COMPARATIVE STUDY OF THE TOXIC EFFECTS OF ENDOSULFAN AND MALATHION ON BLOOD INDICES OF A FRESH WATER TELEOST, TILAPIA MOSSAMBICA

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Abstract: The aim of this study was to determine and compare the acute toxicities of two different categories of pesticides namely organochlorine (endosulfan) and organophosphate (Malathion) which are regularly used in agricultural practices. Juvenile fish irrespective of sex were randomly selected and exposed to these two pesticides. The toxicity tests were performed separately for each pesticide. It was found that fish were more sensitive to endosulfan than Malathion. Median Lethal Concentration (LC50) values of endosulfan were 4.5μg, 4.1 μg, 3.61 μg and 2.75 μg while those for Malathion were 10mg, 8.9mg, 8.1mg and 3.609mg respectively, at 24, 48, 72 and 96 hr respectively. Toxicity assessments were done following static bioassay and probit analyses models. The fish revealed loss of balance and erratic swimming prior to mortality. Both primary and secondary blood indices and behavior is greatly influenced by endosulfan even in fewer amounts when compared to Malathion.

Blood was assayed for selected hematological parameters (haematocrit, hemoglobin, red blood cell counts, erythrocyte sedimentation rate, and total plasma protein and plasma glucose concentration). Haematocrit and Erythrocyte Sedimentation Rate (ESR) values were found high in fish treated with both the pesticides. ESR is negatively correlated with RBC count. Compared to those of the control fish, the Hb content and RBC count of the exposed fish were much lower. The values of MCV, MCH and MCHC of infected fish were also seen to fluctuate compared with those of the normal fish. Plasma level of glucose was also lower in the exposed fish when compared to the control. It could be concluded that these pesticides are extremely toxic and should be used very carefully. More prominent poisoning symptoms were observed in fish treated with endosulfan. Our results conclude that endosulfan is highly toxic than Malathion. The pesticides also caused time and dose-dependent deformities and behavioral abnormalities.

Keywords: Pesticide toxicity, Comparative toxic analysis, Tilapia mossambica

Introduction: The comparative analysis of toxic effects of pesticides is helpful to better understand the comparative ecological risk caused by pesticides. In general, the organophosphate (OP) and organochlorine (OC) pesticides share a common mode of action. Exposure to multiple these pesticides can lead to additive toxicity. On the other hand the different pesticides differ extensively in their potency and how well they are absorbed by the body depending on the route of exposure (Reigart, J. R.; Roberts, J. R. 1999).Fish and other aquatic biota may be injured by pesticide-contaminated water. Pesticide surface runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream [1]. Presence of pesticides in aquatic systems and its effects on can be assessed in fish by using physiological, behavioral and haematological indices [2], [3]. Pesticides provoke different kinds of toxicity in fish, which lead to bring changes in behaviour [4] [5] [6] [7] and haematological changes [7] [8] [9] [10].

Malathion (organophosphate) and Endosulfan (organochlorine) are common in many ecosystems and are used extensively to enhance the yield in agriculture crops. Endosulfan is very toxic to fish, even compared to other organochlorine pesticides [11]. However, the mechanism o toxic effects of both the pesticides are the same as of other pesticide substances as they inhibit entire series of enzymes and mainly of acetylcholinesterase [12] [13] [14] [15]. But endosulfan became an extremely dangerous agrichemical due to its acute toxicity, probable for bioaccumulation, and role as an endocrine disruptor. It is acutely neurotoxic. It is a GABA-gated chloride channel antagonist, and a Ca^{2+}, Mg^{2+} ATPase inhibitor. Both of these enzymes are involved in the transfer of nerve impulses. [16].

The purpose of this study is to determine and compare the acute toxicities of two pesticides Malathion (organophosphate) and Endosulfan (organochlorine) and to establish a relationship between the toxicities of two different classes of pesticides in which mode of action is different. *Tilapia* serves as a natural, biological control for most aquatic plant problems. This study therefore, parallelly evaluates the effects of Endosulfan, an organochlorine insecticide (OC) and Malathion, an organophosphate insecticide (OP) at sub lethal doses on behavior and haematological parameters of *Tilapia mossambica* to contribute to the knowledge of the effects of pesticides on fishes. The most important cause for execution of toxicity tests with fish and other aquatic organisms is to establish at which concentration these substances are harmful to the organisms or which have no noticeable effect. Changes in these blood parameters show toxic stress in animals especially on blood and blood-forming organs [17]. The purposes of this study were to evaluate the chronic toxicity of endosulfan on anemia by determining hematological and biochemical indices, and to establish a possible relationship among alterations in hematological indices, anemia, plasma biochemical profile and changes in behaviors and mortality of Tilapia.

Materials and Methods: *Sample Collection and Maintenance*: Healthy and active *Tilapia mossambica (Peters)* juveniles of same size, length and weight approximately (22gm-25gm) (irrespective of age and sex) were procured from the local fishermen at Jhabua, Madhya Pradesh, India and were maintained for 15 days for acclimatization under natural conditions.

