Environment and Ecology 41 (3C) : 2026—2030, July—September 2023
Article DOI: https://doi.org/10.60151/envec/KEND7676
ISSN 0970-0420

Hematological Responses of Tilapia *Sarotherodon mossambicus* (Peters) Exposed to the Pesticide, Sevin

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Received 27 April 2023, Accepted 20 June 2023, Published on 4 September 2023

ABSTRACT

Since the blood takes part directly or indirectly in various physiological processes of the body, hematological parameters are considered important biomarker of alterations in metabolism or physiology. Indiscriminate use of pesticides has polluted different water bodies with adverse effects on the health of aquatic biota including fishes. Toxicological effects of agrochemicals including pesticides can be observed by monitoring hematological parameters. Present review deals with study of effects of pesticides on the important blood parameters such as erythrocyte count, hemoglobin content, packed cell volume, erythrocyte sedimentation rate, absolute values, leucocyte count, coagulation time and thrombocyte count of freshwater fish species. The review also aims to explain possible mechanism of pesticide induced alterations in the parameters and deleterious consequences on fish health. Tilapia (*Sarotherodon mossambicus*) were exposed to 5, 10, 20, 40, and 60% concentration of the 96 hrs, LC 50 value of Sevin for 7, 14 and 21 days. There was a significant decrease in the levels of hemoglobin and hematocrit of tilapia *Sarotherodon mossambicus* exposed to varied concentrations of Sevin indicating stress at higher concentrations.

Keywords *Sarotherodon mossambicus*, Hematology, Sevin, Stress.

INTRODUCTION

Now-a-days, detrimental ecological consequences posed by indiscriminate use of pesticides in agriculture are of great concern in general. Moreover, the increasing trend of Indian population requires self-sufficiency in food production by improved tools and techniques and effective chemicals. Pesticides are one of those agrochemicals that are widely used in agriculture to control different types of pests e.g., insects, unwanted weeds, parasitic nematodes and fungus (Tudi *et al.* 2021). A carbonate pesticide has increase during the recent past. The increasing interest in bioassay studies of fish responsive to pesticide stress is gaining recognition as there is essentiality of useful tools for monitoring the level of pollutants in the environment. In pesticide production and

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consumption, India is the biggest country in Asia and twelfth in the world while at fourth position for export (Devi *et al*. 2017).

The lethal concentration index LC_{50} is one of the widely used sensitivity tests to quantify chemical toxicants on animals (Sharmin *et al.* 2021). Water pollution is the cause of death of several interdependent aquatic forms of life and also a source of bio-magnification of persistent pesticides. This can result in local effect on environment and mortality of fish. Fishes are particularly sensitive to any change in physico-chemical as well as biological characteristics of aquatic bodies. The toxic chemicals in aquatic environment are proved to be dangerous for the survival of fish (Zacharia 2011). Hematological parameters of fish are becoming indispensable tool to assess the impact of pesticides on fish. Further, it is prerequisite to understand and estimate normal range of hematological parameters of fish under unexposed condition (Rao *et al.* 2017). The present study deals with the determination of hematological response of tilapia, *Sarotherodon mossambicus* exposed to Sevin.

MATERIALS AND METHODS

Fingerlings of *Sarotherodon mossambicus*, measuring 11 to 16 cm in total length (TL) and weighing 6-8g were obtained from wild and reared in fish ponds of College of Fisheries, Mangalore. Later they were stocked in cement cisterns (5x5x1 m) for 15 days in cow dung fertilized at 10,000 kg/ha. Besides from natural food available in the cisterns, the fingerlings were fed once daily with a mixture of rice bran and groundnut oilcake in the ratio of 1:1 by weight at 5% of body weight per day. They were then acclimated in a plastic trough of 20 liter capacity for a week period in the laboratory. During the period, the fishes were fed with standard pelleted feed developed at the rate of 5% of body weight per day. Fresh water drawn from the well situated at the college campus was used for the study. Prior to the start of the experiment, the water was filtered through filter to remove debris. Later, it was stored in plastic troughs and aerated. The Ph of the filtered and aerated water used for the experiment ranged between 7.4 and 7.6 and mean hardness value varied between 50-64 ppm $CaCO_3$. All the tests were performed at room temperature (26 ± 2^0 C). Carbaryl commercially known as Sevin (Manufactured by Gujarat Agro Industries Limited, Ahmedabad) 50% WDP (water dispersible powder) with an active ingredient carbaryl of 50% and 50% adjuvant and carriers were used for the study. The stock solution of concentration of 1000 ppm was prepared and further diluted to the required concentration needed for the both short term and long studies.

