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## Acute and sublethal toxicity of an azole fungicide tebuconazole on ionic regulation and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in a freshwater fish *Cirrhinus mrigala*

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### Abstract

Tebuconazole (TCZ), an azole fungicide has been commonly used in a variety of industry and agriculture products. TCZ is persistent in the environment and thereby posing a risk to aquatic organisms. The present study is aimed to investigate the acute and sublethal toxicity of TCZ on ionic regulation and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in a freshwater fish *Cirrhinus mrigala*. The median lethal concentration of TCZ for 24 and 96 h (with 95% confidence limits) were found to be 9.6 and 7.2 mg L<sup>-1</sup>, respectively. For sublethal toxicity study, 1/10th of 24 h LC<sub>50</sub> value (0.96 mg L<sup>-1</sup>) and 1/5th of 96 h LC<sub>50</sub> value (1.44 mg L<sup>-1</sup>) were taken. In acute treatment, plasma sodium and potassium levels were found to be decreased significantly ( $p < 0.01$ ) in TCZ treated fish whereas a significant increase in plasma chloride level and gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was observed. In sublethal treatment, plasma sodium level was found to be increased up to 7<sup>th</sup> day in both the concentrations. After 7<sup>th</sup> day, plasma sodium level exhibited a significant decrease when compared to control group. Plasma potassium level was found to be decreased in both the concentrations throughout the exposure period when compared to control group ( $p < 0.05$ ). Significant increase in plasma chloride level was observed in fish exposed at 0.96 mg L<sup>-1</sup> for a period of 35 days. However, at 1.44 mg L<sup>-1</sup> concentration, a fluctuation of plasma chloride level was observed. Gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activities were found to be increased throughout the study period in both the concentrations. The findings of the present study indicate that TCZ can change the Na<sup>+</sup>/K<sup>+</sup>-ATPase activity which in turn alter the plasma electrolyte levels. The alterations of these parameters can be used for monitoring of azole fungicides in the aquatic environment.

**Keywords:** Tebuconazole, *Cirrhinus mrigala*, electrolytes, Na<sup>+</sup>K<sup>+</sup>-ATPase

### 1. Introduction

An increase in food demand and agricultural productivity has resulted in a significant increase in the use of pesticides [1-5]. The environmental contamination due to extensive use of pesticides is of great concern in the last years [6-8]. The indiscriminate use of pesticides may enter the aquatic environment and thus pose a potential risk to a range of aquatic organisms, including fish [5, 9, 10]. Among the different types of pesticides, fungicides such as phenylamides, dicarboximides and carboxylic amides are commonly used to control fungal organisms causing crop damage [9].

Triazoles are a class of fungicides largely used in agriculture due to its fungicidal activity [4, 11] and approximately 20% of the triazoles fungicides are used as crop protection products at global market [10, 12]. Tebuconazole ((RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol) is a systemic fungicide, which is effective against various diseases affecting cereals, maize and controls numerous pathogens in various crops including grapevines and vegetable crops [13-16]. As a result, tebuconazole has been detected in many foods [17, 18], in human beings [19] and in aquatic environment [4, 20, 21]. Recently, elevated level of tebuconazole has been detected in the environment with an environmental half-life from 300 to 600 days in soil [22, 23]. Furthermore, due to its aqueous solubility, it has been detected in the aquatic environment from 0.6 to 200 µg/L [24, 25].

In the aquatic environment, tebuconazole has caused adverse effects on aquatic organisms such as disturbance in energy metabolism [26], oxidative stress [27], thyroid endocrine disruption [12], developmental toxicity [23] and alteration in neurochemical parameters [10].

It is classified as toxic to aquatic organisms that may cause long-term adverse effects in the aquatic environment [12, 28, 29]. In addition, tebuconazole has been classified as a possible human carcinogen by United States Environmental Protection Agency. The evaluation of the effects of toxicants on aquatic organisms usually includes chronic toxicity tests as well as acute and sublethal studies [30, 31]. In recent years more attention has been focused on specific and nonspecific biomarkers for monitoring of xenobiotics and its adverse effects on aquatic organisms [32-34]. In this line, the use of biomarkers in fish is widely recommended to acquire the knowledge on the state of the aquatic environment [35].

