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Effect of an Organophosphate Insecticide, Dimethoate, on Antioxidant Enzymes of the Fish Nile Tilapia, (*Oreochromis niloticus)* (L.)

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Abstract: ***Application of synthetic insecticides to save crops from the invasion of pest is a common practice; however unwise use of synthetic insecticides causes health and environmental concerns, including the contamination of aquatic ecosystem. Effect of dimethoate, an organophosphorus insecticide on the antioxidant activity of fresh water fish, Nile Tilapia (Oreochromis niloticus) was studied. The LC_{50} value of dimethoate for 96 h was calculated as 0.234 ppm. The 1/5th of 96 h LC_{50} value was taken as the sublethal dose. The treated fishes were scarificed and the gill, liver and kidney were collected for the antioxidant enzyme analysis. Results showed significant variations in the antioxidant enzymes of fish treated with insecticide. In the gill, SOD and catalase was found reduced (P<0.05), while the GPx, GSH, GR and LPO increased significantly (P<0.05). SOD, GPx, and LPO of activity in liver were increased (P<0.05) but the other enzymes CAT, GSH and GR decreased significantly (P<0.05). In the kidney, only the GR and LPO increased significantly (P<0.05), while the SOD, catalase, GPx and GSH reduced markedly (P<0.05).***

Keywords: Pesticide, dimethoate, *Oreochromis niloticus*, antioxidant enzymes

1. Introduction

Indiscriminate use of harmful chemicals poses threat to aquatic environment. Use of chemical pesticides has a serious concern as often their objective deviates from the targeted pests to non targeted organisms. Leaching of pesticides to water bodies is a serious hazard to aquatic fauna and flora, including fishes.

Oxidative process plays a crucial role in the metabolic responses and cause oxidative stress due to the production of Reactive Oxygen Species (ROS). Many of the xenobiotics, such as chemicals, pesticides and metal ions are responsible for causing oxidative stress in organisms as they influence the formation of ROS and alterations in the antioxidant system. Oxidative stress primarily formed due to the result of an imbalance between the production of reactive O_2 species (ROS) and antioxidant defenses in living organisms [1].

Extreme oxidative stress may lead to the increased production of antioxidant enzymes, DNA damages, protein oxidative and lipid peroxidation products. Cellular antioxidant system, under normal conditions can convert ROS and other pro- oxidants to harmless metabolites as well as protect normal cellular function and metabolism [2]. The major antioxidant enzymes are super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), glutathione reductase (GR) and lipid peroxidase (LPO). Super oxide dismutase is present in almost all the aerobic organisms for catalyzing the dismutation of super oxide to H_2O_2 and O_2. Catalase is an ubiquitous heme enzyme, catalyses the dismutation of H_2O_2 to H_2O and O_2 and involves in Haber-Weiss reaction [3]. GPx is a Selenium-dependant enzyme that scavenges H_2O_2 [4]. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free H_2O_2 to H_2O. In cells, glutathione (GSH) is maintained in reduced form by the enzyme glutathione reductase (GR). GSH can function as an antioxidant in many ways, it can react with singlet O_2, super oxide and hydroxyl radicals, thereby functions as a free radical scavenger. GSH may stabilize membrane structure by removing acyl peroxides formed from the lipid per oxidation reactions [5] and also act as a prevalent substrate for the enzyme GST.

Dimethoate is the commonly used insecticide using against a number of insect pests, including aphids, mites, saw flies and boring insects [6]. The effects of dimethoate on different fishes have already been documented [7], [8], [9]. 96h LC_{50} value of dimethoate were calculated on *Clarias batrachus* and *Heteropneusteus fossilis* were 65 mg $L^{-1}$and 2.98 mg L^{-1}respectively [10], [11].

The present study deals with the toxic effects and the oxidative stress of organophosphate pesticide, dimethoate on fish, Nile Tilapia (*Oreochromis niloticus*).

