EFFECT OF MALATHION ON BLOOD BIOCHEMICAL PARAMETERS (UREA AND CREATININE) IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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ABSTRACT: The present study was conducted to evaluate the serological changes (urea and creatinine) in the kidneys of Nile tilapia (*Oreochromis niloticus*) after acute exposure to Malathion. Four groups of 15 fish were made each and categorized as; Group A: (control group) and Groups B, C and D (experimental groups). The LC_{50} of Malathion was 2µg/L at 96 hours of exposure in Nile tilapia. The fish were exposed to sub-lethal concentrations of 0.5, 1.0 and 1.5 µg/L to group B, C and D respectively. Blood samples were collected after 48, 72 and 96 hours of exposure to Malathion. Serum was separated and analyzed for levels of urea and creatinine that was significantly enhanced after acute exposure to Malathion at 48, 72 and 96 hours. Moreover, morphological and behavioral changes and mortality rates were also recorded. The results revealed that the pesticide (Malathion) has an adverse effect on kidneys. High levels of Malathion result in kidney damage and serological alterations in Nile tilapia.

Keywords: Fish, Nile tilapia, Malathion, serum, creatinine

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INTRODUCTION

Pollution of the aquatic environment is a serious and growing problem for aquatic organisms. The aquatic organisms, particularly fish, start to accumulate pollutants (organophosphorus or chlorinated pesticides) directly from contaminated water and indirectly through the food chain (Pandcy *et al*., 2006; Rao, 2006; Terziev *et al*., 2019). However, effects are not always related to immediate and apparent injuries andsometimes take years to appear (Garcia *et al*., 2012). A wide body of literature supports the effect of various chemicals and insecticides as serious physiological impairment to tissues and organs and health issues of various fish (Velisek *et al*., 2004; Agrahari *et al*., 2007; Matos *et al*., 2007; Banaee *et al*., 2008; Banaee *et al*., 2010; Khoei *et al*., 2018). Similarly, emergences of behavioral changes (swimming, feeding activities, predation, competition, reproduction, and social interactions between species such as aggression) are the most sensitive indicators of potential toxic effects of different insecticides and are reported several studies (Auta *et al*., 2002; Cong *et al*., 2008; Halappa and David, 2009; Patil and David, 2008; Werner and Oram, 2008; Benli and Ozkul, 2010; Banaee *et al*., 2011; Renick *et al.*, 2016; Odo *et al*., 2017; Jasmin *et al*., 2018). A study reported on fish *Labeo rohita* that Malathion is genotoxic and has ability to damage DNA of the gill cells (Ullah *et al*., 2016).

In various pesticides, Malathion is an important ingredient used in malaria eradication programs. It is also used to control the motile stages of mites and some other insects on fruits and vegetables (Kamel *et al*., 2007; Cheng *et al.*, 2018). The use of Malathion as a pesticide in the surrounding area of aquatic ecosystems and its drift to water bodies may exert adverse effects on gills of the aquatic organisms (Subburaj *et al*., 2018). According to an estimate, in developing countries, 60-70 % pesticides are in practice (Faheem *et al*., 2015). Okonya *et al*., (2019) reported that the excessive use of pesticides damages the health of farmers and domestic animals.

Nile tilapia is the most favorite among aquaculturists. The major characteristics that are important for its popularity is its ability to tolerate a wide range of environmental conditions, fast growth, successful reproductive strategies, and ability to feed at different trophic levels. It has been observed the fish exhibits trophic plasticity according to the environment (Bwanika *et al*., 2007). According to Shipton *et al.* (2008) these specific traits of Nile tilapia allow them to be an extremely successful invasive species in subtropical and temperate environments. In Pakistan to fulfill the growing demand of protein there is a need to improve the capacity, productivity and quality of the aquaculture sector. Moreover, due to the high survival rate of Nile tilapia, its growth and market demand have been increasing in Pakistan from the last few decades (Iqbal *et al*., 2012; Khan *et al*., 2014; Chughtai *et al*., 2015). The Government of Pakistan is also focusing on Nile tilapia (an exotic species) due to its most adaptive features and wants to use this fish as a cheap source of food protein for its overgrowing population.

The present study is designed to observe the serological changes like urea and creatinine level in Nile tilapia for an organophosphate insecticide Malathion that affects kidney function. Both Serum creatinine and urea are freely filtered by the glomerulus so the use of

Malathion may affect the efficacy of kidney. This study also analyzed the behavioral and morphological changes in Malathion intoxicated Nile tilapia.

