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Effect of Malathion on physio-biological aspects of *Notopterus notopterus* (Pallas)

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Abstract

During the present experiment, *N. notopterus* were exposed to lethal concentration (0.1 ppm) of malathion for a period of 03 hours in triplicates. A marked reduction in the opercular beat frequency (92.3 to 45.3) and tail beat frequency (7.3 to 4.3) was observed after the exposure. The results on the haematological aspect of the experiment (5 replicas) revealed that significant ($P<0.05$) increase in WBC (6.06 to4.58), and reduction in Hb, RBC, PCV, MCV, MCH, MCHC and other non-specific defense cells. The increase in the WBC count is due to the non-specific immune response of the fish.

INTRODUCTION

As a result of irrigation and rain runoff, the pesticide or pesticide residues are added continuously to the aquatic environment, thereby polluting fish and water bodies. Fish and water bodies around the world offer not only food to people but also act as a means of generating employment and money for thousands. Unfortunately fish and water bodies are in grave danger because of bioaccumulation of pesticides. Many types of fish and different aquatic ecosystems have been adversely affected by pesticides which end up being suspended in water and binding with soil particles thereby making the water and soil particles unavailable to aquatic life.

Fish live closely with their aquatic environment; living epidermal cell membranes have direct contact with all materials carried by water. This intimate contact eases the movement of chemicals into and through the mucous, skin and other external layers and becomes a disadvantage to the fish when nefarious chemicals, pollutants and contaminates enter the aquatic environment. These Chemicals can have adverse effects on the fishes' physiological pathways, including those imported mechanism that help protect the fish against disease; the non-specific defense mechanism and the specific immune response, which may be assessed through the haemo-biochemical alteration.

Blood analysis is crucial in many fields of ichthyological research and fish farming and in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes in fishery management and diseases investigation [1]. Many workers have assessed the effect of various pesticides on the behaviors and haematological responses of various species of fish [2-4] and have found varying responses after exposing the fish to varying sublethal concentrations using the 96 hours acute toxicity tests. As a matter of fact, the present research work on the "effect of malathion on the blood profile of fish *Notopterus notopterus* Pallas" will be an engender in the field of fish toxicology and a value addition to the haematobiochemical profiles of fish exposed naturally and artificially to sub lethal/lethal concentrations of different pesticides.

MATERIALS AND METHODS

Original healthy *Notopterus notopterus* (pallas) fishes weighing 150-170 g with a mean body length of 24-26 cm, were collected from the fish hatchery in Bhopal, M.P. Fish were brought to the wet laboratory and acclimatized for two weeks prior to experimentation. The fishes were fed with balanced diet/pelleted feed with 35% crude protein diet at 2% biomass.

Malathion ($C_{10}H_{19}O_6PS_2$) is manufactured by Shivalic Agro Chemical Industries India. It is manufactured by Shivalic Agro Chemical Industries. The parathion is broad spectrum organophosphate pesticides used to control many insects pests. It has non-systematic, contract stomach and fumigant action. This organophosphate insecticide is cholinesterase (an enzyme that is essential for the proper working of nervous system of both human and insect) inhibitor. Parathion is highly toxic by all routes of exposure. The lethal concentration (0.1 ppm) of the pesticide was prepared by dissolving 1 ml of original concentration of pesticide individually in 10 liter of chlorine free water. 30 L of the diluent water was used as control. The fishes (n = 30) were kept in each aquarium in triplicates for each treatment. The stock solution of 0.1 ppm of the solution was introduced separately in each tank. The fishes were observed for 1-5 hours for any mortality during the exposure time.

Opercular beat frequency (OBF) was calculated by observing the opercular beats before and after the exposure to assess the impact of pesticides on the physiological requirement of oxygen. The OBF was measured using the stop watch, analyzed for one minute after every 20 minutes post exposure. Tail beat frequency (TBF) is an index of calculating the frequency (no. of times) of tail movements of the fish before and after the exposure to pesticides. TBF gives an index about the physiological imbalance/abnormal behavior a fish shows post exposure, owing to the damage to the central nervous system or other physiological processes.

The blood samples from the challenged fishes were taken after every 10, 20 and 30 min. in fishes exposed to mixed solution of the pesticides. Blood samples were collected from the caudal tail vessels with 21 or 23 gauge needles and 1 or 3 cc syringes before ventilatory response was noticeably depressed. PCV (%) was determined by centrifuging the blood for three minutes (3000 rpm). The haemoglobin Content (Hb) of erythrocytes was determined by the hemoglobin cyanide method. After standardization of haemoglobin estimation with the standard cyanmethemoglobin solution of "VEB Berlin-Chemie" or Berlin Chemicals, the hemoglobin content was determined in g/100 ml. RBC value was determined by counting all the cells lying to the left and below the demarcation line of counting chamber. MCV, MCH and MCHC were calculated by the standard formula's [5].