2. Materials and Methods

Oreochromis niloticus (25.8 ± 1.2 g) collected from the hatchery at Alappuzha, Kerala, were treated with 0.1% potassium permanganate solution to remove infection and were reared for one month in a 1000 L fiber tank with aeration and feed. After acclimatization, uniform size fishes were transformed into glass tanks. LC_{50} value for 96 h of dimethoate on the test fish was studied by Probit method [12]. The 1/5th of 96 h LC_{50} value (0.047ppm) was taken as the sublethal dose and its effect on antioxidant enzymes were studied. Untreated fishes were run as control. The hydrographic parameters such as temperature, pH, hardness and dissolved oxygen were calculated [13] and maintained as such throughout the entire experimental period. The experimental fishes were sacrificed by decapitation and the gill, liver and kidney were dissected out and washed in physiological saline (0.9% NaCl) and kept at $^{-}$20°C until

analysis. The tissues were homogenized for 5 min in ice-cold 0.1M Tris-HCl buffer solution at pH 7.2 (115 w/v) using Polytron homogenizer (Polytron Model PT 3000, Kinematica-Switzerland) and centrifuged (Remi-India) at 8000 rpm for 30 min.The resultant supernatant was removed and stored (-400°C) for use in the antioxidant enzyme assays. Superoxide dismutase was determined by the method described by Kakkar *et al*. [14]. Catalase was assayed by the method of Maehly and Chance [15]. The activity of glutathione peroxidase was determined by the method of Lawrence and Burk [16].Glutathione reductase was assayed by the method of David and Richard [17]. Glutathione was determined by the procedure described by Patterson and Lazarow [18]. Lipid peroxidation products were estimated by the Thiobarbituric acid (TBA) assay method as described by Okhawa *et al*. [19].

3. Results

Probit analysis proved that the LC_{50} value of Dimethoate for 96 h for the test fish was 0.234 ppm with ranges 0.225-0.242 (intercept -3.793 ± 0.283, P< 0.001, slope 16.235 ± 1.142, P< 0.001) (Table.1 and fig.1&2). Linear relationship between the probit mortality and the concentration of dimethoate indicated a positive corelation and showed a significant difference (P< 0.001). The hydrographic parameters such as temperature, pH, hardness and dissolved oxygen were observed as 27-30°C, 6.2-7, 20-25 mg L^{-1} and 6-10 mg L^{-1} respectively. Antioxidant enzyme in the gill of treated fish such as SOD (2.73± 0.57 U/ mg protein) and CAT (0.002 ± 0.00 U/mg protein) was significantly reduced (P< 0.05) where as GPx (133.82 ± 4.15U/mg protein), GSH (9.17±0.22 U/mg protein) GR (173.23 ±3.66 U/mg protein) LPO (1.11 ±0.10 U/mg protein) etc. were significantly (P < 0.05) increased than the control group (Fig.3). Antioxidant enzymes in the treated liver such as SOD (4.13±0.35 U/mg protein), GPx (127.01 ± 4.72 U/mg protein), LPO (0.76±0.02 U/ mg protein) etc. were significantly increased (P < 0.05) and CAT (0.002 ±0.00 U/mg protein), GSH (4.79 ±0.15 U/mg protein), GR (15.23 ± 1.37 U/mg protein) etc. were significantly decreased (P < 0.05) than the control batch (Fig. 4). Antioxidant enzymes in the treated kidney such as SOD (1.45± 0.4 U/mg/protein), CAT (0.003±0.00 U/mg protein), GPx (10.41 ± 0.03 U/mg protein), GSH (2.07 ±0.16 U/ mg protein) etc. were significantly (P < 0.05) decreased while GR (312.2 ±6.5 U/mg protein) and LPO (1.07 ± 0.02. U/ mg protein were increased (P < 0.05) than that of the control (Fig. 5).