Maintenance of constant internal ion concentration is essential for the regulation of water influx and ion efflux in aquatic organisms such as fish [36]. Ions such as sodium, potassium and calcium ions play a major role to keep the hyper osmotic properties and normal metabolic functioning in freshwater fishes. Furthermore, these ions are very sensitive to environment stressors [6, 34, 37]. Hence measurement of these ions in the blood of aquatic organisms provides an appropriate biomarker to environmental stressors [38-40] and to monitor the polluted water bodies [37]. Many authors have been reported the alterations in serum/plasma electrolytes in fish exposed to pesticides such as cypermethrin [6], chlorpyrifos [41], lampricide [42], and paraquat dichloride [43]. However, reports on toxic effect of tebuconazole on freshwater fishes are scanty. Assessment of biochemical constituents and enzymes has been explored as potential biomarkers in aquatic organisms under stress conditions. The enzyme adenosine triphosphatase (ATPase) plays an important role in intracellular functions [6, 44].  $\text{Na}^+/\text{K}^+$ -ATPase is a membrane bound enzyme play an important role in the active transport of ions [45, 46] and also play a central role in whole body osmoregulation [47]. These enzymes are located in the cell membrane and their main function is to regulate osmotic pressure [48]. This enzyme eliminates the excess intra and extra-cellular  $\text{Na}^+$  and  $\text{Cl}^-$  in hyperosmotic environment and takes up  $\text{Cl}^-$  in a hypoosmotic environment through chemical gradients [49]. Aquatic contaminants can alter  $\text{Na}^+/\text{K}^+$ -ATPase activity by interacting directly with the enzyme [47]. Inhibition of  $\text{Na}^+/\text{K}^+$ -ATPase has been reported in fish exposed to pesticides such as monocrotophos [50], deltamethrin [6, 51] and chlorantraniliprole [3]. Inhibition and induction of  $\text{Na}^+/\text{K}^+$ -ATPase activity can be used as potential biomarkers for the impacts of pollutants on environmental organisms [52].

India is the leading manufacturer of pesticides in Asia, and ranks twelfth in the world for the use of pesticides [53]. In India, 56.7% of the population is engaged in agriculture and is therefore, exposed to pesticides [54]. Tebuconazole is widely used in Indian agriculture. However, the impact of tebuconazole on freshwater fishes particularly in Indian major carps is scanty. Hence, the aim of this present study is to evaluate the toxicity of tebuconazole on ionic regulation and  $\text{Na}^+/\text{K}^+$ -ATPase activity in a freshwater fish *Cirrhinus mrigala*. The fish *C. mrigala* used in the study is an important candidate species in aquaculture and fisheries in Indian subcontinent and also an edible fish in India. The biological biomarkers may be useful for environmental monitoring programme in order to assess the exposure of environmental pollutants on aquatic ecosystems.

## 2. Research method

### 2.1 Fish maintenance and LC<sub>50</sub> determination

*Cirrhinus mrigala* (average length of 9.5cm and average

weight of 7.5g) were obtained from Tamil Nadu Fisheries Development Corporation Limited; Aliyar Fish Farm, Tamil Nadu, India and they were stocked in a large cement tank containing dechlorinated tap water and acclimatized to test conditions for 20 days. The physicochemical characteristics like water temperature, salinity, dissolved oxygen, pH of tap water which is free from chlorine were analysed [55] and are as follows; temperature ( $28.4 \pm 2$  °C), pH (7.4), dissolved oxygen ( $6.8 \text{ mg L}^{-1}$ ) and total hardness ( $88 \pm 90 \text{ mg L}^{-1}$ , as  $\text{CaCO}_3$ ). Fish were fed ad libitum with rice bran and groundnut oil cake in the form of dough and water was renewed daily.

