Journal of Advanced Laboratory Research in Biology
E-ISSN: 0976-7614
Volume 10, Issue 3, July 2019
PP 95-99
https://e-journal.sospublication.co.in

Research Article

Biochemical effects of Organophosphorus pesticide, Quinalphos on freshwater fish, *Oreochromis niloticus* (L.)

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Abstract: Quinalphos is a synthetic organophosphorus broad-spectrum insecticide, widely used to control insect pests in agriculture. In the present study, an attempt has been made to investigate the biochemical effect of quinalphos on different tissues like liver and brain of the edible freshwater fish *Oreochromis niloticus* (L). The *O. niloticus* were exposed to sublethal concentrations of quinalphos for two different periods of time (1 day and 5 days). From the result, the protein content and acetylcholinesterase enzyme in the tested tissues of *O. niloticus* was found to decline with increasing exposure periods of quinalphos. Activity of antioxidant enzymes glutathione S-transferase and catalase shows a significant change in their level compared to the control. The present study suggests that quinalphos in sublethal dose altered antioxidant balance of the fish which cause severe oxidative stress. Therefore, the use of quinalphos in the agriculture may be a threat to aquatic population and also other organisms including human beings via food chain.

Keywords: *Oreochromis niloticus*, Quinalphos, Glutathione S-transferase, Catalase, Acetylcholinesterase.

1. Introduction

One of the major problems the world facing today is environmental pollution. Every passing year, the rate of pollution increases and that causes severe, irreparable environmental damage. Among the different pollutions, water pollution is the most hazardous one. Water pollution is the biggest threat of urbanization, industrialization and modern agriculture practices. It leads to variation in physical, chemical and biochemical properties of water bodies [1]. Many water pollutants are reported as toxic chemicals, among these the most potential water pollutants are the pesticides. Pesticides are widely used in agricultural field to control insects, pests and disease vectors. Pesticides find their way into aquatic habitats such as ponds, lakes and rivers, thus pollutes the aquatic environment [2]. Approximately less than 0.1% of the pesticide, applied in the fields, approaches the specific target organism while the remaining 99.9% enters into the environment [3]. The extensive use of pesticides leads to water pollution and affects the non-target organisms, especially fish population in the aquatic ecosystem.

Organophosphorus (OP) insecticides are the most considerably used synthetic chemicals for controlling agricultural pests. Quinalphos (*O,O*-diethyl *O*-2-quinoxalinyl phosphorothioate), being an organophosphorus insecticide, is used worldwide to control agricultural pests. Quinalphos acts as a cholinesterase inhibitor and may cause disorders in the physiological state of the nervous system. It causes disruption in the passage of impulses across the neuromuscular junction and neural junction by inhibiting acetylcholinesterase.

Alteration in the biochemical parameters of pesticide-treated fish is a significant tool for the assessment of water quality in the field of environmental toxicology [4]. Therefore, the present study was undertaken to investigate the effect of quinalphos on certain biochemical parameters of the freshwater fish, *Oreochromis niloticus*.

2. Materials and Methods

2.1 Test Organism

The fries of cichlid fish, *Oreochromis niloticus* were collected from local fish farm Kottakkal, Malappuram District, Kerala. The fishes were acclimatized in the laboratory condition for 3 to 4 weeks in dechlorinated tap water and well-aerated glass tank. The physicochemical properties of the tap water were estimated by following the APHA [5]. Healthy

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Received: 02 May 2019
Accepted: 01 June 2019

fishes weighing 3.8±0.5g and 4.8±0.4cm length were selected for the study.

2.2 Experimental design

An acute toxicity test was conducted by static renewal bioassay method to determine toxicity of quinalphos on fish. The fish were exposed to different concentration of quinalphos for 96 hrs and mortality was noted in every 24 hrs. After the 96 hrs of exposure period, the mortality data was subjected to Probit analysis [6].

After the exposure in the sublethal concentrations for 1 day and for 5 days, the live fishes were killed and the tissues like gill, brain and liver were dissected out for the biochemical analysis. 5% tissue homogenate was prepared in isotonic ice-cold solution. Centrifuged at 10,000 rpm for 15 minutes at 4ºC to get the supernatant and this was used for the biochemical analysis. Protein was estimated by the method described by Lowry *et al*., [7] and catalase activity was estimated by the method of Sinha *et al.*, [8]. The activity of acetylcholinesterase and glutathione S-transferase were estimated as per Ellman *et al*., [9] and Beutler *et al.*, [10] respectively.

