

**HEMATOLOGICAL AND BIOCHEMICAL RESPONSE IN THE BLOOD  
OF *ALBURNUS TARICHI* (ACTINOPTERYGII: CYPRINIFORMES: CYPRINIDAE)  
EXPOSED TO TEBUCONAZOLE**

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**Background.** Lake Van, the second-largest soda lake in the world, has a pH 9.8 value. A fish, locally known as Van fish, *Alburnus tarichi* (Güldenstädt, 1814), is an endemic fish of Lake Van and known also as pearl mullet, tarek, or Van bleak. Tebuconazole is a widely used pesticide around Lake Van. In this study, we will focus on the effects of tebuconazole on Van fish blood to provide critical information on the environmental risk assessment of pesticides in various aquatic environments.

**Materials and methods.** The Van fish were exposed to tebuconazole for 24, 48, 72, and 96 h at a concentration of 2.5 mg · L<sup>-1</sup>. Subsequently, the resulting hematological and biochemical parameters were determined.

**Results.** There was a statistically significant decrease in erythrocytes (RBC), hemoglobin (Hb), and hematocrit (Hct) in the blood parameters ( $P < 0.05$ ). The levels of serum, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea, and creatine increased significantly ( $P < 0.05$ ). The serum cortisol level increased significantly at all hours after the administration of tebuconazole ( $P < 0.05$ ).

**Conclusions.** As a result, toxicity caused by pesticides caused negative changes in the biochemical and hematological values of Van fish. Changes in these parameters have shown that it can be used as a biomarker for toxicity.

**Keywords:** toxicity, hematology, fish, cortisol, tebuconazole

## INTRODUCTION

The world population is growing steadily and rapidly, thus, increasing the demand for agricultural products, especially for food. Such increased demand is matched by the increased production and the latter is accompanied by an exponential increase in crop pests development. As the consequence, we witness an unprecedented rise in the production of pesticides used to protect crops. Given the toxicity of pesticides to non-target organisms, these agents create a threat to various ecosystems (Karanasios et al. 2012, Chromcova et al. 2015, Faggio et al. 2018, Fiorino et al. 2018, Plhalova et al. 2018, Çilingir Yeltekin and Oğuz 2018, Blahova et al. 2020, Petrovici et al. 2020, Stara et al. 2020). These pollutants reach the surface and ground waters thus affecting its chemical composition (Kohler and Triebkorn 2013, Çilingir Yeltekin and Oğuz 2017, Çilingir Yeltekin 2018, Alak et al. 2019a).

The popular fungicides throughout the world because of their distinctive mode of action supported the inhibition of the plant ergosterol synthesis pathway. Ergosterol plays an essential role in the structure of the fungal membrane (Bien and Espenshade 2010). These biological properties give triazole fungicides a wide range of field applications: in the protection and treatment of a wide variety of crops (crops, soy, fruits, vegetables, and ornamental flowers) against a broad spectrum of fungal pathogens. The triazoles represent approximately 20% of the global market for systemic fungicides (Pearson et al. 1994). Consumption of nutrients with high amounts of fungicides can cause serious health problems. Tebuconazole, fungal cytochrome P450 (CYP450) enzymes it shows greater affinity and is a potential inhibitor of the sterol14 $\alpha$ -demethylation reaction in CYP51-catalyzed fungi belonging to the CYP450 monooxygenase superfamily (Muñoz-Leoz et

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al. 2011). Systemically effective tebuconazole can cause toxic effects on organisms in the aquatic environment for a long time. Few studies have been conducted on the emergence of fungicides in various waters. These studies (surface, groundwater, or wastewater) almost always showed the presence of tebuconazole and often the highest concentration (Youness et al. 2018, Alak et al. 2019b). Its common name is tebuconazole, its chemical name is (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentane-3-ol. It is the best known and sold systemic fungicide of the triazole family. The racemic form has been commercially available since 1988 and is now used as a growth factor and biocide. Due to its highly lipophilic properties, it is very stable in the soil and takes half-life weeks ( $DT_{50} = 49\text{--}610$  days), depending on the soil properties and the initial concentration of the conjugate (Roberts and Hutson 1999, Čadkova et al. 2012, Sehonova et al. 2017, 2018).