For leucocyte counting, the blood was drawn in to the 0.5 mark in the erythrocyte pipette. After shaking, the counting chamber was filled in the large squares which are present at the four angular points of the Neubauer counting chamber and demarcated by triple lines (1 mm^2). Differential leucocyte count (DLC) included different cell counts. Unna-Ziehl staining was used for differentiating small and large lymphocytes. € granulation staining was used for differentiating neutrophils by the standard method of Romeis (1968). ∂-granulation staining was used for differentiating monocytes as per the standard methods Unna Ziehl staining was done for differentiation of thrombocytes as per the methods of the total serum protein was determined by Gornall's biueret method [6].

RESULTS

The pesticide malathion was observed to have adverse effect on the physio-biological activities of *N. notopterus*, as depicted in the tables 1 and 2. The results of the opercular beat frequency (OBF) for malathion are presented as mean±SE in Table 1. In case of 0.1 ppm malathion exposure to *N. notopterus* (pallas), the OBF rose from 90.7±0.6 (0 minutes) to 93.7±5.5 (01 hour post malathion exposure). The OBF showed further increase to 113.3±2.9 (02 hour post malathion exposure) and reduced to 45.3±5.5 (3 hr post malathion exposure). Tail beat frequency (TBF) showed the same trend. In case of 0.1 ppm malathion exposure to *N. notopterus* (pallas), the TBF rose from 7.7±0.6 (0 minutes) to 9.7±0.6 (01 hr post malathion exposure). The TBF showed further increase to 11.7±2.9 (02 hr post malathion exposure) and 4.3±0.6 (03 hour post malathion exposure).

For investigation into the effect of pesticides on the hematological indices, fifteen blood parameters were studied. The mean±SD value of normal PCV (%) was 25.0±0.83, which rose after 03 hrs of exposure, ranging from 38.0-42.0 with a mean±SD of 40.0±0.98, showing 'variance', 'regression equation' and 'correlation coefficient of 3.7.8, Y=0.245 X +26.4 and 0.97 respectively. The normal haemoglobin (Hb) expressed in g/dL was 8.3±0.23, which decreased after 03 hrs of exposure, ranging from 6.51-6.89 with a mean±SD of 6.7±0.25, with 'variance', 'regression equation' and 'correlation coefficient of 428.3, Y= -0.027 X +8.5 and 0.95 respectively.

The RBC count ($x10^6/\mu L$) was 2.61±0.06, which decreased after 03 hrs of exposure, ranging from 1.58-1.82 with a mean±SD of 1.7±0.69 showing 'variance', 'regression equation' and 'correlation coefficient of 506.8, Y= -0.014 X + 2.62 and 0.98 respectively. Likewise MCV (fL) was 95.8±1.25, which showed an increase after 03 hrs of exposure, ranging from 230.9-239.5 with a mean±SD of 235.2±4.56 showing 'variance', 'regression equation' and 'correlation coefficient of 6744.0, Y= 2.246 X + 94.83 and 0.99 respectively. The normal MCH (pg) was 31.8±0.92, which rose after 03 hrs of exposure, ranging from 36.7-42.1 with a mean±SD of 39.4±1.21 with 'variance', 'regression equation' and 'correlation coefficient of 298.6, Y= 0.112 X + 32.17 and 0.93 respectively. The MCHC (g/dL) was 33.2±1.37, which showed a decrease after 03 hrs of exposure, ranging from 15.5-18.0 with a mean±SD of 16.75±0.95 showing 'variance', 'regression equation' and 'correlation coefficient of 289.6, Y= 0.112 X + 32.17 and 0.93 respectively.