3. Results and Discussion

Organophosphorus insecticides are recognized and marked as one of the major pollutants in the aquatic ecosystem [11]. Organophosphorus insecticides in the water bodies can easily be absorbed through gills and get accumulated in different organs of the fish like liver and brain. This may cause physiological and biochemical alteration on fish. The 96 hrs LC_{50} value of quinalphos was assessed to be 6.53µl/L. A sublethal concentration of 0.65µl/L was selected for the present study. The observation of various biochemical enzyme activities in liver and brain tissues of *Oreochromis niloticus* exposed for 1 day and 5 days of sublethal concentration of Quinalphos has been presented from Fig. 1 to 4 and discussed here.

In fish, proteins are the primary energy source and are involved in regulating physiological and metabolic processes in the body. It is the major source of energy during stress conditions apart from carbohydrate and lipids [12]. A significant difference was observed in the protein content of liver and brain tissues in *Oreochromis niloticus* on different exposure periods (Fig. 1). The highest decrease of protein was seen in liver tissue. When the period of exposure of pesticide increases the decrease in the protein content was also seen. Maximum decrease was seen in 5th day of exposure. The reduction in the protein content may be due to increased utilization of protein to meet the high energy demand when the fish is under stress condition [2].

A study by Agrahari *et al.*, [13] shows that the protein content was decreased in different tissues exposed to monocrotophos in a freshwater fish *Channa punctatus.* Significant decrease of total protein content on exposure of different pesticides was reported in many studies [4,14,15,18].

Inhibition of AChE is the main mechanism of action of organophosphorus compounds. Being the main target organophosphorus toxicity, AChE may serve as a valuable diagnostic tool for organophosphorus exposure [16]. At neuron junction, AChE enzyme modulates the amount of neurotransmitter substance. In the present study acetylcholinesterase enzyme activity in response to quinalphos was analyzed in the liver and brain tissues of fish and presented in Fig. 2. The data showed that changes in the activity of the enzyme acetylcholinesterase were statistically significant in relation to respective controls. In both liver and brain tissues, the level of enzyme decreased compared to control. Inhibition of the enzyme activity by quinalphos was dependent on the duration of exposure (Fig. 2a and 2b). In the present study, the maximum decrease of enzyme is seen in the 5th day treatment.

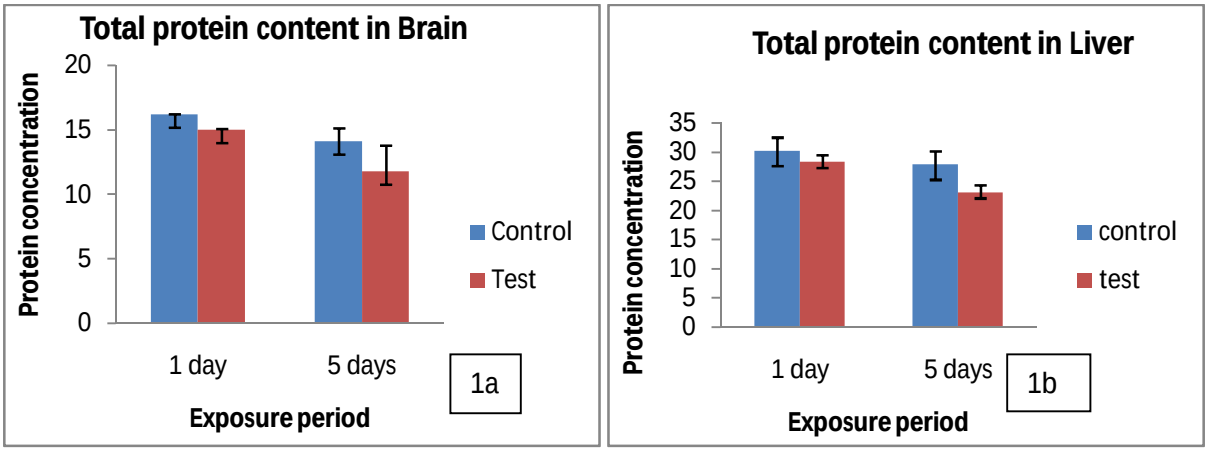

Fig. 1: Effect of Quinalphos on Total protein content in *O. niloticus*. Fig. 1a: Shows the total protein Content in brain and Fig. 1b: Shows total protein content in liver.