Fishes are used as effective indicators of aquatic pollution due to their high sensitivity to environmental contaminants (Çilingir Yeltekin and Sağlamer 2019). In addition, pesticide residues in fish reach people in a short time because fish are consumed too much by humans (Xu et al. 2019). Blood parameters are used as sensitive toxicity indicators. In addition, blood parameters are used to determine the biological effect of pesticide species on fish (Wu et al. 2014).

Van fish, *Alburnus tarichi* (Güldenstädt, 1814), is an endemic species living in the Lake Van, the largest inland water lake of Turkey. In addition, this fish species is an important source of protein for the people of the region. Tebuconazole is a widely used pesticide around Lake Van. In this study, we will focus on the effects of tebuconazole on Van fish blood to provide critical information on the environmental risk assessment of pesticides in various aquatic environments.

## MATERIALS AND METHODS

**Experimental design.** Since there were no live fish sold, a total of 80 *Alburnus tarichi* (male and female) were obtained from the Karasu River flowing into Van Lake (Turkey). The sampling time of the study covers the date range of 1 May–10 May 2018. The mean weight of the fish was  $111.2 \pm 0.52$  g and the mean length was  $20.39 \pm 0.36$  cm. The fish were transferred to fiberglass tanks (300 L) in an animal farm of the Van Yüzüncü Yıl University. The fish were kept for 7 days to adapt to the environmental conditions. During the study; under normal lighting (14:10 h light:dark), the water was continuously vented and the fish were fed to a specific saturation with a commercial feed. Mean values of some parameters of water measured daily during the experiment: temperature  $12.9 \pm 2^\circ\text{C}$ , pH  $8.62 \pm 0.3$ , dissolved oxygen  $6.42 \pm 0.15$  mg · L<sup>-1</sup>, oxygen saturation 60.1% L, conductivity 730 μS · cm<sup>-1</sup>, and the salinity 49%. The study was conducted with permission from the Local Ethics Committee of Animal Experiments of Van Yüzüncü Yıl University (YUHADYEK (2018/08)).

The Van fish were divided into eight groups and ten fish were distributed randomly in each tank. Since the effect of chemicals on metabolism in living organisms mostly occurs one day later, sampling started at 24 h in this study. Group I fish were used as a control group in 24, 48, 72, and 96 h periods and no chemical treatment was performed. Group II fish were used as a chemical group in 24, 48, 72, and 96 h periods. Tebuconazole was applied to fish at a concentration of 2.5 mg · L. The water was changed every 2 days to minimize possible changes in the tebuconazole concentration. Proven fungicide concentrations the current reference for the LC<sub>50</sub> value was selected based on the literature (Lutnicka et al. 2016) and was less than 25% of this value. The available literature on the tested concentrations of fungicides detected in surface waters is insufficient. At the end of the application period of each group, the Van fish was anesthetized using aminobenzoate methanesulfonate (MS-222, 100 mg · L<sup>-1</sup>). Blood was then collected from the caudal veins of the fish with a heparinized plastic disposable syringe.

**Determination of hematological and biochemical parameters.** Erythrocytes were detected using a hemocytometer (Rusia and Sood 1992). Hb concentrations were determined by cyanmethemoglobin method (Drabkin et al. 1946) and Hct values were determined by microhematocrit method (Nelson et al. 1989). Biochemical parameters in the serum samples were analyzed using biochemical analyzers (Architect ci-16200, Architect i-2000 SR, Abbott Laboratories, Diagnostic Division, Abbott Park, IL, USA). The following procedures were used to measure the serum parameters: UV assay technique for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activity, creatinine and urea concentrations were measured; electrochemiluminometric technique was used for determining the cortisol level.

