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Research Article

Bioclinical stress of Rogor pesticide in the fish *Amphipnous cuchia*

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ABSTRACT

Background: The impact of acute and sub lethal toxicity of synthetic Rogor on some biochemical and hematological parameters of a fish *Amphipnous cuchia* was estimated aquatic environment by various pollutants like pesticides, detergents, discharge of effluents and heavy metals induce changes in the biochemical, hematological and behavioral aspects of inhabitants. These pollutants cause serious effects on growth, biochemistry, physiology, neurochemistry and survival rate of the aquatic organism.
Methods: The present research work is one such attempt to investigate the effect of organophosphorous pesticide Rogor on biochemical and hematological parameters of fish *Amphipnous cuchia*. The main objective of this study was to investigate the level of AST, ALT, hematocrit and TLC in fish *Amphipnous cuchia* exposed 24 to 96 hours to four different concentrations of Rogor pesticide.
Results: Rogor toxicity resulted in a significant maximum increase (89.15%) in level of aspartate amino transferase (7.87±0.14/18.31±0.09) and alanine amino transferase (76.65%, 5.28±0.11/3.03±0.14) enzyme. Regarding to hematological parameter, significant maximum increase (77.12%) in total leukocyte count (27,400±298/15,470±286) and maximum decrease (66.07%) in hematocrit (6.20±0.04 /18.31±0.09) level was observed.
Conclusions: In this study it is concluded that exposure to sublethal/lethal concentration of Rogor results in a significant alterations in different biochemical and hematological parameters and this kind of biochemical and physiological changes may directly affect the survivability of these fishes in these natural habitat.

Keywords: Rogor pesticide, Pesticide toxicity, Aspartate transferase, Alanine transferase, TLC, Hematocrit

INTRODUCTION

Use of pesticides in India began in 1988 when DDT (dichloro diphenyl tetrachloroethane) imported for malaria control and BHC (benzane hexachroride) for locust control currently, there are approximately a total of 215 pesticides are registered for manufacture and use of India (source: Central Insecticides board and Registration committee ministry of agriculture, 2008). These pesticides are will recognized as on economic approach to controlling pests, and to yield higher production of agricultural products.[1]

Though rampant use of these pesticides has given rise to several short term and long term adverse effects of these chemicals. Now there is growing concern worldwide over the indiscriminate use of such chemicals which result in environmental pollution and toxicity risk to organism even in human beings also. By so many studies on the toxicity of pesticides on different organism it is indicated that these chemical act as a potent neurotoxicant.[2]

In India, 51% of food commodities are contaminated with pesticide residues and out of these 20% have pesticide residues above the maximum residue level values on a worldwide basis. It has been observed that their long term low dose exposure are increasingly linked to human health effects such as immune suppression, hormone disruption, reproductive abnormalities and concern.[3] The chemical name of Rogor is Dimethoate, one of the most

widely used insecticides in the world; it is a particular concern to those exposed occupationlly during manufacture, formulation and use.[4] It was introduced in the 1950's, originally patented by American Cyanamid, and is now produced by 39 companies around the world.[5] Rogor is used against a broad range of pests such as mites, aphids, thrips, white flies etc and on a number of crops including fruits, cotton, potatoes, tea, vegetables, citrus and cereals.[6]

Like all organo phosphorus, Rogor acts by interfering with the activities of cholinesterase, an enzyme essential for the proper functioning of the nervous system of insects and human. Responses to OP insecticides by aquatic organism, not only depends on the quality and quantity of chemical, thought it also depends on exposure time of OP, water quality / characteristics, and the species of organism.[7,8] Acetylecholinesterase (Ache E.C. 3.1.1.7) enzyme is widely used for rapid detection to predict early warning of toxicity of pesticides.[9] Organo phosphorus pesticides are known to inhibit AchE, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter AchE to choline and acetate.[10]

The inhibitory effects of OP insecticides are dependent on their benching capacity to the active site of AchE and by their rate of phosphorylation in relation to the behavior and age.[11] Teratogenicity (birth defects), reproductive effects, mutagenicity, cancer may be chronic effects of Rogor along with acute toxic effects.[12]