Table 1: Lethal toxicity of Oreochromis niloticus exposed to dimethoate

Treatment (ppm)	Percentage mortality (%) Hours after treatment (h)			
	24	48	72	96
0.15	0	0	0	0
0.20	10	20	20	30
0.25	20	30	40	60
0.30	30	30	60	80
0.35	40	60	80	100
0.40	50	100	100	100
0.45	80	100	100	100

Number of fishes (n) = 8

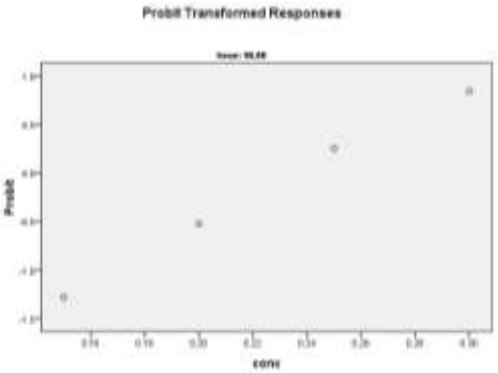

Figure 1: Percentage mortality of the fish *O. niloticus* after 96h exposure to different concentration of dimethoate (ppm).

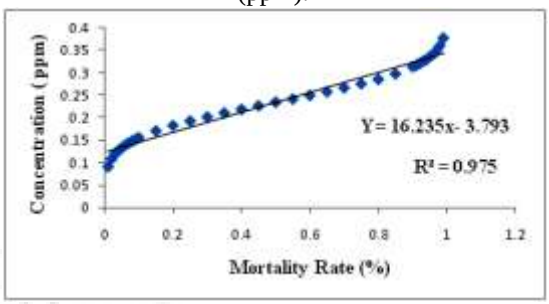

Figure 2: Scatter plot indicating the LC_{50} value of dimethoate after 96h on fish *O. niloticus*.

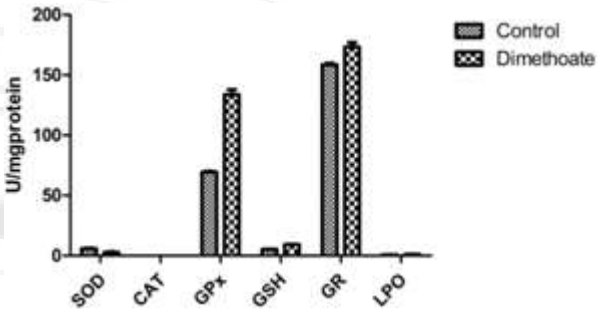

Figure 3: Activity of antioxidant enzyme in gill of *O.niloticus* due to the sub lethal concentration of dimethoate.

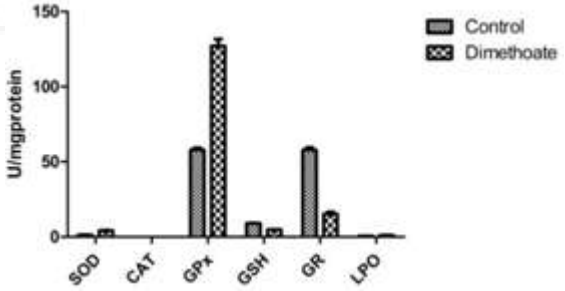

Figure 4: Activity of antioxidant enzyme in liver of *O.niloticus* due to the sub lethal concentration of dimethoate.

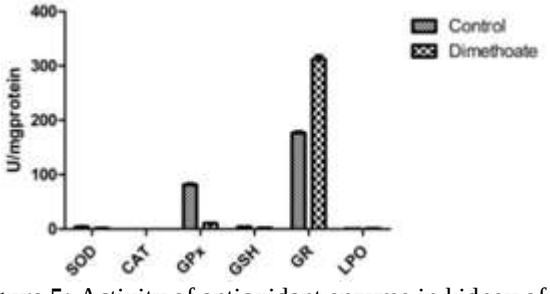

Figure 5: Activity of antioxidant enzyme in kidney of *O. niloticus* due to the sub lethal concentration of dimethoate.