To find out the median lethal concentration of tebuconazole for 24 and 96 h to the fish *C. mrigala*, circular plastic tubs of 20 litre capacity were taken and different concentrations of tebuconazole were added and ten healthy fish were introduced to each tub. Tubs with 20 liters of water with 10 fish each were also kept simultaneously as control. Three replicates were also maintained. The concentration at which 50 % kill of fish occurred after 24 and 96 h treatment was taken as the median lethal concentration (LC<sub>50</sub>). LC<sub>50</sub> concentrations for 24 h and 96 h were calculated by probit analysis method of Finney [56]. The median lethal concentration of tebuconazole for 24 and 96 h were found to be 9.6 and 7.2  $\text{mg L}^{-1}$ , respectively. One-tenth and one-fifth value of the LC<sub>50</sub> concentration of tebuconazole for 24, 96 h (0.96  $\text{mg L}^{-1}$ , 1.44  $\text{mg L}^{-1}$ ) was taken as the sublethal concentration.

### 2.2 Acute and sublethal toxicity studies

For the purpose of acute toxicity test 60 healthy fingerlings of *C. mrigala* (20 in each batch) were selected and exposed to the tebuconazole ( $7.2 \text{ mg L}^{-1}$ ) toxicant. Controls groups (without toxicant) were also maintained. At the end of 96 h period fish from the control and tebuconazole treated groups were used for the assay. For sublethal toxicity studies, 100 fingerlings of *C. mrigala* were collected from the stock and grouped into two with 50 fish in glass aquarium. Then, 0.96 and 1.44  $\text{mg L}^{-1}$  of tebuconazole was added to glass aquarium. A common control (without toxicant) was also maintained. Three replicates were also maintained for each concentration and control groups. Experiment was conducted for 35 days and no mortality was recorded during the above exposure period. At the end of the 7, 14, 21, 28 and 35<sup>th</sup> days of exposure, fish were randomly selected from experiment and control aquarium for the analysis.

### 2.3 Analysis of plasma electrolytes and gill $\text{Na}^+/\text{K}^+$ -ATPase activity

Blood from control and tebuconazole treated fish was collected from cardiac region and centrifuged for 20 min. at 10,000 rpm and plasma was collected for the estimation of plasma electrolytes. Sodium level was estimated following the method of Maruna [57]. Chloride was estimated by the modified method of Tietz [58] and Young *et al.* [59]. Potassium level was estimated following the method of Young and Chan [60] and Tietz [61]. All the assays were carried out using standard kits and the values were expressed as mmol/L.

For the estimation of gill  $\text{Na}^+/\text{K}^+$ -ATPase, 100 mg of the gill tissue was collected, homogenized with ice-cold 1.0 ml of 0.1 M Tris-HCl buffer (pH 7.4), centrifuged at 1000 rpm at 4°C for 15 min. and the supernatant was used for the estimation of  $\text{Na}^+/\text{K}^+$ -ATPase activity (Shiosaka) [62]. To 0.1 ml of tissue extract (gill) of control and tebuconazole treated groups, 0.3 ml of Tri-HCl buffer (pH7.5) was added followed by 0.1 ml

of 0.02 M ATP, 0.1 ml of 100 mM NaCl and 0.1 ml of KCl solutions. A blank was also kept with 0.1 ml of distilled water. The contents were incubated in water bath at 37 °C for 15 min and the reaction was terminated with 2.00 ml of 5% TCA, kept at 4° for 30 min. and centrifuged for 5 min. at 500 rpm. Then, 1 ml of ammonium molybdate and 0.4 ml of ANSA reagent was added to the supernatant and allowed to stand for 10 min at room temperature. The intensity of the blue color developed was read at 680 nm against reagent. Suitable standards were also run through each batch of assays. The enzyme activity was expressed in terms of micrograms of inorganic phosphorous formed per gram of tissue.