Biochemical and hematological analysis can provide us valuable knowledge for assessing the health and conditions of aquatic fishes and it can correlate the health of human also because a major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes.[13,14] Normally, alanine aminotransferase (ALT/SGPT) is found in side liver cells. If liver is inflamed or injured SGPT is released in to blood stream. Thus the ALT is liver specific enzyme and it measures the level of hepatotoxicity. Increase in ALT indicates the tissue damage in liver.[15,16] Elevated SGOT/AST (aspartate aminotraneferase) activity serves as marker enzyme for cardiac tissue damage in fish and humans. Hematological parameters of diagnostic importance are absolute erythrocyte count, hemoglobin, total leukocyte count and hematocrit. The present research study is designed to study the toxic effects of Rogor on fish *Amphipnous cuchia* with regard to biochemical and hematological parameters.

METHODS

Animal maintenance

Amphipnous cuchia average length (60-68 cm) and weight (135-450 gm) procured from NBFGR, Lucknow were brought to biochemical laboratory in large aerated drum, washed three times in tap water and treated with 2% KMnO4 to remove any parasitic infections, and they were transferred to large glass aquaria and acclimatized for 96 hours. The average values for water characteristics data holding in aquaria were temperature 26±2^{o}C, p^{H} 7.10±0.05, dissolved oxygen 8.15±0.064 mg/L, total hardness 114.51±1.29 and alkalinity 118.50±2.63 mg/L.

Collection of samples for biochemical studies

Blood was taken from the caudal vein with heparinized syringes. The collected blood was transferred to clean dry test tube and allowed to clot at 10°c. The samples were centrifuged at 1000 g for 7 min to separate blood serum and it was transferred to another clean dry test tube for investigation of biochemical parameters i.e, SGOT/AST and SGPT/ALT Activity of enzyme was expressed as µmol pyruvate formed /ml/hour.

Estimation of aspartate aminotransferase (AST/SGOT)

Activity of AST was estimated according to method of Reitman and Frankel (1957) as given by Wootton (1964). 0.9 ml DL-asparatic acid solution (222 mm) and 0.1 ml α-ketogluteric acid solution (20 mm) were mixed to make the substrate. The substrate was taken into two separate clean dry test tube one for test and other for control. 0.2 ml serum was added in the test and incubated at 30^{o}C for 60 minutes. 1.0 ml of 2,4-dinitrophenyl hydrazine solution (1 mm) was added in each test tube, 0.2 ml serum was then added to 'control' and mixed thoroughly , then 10.0 ml 0.4 N NaOH was added and mixed . Optical density was determined at 505 nm against water blank and standard were also prepared as given in the method. Sodium pyruvate was used in standard and volume of serum was replaced by water. SGOT level was calculated as micro mole pyruvate formed /hour /ml serum.

Estimation of alanine aminotransferase (ALT/SGPT)

The method of Reitman and Frankel (1957) as given by Wotton (1964) was also adapted for the estimation of this enzyme. The same procedure was followed like AST except that DL- alanine was used in place of DL- aspartic acid in substrate and incubation time was kept only 30 minutes. Activity of ALT was calculated as µmole pyruvate formed/hour/ml of serum

Collection of samples for hematological studies

Two parameters for hematological studies had been taken during research study i.e, hematocrit and total leucocyte count. Blood was collected from caudal vessels and blood films were prepared for confirming the protozoan and other infections. For this purpose standard glass slides washed with 90% ethyl alcohol, were taken and then uniform film was immediately prepared and it was stained in giemsa's leishman's according to methods of Grandwhol (1943) and Wintrobe (1957).

Haematocrit

Electrical conductivity method was used for the analysis of hematocrit. It was calibrated directly in terms of percentage.

Total leukocyte count

Leukocytes were counted by electrical conductivity method of cell counting. A 1:250 dilution of the whole blood along with lysing and hemoglobin reagent was used for leukocyte count. The transducer was adjusted such a way that 0.3125 ml of sample was counted. The displayed readings for leucocytes were in thousands of cells per cubic millimeter of whole blood.