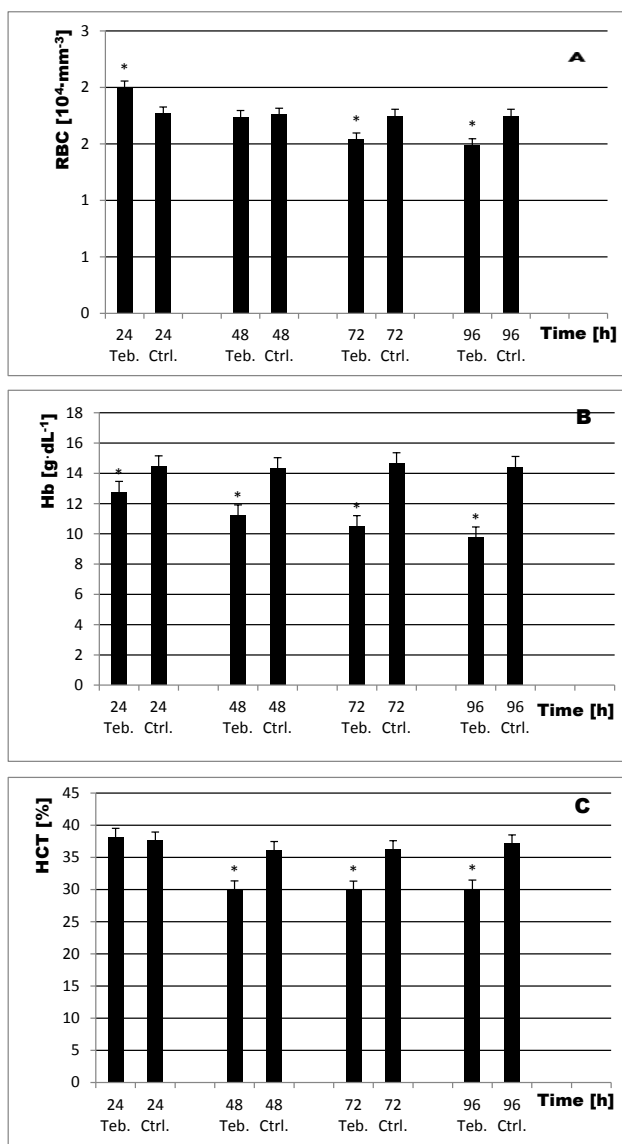
**Statistical analysis.** The results are expressed as mean ± standard error. Student's *t*-test was used to compare study groups. Statistically significant differences were considered significant at  $P < 0.05$ . Statistical analysis of all data was performed using SPSS Statistics version 23.0.

## RESULTS

The hematological data of the fish exposed to tebuconazole were found to be statistically significant by the end of 24, 48, 72, and 96 h, according to control group ( $P < 0.05$ ) (Fig. 1). Biochemical parameters of ALT, AST, LDH, urea, and creatine were studied in the blood of Van fish exposed to tebuconazole. Statistically significant changes were recorded in all these parameters ( $P < 0.05$ ) (Fig. 2). Serum cortisol levels were significantly increased at 24, 48, 72, and 96 h of the Van fish exposed to tebuconazole compared to the control group (Fig. 3).

## DISCUSSION

In many agricultural, garden, and industrial environments, a fungicide with a pesticide is used to destroy or prevent undesired growth of fungal microorganisms. The negative effects of various fungicides on all



**Fig. 1.** Changes in some hematological parameters of Van fish exposed to tebuconazole; (A) Red blood cell; (B) hemoglobin; (C) hematocrit;  $\text{RBC} = 10^6 \cdot \text{mm}^{-3}$ ,  $\text{Hb} = \text{g} \cdot \text{dL}^{-1}$ ; Teb = tebuconazole, Ctrl. = control; all values are mean  $\pm$  SD, \* $P < 0.05$

organisms are known to cause, at least, stress (Jaleel et al. 2007, Manivannan et al. 2007, Burgos Aceves et al. 2018). Since they are exposed to pesticides in aquatic organisms, they form chain reactions one after another in their metabolism. In fish, pesticides are taken from the gills and gastrointestinal tract and then transported to the tissues and organs by blood (Srivastava and Choudhary 2010). The stress condition then occurs and its destructive effects are first seen by blood cells. This changes the physiological and biochemical parameters of the fish (Beyea et al. 2005, Aliko et al. 2018, 2019, Vajargah et al. 2019).

