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GENOTOXIC AND HAEMATOLOGICAL EFFECT OF COMMONLY USED INSECTICIDE ON FISH *HETEROPNEUSTES FOSSILIS*

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ABSTRACT

During last three to four decades application of synthetic pesticides in agricultural field to increase agricultural production, has been increased tremendously. Pesticides in agricultural runoff affect all the aquatic organisms. Acephate is an organophosphate insecticide applied to food crops, citrus trees, on golf courses. The result from this study reveals that Acephate had some genotoxic and haematological effect on *Heteropneustes fossilis.* The fishes were exposed to sublethal concentrations (90% of LC_{50} of 24hr) of Acephate. Micronucleus assay was carried out after 24, 48, 72and 96hours to analyze micronuclei. The number of micronuclei at 72h was maximum. For haematology fishes were exposed to the chemical for one week. It was determined that Acephate caused a decrease in haemoglobin(Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cells(RBC) levels and increase in white blood cells level.

KEYWORDS: *Heteropneustes fossilis,*Micronucleus assay, haematology, Acephate, Genotoxicity.

1. INTRODUCTION

Development of the industries has been witnessed with the production of various types of the chemicals during last 3 to 4 decades. Due to the evolution of the different types of the chemicals, there is an increase in use by the framers of the various countries from day to day. With a limited availability of arable land supporting an ever-increasing human population, the threat of crop loss is more acute. Thus pesticides have become more essential component of modern agriculture. Astaf (Acephate®) is a broad-spectrum commercial grade organophosphate insecticide, active on moths and aphids, used for protection of variety of vegetables and fruits. Acute toxic effects of acephate on freshwater fish *Puntius sophore* are the subject of a study by GavitandPatil (2016).[1] *Channa punctata*'s haematological changes were covered by Satish et al. (2018) due to acephate.[2] According to Shahi& Singh, (2014) pesticides decrease a number of haematological parameters in *Clariasbatrachus* fish, including Hb, RBC, and WBC,[3] According to Jagyanseni et.al (2022) Acephate causes DNA damage and nuclear abnormalities in *Clarias batrachus.*[4] Various insecticides have been shown to have genotoxic potential on different freshwater fish by Campana et al. (1999), Cavas et al.(2003), and Ismailetal.(2018).[5,6,7]

2. MATERIALS AND METHOD

2.1. Test chemical: In experiment were used Acephate, trade name Astaf insecticide manufactured by Rallis India Ltd.,and a widely used insecticide. The solvent used was glass double distilled (g.d.d.) water.

2.2. Experimental animal: Fresh water fishes were collected from local outlets of Cuttack district. All the fishes were kept in glass aquaria containing 40L dechlorinated tap water for acclimatization to the laboratory condition for fifteen days prior to the initiation of the experiment. The fishes were fed twice daily on commercial fish food. The aquarium water was aerated properly and changed every day. The feed remains, excretory waste and dead animals were removed from aquaria to avoid any stress and contamination. The physio-chemical properties of experimental water are temperature 28^0C, pH 6.8 -7.05, dissolved oxygen 6.5-7.3 mg/L.

2.3. Haematological study: Fishes were kept in two groups, 15 fish in each group group 1 served as control, fish in group 2 were exposed to sublethal concentration of Acephate,90% of LC_{50} of 24h at 24h intervals for one week.The peripheral blood was collected by puncturing the caudal vein with heparinized disposable needle fitted with 2ml syringe as per the standard protocol of Al-Sabti (1986) and Das and Nanda (1986).[8] The collected blood

samples were transferred to microtubes, containing EDTA and were gently mixed by carefully turning it upside down and used for haematological analysis. All haematological parameters such as red blood cells (RBC), white blood cells (WBC), packed cell volume(PCV), haemoglobin(Hb), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were estimated by automated hematological machine.