RESULTS

Aspartate aminotransferase (AST)

The results obtained on aspartate aminotransferase (AST) enzyme levels of the fish *Amphipnous cuchia* exposed for 24 to 96 hours, to four different concentration of Rogor, have been summarized in Table 1. Generally the AST activity was elevated at all concentrations and exposure to the pesticide Rogor. Maximum elevation of AST of 89.15% above control was observed at the highest concentration of 1.35 mg/L while minimum was at terminal exposure to the 1.05 mg/L concentration. 50% fishes did not survive at pesticide 1.05 mg/L under 96 hours exposure, same with 1.25 mg/L concentration after 48 hours exposure and at 1.35 mg/L pesticide concentration, dose became lethal for 48 hours and onwards exposure.

Alanine aminotransferase (ALT)

The results obtained on alanine aminotransferase (ALT) levels of the fish *Amphipnous cuchia*, exposed for 24 to 96 hours, to four different concentration of Rogor, have been summarized in Table 2.

The maximum elevation of ALT level of 76.65% above control was observed after prolonged exposure of 96 hours at lowest concentration of Rogor 1.00 mg/L. while minimum elevation of 5.98% was seen after 72 hours at the same concentration. Generally, it was observed rise in ALT levels at each and every concentration and at very high concentration and long exposure duration 50% of fishes did not survive.

Table 1: Effect of pesticide Rogor on AST levels of fish *Amphipnous cuchia.*

Pesticide conc. (mg/L)*	AST µmoles formed/ml/hour (Mean±S.D); Range in paranthesis; time of exposure in hours			
	Control values 4.15±0.21 (3.15 – 4.72)			
	24	48	72	96
1.00	7.64±0.24 (7.40 – 7.87)	6.76±0.12 (6.64 – 6.90)	6.10±0.13 (5.95 – 6.23)	6.61±0.32 (6.17 – 6.88)
1.05	7.80±0.48 (7.05 – 8.11)	6.81±0.50 (6.06 – 7.17)	6.34±0.14 (6.11 – 6.43)	------
1.25	7.77±0.18 (7.57 – 7.97)	6.71±0.39 (6.14 – 7.60)	------	------
1.35	7.87±0.14 (7.67 – 7.99)	------	------	------

* no. of observations 10 in each case

Table 2: Effect of Rogor pesticide on ALT levels of fish *Amphipnous cuchia.*

Pesticide conc. (mg/L)*	ALT µmoles formed/ml/hour (Mean±S.D); Range in paranthesis; time of exposure in hours			
	Control values 3.03 ± 0.14 (2.90 – 3.16)			
	24	48	72	96
1.00	3.79±0.11 (3.67 - 3.92)	4.67±0.34 (4.18 – 4.94)	3.21±0.09 (3.10 – 3.27)	5.28±0.11 (5.20 – 5.40)
1.05	3.99±0.16 (3.88 - 4.11)	3.48±0.41 (3.07 – 3.87)	3.58±0.11 (3.49 – 3.68)	------
1.25	4.24±0.13 (4.09 – 4.38)	5.00±0.09 (4.88 – 5.11)	------	------
1.35	4.27±0.16 (4.12 - 4.40)	------	------	------

* no. of observations 10 in each case

Table 3: Effect of pesticide Rogor on hematocrit of fish *Amphipnous cuchia.*

Pesticide conc. (mg/L)*	Hematocrit (%) (Mean±S.D); Range in paranthesis; time of exposure in hours			
	Control values : 18.31±0.09 (18.20 – 18.43)			
	24	48	72	96
1.00	17.49±0.08 (17.38 – 17.62)	12.32±0.12 (12.13 – 12.50)	10.31±0.08 (10.19 – 10.40)	9.08±0.03 (9.02 – 9.14)
1.05	11.40±0.09 (11.30 – 11.58)	9.17±0.03 (9.12 – 9.22)	8.65±0.04 (8.59 – 8.72)	-----
1.25	10.70±0.05 (10.62 – 10.78)	7.38±0.08 (7.28 – 7.50)	-----	-----
1.35	6.20±0.04 (6.14 – 6.26)	-----	-----	-----

* no. of observations 10 in each case

Table 4: Effect of pesticide Rogor on total leucocyte count of fish *Amphipnous cuchia.*