## 2.4 Data analysis

The LC<sub>50</sub> Concentration for 24 and 96 h was calculated by using SPSS software (Ver. 16). The data were analyzed statistically at  $p < 0.05$ . For acute study, students 't' test was used. For sublethal studies, data were expressed as means and analyzed by analysis of variance (ANOVA), followed by a Duncan multiple range test (DMRT).

## 3. Results

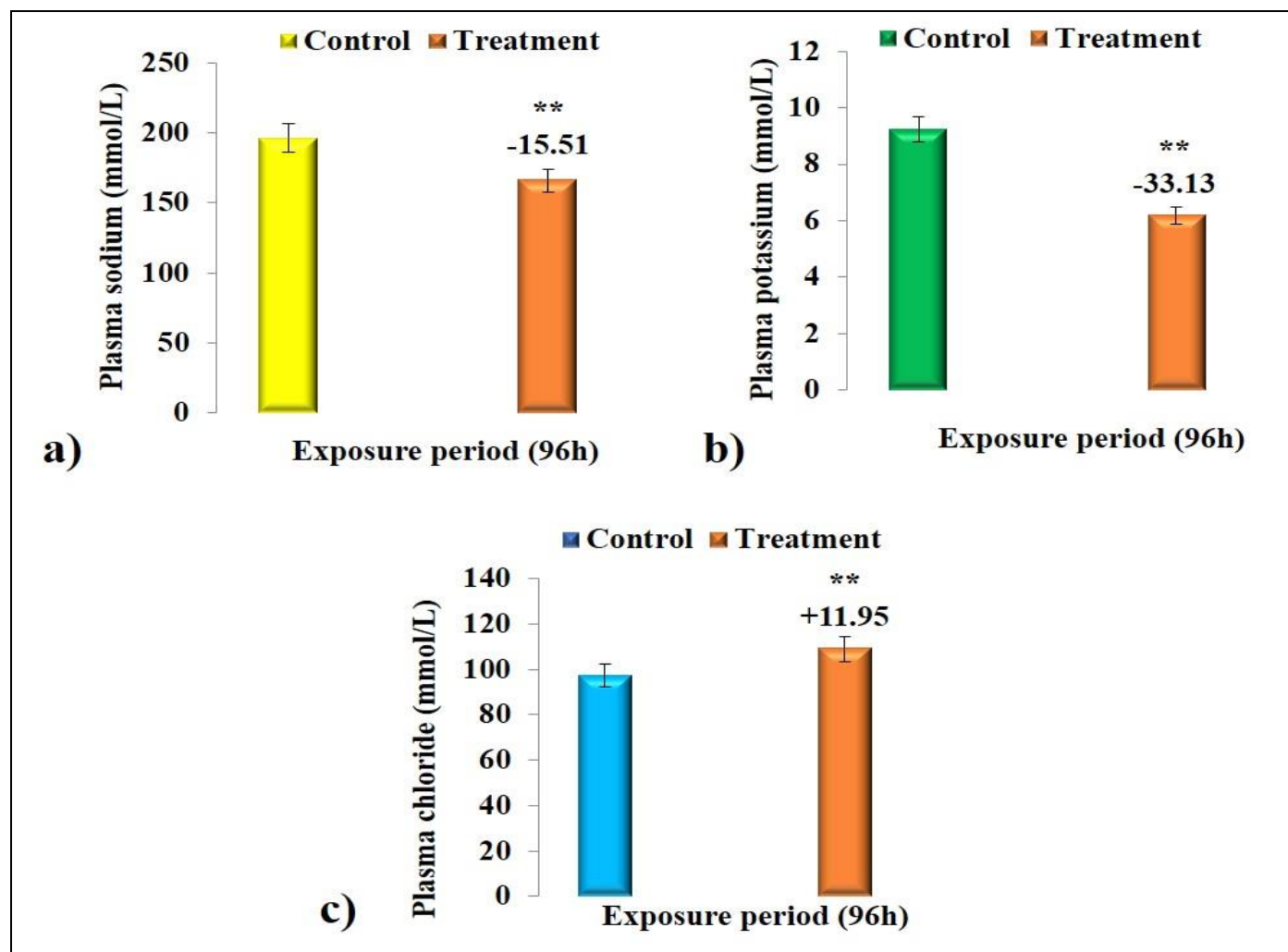
### 3.1. LC<sub>50</sub> Values

The LC<sub>50</sub> values for tebuconazole at 24, and 96 h were 9.6

and 7.2 mg L<sup>-1</sup>, respectively. The observed data clearly indicate that there was a significant decrease in LC<sub>50</sub> values as the exposure period to the fungicide tebuconazole increased. For sublethal toxicity study, 1/10th of 24 h LC<sub>50</sub> value (0.96 mg L<sup>-1</sup>) and 1/5th of 96 h LC<sub>50</sub> value (1.44 mg L<sup>-1</sup>) were taken.

### 3.2. Ionic regulation

Plasma sodium and potassium level of fish *Cirrhinus mrigala* exposed to tebuconazole for 96 h exhibited a significant decrease (Fig. 1a, 1b). However, plasma chloride level on exposure to the fungicide recorded a significant increase at the end