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Effect of Organophosphate Pesticides Malathion on Excretory Function of the Channel Cat Fish, *Heteropneustes fossilis* (Bloch)

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Abstract

Present investigation is aimed to study the 'Effect of organophosphate pesticide malathion on excretory function of the channel catfish, Heteropneustes fossilis (Bloch). A sub-lethal concentration of 0.1 ppm and 0.2 ppm Malathion were prepared and used in this investigation process. In this study, acclimatized fishes were divided into a control group (Group 1) and two experimental groups (Group 2.1 and 2.2). Experimental groups were treated with sub lethal dose of the pesticide malathion whereas control group was being freed from the treatment of the pesticide. Level of Ammonia and Urea in Serum and GLDH and Arginase activity in liver tissues were estimated at the interval of 10, 20 and 30 days in the fishes under investigation. A dose and duration dependent alteration was observed in the level of serum ammonia, serum urea, serum creatinine, and GLDH activity in the liver tissue among the experimental group of fishes exposed to malathion.

Key Words: *Ammonia; Creatinine; GLDH; Malathion; Urea.*

INTRODUCTION

Owing to the fact of harmful effect of Organophosphate pesticide to the non-target species, in recent years, a considerable research work has been carried out on a variety of alternative pest control measures. Yet, the use of organophosphate pesticide is interestingly remained the main stay in the control of insect pests in agricultural sites, commercial, institutional and industrial sites; in and around homes; and on pets [1]. The main mechanism by which the organophosphates exert a toxic effect is the inhibition of cholinesterases (ChEs), an important group of enzymes of the nervous system of both vertebrates and invertebrates [2-3].

Pesticides are also well known for causing more toxic effects in teleost fishes. The magnitude of pesticide pollution was studied in the Indian fishes by various workers [4-14].

Malathion, an organophosphate pesticide is being extensively used as dust, emulsion, and vapour to control wide variety of insect pests under different conditions. Like other pesticides, lethal and sub-lethal treatment of Malathion exerts various toxic effects on fish. Malaoxon, a primary metabolite of Malathion is 60 times more toxic than Malathion and it seriously and chronically poison the occupants living in the environment [15].

Present investigation is aimed to carry out a study on the effect of organophosphate pesticides malathion on excretory function of the channel cat fish *Heteropneustes fossilis* (Bloch).

MATERIALS AND METHODS

Specimen

Healthy and sexually mature specimen of *Heteropneustes fossilis* of equal size group (12 ± 3 cm) and average weight (12 to 15 gm) are procured from the local market and the fishes were kept for 15 days in glass aquarium containing 80 litres of fresh water in the laboratory at about water temperature 25 ± 3º c for acclimatization. Fishes are starved for 24 hours prior to the experiment and are not fed during the period of experiment [4].

Pesticide

The organophosphorus pesticide malathion (50%vEC) were procured from the local market for present investigation purpose.

LC_{50} Calculation

A pilot experiment was done to find out the LC_{50} value of dichlorvos and malathion by probit analysis [16] and LC_{50} for 96 hours is found to be 0.98 ppm for malathion. Sub-lethal concentrations of 0.1 ppm and 0.2 ppm for malathion were prepared by using standard technique [17].

Design of the experiment

In this experiment, the specimens were kept mainly in Control Group (Group I) and Experimental Group (Group 2) as follows

Investigation group	Sub group	Sub-lethal dose
Control Group	Nil	No treatment of malathion
Experimental Group 2	Group 2.1	Treated with 0.1 ppm malathion
	Group 2.2	Treated with 0.2 ppm malathion

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Collection of sample

Blood were collected from the fishes of both the groups and serum was separated by centrifugation technique. Tissue homogenate was prepared from the collected liver tissue and was centrifuged based on the protocol of the individual experiments.