Pesticide conc. (mg/L)*	Total leucocytes count/cu mm (Mean±S.D); Range in paranthesis; time of exposure in hours			
	Control values 15,470±286 (15,100 – 15,800)			
	24	48	72	96
1.00	15,891±287 (15,550 –16,300)	18,669±273 (18,300 – 18,950)	21,500±366 (21,000 – 21,900)	25,440±242 (25,300 – 25,900)
1.05	18,750±167 (18,500 – 18,900)	19,460±321 (19,000 – 19,800)	21,940±230 (21,700 – 22,300)	-----
1.25	23,480±285 (23,100 – 23,800)	26,540±207 (26,300 – 26,800)	-----	-----
1.35	27,400±298 (27,000 – 27,800)	-----	-----	-----

* no. of observations 10 in each case

Hematocrit

Table 3 showed the result and observation of hematocrit parameter on fish *Amphipnous cuchia* exposed under different concentrations of Rogor at 24 to 96 hours of exposure. Over all observation regarding to hematocrit parameter was found to decease in its level at all concentrations and time intervals. The maximum fall of hematocrit level was noticed as 66.07% below control within 24 hours of exposure at the highest concentration of 1.25 mg/L of Rogor. The hematocrit levels decreased gradually with increasing time intervals and were minimum at the end.

Total leucocytes count

Result of TLC represented and summarized in Table 4. Leucocytosis was evident at all concentration and exposures to the pesticide Rogor. The maximum increase of TLC 77.12% above control was observed at the highest concentration of 1.35 mg/L within 24 hours while minimum rise was 2.7% above control at the lowest concentration of 1.00 mg/L of Rogor pesticide.

DISCUSSION

In the present research article we studied the adverse effects of the organophosphate pesticide Rogor. These pesticides are responsible to degrade the environment frivolously. The biochemical and hematological investigations may act as indicators or monitors of prevailing aquatic pollution under natural ecological condition and in experimentally created aquatic environment polluted with contaminants such as pesticides, insecticides, toxic metals etc.

In the present study it was found the increasing tendency of aspartate transaminase (AST) and alanine transaminase (ALT) enzymes in experimented fishes exposed under the Rogor pesticide, suggesting that toxicity of OP Rogor pesticide induce its changes because of their adverse action on respective tissue sites of *Amphipnous cuchia* and the elevated levels of AST and ALT is due to combat the toxic effects of Rogor pesticide toxicity. Similar changes had been observed on fresh water fishes due to other pesticides by Pandey et al, Sethi and singh, Tandon et al.[17,18,19]

Regarding to hematological parameters total leukocyte counts were observed in increasing manner. The significant elevation of TCL in present study indicate the sensitivity of TLC count of Rogor pesticide and these alterations could be due to immunological reaction to produce antibodies to cope up with stress triggered by Rogor pesticide. Similar finding was also noticed by Ramesh and Sarvan, 2008. Hematocrit was another parameter of clinical chemistry and fall in hematocrit level was observed. These observations are similar with their findings of Darbal et al, Koundinya RP et al.[20,21]

CONCLUSION

The finding of present research revealed the parameters AST, ALT and TLC are in increasing trends, suggesting that the organo phosphorous pesticides induce it's changes because of their adverse action on respective systems of fish *Amphipnous cuchia*, and the level of AST and ALT enhanced to combat the effect of pesticides toxicity. Similar significant changes in biochemical parameter of fresh water fishes were observed due to effects of pesticides (Pandey et al, Sethi and Singh, Tandon et al and Mishra BP et al). 50% fishes did not survive at higher and long time exposure because of asphyxiation, caused by toxicity of pesticide. significant increase in TLC i.e. leucocytosis in the present study indicate the hypersensitivity of total lecucocytes count to Rogor pesticide and these changes may be due to immunological reactions to produce antibodies to cope up with stress induced by Rogor (Ramesh and sarvanan). Under the light of this toxicity study it is concluded that exposure to sublethal/ lethal concentration of Rogor results in a significant alterations in different biochemical and hematological parameters and this kind of biochemical and physiological changes may directly affect the survivability of these fishes in these natural habitat.

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