of 96 h (Fig. 1c). During sublethal treatment, plasma sodium level was found to be increased at the end of 7<sup>th</sup> day in both the concentrations (Fig. 2). After 7<sup>th</sup> day, plasma sodium level exhibited a significant decrease when compared to control group. Plasma potassium level was found to be decreased in tebuconazole treated (0.96 mg L<sup>-1</sup> and 1.44 mg L<sup>-1</sup>) fish throughout the exposure period when compared to control group ( $p < 0.05$ ) (Fig. 3). Significant increase in plasma chloride level was observed in fish exposed at 0.96 mg L<sup>-1</sup> for a period of 35 days (Fig. 4). However, at 1.44 mg L<sup>-1</sup> concentration, a fluctuation of plasma chloride level was observed.



**Fig 1 (a-c):** Plasma electrolytes (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>) level of *Cirrhinus mrigala* treated with acute concentration (7.2 mg L<sup>-1</sup>) of tebuconazole for 96 h.

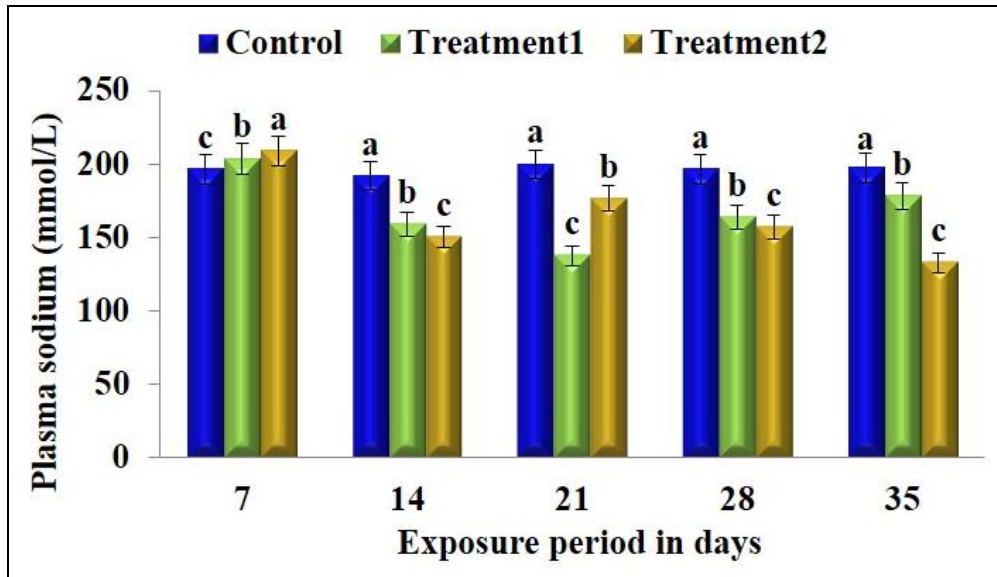


Fig 2: Plasma sodium level of *Cirrhinus mrigala* exposed to sublethal concentrations (0.96 mg L<sup>-1</sup>; Treatment-I, 1.44 mg L<sup>-1</sup>; Treatment-II) of tebuconazole for 35 days.