Methods of Estimation of biochemical parameters

Ammonia in serum was estimated by the method of Anken and Schiphorst [18] at the wavelength of 340 nm in spectrophotometer. Urea in Serum was estimated through Modified Berthelot Method by Fawcett and Scott [19]. GLDH activity in liver tissue was determined spectrophotometrically by adoption of the method of Doherty [20]. Arginase activity in liver tissue was estimated by the method of March et al. [21] and Creatinine was assayed spectrophotometrically using modified Jaffe Method [22].

Duration of treatment

The investigation parameters were studied in control and experimental groups after 10, 20 and 30 days of experimental period.

RESULTS

Serum ammonia

Changes in the mean ± SD values of serum ammonia in the *Heteropneustes fossilis* are shown in the table-1 and the percentage deviations of different experimental groups from the mean values of control group are presented in the fig-1. Serum ammonia was found to be decreased slightly than the control group of fishes on 10th, 20th and 30th day at sub-lethal dose of 0.1 ppm and 0.2 ppm malathion.

Table 1: Presenting the mean ± SD values of serum ammonia (mg/dl) in control and experimental group of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of malathion.

Investigation Group of fish	Mean ± SD Values		
	10 days	20 days	30 days
Control Group	5.29±0.03	5.64±0.02	5.46±0.04
0.1 ppm malathion	5.12±0.11	4.81±0.06	4.96±0.08
0.2 ppm malathion	4.91±0.12	4.75±0.07	4.81±0.04

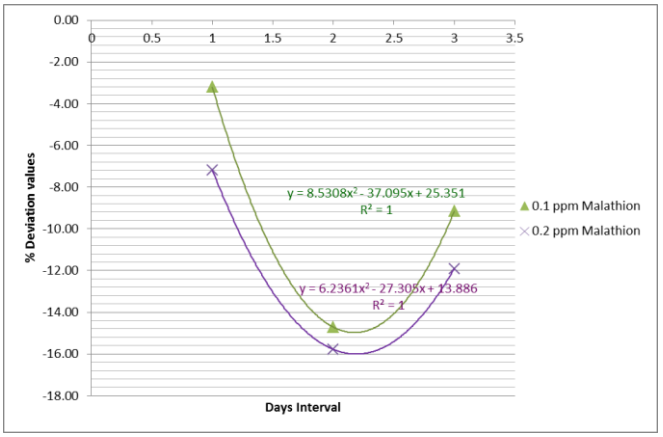

Figure 1: Presenting the % deviation values of serum ammonia (mg/dl) in control and experimental group of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of dichlorvos and malathion.

Serum urea

Alterations in the mean ± SD values of Serum urea in the *Heteropneustes fossilis* are shown in the table 2 and the percentage deviations of different experimental groups from the mean values of control group are presented in the fig-2. A significant ($p < 0.01$) increase in the level of serum urea was recorded at sub-lethal dose of 0.1 ppm and 0.2 ppm from malathion throughout the experimental period of 30 days in comparison to the control group of fishes. Fishes exposed to 0.2 ppm malathion exhibited higher level of serum ammonia than the fishes administered with 0.1 ppm malathion.

Table 2: Presenting the mean ± SD values of Serum urea (mg/dl) in control and experimental group of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of malathion.

Investigation Group of fish	Mean ± SD Values		
	10 days	20 days	30 days
Nil	23.9±1.09	24±1.08	33.8±2.1
0.1ppm malathion	40.1±3.08	40.8±2.89	40±2.45
0.2ppm malathion	41±2.95	41.1±2.77	41.2±2.86

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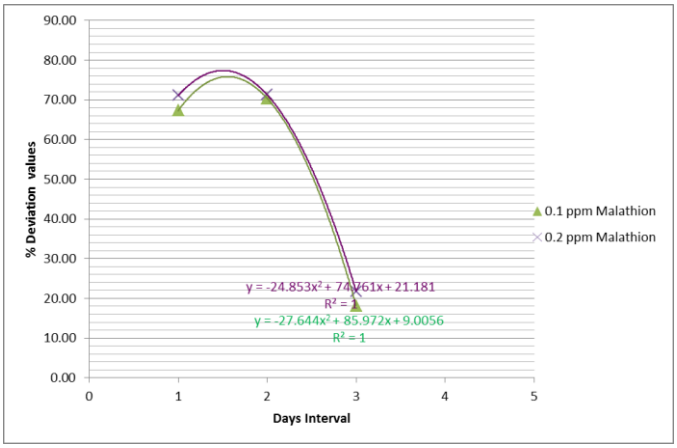

Figure 2: Presenting the % deviation values of Serum urea (mg/dl) in control and experimental group of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of malathion.