4. Discussion

The assessment of toxicity of insecticide with reference to aquatic biota, especially fish is crucial in establishing the toxicity evaluation [20]. Exposure of fish to xenobiotics are known to interfere with the enzyme activity in vital organs [21]. In the present study, the 96 h LC_{50} value detected in *O. niloticus* due to the exposure of dimethoate was 0.226 ppm. Kumar and Singh [22] observed the LC_{50} value of *Catla catla* on 96 h was as found to be 0.007 mg L^{-1},whereas Singh *et al.* [23] recorded LC_{50} as 1.60 mg L^{-1} in the case of *C. carpio*. According the nature of the fishes, there are wide ranges in the LC_{50} value due to the exposure of dimethoate. The LC_{50} value of certain fishes are as high as *Channa punctatus* (17.9 mg L^{-1}) [24]. The variations in the LC_{50} value is might be due to its various factors such as sensitivity to the toxicant, its concentration and duration of exposure and type and size of the test animal [25].

In the current investigation the SOD-CAT activity in the gill and kidney tissues was found reduced. Bagnyukova *et al.* [26] observed that exposure of glyphosate-based herbicide to the fish, *Prochilodus lineatus* caused transient reduction in superoxide dismutase and catalase activity 6 h after exposure. It is presumed that it might be due to the inhibition of the excess hydrogen peroxide or due to the excess $O2^{-\cdot}$. Exposure of dimethoate to the experimental fish showed an increased SOD activity in the liver. This result is in corroboration with the findings of Sulfath *et al.* [27] in *O. mossambicus* who concluded that the increased activity of SOD might be the due to the removal of toxic H_2O_2. Increased mRNA level of SOD and CAT were found in the liver of *Danio rerio* after the treatment of 10 µg/ L of atrazine for 14 days [28].

Catalase along with GPx removes the H_2O_2 produced by dismutation of $O_2^{-\cdot}$ (Superoxide radical) by Superoxide dismutases (SODs) and the hydroperoxides [29].The present study reveals the GPx activity was significantly increased in all the tissues except the kidney. Increased activity of GPx was also noted in *Prochilodus lineatus* due to the effects of herbicide (roundup) and it might be due to the elevated production of oxidative stress which will break the balance of ROS- antioxidant system [30]. Decreased GPx activity seen in the kidney of current study was similar to the studies of Atli and canli [31] in *O.niloticus* due to the exposure of Cu, Zn and Fe. Significant decline of GPx was also seen in the fish *C.carpio* due to the toxic effect of Simazine [32].
In dimethoate treated fishes, both the liver and kidney showed decreased activity of GSH but the gill showed an increased activity. Barbary [33] also found a reduction in the GSH in *O. niloticus* contaminated by aflatoxin. In the case of glutathione reductase (GR), an increased activity was found in the gill and kidney, but it found decreased in the liver. Similar result was reported in *Prochilodus lineatus* due to the exposure of atrazine by Santos and Martinez [34]. Zhang *et al.* [35] observed an increase in GSH in *Carrasius auratus* due to the effects of 2, 4-dichlorophenol, and it was explained as an adaptive mechanism under the oxidative stress. The reports of Kozer *et al.* [36] revealed that the decreased level of GSH may be due to the consumption of GPx -GR as they have direct association with GSH and the increased level of GR might be due to an attempt of maintenance of normal level of GSH.

Exposure of the fish to dimethoate caused significant increase in the level of LPO in the gill, liver and kidney. Gad [37] observed similar phenomenon in *O. niloticus* due to the contamination of crude oil and arrived the conclusion that free radical induced oxidative cell injury results in the increased production of LPO. Higher level of LPO was also reported in *O. niloticus* and *C. carpio* due to the effect of diazinon for 30 days [38], [39] and in Atlantic cod exposed to alkyl phenol for 15 days [40].

5. Conclusion

Exposures of dimethoate to *Oreochromis niloticus* caused variations in the major antioxidant enzymes. While a decrease in the superoxide dismutase (SOD) and catalase (CAT) was observed in the gill, the other enzymes like glutathione peroxidase (GPx), glutathione (GSH), glutathione reductase (GR) and lipid peroxidase (LPO) were found increased. In the case of kidney, along with SOD-CAT., GPx - GSH were also decreased, but an increase in GR-LPO was observed. The titre of SOD, GPx and LPO in the liver increased and CAT, GSH and GR decreased. The result concludes that antioxidant enzyme productions in *O. niloticus* due to the exposure of dimethoate were tissue depended.

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