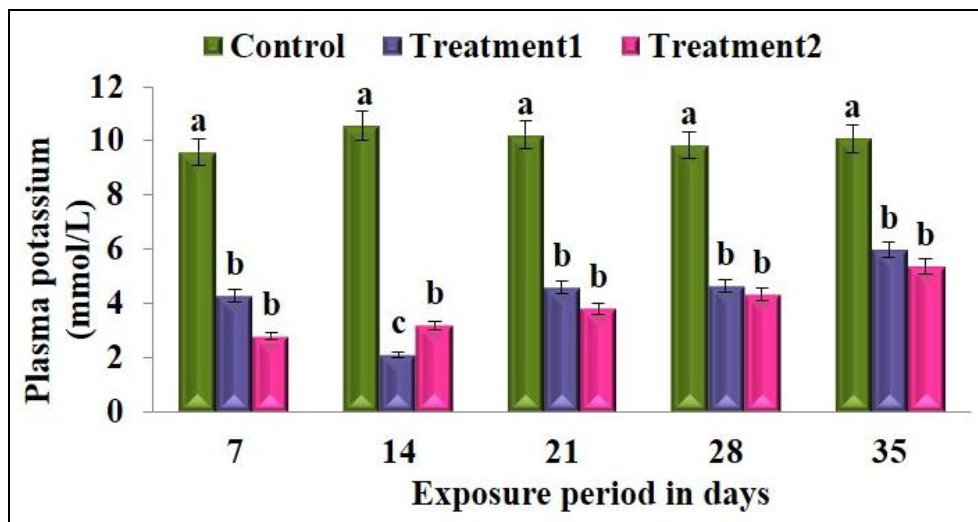


Fig 3: Plasma potassium level of *Cirrhinus mrigala* exposed to sublethal concentrations (0.96 mg L<sup>-1</sup>; Treatment-I, 1.44 mg L<sup>-1</sup>; Treatment-II) of tebuconazole for 35 days.

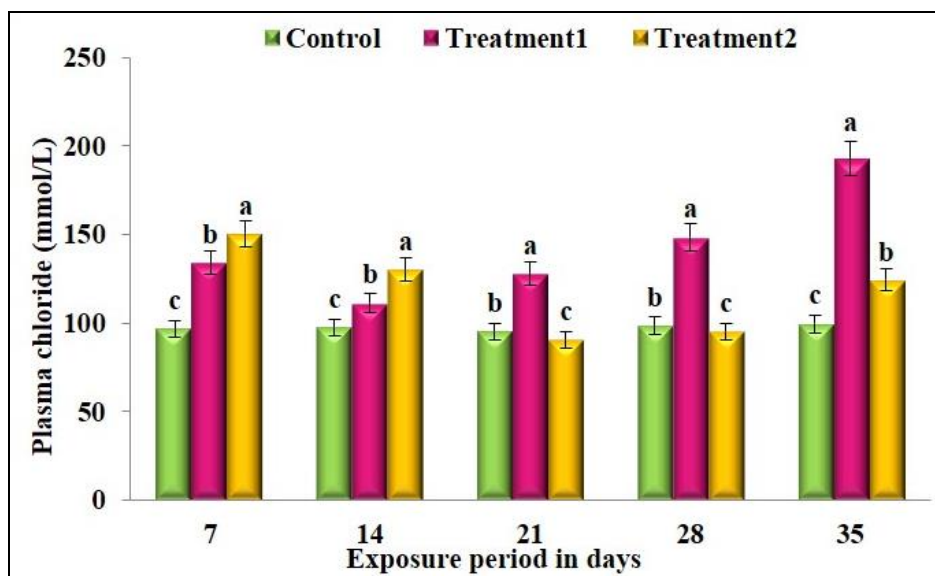


Fig 4: Plasma chloride level of *Cirrhinus mrigala* exposed to sublethal concentrations (0.96 mg L<sup>-1</sup>; Treatment-I, 1.44 mg L<sup>-1</sup>; Treatment-II) of tebuconazole for 35 days.