The normal WBC ($x10^3/\mu L$) was 6.06±0.24 which showed an increase after 03 hrs of exposure, ranging from 4.38-4.78 with a mean±SD of 4.58±0.10 with 'variance', 'regression equation' and 'correlation coefficient of 318.47, Y= -0.269 X + 31.85 and 0.97 respectively. The small lymphocytes count ($x10^3/\mu L$) was 25.3±0.02, which showed an increase after 03 hrs of exposure, ranging from 34.4-37.2 with a mean±SD of 35.8±0.08 with 'variance', 'regression equation' and 'correlation coefficient of 440.17, Y= 0.027 X + 5.955 and 0.97 respectively. The large lymphocyte count ($x10^3/\mu L$) was 1.5±0.02, which later showed an increase after 03 hrs of exposure, ranging from 2.24-2.56 with a mean±SD of 2.4±0.018, with 'variance', 'regression equation' and 'correlation coefficient of 290.23, Y= 0.015 X + 27.4 and 0.14 respectively. The normal neutrophil count ($x10^3/\mu L$) was 1.9±0.014 which showed an increase after 03 hrs of exposure, ranging from 3.18-3.42 with a mean±SD of 3.3±0.010 with 'variance', 'regression equation' and 'correlation coefficient of 501.16, Y= 0.024 X + 1.83 and 0.99 respectively.

The monocytes count ($x10^3/\mu L$) was 1.65±0.002 which later showed an increase after 03 hrs of exposure, ranging from 4.32-4.68 with a mean±SD of 4.5±0.010 with 'variance', 'regression equation' and 'correlation coefficient of 496.4, Y= 0.047 X + 1.455 and 0.98 respectively. The eosinophils count (0.5±0.02) showed an increase after 03 hrs of exposure, ranging from 0.8-1.0 with a mean±SD of 0.9±0.001 with 'variance', 'regression equation' and 'correlation coefficient of 530.6, Y= 0.007 X + 0.515 and 0.97 respectively. The thrombocyte like cells (1.8±0.021.) showed an increase after 03 hrs of exposure, ranging

Table 1. Summary of OBF values of *N. notopterus* exposed to 0.1 ppm of malathion

Pesticide	Exposure Duration			
	00 hour	01 hour	02 hours	03 hours
OBF	92.3±2.5	93.7±5.5	113.3±2.9	45.3±5.5
	00 hour	01 hour	02 hours	03 hours
TBF	7.7±0.6	9.7±0.6	11.7±2.9	4.3±0.6

Table 2. Mean haematological parameters of *Notopterus notopterus* exposed to five trials of 0.1 ppm Malathion

Parameter	Control	01 hr.			02 hrs.			03 hrs.		
		Min.	Max	Mean±SE	Min.	Max	Mean±SE	Min.	Max	Mean±SE
PCV (%)	25.0±0.83	31	35	33.0±0.25^{b}	35.8	38.2	37.0±2.11^{b}	38	42.0	40.0±0.98^{b}
Hemoglobin (g/dL)	8.3±0.23	8.05	8.35	8.20±0.12^{b}	6.9	8.10	7.50±1.25^{b}	6.51	6.89	6.7±0.25^{a}
RBC (X 10^6/µL)	2.61±0.06	2.15	2.45	2.30±0.03^{a}	1.89	2.35	2.12±0.92^{ab}	1.58	1.82	1.7±0.69^{ab}
MCV (fL)	95.8±1.25	141	145.8	143.4±5.63^{a}	166.5	182.5	174.5±4.21^{a}	230.9	239.5	235.2±4.56^{ab}
MCH (pg)	31.8±0.92	34.52	36.78	35.65±1.95^{a}	32.54	38.2	35.37±1.12^{ab}	36.7	42.1	39.40±1.21^{b}
MCHC (g/dL)	33.2±1.37	23.43	26.25	24.84±0.29^{a}	18.04	22.5	20.27±0.85^{ab}	15.5	18.0	16.75±0.95^{ab}
WBC (X 10^3/µL)	6.06±0.24	5.73	6.85	6.29±0.12^{ab}	6.95	7.31	7.13±0.05^{a}	6.91	8.25	7.58±0.10^{b}
Small lymphocytes (X 10^3/µL)	25.3±0.02	27.3	31.5	29.4±0.08^{ab}	29.7	32.1	30.9±0.02^{a}	34.4	37.2	35.8±0.08^{b}
Large lymphocytes (X 10^3/µL)	1.5±0.020	1.45	1.75	1.6±0.018^{ab}	1.68	1.92	1.8±0.010^{a}	2.24	2.56	2.4±0.018^{b}
Neutrophils (X 10^3/µL)	1.9±0.014	2.05	2.35	2.2±0.010^{a}	2.65	2.95	2.8±0.010^{a}	3.18	3.42	3.3±0.010^{b}
Monocytes (X 10^3/µL)	1.65±0.02	2	2.40	2.2±0.020^{ab}	3.05	3.35	3.20±0.20^{a}	4.32	4.68	4.5±0.010^{b}
Eosinophils (X 10^3/µL)	0.5±0.020	0.45	0.85	0.65±0.01^{b}	0.78	0.92	0.85±0.02^{b}	0.8	1.0	0.9±0.001^{b}
Thrombocyte like cells (X 10^3/µL)	1.8±0.021	1.2	1.6	1.4±0.014^{b}	1.98	2.42	2.2±0.020^{b}	3.3	3.9	3.6±0.014^{b}
Thrombocytes (X 10^3/µL)	34.9±0.02	31.6	34.2	32.9±0.04^{ab}	40	44.2	42.1±0.15^{ab}	44.5	46.3	45.4±0.01^{b}
Plasma protein (g/dL)	3.8±0.024	3.5	4.1	3.8±0.010^{a}	2.35	2.65	2.5±0.010^{a}	1.6	2.0	1.8±0.020^{ab}