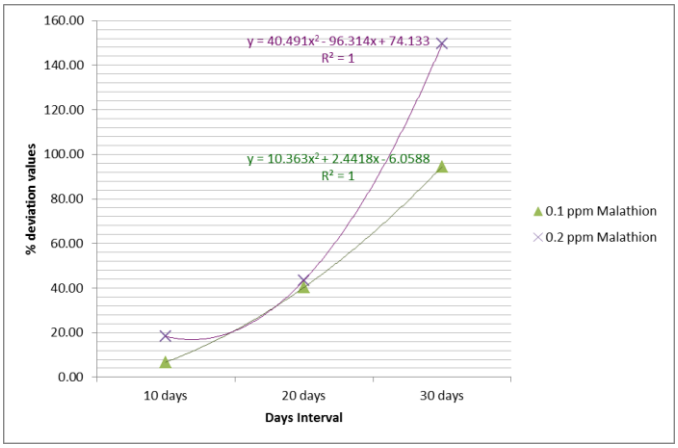

Figure 3: Presenting the % deviation of serum Creatinine (mg/dl) in control and experimental groups of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of malathion.

Creatinine

Alterations in the mean ± SD values of creatinine in the *Heteropneustes fossilis* are shown in the table 3 and the percentage deviations of different experimental groups from the mean values of control group are presented in the fig-3. A significant increasing ($p< 0.01$) trend in the mean ± SD values serum creatinine was observed in the group of fishes administered with sub-lethal dose of 0.1 ppm and 0.2 ppm malathion from 10th day onwards and reached maximum value on 30th day compared to control group of fishes.

Table 3: Presenting the mean ± SD values of serum Creatinine (mg/dl) in control and experimental groups of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of malathion.

Investigation Group of fish	Mean ± SD Values		
	10 days	20 days	30 days
Nil	0.67±0.08	0.69±0.08	0.64±0.08
0.1 ppm malathion	0.72±0.03	0.97±0.03	1.25±0.04
0.2 ppm malathion	0.80±0.01	0.99±0.08	1.60±0.09

Glutamate Dehydrogenase (GLDH) activity

Variations in the mean ± SD values of GLDH activity in the *Heteropneustes fossilis* are shown in the table 4 and the percentage deviations of different experimental groups from the mean values of control group are presented in the fig-4. In the group of fishes exposed to 0.1 ppm malathion, the mean ± SD values of GLDH activity was more or less found to be similar with the control group of fishes. A slight elevation in the mean ± SD values of GLDH activity was recorded in the group of fishes treated with 0.2 ppm malathion compared to control group of fishes.

Table 4: Presenting the mean ± SD values of GLDH activity (U/mg) in control and experimental group of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of malathion.

Investigation Group of fish	Mean ± SD Values		
	10 days	20 days	30 days
Nil	1.2±0.05	1.23±0.02	1.24±0.02
0.1ppm malathion	1.26±0.04	1.21±0.02	1.25±0.03
0.2ppm malathion	1.38±0.03	1.26±0.03	1.31±0.02

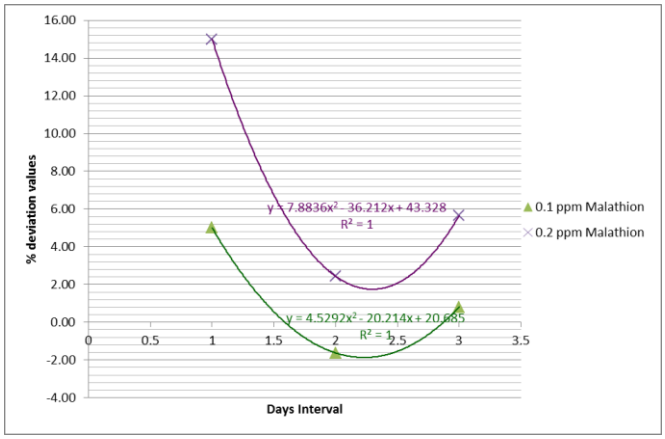

Figure 4: Presenting the % deviation values of GLDH activity (U/mg) in control and experimental group of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of dichlorvos and malathion