Acute toxicity test: Acute toxicity tests for both endosulfan and Malathion were performed as in our earlier experiments [18] [19]. The 96h LC_{50} value of Malathion and endosulfan was found to be 3.609 mg/L and 2.71 µg/L respectively. One tenth of the LC50 values were selected for sub lethal studies. Behavioral patterns were observed in all experimental and control groups. During the experimental period the control and exposed fish were kept under constant observation to study swimming behavior.

Haematological profile: After the biometric test period, brood stocks were anesthetized and blood samples were acquired through caudal vein puncture. Haematological indices were determined using different experimental techniques in the laboratory [20],[21].The haematological parameters tested were erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and Leucocyte count (WBC).

Coagulation test*:* The blood in the tube was maintained at room temperature for coagulation. (INR) The International Normalized Ratio (INR) was measured on the basis of blood clotting time.

Erythrocyte sedimentation: Erythrocyte sedimentation rate and clotting time were also determined. The data obtained from this study was subjected to various statistical tools. The differences in the means (±SEM) between groups were assessed using Independent Samples-t test, adjusted to 95% confidence limits some important.

Biochemical parameters: Blood glucose was determined using the Folin Malmros micro procedure as modified by Murrell and Nace (1958) [22]. The total Protein content of the pesticide exposed tissue samples were estimated according to modified standard method of Lowery et al (1951) [23].

Statistical analysis: The results were computed by Stat Plus® version 2009 and XLSTAT computer software. The differences in the means (±SEM) between groups were assessed using Independent Samples-t test, adjusted to 95% confidence limits some important. Excel 2010 used for drawing charts.

Results and Discussion

Comparison of Behavioral studies: Behavioral interactions are mainly linked with predator avoidance, reproductive, and social behaviors. It form an important part of a successful, adaptive life history strategy of any organism. Variation to normal behavioral patterns caused by exposure to pollutants therefore poses serious risks to the success of fish populations. Environmental contaminants such as metals, pesticides, and other chemicals create severe risks to many aquatic organisms including fish. For that reason, a great deal of earlier research has described physiological mechanisms of toxicity in animals exposed to contaminants. In contrast, effects of contaminants on fish behavior are less studied. Because behavior associated neuro physiological function with ecological processes, hence behavioral indicators of toxicity appear ideal for assessing the effects of aquatic pollutants on fish populations.

Tilapia when exposed to the different concentrations of the pesticides, showed abnormal behavioral changes. When treated with sub lethal concentrations (1/10) of both Malathion and Endosulfan, the exposed fish were aggregated at the corner of the aquarium resting at the base and frequently come to the surface followed by heavy breathing with stronger opercular movements and loss of equilibrium. The fishes were jumping inside the aquarium so that it would get out of the aquarium. It is maybe due to irritation on the skin. Their body became slimy and lots of mucus secretion from the skin. After 48 hours in both experimental groups they lost their balance and the body is obliquely placed from the horizontal position. After 72 hours they stop moving and settled in a particular place and also not take food at all. Moreover over-secretion of mucus was observed from the body surface. Body colour also darkened, pectoral and pelvic fins got stretched and the fish revolved vertically prior to death. These effects are much severe in fish treated with Endosulfan than Malathion. The abnormal behavioral pattern in exposed fish may be due to which is due to inhibition of acetyl cholinesterase (AChE) activity leading to accumulation of acetylcholine in cholinergic synapses causing hyper stimulation [24]. Especially it was observed that after the exposure to endosulfan, the intoxicated fish exhibited erratic, jerky and abrupt swimming, frequent surfacing and gulping. This could be due to the skin irritation, respiratory rate impairment or a response to the altered locomotor activity which is an indication of the effect of endosulfan on the nervous system of the fish (Hii, Y [25].

Haematological studies: Blood offers important profile to study the toxicological impact on animal tissues. These indices have been effectively employed in monitoring the responses of the fish to the stressors and evaluating its health status under such adverse conditions. Haematological parameters of fish are highly variable between and within species and seasons [26]. It also differs with other individual factors like temperature, season, sex, food, and the type of culture [27], [28].

Results of haematological profile are summarized in table 1. Results the present study clearly demonstrate that a significant decrease in erythrocytes counts (RBCs) and haemoglobin in fish treated with both Endosulfan and Malathion which may be due to suppressive and toxic effect on bone marrow and subsequently on haematopoiesis [29]. Results also indicate that sub lethal concentration of 1/10 of LC50 of Endosulfan and Malathion induced significant decrease in hemoglobin content in treated animals compared with normal. However the decrease is much higher in fish treated with Endosulfan than Malathion. The decrease in other haematological variables (PCV, Hb and RBC) of the exposed fish may be due to haemolysis of red blood cells by both pesticides leading to significant decrease in haematocrit value which results in fish anaemia. Similar observations were reported for juvenile *C. gariepinus* separately treated with endosulfan and with other pesticides separately [30]. The mean corpuscular hemoglobin (MCH) values in both treated animals did not showed many significant differences. But, the sub lethal doses of both the pesticides induced significant dissimilarities in mean corpuscular hemoglobin concentration (MCHC). Haematocrit values were decreased but MCV values were elevated in fish exposed with both pesticides. Reduction in Heamoglobin was accompanied by lowest haematocrit value (Tab.1).Reduction in Hb concentration was due to reduction in total number of RBCs. The decrease in HCT and increase in MCV are characteristic of an anemic condition [31].The significant decrease in RBC count in the present study might be due to haemolysis and shrinkage of blood cells by the toxic effect of insecticide. Chakraborty and Banerjee (1988) [32]. Mishra, J. and Srivastava, A.K., (1983.) [33]. and Adhikari et al (2004) [34]. also observed in a similar findings in fish treated with pesticides and chemicals.