Note: Values are mean±SD of five replications (d.f. 5, 30). Means in the same row having different superscripts are significantly different ($P < 0.05$) and values in the same row with same superscript are not significantly different ($P > 0.05$).
* The values of the MCV, MCH and MCHC are calculated by the formulae, corresponding to the appropriate values of Hb, PCV and RBC. Sampling time is 1 hr., 2 hrs. and 3 hrs.

from 3.3-3.9 with a mean±SD of 3.6±0.014 with 'variance', 'regression equation' and 'correlation coefficient of 506.1, Y= 0.031 X + 1.32 and 0.83 respectively. The thrombocytes (34.9±0.02) showed an increase after 03 hrs of malathion exposure, ranging from 44.5-46.3 with a mean±SD of 45.4±0.01 with 'variance', 'regression equation' and 'correlation coefficient of 322.8, Y= 0.203X + 32.72 and 0.88 respectively. The normal plasma protein content (g/dL) was 3.8±0.024, which showed a decrease after 03 hrs of malathion exposure, ranging from 1.6-2.0 with a mean±SD of 1.8±0.02 with 'variance', 'regression equation' and 'correlation coefficient of 498.8, Y= -0.036 X + 4.07 and 0.94 respectively.

DISCUSSION

The observed increase in OBF and TBF during the exposure to various pesticides either solitary or in combinations had been reported earlier by [7]. The initial increases in OBF and TBF may be associated with the sudden response to shock. In addition, the behavioral response to pesticides with marked deviation in the rate of OBF and TBF from reference sample (control) imputes an adjustment in physical fitness as a result of the stress condition [8] and reported that organisms exhibit behavioral responses to chemical stress both at acute and sub lethal toxicity. This elicits the potency and sensitivity of the fish *N. notopterus* to the test chemical [9-10].

The *Notopterus notopterus* exposed to 0.1 ppm of malathion for 03 hrs in triplicates, with repetition of 5 sets revealed an impact on haemoglobin, RBC and plasma proteins. Hb showed an decrease from 8.3 to 6.7, with RBC's decreasing from 2.61 to 1.7. However, plasma proteins (g/dL) showed a decrease from 3.8 to 1.8. The earlier toxicity tests of malathion has been carried out by [11], (mosquito fish)[12], (*Carassius auratus*)[13], (fathead minnows)[14], (*Clarias batrachus*) [15] and (*Tilapia nilotica*)[16].

During the present research, 0.1 ppm of malathion was tested against *N. notopterus* to observe the haemo-biochemical changes associated with the stress caused by the pesticide. The results revealed decrease in values (after 3 hours post exposure) of Hb (g/dL) from 8.3 to 6.7; RBC (x 10^6/µL) from 2.61 to 1.7; MCHC (g/dL) from 33.2 to 16.75; and plasma proteins (g/dL) from 3.8 to 1.8. The other parameters which showed an increase include PCV (%) from 25.0 to 40.0; MCV (fL) from 95.8 to 235.2; MCH (pg) from 31.8 to 39.40; small lymphocytes (x 10^3/µL) from 25.3 to 35.8; large lymphocytes (x 10^3/µL) from 1.5 to 2.4; neutrophils (x 10^3/µL) from 1.9 to 3.3; monocytes (x 10^3/µL) from 1.65 to 4.5; eosinophils (x 10^3/µL) from 0.5 to 0.9; thrombocyte like cells (x 10^3/µL) from 1.8 to 3.6 and thrombocytes (x 10^3/µL) from 34.9 to 45.4.

Our results are well illustrated and backed by the work of [17], who reported the haematological increase of non specific cells as immune defense against the malathion in channel catfish, *Ictalurus punctatus*. worked on the malathion induced changes in the serum proteins and hematological parameters of an Indian catfish *Heteropneustes fossilis* (Bloch). The authors reported an increase in differential leukocyte count and decrease in blood parameters like RBC (3.8 to 2.2); Hb (5.9 to 3.9); and plasma proteins (3.5 to 2.1) on exposure of fish to 30 days trial against various concentrations of malathion. The findings of the above authors lend complete support to the results obtained during the present study.