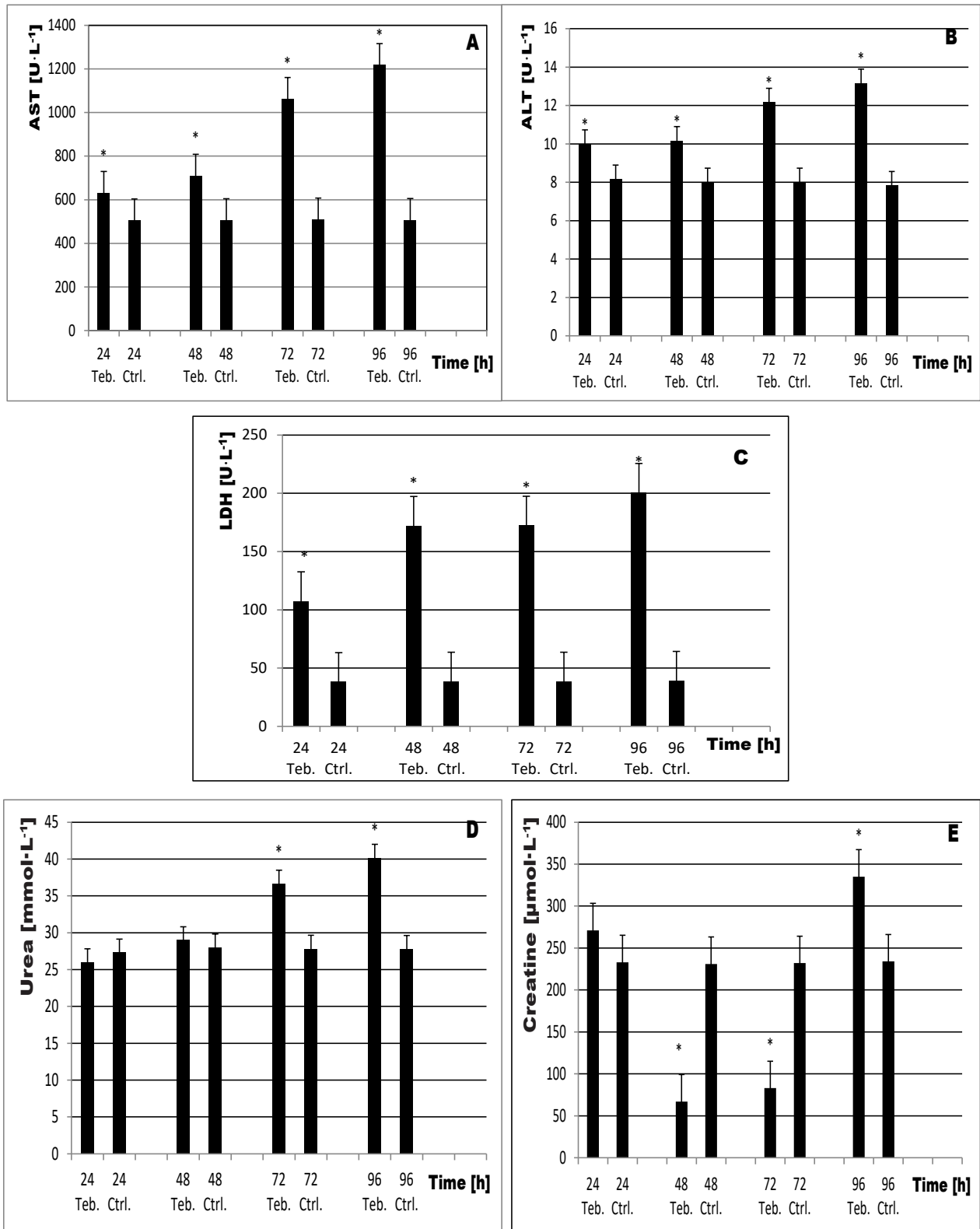
In this study, a significant reduction in RBC, Hb, and HCT in Van fish was observed. This may be due to hematopoietic insufficiency. Blood indices of fish exposed to toxic substances are commonly used as bioindicators. The decrease in hemoglobin, an indicator of anemic

conditions, can be considered to be the result of hemolysis caused by stress caused by toxic substances (Ucar et al. 2019). The erythrocyte numbers, Hb content, and Hct levels decrease in fish exposed to toxic substances may be due to inhibition of erythrocyte synthesis (Özok et al. 2018). A significant decrease in Hb and RBC values may have occurred due to the insufficiency of the hematopoietic system in fish under stress (Shahjahan et al. 2018). The blood RBC, Hb, and HCT levels in this study also support these conditions.