DISCUSSION

A decrease in the level of serum ammonia and increase in the level of serum urea was observed in the present study. Similar observations were made by Venkataramana *et al.* (2005) on *Glossogobius giuris* (Ham) and Vellas and Serfaty (1974) on *Cyprinus carpio* [23-24]. The exit of ammonia from mitochondria of liver in ammonotelic fishes is an enigma for long time; yet, the gills are the main sites of ammonia excretion in fish. On the other hand, Kidney may also excrete smaller quantities of ammonia [25]. Ammonia is considered as one of the toxic substance which cannot be retained in the body for longer period and could be converted into less toxic substance like urea [23, 26]. According to Kumar *et al.* (2012), decrease in ammonia in serum and increase in urea and GLDH activity are indicative of activation of a second mechanism of ammonia detoxification, that is, ureogenesis by the fishes under pesticide-induced stress [27]. Common cause for increase in blood urea is atypical nitrogen excretion usually due to kidney damage and urinary obstruction [28]. However, under some circumstances as stress or enhanced ammonia level in the surrounding, fishes are reported to change their nitrogen excretion mechanism by forming urea as the end product for nitrogen excretion. Increased urea in the experimental fish was also reported to be due to the inability of the damaged kidney to filter the urea up to the normal levels [29].

Present investigation finds an elevation in the activity of Glutamate dehydrogenase (GLDH) activity in the experimental fishes treated with malathion. Glutamate dehydrogenase catalyse the reversible reaction of oxidative deamination of glutamate to ά- ketoglutarate and ammonia and play an important role in the catabolism and biosynthesis of amino acid [30-31]. The increase in GDH activity at the sub lethal concentration could lead to increased production of glutamate in order to eliminate ammonia [32]. Enhancement in GLDH activity helps in supplying keto acids to the TCA cycle in order to compensate the energy crisis in liver tissue during ammonia toxicity [33]. Its activity increases with hepatocellular damage [34]. The increased GLDH activity may indicate increased rapid utilization of amino acids [35-37] and onset of detoxification mechanism [38-39]. GLDH in extra-hepatic tissues could be utilized for the channelling of ammonia for its detoxification into urea in the liver. Hence, the activities of GDH are considered as sensitive indicators of stress [40].

In this study, Creatinine was found to be increased significantly in *H. fossilis* exposed to sub-lethal doses of malathion. Similar observation of increasing creatinine was made by Mastan and Ramayya (2009) in Channa gachua (Ham.), Mossa *et al.* (2015) on male albino rats [41-42]. Increased level of creatinine also reveals the malfunctioning of kidney under stress after pesticide exposure [43]. Creatinine is liberated as an end product of creatine degradation in skeletal muscles. Creatinine is solely considered a waste product and is then diffuses into the bloodstream from the muscle and enters to the renal parenchyma where it is filtered by the glomerulus and excreted in the urine. Creatinine is found to be high in blood when it is not reabsorbed by the renal tubules. The constancy of creatinine formation and excretion makes creatinine a useful index of renal function, primarily glomerular filtration [44].

CONCLUSION

A significant ($p < 0.01$) decrease in the level of serum ammonia in *Heteropneustes fossilis* (Bloch) exposed to sub-lethal doses of malathion were observed in the present investigation. On the other hand, a significant increase ($p < 0.01$) in the level of serum urea, serum creatinine and GLDH activity were observed in *Heteropneustes fossilis* (Bloch) administered with sub-lethal doses of malathion.

CONFLICT OF INTEREST: None

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