The results of [16], support our findings partially, who injected *Tilapia nilotica* against *Staphylococcus aureus* and exposed them to various concentrations of pesticides. Their results revealed that the mean total RBCs, WBCs counts, PCV, Hb, MCV, MCH, MCHC values were lower in vaccinated groups than in the groups exposed to the tested pesticides. The total protein and globulin values were lower in vaccinated as compared to the non-vaccinated groups. The alteration in blood values could be due to resistance/vulnerability of test species against the pesticides. On contrary [18] reported the regular feature of changes in haematological characteristics of a bony fish, *Tilapia guineensis*, exposed to common pesticides. The authors reported an increase in values of PCV (28 to 43); MCV (88.0 to 120.5); and MCH (30.5 to 59.6) and cited the cell enlargement as the cause for the increase in the values of above parameters.

The haematology of fingerlings of *Labeo rohita* exposed to two sub lethal concentrations i.e., 10.3 ppm and 2.06 ppm of nuvan was studied by [19]. The authors revealed an increase in blood glucose level (7.63-27.02%) over the control and elevation of total leukocyte count from 5.51 to 40.92% over 45 days period in comparison to control value. The serum protein value decreased from -2.21 to -22.51%, the total erythrocyte count decreased from -3.35 to -8.49% and haemoglobin percentage decreased from -21.49 to -35.74. The findings of the above authors are in agreement with the results obtained by us. Likewise the work of [20] lends support to our findings, who worked on the effect of diazinon on haematological indices of common carp (*Cyprinus carpio* L.). The authors revealed that the experimental group of one- to two-year-old common carp showed significantly lower values ($p < 0.01$) of erythrocyte count (RBC), haemoglobin content (Hb) and haematocrit (PCV) compared to the control group. Values of MCV, MCH and MCHC were comparable in both groups during the study. In contrary, there was a significant decrease in leukocyte count ($p < 0.01$), as well as in both the relative and absolute lymphocyte count ($p < 0.01$) and a significant increase in both the relative and absolute count of developmental forms of neutrophil granulocytes: myelocytes ($p < 0.01$) and metamyelocytes ($p < 0.05$) in the experimental group.

Experiments on the effect of malathion on biochemical and haematological changes in freshwater catfish, *Heteropneustes fossilis* by [21] revealed significant reduction in RBC and Hb following the 4-day exposure; however, no apparent change in their level was observed after 8 and 16 days' exposure. The authors justified their statement with the fact that after prolonged exposure, *H. fossilis* develops a tolerance and partially recovers from the stress condition. The decrease in haematological parameters like RBC, Hb and haematocrit on exposure to pesticide are due to the disruptive action on erythropoietic tissue. This was documented by [22], who worked on change of acute toxicity and haematological parameters of *Pseudobagrus fulvidraco* exposed to lindane.

The effects of pesticides on blood characteristics and histological changes in erythrocytes of the fish species *Cyprinus carpio* and *Puntius ticto* were studied by [23]. The results obtained by the authors are in complete agreement with our findings.

Worked on the responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf) [24]. While as evaluated the immunosuppressive action of nuvan (dichlorovos) in the banded pearl spot, *Etroplus suratensis* and evaluated the effect on haematology and humoral immune response [25]. The authors revealed that there was significant ($P < 0.05$) reduction in hemoglobin content and total serum protein in the test groups compared to the control fish. The TEC, PCV and ESR values were lower in the test groups compared to the controls but the differences were not statistically significant ($P < 0.05$). The total leukocyte counts were significantly higher ($P < 0.05$) in nuvan treated groups compared to control fish, which is in complete agreement with our findings.

Static bioassay experiments were conducted to ascertain the acute effects of water borne chlorpyrifos-ethyl at 0.64, 0.80, 0.96, 1.12 and 1.28 mg/l on a freshwater fish, *Clarias gariepinus* after 96 h by [26]. The authors reported that the exposure to chlorpyrifos-ethyl caused a significant dose dependent ($P<0.05$) inhibition in haematocrit (PCV) and Hb values. There was also a significant decrease ($P<0.05$) of RBC. The WBC elevation was significantly dose dependent ($P<0.05$) in all concentrations. The inhibition of MCH and elevation of MCHC were both non-significant (NS), but the inhibition of MCV was significant between control and exposed group ($P<0.05$). More or less similar results have been obtained by [27].

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