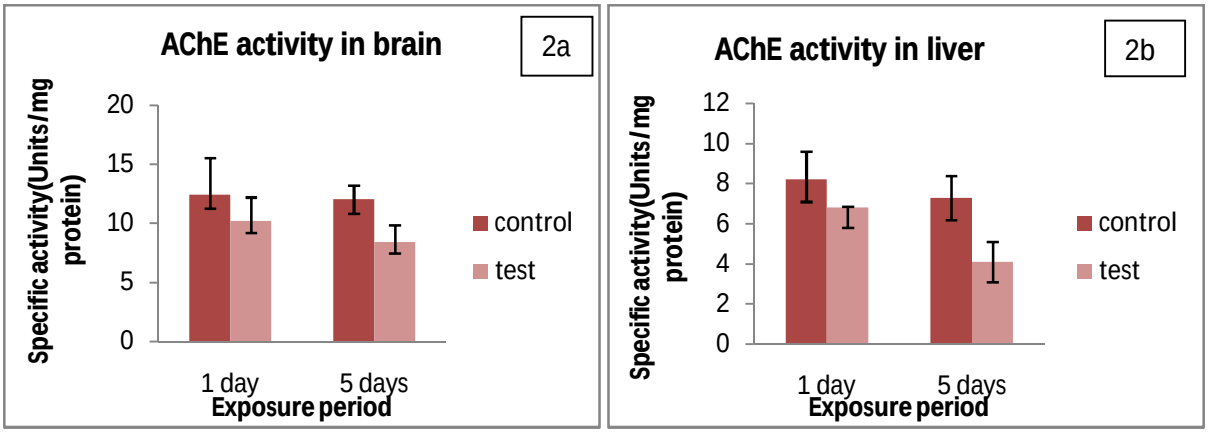

Fig. 2: Effect of Quinalphos on acetylcholinesterase enzyme activity in *O. niloticus.* Fig. 2a: Shows AChE activity in brain and Fig. 2b: Shows AChE activity in liver.

The present observation is in agreement with the investigation of Sahib *et al*., [17] and revealed a decrease in the AChE in Tilapia exposed to malathion and it is reported that AChE was found to be decreased in liver tissues exposed to sublethal concentration of quinalphos on common carp, *Cyprinus carpio.* The present findings on AChE are compared and are on par with several reported works like Naveed *et al*., [18] observed the decrease in the level of AChE in different tissues of *C. punctatus* exposed to sublethal concentration of triazophos.

The antioxidant enzymes are important components in preventing oxidative stress. In aquatic organisms, pesticides caused oxidative stress may lead to the production of ROS and alterations in antioxidant enzymes. ROS production may attack adjacent molecules resulting in function or dysfunction and damage molecular structure of many organs and systems [19]. Recently in toxicology research, oxidative stress induced by organophosphorus insecticide has widely been used to demonstrate the toxicity mechanism [20].

Catalase is an important enzyme in the antioxidant defense system that protects animals from oxidative stress. It eliminates hydrogen peroxide a non-radical reactive oxygen species (ROS) which can penetrate through all biomembranes and directly inactivates few enzymes. The enzyme GSTs are multifunctional enzymes involved in the detoxification of many xenobiotics and this play an important role in the protecting tissues from oxidative stress. GST plays a major role in diminishing oxidative stress in all life forms. CAT and GSTs are considered as a sensitive biomarker of oxidative stress before major deleterious effects occur in fish [21].

Effect of sublethal concentration of quinalphos induced catalase activity in different tissues was represented in Fig. 3a & 3b. An increase was found in catalase activity in the liver and brain tissues in 1 day exposure of sublethal concentration of quinalphos. This may be due to overcoming oxidative stress. The highest activity was observed in the liver tissue than brain after 1 day exposure to the quinalphos. The brain tissue responding immediately to toxic exposure, these enzymes might be protecting the tissue from pesticide toxicity as other previous reports [22]. After 5 days of treatment, the level of enzyme reduced on both control and test compared to the 1 day exposure to the sublethal treatment. Ansari and Ansari [23] also observed time and concentration-dependent decrease in the level of catalase enzyme in the liver tissue exposed to alphamethrin on Zebrafish. Decrease in the level of CAT might be due to binding of the toxicant to the enzyme molecule or inhibiting the enzyme synthesis [24].

GST activity in response to short-term exposure of quinalphos was analyzed in the liver and brain tissues of *O. niloticus* and the data were graphically represented in Fig. 4a & 4b. After 1 day of exposure liver and brain shows increased level of GST. This may be due to overcoming the pesticide-induced oxidative stress.

In comparison with the control group, a significant increase in GST activity was observed. GST activity has also been reported in the studies with common carp, *Cyprinus carpio* exposed to azinphosmethyl [25]. GST activity either has a significant increase or decrease with different patterns according to the exposed elements or exposure conditions [21].