Mortality test were conducted in 20 liters capacity glass troughs filled with 15 liters of test media. A day prior to the start of the experiment, feeding of fish was stopped. Later fishes were exposed to varied concentrations of Sevin ranging from 11 to 16 ppm with uniform increment of 1 ppm. The median lethal concentration (LC 50) of Sevin on tilapia for 96h exposure was estimated using Thompson moving average method. Sub lethal toxicity studies were carried out for short term (14 days) and long term duration (21 days). Three different concentrations of Sevin viz., 20%, 40% and 60% of 96h LC 50 were set up for long term duration experiment while 5%, 10% and 20% and 96 h LC 50 were set up for long term duration experiment samples of three fish each in experimental cisterns were drawn every 24 hrs on 0, 7 and 14^{th} day for the estimation of hematological parameters. Feeding was resorted to every alternate day in order to avoid starvation.

Heparinized syringes were used to draw the blood for the estimation of red blood corpuscles, hemoglobin content and hematocrit values. As the blood from each fingerling was limited, blood samples obtained from several individuals were pooled and used for the estimation of the above mentioned parameters. The RBC count was determined using a Neubauler's chamber. The RBC cells were counted in 80 smallest squares and they were expressed in 106 /mm^3. The estimation of hemoglobin content in fish blood was made using the "acid hematin method" and it was expressed in gram percent (%) of volume and hematocrit values by the "micro hematocrit method" calculated as volume of the erythrocytes as a percentage of plasma volume (Ht %).

RESULTS AND DISCUSSION

Sevin is considered to be a safe pesticide and its

transport to the aquatic environment in considerable amount may prove to be an environmental hazard. Carbaryl bioassays of 96 h or less have been completed with TL 50 (medium tolerance limit) values determined for a number of fish species.

Lethal toxicity studies

In the present study, results obtained with *Sarotherodon mossambicus* for the 96th day LC 50 (Table 1) shows a value of 13.00 ppm as its median toxicity level. The differences in LC50 are attributed to type of species used, their size and age, physico-chemical properties of the rearing environment and formulation of pesticide administered (Sharmin *et al*. 2021). The LC 50 values of Sevin at 24, 48 and 72h exposures were 13, 10 and 8 ppm respectively. The observation of advanced test showed that the higher the concentration of pesticides given the higher fish mortality. It conformed to the previous study that mentioned the percentage of survival of tilapia fish (*Oreochromis niloticus*) decreases with increasing concentrations of the pollutants Rudiyanti and Ekasari (2009).

Sub lethal toxicity studies

Hematological responses

Hematology parameters are important in the health status of any organisms. In fishes, they are used for clinical diagnosis of fish physiology which is determined by the effect of the internal and external physical environment (Adeyemo 2005). Several studies on hematological changes in fish have been employed in a number of toxicological studies which are considered as possible indicators in environmental monitoring and also in the diagnosis of abiotic fish diseases.