### 3.3. Na<sup>+</sup>/K<sup>+</sup>-ATPase activity

In acute treatment, gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity registered 138.15% increase over that of the control group (Fig. 5). In sublethal treatment gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was found to

be increased in both the concentrations of tebuconazole treated fish (except at the end of 21<sup>st</sup> day in 1.44 mg L<sup>-1</sup> concentration) when compared with the control groups ( $p < 0.05$ ) (Fig. 6).

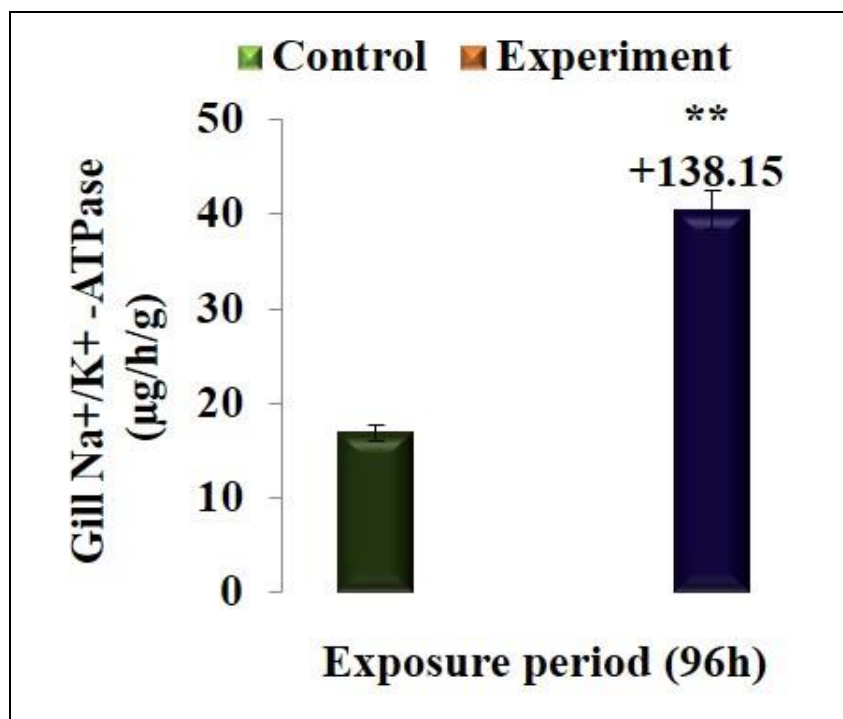


Fig 5: Gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity of *Cirrhinus mrigala* treated with acute concentration (7.2 mg L<sup>-1</sup>) of tebuconazole for 96 h. Bar represent the mean ± SE

