Tebuconazole Induced Biochemical Alteration in Serum of *Cyprinus carpio* (L.1758)

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Abstract: ***In the present study we investigate the effect of azole fungicide tebuconazole on the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activity and protein level in Cyprinus carpio (L., 1758). Tebuconazole commonly known as Folicur, a triazole fungicide is a broad - spectrum systemic fungicide widely used in agricultural fields across the world. Tebuconazole causes detrimental impacts onthe aquatic environment over a long period andhas toxic effects on aquatic organisms. The fish C. carpio (L.1758) have been used as indicator organisms for the determination of toxicity in an aquatic environment. After acclimatization in laboratory conditions, the fish had been divided into three groups, each of which include 6 fish. Group I were kept in a normal water environment serve as control and those in Groups II and III were in 6.47 and 8.09 µL/L tebuconazole added water tanks, respectively, for 20 days. Blood samples were taken from the anal vein of thefish and serum were collected. Then, ALT, AST, and ALP activities and protein levels were analyzed. While protein level decreased in tebuconazole treated groups compared to Group I, increases were determined in terms of ALT, AST, and ALP activity. In conclusion, it was found that fungicide tebuconazole exposure caused significant changes in serum enzymatic activities and protein levels of Cyprinus carpio. The increase in AST and ALT levels could be associated with liver function changes as a result of fungicide exposure to the fish.***

Keywords: *Cyprinus carpio*, Tebuconazole, ALT, AST, phosphatase

1. Introduction

Agriculture is an important economic sector in India. Over 58 percent of rural households depend on agriculture as their principal means of livelihood [1], [2]. Asia is where more than half of the pesticides utilized globally. India ranks third in Asia, behind China and Turkey, in terms of the global use of pesticides. [3]. Pesticides and fertilizers are an integral part of modern agriculture. Commonly used pesticides include insecticides, fungicides, and herbicides for the management of uncontrolled weeds and pests on agricultural sites [3]. In order to increase agricultural productivity Pesticides are used, but they are applied indiscriminately and pollute the biota. The environmental transfer of pesticides causes harm to non - target species. Some insecticides have the potential to be harmful to the environment and human health. World Health Organization estimates 500, 000–1, 000, 000 people per year worldwide experience health effects as a result of pesticide poisoning [4]. Only around 0.1 percent of pesticides are believed to reach the intended organisms, with the rest polluting the environment and causing environmental harm [5], [6].

Water pollution, contamination of water bodies may be defined in terms of the undesirable changes in the chemical and physical properties of water which are not favourable to all those living things utilizing water for their lives [7]. Water pollution comes from a variety of sources and takes many different forms. water pollution from feedlots, pastures, and croplands may be caused by agriculture. Landfills, oil drilling, and mining could all be significant sources ofwater pollution. Other water pollution sources, related to humans, are sanitary sewers, storm sewers, industry, and construction [8]. The leaching and mixing of chemicals from agricultural practices is responsible for > 50% of the water pollution in streams and rivers according to Environment Protection Agency (EPA) report, 1990 [8]. Fungicides are widely regarded as substances having a variety of effects on the aquatic environment. Tebuconazole, a fungicide with systemic effect, has an influence in such a way as to inhibit ergosterol synthesis within the fungus body. Tebuconazole causes detrimental impacts on the aquatic environment over a long period and has toxic effects on aquatic organisms [9]. Fish are useful bioindicators in determining stress factors against environmental contaminants [10]. Changes in haematological and biochemical parameters of fish blood are frequently used as markers of physiological and sublethal stress responses to environmental contaminants [11].

Common carp (*Cyprinus carpio*), a warm freshwater species found all over the world and must be produced sustainably through intensification in order to meet rising global demand. It is a widely cultured fish and contributes 7.7% of the world's total finfish aquaculture production [12]. *Cyprinus carpio* (L.1758) is used as a model organism in our study. The present study was performed to examine the effect of tebuconazole on serum marker enzyme activities and protein, and cholesterol levels of *Cyprinus carpio* (L.1758).

