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## Numerical and Morphological Alterations in Erythrocytes of Pesticide Induced Indian Catfish *Heteropneustes fossilis* (Bloch)

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### ABSTRACT

The objective of the present study was to detect the erythrocyte alterations in freshwater teleost fish *Heteropneustes fossilis* (Bloch) exposed to different sub-lethal concentrations of organophosphate pesticide Nuvan on different time intervals. The 96 h LC<sub>50</sub> value of Nuvan was estimated by using log-dose probit regression line method. On the basis of LC<sub>50</sub> value, the sub-lethal concentrations were determined as 0.26 mg/L, 0.32 mg/L and 0.43 mg/L which are 1/25, 1/20 and 1/15 of LC<sub>50</sub> respectively. Well acclimated fishes were exposed to these different concentrations of Nuvan for 60 days. The fishes from both control and treated group were sacrificed after 7, 15, 30 and 60 days and blood samples were collected. Total erythrocyte count (TEC) was calculated by standard method and blood smear slides were prepared in both the groups. It was observed that total erythrocyte count (TEC) in pesticide treated fish decreases besides they show morphological changes when compared to their control sets.

### 1. Introduction

Nuvan (2,2-dichlorovinyl dimethylphosphate) is an organophosphate insecticide used for pest control on wide variety of crops. Pesticides by their nature are toxic compounds and as such besides controlling pests they also have potentialities of affecting the life and environment adversely. Organophosphate pesticides are finding increasing use in recent years since they are biodegradable and short time, because of their low persistence, repeated applications of this pesticide are being practiced for the control of pests in agriculture fields and there by large quantities find their way into water bodies. It is known that the organophosphate insecticides have antiacetylcholinesterase activity. The anaemic response could be as a result of destruction of inhibition of erythrocyte production, haemodilution and it could be as a result of the destruction of intestinal cells by the toxicant [1]. Large scale application of pesticides are hazardous because they enter the aquatic ecosystem as run-off and affect the aquatic organisms, especially fish [2].

Aquatic pollution due to pesticide needs considerable attention because of its harmful effects on aquatic organisms which may cause fish mortality. In this study, the acute and chronic effects of Nuvan stress on quantitative and qualitative aspects of erythrocytes in the freshwater fish, *Heteropneustes fossilis* were determined. This study also aimed to evaluate dose and duration dependent effects of Nuvan in the experimental fish.

### 2. Experimental Methods

#### 2.1 Experimental Fish

The healthy and active specimens of *Heteropneustes fossilis*, (Bloch) selected as a model for the present investigation were procured from local outlets of river Yamuna (UP, India) in the month of December, when room temperature ranged 18 °C - 25 °C.

#### 2.2 Maintenance of Experimental Fishes

The mean weight of fishes used was 60 ± 5 g and the mean length was 18.5 ± 5 cm. The fishes were transferred to the departmental laboratory and were put in to the stock aquarium containing tap water for two weeks so that they become acclimated to the new laboratory conditions. The fishes were carefully analyzed and treated with 0.2% KMnO<sub>4</sub> solution for

2 minutes before stocking to get rid of any dermal infection. The water was changed every alternate day. Fishes were fed properly with commercial food manufactured by Aerosol Chemicals Private Ltd., India. The temperature of the experimental room was maintained constant (21 ± 2 °C) and lighted for 12 hours. The water used throughout the experiment has pH value of 7.1 ± 0.1 and total hardness of 230 ± 2 CaCO<sub>3</sub> mg/L. The experiments were conducted in clean large glass aquaria measuring 75 cm x 37 cm x 37 cm.

#### 2.3 Experimental Chemical

The technical grade insecticides Nuvan (2,2-dichlorovinyl dimethylphosphate) selected for the present investigation is the trademark of AMVAC Chemical Corporation of India.

#### 2.4 Sub Lethal Toxicity

LC<sub>50</sub> for 96 hours of Nuvan to *Heteropneustes fossilis* was calculated as 6.45 mg/L by using log-dose probit regression line method [3]. After estimating the 96h LC<sub>50</sub> value, three different sub-lethal concentrations of Nuvan 0.26 mg/L, 0.32 mg/L and 0.43 mg/L which corresponds 1/25, 1/20 and 1/15 of LC<sub>50</sub> respectively was chosen to study their long-term and sub-acute effect on blood haematological component. Three sets of about twenty fishes were kept in three different aquaria each containing 20 L test solution. Simultaneously a control set was run parallel to the treated ones. Feeding was stopped two days prior to the commencement of experiments to keep the test animals more or less in the same state of metabolic requirements. Fifteen to twenty control as well as treated fishes were sacrificed after 7, 15, 30 and 60 days from each aquarium. Slime and water present on the body surface of the fishes were removed by using blotting paper. The blood was collected from their cut caudal vein in to a test tube containing an anticoagulant EDTA (1%).