MATERIALS AND METHODS

Selection and experimentation place: The present study was carried out to determine the serological changes in Malathion intoxicated Nile tilapia (*Oreochromis niloticus*). The experiment was performed in the toxicology laboratory, Zoology Department, Government Postgraduate Islamia College for Women, Cooper Road, Lahore, Pakistan.

Animal source, maintenance and acclimatization: Over 130 specimens of fresh water farmed fish Nile tilapia (*Oreochromis niloticus)* fingerlings were obtained alive through the courtesy of the Department of Fisheries, Manawa Hatchery, Lahore. The fish was carried to the laboratory in polythene bags and were immediately transferred to glass aquaria containing 45 liters of water with a total capacity of 65 liters. The aquaria were covered with a mosquito net to prevent fish escaping and predators. The fish were allowed to acclimatize for one week prior to the experiment. The water was replaced daily during acclimatization and the aquaria were artificially aerated using air pumps. An optimum fish food (Nova feed) was used; available for all kinds of fish in the market. The feed contained rice polish, fish meal, protein and starch.

Experimental chemical and grouping of animals: The technical grade 'Malathion' is a domestic insecticide selected to see its effect on the renal functioning activities of fish Nile tilapia. Fish were categorized into four main groups; A, B, C and D with fifteen fish in each group. Group A was control or untreated group (No dose was given) while group B, C and D were experimental groups. The average body weight of each group was recorded. Each group of fish B, C and D was provided with three sub lethal doses 0.5, 1.0 and 1.5µg/L respectively and exposed to 48, 72 and 96 hours of Malathion. During the exposure, mortality rate, morphological, physical and behavioral changes were observed in fish, by monitoring the swimming and feeding activities. LC_{50} of Malathion was evaluated according to the Probit analysis method, cited by Al-Ghanim, (2012).

Blood sampling, Serum separation and Storage of serum: The blood samples were collected in Eppendorf, allowed to settle at room temperature and centrifuged at 3000 rpm for about 15 minutes. After centrifugation light straw color serum was separated and stored at 4^oC prior to analysis. For analysis, the tubes were deforested at $37^{\circ}C$ and then centrifuged again. The creatinine and urea were measured in the serum of fish using commercially available assay kits (SKT) in Lifeline Laboratories and Diagnostic Center, Lahore.

Statistical Analysis: Statistical Package of Social Sciences (SPSS) version 21 was used to compute descriptive statistics and One-way ANOVA.

RESULTS AND DISCUSSION

In the current study, Nile tilapia (average weight of 31.2 ±2.0 g) was used to study the effect of Malathion on renal functions. The levels of Creatinine and urea in blood serum were analyzed in fish after exposure to various sub-lethal concentrations of Malathion. During the experimental period, certain morphological and behavioral changes were also recorded in the exposed fish and findings agreed with Beauvais *et al*. (2000).

The morphological changes, like bulging of the eye, excessive mucus secretion, eye and body hemorrhage, scale erosion, tail rotting and mortality were noticed during the study (Table: 1 and 2). The symptoms were more pronounced and enhanced with the concentration of dose and time as also reported by Hussain *et al*. (2016). Furthermore, behavioral changes were noticed in respect to the mobility of experimental fish. It was observed that the exposed fish suffered a shock at the beginning and showed respiratory distress manifestations, including hyper-excitability by erratic movements and active swimming. Similarly, the effect of different chemicals on physiological and behavioral features of fish has been reported in various literature (Kavitha and Rao, 2008; Ullah *et al*., 2014; Zeid and Khalil, 2014; Sharafeldin *et al*., 2015). Subburaj *et al*. (2018) also reported that Malathion has shown significant damage in gills of fish tilapia.

Group A was control and the mean concentration of urea and creatinine was 9.0 ± 0.1 and 0.62 ± 0.2 mg/dl respectively. The experimental groups B, C, and D were exposed to 0.5, 1.0 and 1.5µg/L concentration of Malathion respectively and the mean concentration of urea and creatinine in serum of Nile tilapia were recorded in each group. The concentrations of urea in experimental groups (B, C and D) were recorded after 48, 72 and 96 hours of exposure to Malathion. It was noticed that the level of urea and creatinine in all groups like B, C and D was increased with the increase in exposure time. Furthermore, it was noticed by one-way ANOVA that the level of urea and creatinine showed a considerable ($P<0.05$) increase in fish exposed to Malathion (Table: 3 and 4). The increase in urea level on exposure to some insecticide in *Heteropneustes fossilis* (Bloch) was reported by Deka and Dutta, (2015). However, elevation in both urea and creatinine in different fish on exposure to different chemicals and insecticide was described in literature by

Harabawy and Ibrahim, (2014) and Sayed and Hamed, (2017).