2. Materials and Methods

a) Animal Collection

The fish *Cyprinus carpio* of weight 150 - 200gm and length up to 20±2cm were collected from Fish Seed and Breeding Farm Deoli, District Bilaspur, Himachal Pradesh and were safely brought in oxygen water packed polythene bags to the laboratory. The fish were checked against injury or infection by keeping in 0.2% of potassium permanganate solution for 2 - 4 min. The experimental procedure were conducted after the approval of Fisheries Department of Himachal Pradesh.

b) Acclimatization

The fish were acclimatized to the laboratory conditions for 15 days in a glass aquarium filled with 80 L of dechlorinated tap water. The water was dechlorinated using an anti - chlorine solution and then exposed to air for 24 hours. All the aquaria were equipped with aerators and filters. Only 40% of the volume of the water was changed after every 24 hours. They were fed with commercial supplementary feed twice a day (10: 00 and 17: 00 h). Unconsumed feed and excreta were siphoned daily. The physicochemical properties of water were temperature 22ºC, pH 7.8±0.2, D. O.8±2mg L - 1, TDS 150±8 ppm (water analyzer kit), total alkalinity170±10 mg L - 1 (titration method).

c) Chemicals

Tebuconazole (25.9% EC), a commercial grade was purchased from the local market. The solution of desired concentration was prepared by diluting it with distilled water.

d) Experimental design

After acclimatization, all fish were totally divided into 3 groups based on the exposure to tebuconazole (25.9% EC). During the experiment, the water in each aquarium was aerated and the temperature was 22ºC. Group I were kept in a normal water environment served as control and those in Groups II and III were in 6.47 and 8.09 µL/L tebuconazole added water tanks, respectively, for 20 days. No water changed during the experimental procedure.

e) Determination of biochemical parameters

Blood was collected in a clean and dry 1.5 ml Eppendorf tube (without anticoagulant). This was allowed to clot for 1 h in an inclined position at room temperature. Centrifuged at 3000 rpm at 4 ºC for 10 min. The supernatant (serum) was collected and stored at - 20ºC for further analysis. Protein, glucose, cholesterol, and marker enzymes viz. SGOT, SGPT & ALP were analysed. Total protein was analyzed by the Coomassie blue method of Bradford, (1976) [13]. The estimation of glucose and cholesterol was done according to the Anthrone method given by Seifter*et al*. (1950) [14] and Zlatkis*et al*. (1953) [15] respectively. The enzymes (SGPT and SGOT) were estimated by the method of Bergmeyer and Bemt (1965) [16] and alkaline phosphatase activity by the method of Weil and Russel (1940) [17].

f) Statistical analysis

Data were expressed as mean ± S. E. M. The statistical significance was determined by applying one - way ANOVA and was significant at $p<0.05$.

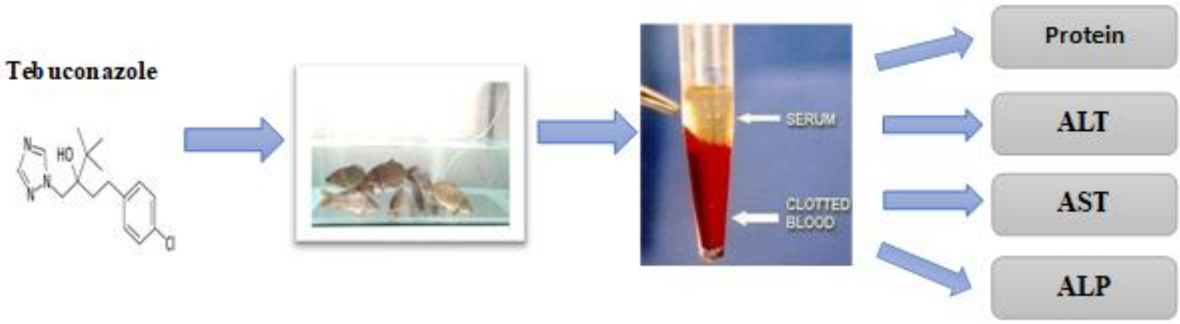


Figure I: Diagrammatic representation of the experimental plan

3. Results

The alterations in the biochemical parameters of serum of the control and tebuconazole treated *C. carpio*were presented in Table I and Figure II. The fish after exposure to tebuconazole revealed an alteration in the level of total protein in the serum. The protein level diminished to 27.41% and 34.33% in groups II and III respectively in comparison to the control. Serum total cholesterol of the fish increased highly significantly after 20 days in both concentrations as compared to the control groups. An increase of 106.39% and 140.34% had been observed with respect to control. Hyperglycaemia was observed in the tebuconazole treated fish.