#### 2.5 Haematological Study

Blood smears were prepared on glass slides for morphometric study of erythrocytes. T.E.C was counted with the help of standard improved Neubauer haemocytometer [4]. The morphometry of RBC's was studied by oculometer method.

#### 2.6 Statistical Calculations

Student's t-test was employed to calculate the significance of the difference between control and experimental means. P values of 0.05 or less were considered statistically significant [5].

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3. Results and Discussion

3.1 Total Erythrocyte Count (TEC)

The changes in the level of TEC in the blood of *Heteropneustes fossilis* exposed to different concentration of Nuvan are shown in Fig. 1. TEC decreased non-significantly ( $p>0.05$ ) at 0.26 mg/L on 7<sup>th</sup> day of exposure however at the same concentration significant decrease of TEC were seen on 15<sup>th</sup>, 30<sup>th</sup> and 60<sup>th</sup> day of exposures. Significant ( $p< 0.05$ ) decrease of TEC was observed on 7<sup>th</sup> day of exposure at 0.32 mg/L where as highly significant ( $p\leq 0.01$ ) anaemic condition was observed on 15<sup>th</sup> and 30<sup>th</sup> day at 0.32 mg/L with respect to their control sets. Very highly significant ( $p<0.01$ ) depletion of TEC in the experimental fish was recorded in the experimental fish on 15, 30 and 60<sup>th</sup> day of exposure at 0.43 mg/L.

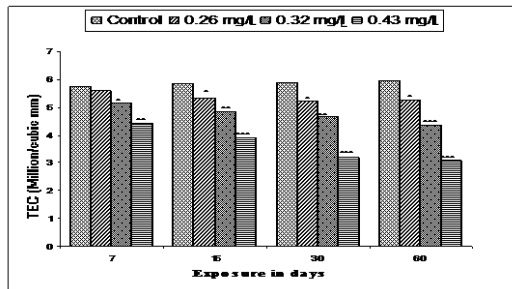


Fig. 1 Alterations in the TEC in *Heteropneustes fossilis* exposed to 0.26 mg/L, 0.32 mg/L and 0.43 mg/L for 7, 15, 30 and 60 days. Note: \*significant, ( $p<0.05$ ); \*\*Highly significant, ( $p\leq 0.01$ ); \*\*\* Very highly significant ( $p<0.01$ )

3.2 Morphometric Alterations in Erythrocytes

Mean length, breadth and surface area of erythrocytes of *Heteropneustes fossilis* exposed to 0.26 mg/L, 0.32 mg/L and 0.43 mg/L for 7, 15, 30 and 60 days are shown in the Table 1 and Fig. 2. The treated fish showed no statically significant variation ( $p>0.05$ ) after 7<sup>th</sup> day of exposure, some significant variations ( $p<0.05$ ) were shown on 15<sup>th</sup> day of exposure at highest concentration, however after 30 and 60 days the variation in the average length, breadth and surface area of erythrocytes was found statistically significant ( $p<0.05$ ) and highly significant ( $p\leq 0.01$ ).

The Nuvan exposed erythrocytes became elliptical and diminished and showed anisocytosis, however at the end of experiment, cells became hypochromic, small and large vacuoles were observed in most of the cells (Fig. 3). The cell membrane became diffused.

Table 1 Alterations in the erythrocyte morphometry of *Heteropneustes fossilis* (Bloch) exposed to the different concentrations of Nuvan for different time intervals

