia* (HAM). *J. Fish Biol.*10(6): 56–573.

Narra, M. R. (2016). Single and cartel effect of pesticides on biochemical and haematological status of *Clarias batrachus:* A long–term monitoring *Chemosphere,* 144, 966–974.

Neelima, P.; Sunitha, K.; GopalaRao, N. and Chandra Sekhara Rao, J. (2015). Haematological alterations in *Cyprinus carpio*as biomarkers of cypermethrin toxicity. *Int. J. Current Res.,*Vol.7 (8) : PP. 1886–18870.

Ojutika, R. O.; Asuwaju, F. P.; Kolo, R, J.; Obande, R. A and Agbelege, O. O. (2013). Haematological effect of acute concentration of cypermethrin on juveniles of *Clarias gariepinus. Int. J. Eng. Sci. Invent.,* Vol.2 (3): PP. 433–442.

Olufayo, M. O. (2009). Haematological characteristics of *Clarias gariepinus* (Burchell, 1822) juveniles exposed to Derris elliptica root powder. *Afr. J. Food Agric. Nutr. Dev.* 9(3): 920-933.

Ramesh , M.; Narmadha, S. and Poopal, R. K. (2015). Toxicity of furadan (Carbofuran 3%g) in *Cyprinus carpio*:

Haematolofical , biochemical and enzymological alterations and recovery response. *Journal of Basic and Applied Sciences*, 4: 314–326.

Sahli (1962). Determination of haemoglobin by acid haematin method. *In: Practical haematology* (Ed: Dacie J.V. and Lewis S.M.) 5thEdn. Churuchill, London.

Sancho, E.; Ceron, J. J. and Ferrando, M. D. (2008). Cholinesterase activity and haematological parameters as biomarkers in sublethalmolinate exposure in *Anguilla anguilla. Ecotoxicology and Environmental Safety,* 46 (1): 81–86.

Schalm, O.W.; Jain, N.C. and Carroll, E. J. (1975). *Veterinary haematology*, 3rd ed. Lea & Febiger, Philadelphia

Thirumavalavan, R. and Ganesan, J. (2016). Effect of insecticide cypermethrin on haematological alterations in the fish *Catla catla . Int. J. Current Res.,* Vol.8 (10): PP. 40489–40493.

Usmani, K. A. and Knowles, C. O.(2001). Toxicity of pyrethroids and effect of synergists to larval and adult *Helicoverpa zea, Spodoptera frugiperda, and Agrotis ipsilon (Lepidoptera :Noctuidae). J. Econ. Entomol.,*94: 868–873.

Wintrobe, M. M. (1967). *Clinical haematology* (6th Eds.) Lea and Febiger, Philadelphia, Library of Congress, Print USA.

Table-1: Water quality parameters during the exposure of *Clarias gariepinus* to various sub-lethal concentrations of cypermethrin.

Parameters	Control	0.2 µl	0.3 µl	0.4 µl
Length	33.4±1.17	33.2±1.10	32.8±1.34	32.3±1.12
Weight	138.8±7.98	123.8±9.55^{a}	116.5±9.33	128.3±9.34^{a}
pH	6.8±0.13^{a}	6.9±0.11^{a}	6.8±0.14^{a}	6.8±0.12^{a}
Temperature	26.38±0.49^{a}	27.1±0.51^{a}	27.3±0.44^{a}	27.8±0.34^{a}
Dissolved O2	7.58±0.30^{a}	7.40±0.44^{ab}	7.20±0.23^{bc}	7.10±0.52^{c}
Hardness	221.56±1.46^{a}	223.32±1.98^{a}	226.70±2.42^{a}	228.10±1.78^{a}
Akalinity	168.76±3.64^{a}	175.30±2.40^{a}	184.11±3.14^{a}	198.18±3.29^{a}

Means ± SE followed by the same letter with in a row are not significantly different at p≤ 0.05 (ANOVA followed by LSD post-test), n=5.

Table-2. Behavioural study of the *Clarias gaiepinus* under the influence of the chemical pesticide cypermethrin.

Behaviours	Control	0.2µl	0.3µl	0.4µl
Surfacing	+	+	++	+++
Threat	+	+	++	+++
Movement of gills	+	+	++	+++
Hanging in water column	+	+	++	+++

OPEN ACCESS

e-ISSN 2347 – 517X
Original Article

Table-3: Variations in haematological parameters of *Carias gariepinus* exposed to sub-lethal concentrations of cypermethrin for 96hrs.

Parameters	**Control**	**0.2µl**	**0.3µl**	**0.4µl**
RBC (cell x 10^6/ µl)	1.02±0.02	1.35±0.03	1.67±0.10	2.16±0.05
WBC (cell x 10^3/ µl)	48.41±0.46	64.28±0.43	75.46±0.53	101.4±0.16
Hb (g/dl)	4.61±0.26	6.22±0.30	7.74±0.20	9.29±0.26
Haematocrit (%) PCV	13.56±0.42	20.56±0.62	24.02±0.99	28.90±0.89
MCH (Pg/cell)	48.23±1.67^{a}	46.48±0.59	43.24±1.14^{ab}	41.28±1.09^{ab}
MCV (fl/ cell	134.8±5.21^{ab}	150.62±2.76	138.16±3.39^{a}	132.3±3.25^{b}
MCHC (%)	33.96±1.08	29.11±1.49^{a}	29.50±1.78^{a}	30.06±0.94^{a}

Means ± SE followed by the same letter within a row are not significantly different atp ≤ 0.05 (ANOVA followed by LSD post-test), n=5.