Variations in the hematology of fish have been observed under stress conditions due to pesticide pollution. Several studies on fish exposed to pesticide indicating the increase in the hematological parameters viz., RBC, PCV and Hb have been reported. Hematology results corresponds to a better measurement of fish health and environmental status (Eissa and Abou-Elgheit 2014) and they can be substantially manipulated by animal's age, size, physiology, diet component and surrounding hydrology (Parrino *et al.* 2018). The hematology counts in current research demonstrated significant alternation between the animals in control group and treatment groups. However, in the present study during 7 days of exposure the mean control count of RBC of 2.37, million/mm^3 was recorded in the short exposure period and a considerable, decrease in the RBC values of 1.18, 1.17 and 1.14 million/mm^3 were reported in 5%, 10% and 20% Sevin concentration respectively on 14th day. The details on the RBC, Hemoglobin content and hematocrit indices are presented in (Figs. 1- 2).

Hemoglobin content

Haemoglobin is a respiratory pigment present in blood RBC. In fish, it carries oxygen from gills to the tissues and thereby helps in cellular respiration

Table 1. Cumulative percentage mortality of *Sarotherodon mossambicus* (11-16 cm) exposed to different concentrations of sevin at room temperature.

Time of exposure concentration in ppm	0	12	24	36	48	60	72	84	96
Control	-	-	-	-	-	-	-	-	-
11						-	10	10	10
12	-	-	-	-	-	10	10	20	20
13				-	20	20	30	40	50
14	-	-	-	20	20	40	50	60	70
15	-	-	20	20	30	70	90	100	-
16	-	10	20	30	50	80	100	-	-

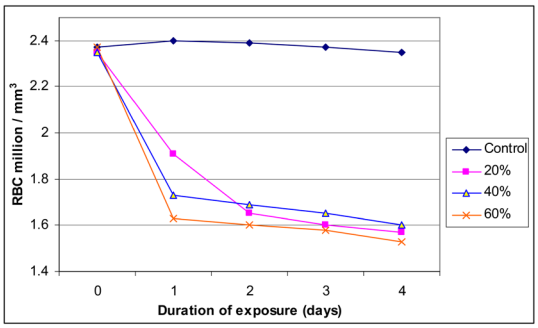

Fig.1. Total RBC (10^6) count of *Sarotherodon mossambicus* exposed to varying concentrations of Sevin over a period of 4 days.

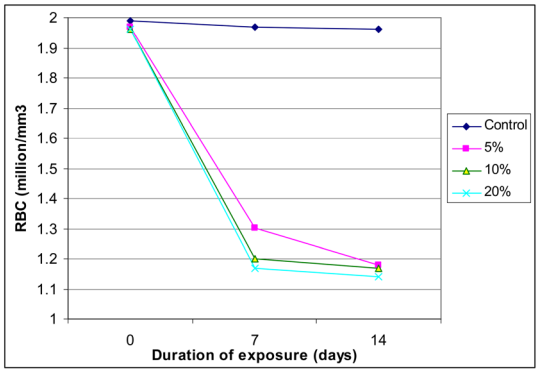

Fig.2. Total RBC (10^6) count of *Sarotherodon mossambicus* exposed to varying concentrations of Sevin over a period of 14 days.

(Brajesh *et al.* 2022). In recent decade, organophosphate induced anemia due to significant decrease in Hb content has been reported in *Barbonymus gonionotus* exposed to quinolphos (Mostakim *et al*. 2015).

In short exposure treatment, the hemoglobin content of 10.00, 7.12, 7.2 and 7.05 g% was registered in 1 day exposure to 0, 20, 40 and 60% Sevin concentration respectively. In long term study, a sharp decline in hemoglobin was noticed following 7 days of exposure and thereby marginally reducing to 6.40, 6.20 and 6.10 g% in 5%, 10% and 20% concentrations of Sevin respectively on 14th day (Figs. 3-4). Hemoglobin content and total leukocytic count in Fipronil exposed fish showed significant ($p<0.05$) decreases compared with the control group. This might be due to the fast oxidation of hemoglobin to methemaglobin or release of oxygen radical due to the toxic effect and