Days	Parameter	Control group (microns)	Experimental groups		
			0.26 mg/L	0.32 mg/L	0.43 mg/L
7	Length	10.94 ± 1.5	10.92 ± 1.8	10.79 ± 1.1	10.40 ± 1.2
	Breadth	7.88 ± 1.62	8.2 ± 1.29	8.04 ± 1.31	7.60 ± 1.17
	Surface area	86.06 ± 4.1	89.43 ± 5.4	86.15 ± 4.6	99.77 ± 3.4
15	Length	10.27 ± 1.9	10.0 ± 1.3*	9.8 ± 1.49	9.6 ± 1.41
	Breadth	7.43 ± 1.67	8.13 ± 1.57*	7.7 ± 1.23*	7.4 ± 1.42
	Surface area	76.31 ± 5.4	82.34 ± 7.3	76.97 ± 6.2*	72.85 ± 5.9*
30	Length	10.34 ± 1.9	10.38 ± 1.4*	10.86 ± 1.3*	9.84 ± 1.31**
	Breadth	7.67 ± 1.80	7.52 ± 1.44*	7.32 ± 1.5**	7.50 ± 1.41**
	Surface area	73.62 ± 4.7	81.82 ± 7.1	97.93 ± 5.7*	73.83 ± 6.2*
60	Length	10.42 ± 1.3	10.70 ± 1.3*	10.4 ± 1.30*	9.72 ± 1.45*
	Breadth	7.75 ± 1.33	8.37 ± 1.35*	7.8 ± 1.59*	7.73 ± 1.50*
	Surface area	80.75 ± 6.5	88.49 ± 6.3*	81.12 ± 8.1*	75.13 ± 7.2**

Mean ± S.Em = Standard error of mean, \* Significant ( $p<0.5$ ), \*\* Highly Significant ( $p\leq 0.01$ )

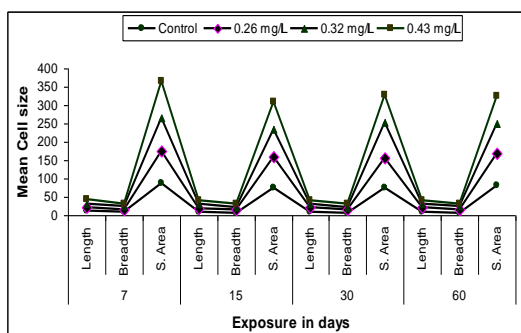


Fig. 2 Alterations in the erythrocyte morphometry of *Heteropneustes fossilis* (Bloch) exposed to the different concentrations of Nuvan for different time intervals <https://doi.org/10.30799/jespr.200.20060203>

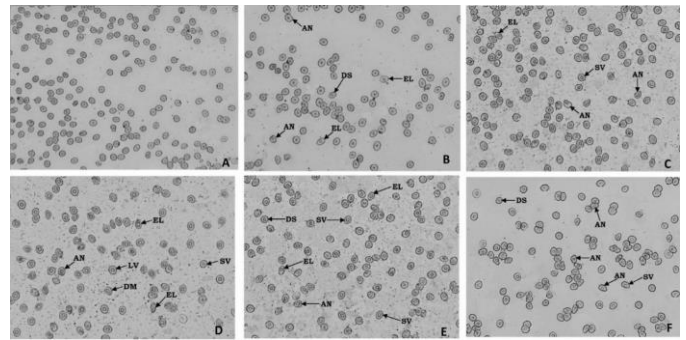


Fig. 3 A-F Micrographs showing erythrocytes of *Heteropneustes fossilis* stained with Geimsa (400X). (A) Shape of erythrocytes at 0.00 mg/L; (B) Shape of erythrocytes on 7<sup>th</sup> day of exposure at 0.26 mg/L; (C) Shape of erythrocytes on 15<sup>th</sup> day at 0.43 mg/L; (D) Shape of erythrocytes on 30<sup>th</sup> day at 0.32 mg/L; (E) Shape of erythrocytes on 60<sup>th</sup> day at 0.32 mg/L; (F) Shape of erythrocytes on 60<sup>th</sup> day at 0.43 mg/L) AN=Anisocytosis, EL=Elliptical, LV= Large vacuoles SV= Small vacuoles DM=Diffusing membrane