Table I: Alterations in serum protein, cholesterol, and glucose level and marker enzymes of tebuconazole treated *C. carpio* in comparison to control for 20 days

Parameters	Control	6.47µL/L TBZ	8.09µL/L TBZ
Total Protein (gm/dl)	15.76±0.02	11.44±0.009 [a]	10.35±0.008 [a]
Cholesterol (mg/dL)	18.94±1.7	39.09±1.73 [a]	45.52±1.63 [a]
Glucose (mg/dL)	30.16±0.71	44.09±0.26 [a]	48.63±0.35 [a]
Serum GPT (µ moles of sodium pyruvate formed /ml of serum/minutes)	25.36±0.86	40.02±0.81 [a]	58.16±1.02 [a]
Serum GOT (µ moles of sodium pyruvate formed /ml of serum/minutes)	54.25±0.02	98.09±0.03 [a]	102.3±0.06 [a]
Alkaline Phosphatase (µM Pi/mg protein)	50.04±0.09	59.57±0.15 [a]	64.00±0.03 [a]

SGOT, and SGPT are also known as aspartate transaminase (AST), and alanine transaminase (ALT) respectively. Increased activity of aminotransferases and alkaline phosphatase were observed in tebuconazole treated fish.80.81% and 88.57% increases in SGOT activity were observed. A highly significant increase of 129.37% in SGPT activity was observed in 8.09µL/L tebuconazole treated fish.80.81% and 88.57% increases in ALP activity of group II and group III were observed as compared to the control group. The increase in AST and ALT levels could be associated with liver function changes as a result of fungicide exposure to the fish.