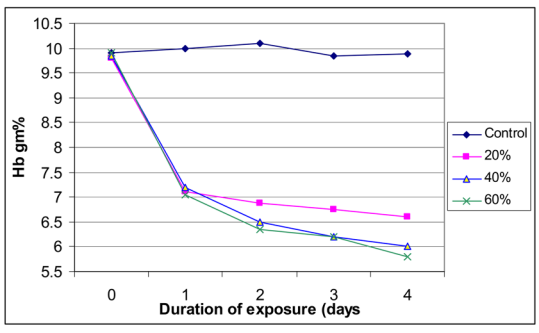

Fig.3. Hemoglobin (Hb) content (gm %) of Sarotherodon mossambicus exposed to varying concentrations of sevin over a period of 4 days.

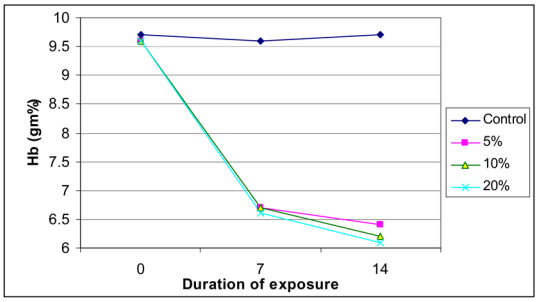

Fig.4. Hemoglobin (Hb) content (g %) of *Sarotherodon mossambicus* exposed to varying concentrations of Sevin over a period of 14 days.

oxidative stress induced by Fipronil as observed by Clasen *et al*. (2012).

Hematocrit indices

In aquatic environments, several hematological indices, including Hb, Hct, RBCs, have been used as indicators of metal pollution and are often used to assess the functional status of the oxygen-carrying capacity of the bloodstream (Fazio 2019). Increase in time of exposure of pesticide, generally in acute levels is associated with the complete destruction of hematopoietic system as an indicative of hematotoxicity in fishes (Ali and Rani 2009). In the present study, hematocrit value showed a linear decreasing trend with a mean control H1 value being 23.97%. Hematocrit values in the short term study sharply

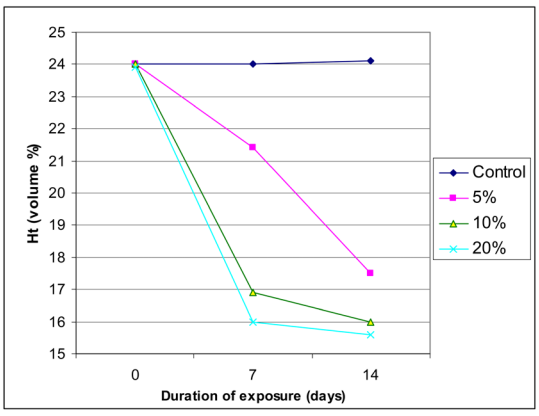

Fig.5. Hematocrit content (Ht) (volume %) of *Sarotherodon mossambicus* exposed to varying concentrations of Sevin over a period of 14 days.

declined to 17.50%, 16.00% and 15.66 in 5%, 10% and 20% concentrations of Sevin respectively (Fig. 5). Blood in fish exhibits the early effects of as toxicity because it enters the blood primarily through the vast gill surface area, at which the barrier between the blood and the metal salt is much thinner, and also through the buccal cavity (Kumar and Banerjee 2012).

CONCLUSION

The rapid fluctuations of these blood parameters due to sub lethal concentration of the pesticides indicate that continuous drainage of this pesticide to fishery waters may be fatal to fishes and hence this chemical should be used with great caution. In general, there is no fixed pattern of ill effect of different classes of pesticides on hematological parameters. However, toxicity of most pesticides has resulted into anemia and leukocytosis in fish. Altered hematological parameters in turn detrimentally change vital physiology of fish e.g. respiration, feeding, reproduction. Any change in the physiology of fish could have catastrophic consequences like decrease in productivity of fish.

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