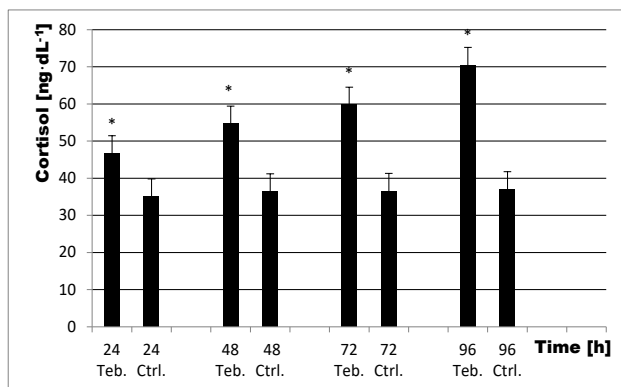
The increase in the activities of ALT, AST, and LDH can be caused by damage to the liver tissue as a result of the toxic effects of pesticides, as well as some diseases (Bhardwaj et al. 2010, Vahedi et al. 2017, Ucar et al. 2019). In this study, statistically significant increases were observed in serum levels of AST, ALT, and LDH at all times of exposure to tebuconazole (24, 48, 72, and 96 h). This may be due to the mixture of these enzymes with the blood as a result of liver damage caused by toxic substances. The study presented is compatible with previous studies on toxic substances. Previous studies have reported that changes in the serum creatinine level are indicators of toxicity and mortality (Muddana et al. 2009, Liew et al. 2019, 2015, Santos Silva et al. 2018, Wan et al. 2019). In one study, the serum creatinine level at 48 h was considered an extremely accurate determinant of pancreatic necrosis (Wan et al. 2019). The presently reported study supports this situation because the creatinine level increased significantly after 48 h. When the kidney or liver is damaged, the urea level may begin to rise (Llopis-Lorente et al. 2019). The urea found in the plasma can be a marker for the diagnosis of nitrogen poisoning under known toxicity conditions (Santos Silva et al. 2018). In the presently reported study, the level of urea increased as time progressed. In addition, statistically significant increases were observed after the 48 h.

In our study, only cortisol level change was investigated from cortisol, lactate, and glucose parameters, which are important stress indicators. It is considered that cortisol is an indicator of the first stress hormones released by the pituitary gland in the hypothalamic–hypervisor axis (Kumar et al. 2018). The structure of cortisol is highly conserved in all vertebrates, including fish (Hontela 2005). The results of the study showed that exposure to toxic substances caused an increase in the level of cortisol similar to that observed in other fish species. The results of the study showed that exposure to toxic substances caused an increase in the level of cortisol similar to that observed in other fish species (Jia et al. 2015). In our study, it was observed that the level of cortisol increased continuously. This shows that the level of cortisol increases in exposure to toxic substances which is consistent with other studies.

This study shows that tebuconazole has a toxic effect on Van fish and that metabolism is affected at different levels over time. In the study, the hematological parameters of Van fish decreased as the application of tebuconazole continued. In the same way, the values of ALT, AST, and LDH increased rapidly in the biochemical parameters and



**Fig. 2.** Biochemical parameters of Van fish exposed to tebuconazole; (A) aspartate aminotransferase; (B) alanine aminotransferase; (C) lactate dehydrogenase; (D) urea; (E) creatine; Teb. = tebuconazole, Ctrl. = control; all values are mean ± SD, \* $P < 0.05$



**Fig. 3.** Serum cortisol levels of Van fish exposed to tebuconazole; Teb. = tebuconazole, Ctrl. = control; all values are mean  $\pm$  SD, \* $P < 0.05$

this may indicate necrosis in the liver. The increase in urea and creatinine levels is compatible with this condition. The increase in the level of cortisol also indicates the severity of stress caused by Van fish in exposure to pesticides.

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