The increasing trend of urea and creatinine was also reported by Zeid and Khalil, (2014) who treated tilapia with di-N-butyl phthalate (DBP) pesticide for 8 weeks and observed the increase in urea and creatinine. According to Saleh *et al.* (2007) elevation of serum urea and creatinine may be due to the decrease of glomerular infiltration rate of the kidneys and tubular dysfunction. The alteration of blood urea in fresh water fish, *Mystus vittatus* was investigated after chronic exposure to metasystox and sevin and it was noticed that blood urea in the present study showed a similar increasing trend. The findings of John, (2007) were also in agreement to the present study. Moreover, Ahmad and Gautam, (2014) treated water catfish (*Heteropneustes fossilis*) with sublethal doses of nuvan and a significant increase was noticed for creatinine. These findings were also supported by Soufy *et al*. (2007) who worked on *Monosex tilapia* by treating it with cabofuran. Depletion of serum creatinine was observed in *Clarias gariepinus* after paraquat dichloride toxicity (Ogamba *et al*., 2011). However elevated levels of serum urea were recorded in *Clarias albopunctatus* due to roundup toxicity Okonkwo *et al*. (2013). These findings are in consistent with current observations.

Similarly a study of Jalili *et al*. (2018) was parallel to our existing findings and showed Malathion ability to alter urea and creatinine levels in rats and Yokota *et al*. (2017) also reported nephrotic syndrome, and higher serum creatinine level in a man who inhaled Malathion. However, Malathion effect can be reduced as reported by Kandiel *et al.* (2014) and honey bee pollen and propolis was used as feed additive. Both (bee pollen and propolis) worked well to control genotoxic and endocrine disruptive effects of malathion in *Oreochromis niloticus*.

Table-1: Morphological symptoms in Nile tilapia (*Oreochromis niloticus*) noticed in control and different experimental groups.

Symptoms	A			B			C			D		
	48	72	96	48	72	96	48	72	96	48	72	96
Bulging of the eye	-	-	-	-	+	+	+	+	++	+	++	++
Mucus secretion	-	-	-	-	+	+	+	+	++	+	++	++
Eye hemorrhage	-	-	-	-	+	+	+	+	++	+	++	++
Body hemorrhage	-	-	-	-	+	+	+	+	++	+	++	++
Scale erosion	-	-	-	-	+	+	+	+	++	+	++	++
Tail rotting	-	-	-	-	+	+	+	+	++	+	++	++

No Symptoms (-); moderate symptoms (+); clear symptoms (++)

Table-2: Mortality of Nile tilapia (*Oreochromis niloticus*) noticed in control and different experimental groups.

	A	B	C	D
48	0	0	1	1
72	0	1	1	2
96	0	1	2	2

Table-3: Mean concentration of serum urea (mg/dl±SD) in Nile tilapia (*Oreochromis niloticus*) in experimental groups B, C and D (treated with 0.5, 1 and 1.5 µg/g Malathion).

Serial No.	Exposure time (hours)	Urea concentration (mg/dl)			
		A	B	C	D
1	**48**	9.0 ± 0.10	9.5 ± 0.17*	13.0 ± 0.76*	12.0 ± 0.14*
2	**72**	9.1 ± 0.15	11.0 ± 0.45*	14.0 ± 0.45*	15.0 ± 0.68*
3	**96**	9.0 ± 0.10	12.0 ± 0.15*	15.0 ± 0.68*	16.0 ± 0.15*

Mean±SD, P<0.05 (n=3); SD= Standard Deviation

Table-4: Mean concentration of serum creatinine (mg/dl± SD) in Nile tilapia (*Oreochromis niloticus*) in experimental group B, C and D (treated with 0.5,1 and 1.5 µg/g Malathion.

Serial No.	Exposure time (hours)	Creatinine concentration (mg/dl)			
		A	B	C	D
1	**48**	0.60 ± 0.04	0.68 ± 0.02**	1.05 ± 0.02**	2.30 ± 0.05*
2	**72**	0.61± 0.02	0.99 ± 0.01**	1.54 ± 0.03**	3.60 ± 0.03*
3	**96**	0.64 ± 0.03	1.22 ± 0.05**	2.00 ± 0.04**	3.99 ± 0.04*

Mean±SD, $P<0.05$ (n=3); SD: Standard Deviation

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