4. Conclusion

The findings of the present study show that sublethal exposure of the organophosphorus insecticide quinalphos induces significant changes in the enzymatic profile of freshwater fish, *Oreochromis niloticus.* Therefore, the use of quinalphos to control insect pests in agriculture may be a threat to fish population and also other organisms including human beings via food chain. The biochemical studies can be used as biomarkers for assessing pesticide toxicity in aquatic environment.

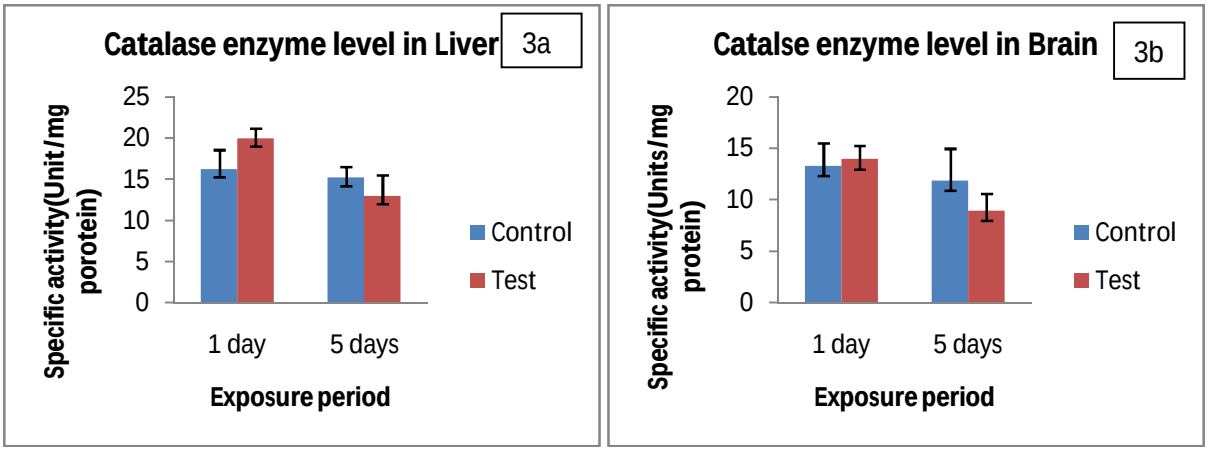

Fig 3: Effect of Quinalphos on Catalase enzyme activity in *O. niloticus.* Fig. 3a: Shows catalase enzyme level in liver and Fig. 3b: shows catalase enzyme level in brain.

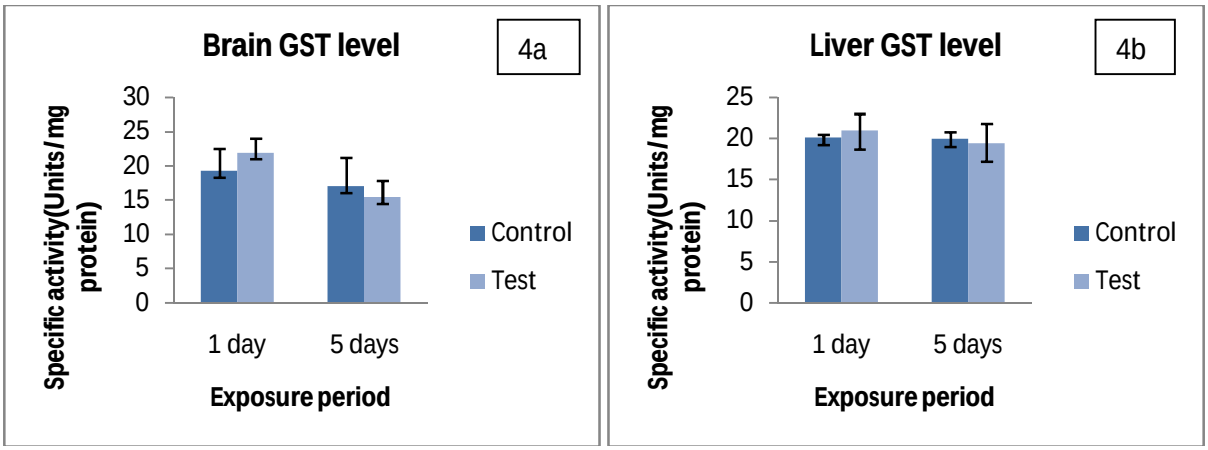

Fig. 4: Effect of Quinalphos on Glutathione S-transferase enzyme activity in *O. niloticus.* Fig. 4a: Shows GST activity in the brain and Fig. 4b: Shows GST activity in the liver.

Acknowledgment

We are thankful to the Department of Biotechnology, Ministry of Science and Technology, New Delhi for their financial support through the Major Research Project.

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