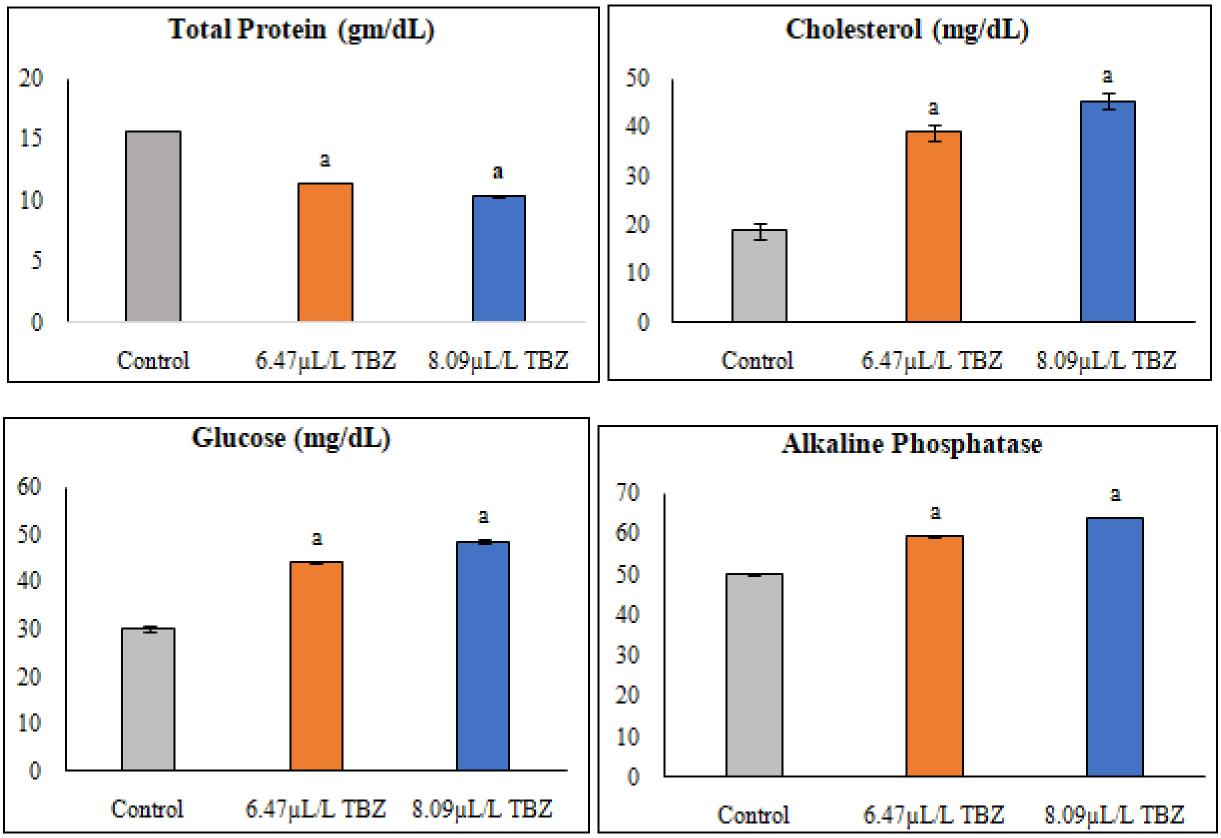
4. Discussion

The overuse of pesticides causes adverse impacts on all living organisms including commercially important fish. It is important for human health to examine the effects of pesticides on the fish playing an important role in the food chain [18]. The total serum protein content is considered a broad clinical index of health, stress status as well as nutritional condition in fish because this index includes a wide spectrum of proteic components [19]. The fluctuation in blood serum protein concentrations is associated with physiological and pathological conditions [20]. The hypercholesterolemia following cypermethrin exposure was observed in *Rhamdiaquelen* [21]; in *Oreochromis niloticus* exposed to short - and long - term treatment of copper, lead, and cypermethrin [22]. Oner et al (2008) [23] found that cholesterol of metal - intoxicated *Oreochromis niloticus* increased compared to corresponding control value. They concluded that the concentration of cholesterol may increase due to hepatic and renal failure causing the release of cholesterol into the blood. Contrary, Vetriselvi, 2016 witnessed a significant reduction in free and total cholesterol, lipids, and phospholipid content of the Brain, Gill, intestine, liver & Muscle tissues of the tebuconazole exposed *Labeorohita* [24]. In the present study, increase in glucose level observed with increasing concentration of tebuconazole. The hyperglycemia indicates disrupted carbohydrate metabolism probably after the elevated glycogenolysis. Increase in whole body glucose and glycogen levels was observed in treated fish, when compared with the control group. Contrarily, the protein levels decreased [25]. Similarly, Sancho et al. (2010) [26] reported an increase in the whole - body glucose levels of *D. rerio* exposed to the same fungicide used in this study. An increase in the whole - body glycogen levels was observed in R. quelen after exposure to clomazone (Crestani et al., 2006) [27].

Increased activities of glutamate–oxalacetate transaminase (GOT), glutamate–pyruvate transaminase (GPT), acid and alkaline phosphatase of blood plasma indicated hepatic tissue damage [28]. Nur et al observed the increased activity of AST and ALT compared to control group, while PON1, and HDL levels decreased in *C. carpio* exposed to a similar pesticide [29]. Increased activity of acid phosphatase and alkaline phosphatase in blood plasma as an indicator of hepatic tissue damage and dysfunction have been shown in fish following the exposure of fungicides [30].

5. Conclusion

In conclusion, the most significant changes in serum enzyme activities and protein cholesterol parameters were found. Hyperglycaemia, decreased serumprotein, and hypercholesterolemia are indicators of altered carbohydrate, lipid, and protein metabolism in fish due to azole fungicide tebuconazole exposure. Increased activity of transaminases and alkaline phosphatase in blood serumis an indicator of hepatic tissue damage. The changes reflect organ dysfunction due to fungicide exposure. This study clearly indicates that the presence of tebuconazole may cause deleterious effects on fish physiology and may potentially disturb their survivability.



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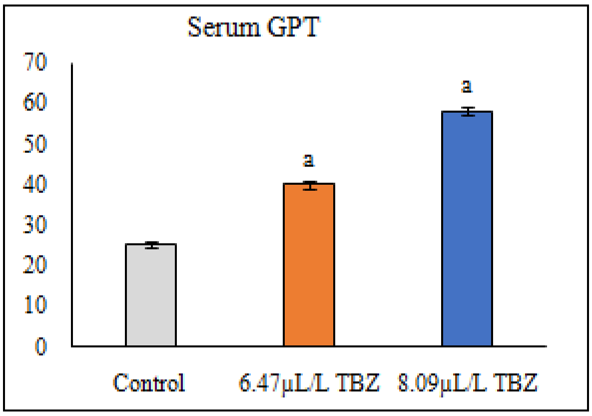

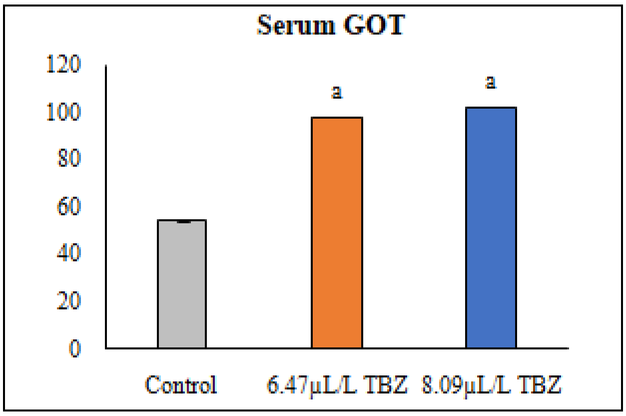

Figure II: Biochemical parameters of control and tebuconazole exposed *C. carpio* (L., 1758) (^{a}p<0.05)

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