The significant reduction in the values of TEC as the concentration of Nuvan increases is an indication of severe anaemia caused by pesticide on the exposed fish. The anaemic response could be as a result of destruction of inhibition of erythrocyte production, haemodilution and it could be as a result of the destruction of intestinal cells by the toxicant [1]. Reduced TEC may be due to swelling erythrocyte or haemolysis which may be due to the increased protein carbon IV oxide in the blood. It may also be as a result of anaemia which is possibly due to haemodilution resulting from impaired osmoregulation across the gill epithelium as reported by Svoboda *et al.* [6], Scheck, Weber and Spieler [7, 8]. Varadaraj *et al.* also observed similar results in *Oreochromis mossambicus* exposed to different pesticides. This is further confirmed by Chindah *et al.* in *Tilapia guineensis* exposed to chloropyrifos [9, 10]. Reduction in the erythrocyte count in the present study can be attributed to: 1. Haemodilution due to the damage of fish organs [11, 12]; 2. Alterations of haematological parameters, whose changes can be interpreted as a compensatory response that improves the O<sub>2</sub> carrying capacity to maintain the gas transfer [13-15]. Similar changes were also reported in carp following the acute poisoning with atrazine by Svobodova and Pecena; and Velisek *et al.* [6, 16]. However, the decrease in the erythropoietic activity of the kidney [17] or the haemodilution may be the cause of decrease in TEC level of *Clarias gariepinus* exposed to Paraquat concentrations [18]. Similar findings were also reported by John in *Mystus vittatus* after chronic exposure to metasytox and sevin [19]. Ramesh and Sarvanan recorded similar results in *Cyprinus carpio* subjected to Chlorpyrifos [20]. Similar findings which support our study has also reported by Gautam and Kumar who studied haematology of *Channa punctatus* subjected to Nuvan [21]. Recently, Ponsen *et al.* studied haematological parameters and blood morphology of Asian eel, *Monopterus alba* and the results recorded are in agreement with our study [22]. Sasikala *et al.* observed similar results in metasytox induced *Channa striata* [23]. Further Alireza *et al.* findings support our study by assessing the heamatological parameters of Benny fish treated with herbicide [24]. Recent reports of Shankar Murthy *et al.* are completely in agreement with our findings [25].

Significant reduction in the size of erythrocytes of treated fish is due to the toxicity of Nuvan which interferes with the physiology of red blood cells with the result their morphology might have got also altered. The red blood cells in Nuvan treated fishes shrink when compared with their respective control groups. Lone and Javid analyzed short term exposure of *Channa punctatus* to sublethal concentrations of three organophosphate pesticides and reported progressive decrease in blood cell diameter which supports to our study [26]. Anees had earlier reported parallel findings while studying the morphology of fish erythrocytes he deduced that methyl parathion and dimethoate caused a consistent decrease in cellular and nuclear diameter of erythrocytes [27]. Mean cell volume and Packed cell volume has been reported to provide information on the size and status of erythrocytes [28], thus the inhibition of mean cell volume (MCV) and packed cell volume (PCV) as observed in the current investigation indicate that Nuvan may have interfered with the normal physiology. Similar findings has been mentioned by Okechukwu *et al.*, while studying the haematological indices of African catfish *Clarias gariepinus* under the acute nominal doses of chlorpyrifos-ethyl [29]. Das and Mukherjee reported similar findings in *Labeo rohita* after exposed to quinalphos in which they observed non-significant decrease in mean length, breadth and surface area of erythrocytes after 15 days exposure whereas after 30 days exposure the variation in mean length, breadth and surface area of erythrocytes was statistically significant [30]. In a similar study, Yii Siang *et al.* investigated the haematological parameters of Asian swamp eel,

*Monopterus albus* (Zuies.) treated with acute toxicity of organochlorine insecticide endosulfan, in which he reported an increasing trend in the size of erythrocytes when compared with control group [31].

In the present investigation the experimental fish exposed to various concentrations of Nuvan showed wide variation in the shape of erythrocytes. Present findings gain support from the observations which reported that various organic pollutants containing pesticides as well, directly affects the erythrocyte membrane and change the cell shape with the result the cell membrane become spiny or takes an echinocyte (cell with spicules) structure [32]. Similar results were also seen in blood samples of *G. niger* from Aliaga Bay where the water is polluted due to domestic and industrial factors. In the blood samples of *G. niger* from Aliaga refinery stations hypochromia (loss of colour), cell membrane became spiny and vacuoles were detected in the cytoplasm of the erythrocytes. In the similar study the structure of red blood cells of fish exposed to toxic elements and environmental pollution had deformed and increased in number [33]. However Patnaik and Patra observed that blood cells became elliptocyte i.e., more cigar like shape and some cells showed variation in size which confirms anisocytosis due to carbaryl effect on blood cells [15].

#### 4. Conclusion

Nuvan, a commonly used organophosphate insecticide in the agricultural fields reached the water bodies through the runoff affects the quality and quantity of erythrocytes in the fishes exposed to it. This can in turn affect the human life directly or indirectly as well. It is concluded that the pesticide has deleterious effects on the number and morphology of erythrocytes in the fish as observed through the present study.

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