Table.1. Comparative analysis of toxic effects of Malathion (Organophosphate) and Endosulfan (Organochlorine) pesticides on *Tilapia mossambica*.

Parameter	Malathion	Endosulfan
LC_{50} value	3.609 mg/L,	2.71 µg/L
Total protein.mgL	1.71±0.1*	1.9±0.1*
Plasma Glucose (mg/L)	166.1±3.4***	168.1±3.4***
Haematocrit (%)	19.2±0.18**	19.2±0.18**
Hemoglobin(g/100ml)	8.81±0.42**	8.11±0.42**
RBC(106 mm-3)	1.83±0.23**	1.53±0.23**
ESR (mm/h)	16.1±0.9	16.1±0.9
MCHC (%)	21.3±11**	21.3±11**
MCH (g)	59.8±3.51**	59.8±3.51**
MCV (ug)	148.4±5.3	148.4±5.3
CT (Sce)	44.74±0.444	41.74±0.446

NS= Not significant * = P>0.05 Significant * * = P>0.01 *** = P>0.001

These alterations were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in hemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to lead [35].The decrease in RBC and Hb content indicates acute anemia. In the present investigation, haemolysis might have been one of the causes for reduction in Hb, RBC and PCV values. Another reason for RBC suppression could also damage the haemopoietic tissue. PCV appears to be positively correlated with RBC counts and consequently a decrease in PCV is observed.

But in contrast, a single spray of monocrotophos insecticide (Nuvacron 40) did not affect significantly the count of erythrocytes (RBCs) and leucocytes count (WBCs) and haematocrit values in volunteers. Decreased heme synthesis in bone marrow, increased rate of destruction or reduction in the rate of formation of RBCs, and increased erythrocyte lipid peroxidation could be the possible reasons for such reduced hematological levels [36].

The MCV gives an indication of the status or size of the erythrocytes and reflects an abnormal or normal cell division during erythropoiesis. On the other hand, the decrease in MCV that observed in this study after 96hours of pesticide exposure coupled with low hemoglobin content indicated that the erythrocytes have shrunk, either due to hypoxia or a microcytic anemia. Thus, microcytosis might be due to the decrease in the haematocrit during exposure. Similar pattern has been detected in Moggel fish *(Labeo umbratus)* after exposure to various pollutants [37]. (Nussey *et al.,* 2000). In this study the fluctuation in the MCH was observed but MCHC had increment .It was indicated that the concentration of hemoglobin in the red blood cells were much lower in the infected fish than in the control group, thereby, depicting an anemic condition. Anaemia can be caused by a number of pathological conditions. The macrocytosis is probably an adaptive response through the influx of immature erythrocytes from the hematopoietic tissues to the peripheral blood to make up the reduced erythrocytes number and decreased hemoglobin concentration [38]. (De Pedro, N., Guijarro).

Results indicate that total protein in both group I and II (exposed) were elevated while glucose values of fish of Group I (malathion treated) and Group II (endosulfan treated) were decreased ($P < 0.05$). The elevated level of plasma Total protein in both experimental fish may be due to increase in immunoglobulins to fight with toxicants and toxicant induced diseases. Each protein has a potential for a variety of post-translational and metabolic modifications, both in normal and diseased cells. Glucose is the primary source of energy to carry out the metabolism. The body naturally strongly regulates blood glucose levels as a part of metabolic homeostasis. Results from table.3 clearly indicate that the glucose level was declined in both Group I and II significantly. The low levels of plasma glucose in the present experiment may be due to pesticide induced hypoglycemia which is fatal to the fish. This condition can produce a variety of signs and effects, but the major problems arise from an inadequate supply of glucose to the brain, resulting in impairment of function (neuroglycopenia).

Conclusions: Variations in the degree of toxicity of different pesticides have also been reported by other workers ([38], [39]. Thus the present study also points out the possibility that although organophosphorus pesticides may have less long term residual effects than that of organochlorines like endosulfan, their short term acute toxicity up to 96 hr is even more hazardous. Under the light of this study, it is concluded that Malathion is moderately toxic while Endosulfan is highly toxic to *Tilapia mossambica.* Exposure to low concentrations of these pesticides results in significant changes in hematological alterations and behavioral changes and these changes may be potentially disruptive for the survivability of the fish in their natural environment. This fact should be taken into consideration when it is used for pest control in the agricultural fields surrounding their natural freshwater reservoirs. The analysis can be converted into decisions in risk management and decisions to minimize the toxic impacts.

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