2.4. Genotoxic study by means of micronucleus assay: Micronuclei are the small chromatid bodies formed by the condensation of chromosomal fragments or whole chromosomes that are not included in the main nucleus following the anaphase. Micronucleus assay is widely used in fishes since, it is the most simple and reliable technique to detect the effect of chemical. The fishes were kept in two groups, each group had 10 fish , group 1 served as control and group 2 were exposed to sublethal concentration of Acephate,90% of LC50 of 24h for 24, 48, 72, 96h. . The peripheral blood smear slides were prepared from the blood collected by caudal incision in accordance with Al-Sabti (1986) and Das and Nanda (1986) with some modification. The slides were analysed for 1000 cells with micronuclei.

2.5. Statistical analysis: Results were presented as mean with standard error of three replicates and differences between means were considered to be significant when $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Hematological observation: The haematological parameters for the fish from the control medium and those of the treated fish after one week were observed. The blood parameters of *Heteropneustes fossilis*, namely the hemoglobin (Hb), red blood cells (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration(MCHC) levels decreased significantly, where as white blood cell (WBC) level increased and no significant change is observed in mean cell volume (MCV) with exposure to Acephate (Table 1).

3.2. Frequency of micronuclei: The table-2 shows peripheral blood erythrocytes with micronuclei induced due to exposure to2.5 mg/L of Acephate. The numbers of micronuclei at 72h were maximum than the control fish.

Table 1: Changes in the haematological parameters of *Heteropneustes fossilis* exposed to Acephate at a 24h interval for 1 week.

Parameters	Control	Acephate
RBC($x10^6/\mu l$)	2.75±0.19	2.09±0.15
Hb(g/dl)	9.39±0.75	6.27±0.08
MCV(µg)	89.23±1.01	89.19±1.02
MCH(Pg)	35.48±3.32	30.12±2.98
MCHC(g/dL)	36.19±1.09	33.07±0.89
WBC($x10^3$/µl)	1.45±0.99	2.01±0.39

Significance at 0.05 levels

Table 2: Frequency of micronuclei in 1000 blood cells of *Heteropneustes fossilis* with Acephate at different duration.

Duration	Slide no.	No of blood cell examined	Cell with micro nuclei		% of cell with micronuclei	
			Control	Acephate	Control	Acephate
24h	1	1000	0	2	0%	0.2%
	2	1000	1	2	0.1%	0.2%
48h	1	1000	1	3	0.1%	0.3%
	2	1000	1	4	0.1%	0.4%
72h	1	1000	2	6	0.2%	0.6%
	2	1000	2	8	0.2%	0.8%
96h	1	1000	0	5	0%	0.5%
	2	1000	0	4	0%	0.4%

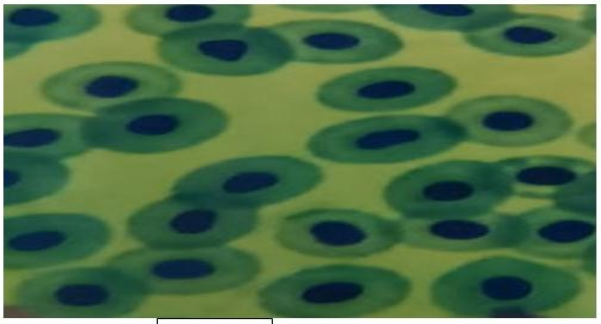

Fig-1

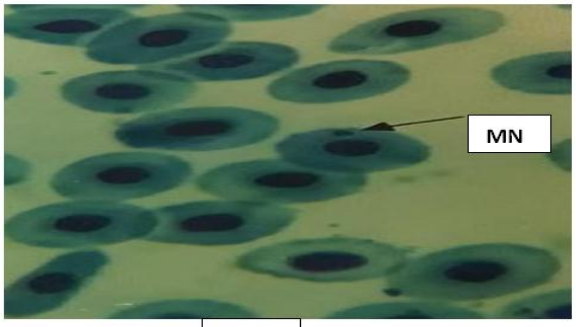

Fig-2

Fig 1: Showing blood cells of *Heteropneustes fossilis* without treatment and Fig 2. showing micronucleus after exposure to Acephate.

4. CONCLUSION

The studies clearly reveal the genotoxic and haematological potential of Acephate,which raises serious concern towards serious health implications for humans and other aquatic organisms that depend on aquatic ecosystems and its use in agriculture.

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