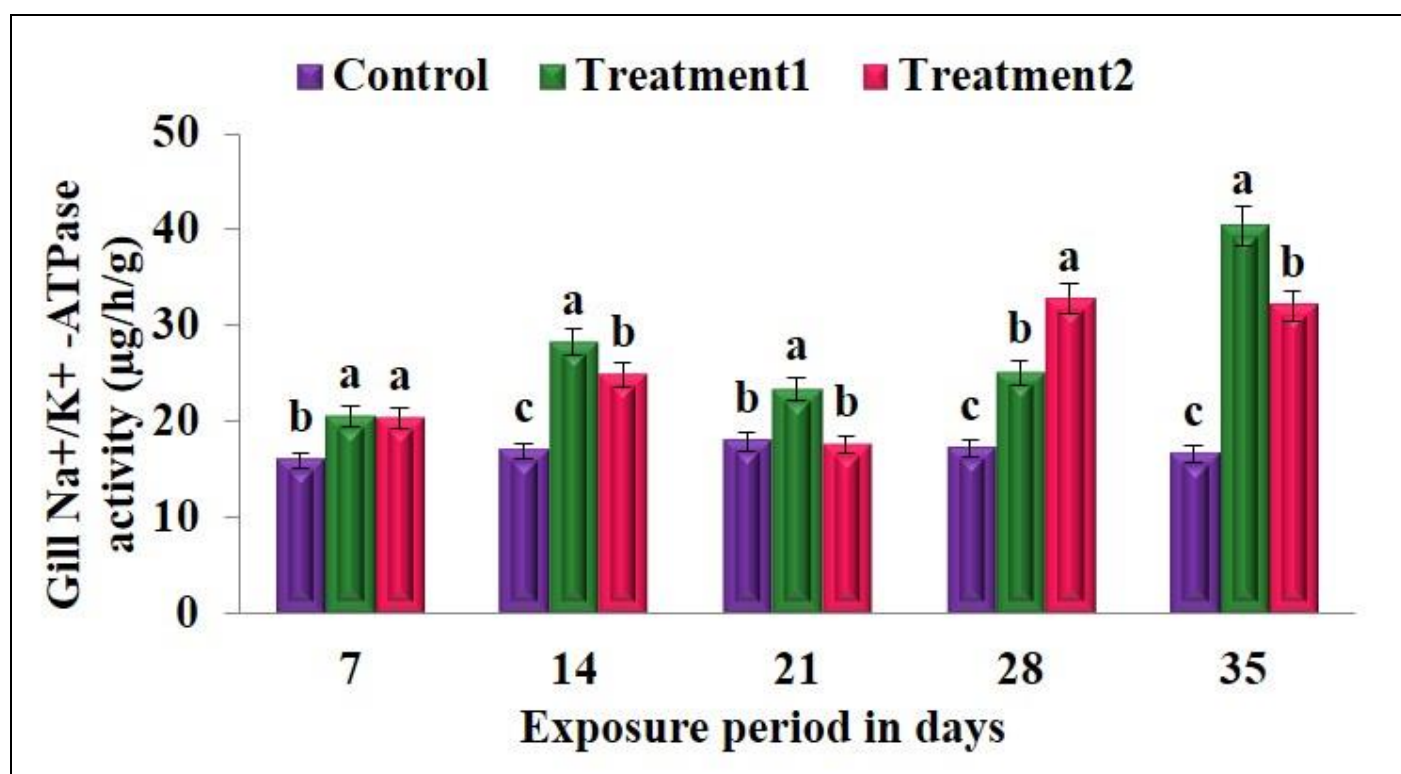


Fig 6: Gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity of *Cirrhinus mrigala* exposed to sublethal concentrations (0.96 mg L<sup>-1</sup>; Treatment-I, 1.44 mg L<sup>-1</sup>; Treatment-II) of tebuconazole for 35 days.

### Discussion

The LC<sub>50</sub> values for tebuconazole at 24, and 96 h to the fish *Cirrhinus mrigala* were found to be 9.6 and 7.2 mg L<sup>-1</sup>, respectively. In the present study the mortality of fish was

increased as the exposure period extended. The decrease in LC<sub>50</sub> value may be due to the resistance of the fish as the exposure period extended [63].

The 24 h LC<sub>50</sub> for the pesticide tebuconazole to the fish *Cyprinus carpio* was 45.6 ppm<sup>[64]</sup>. The 96 h LC<sub>50</sub> to the fish silver catfish *Rhamdia quelen* was determined as 5.3 mg L<sup>-1</sup><sup>[65]</sup>. Similarly the 96 h LC<sub>50</sub> of tebuconazole to the fish *Cyprinus carpio* was found to be 2.37 mg L<sup>-1</sup><sup>[66]</sup>. The LC<sub>50</sub> value for tebuconazole to the fish *C. carpio* was 2.37 mg L<sup>-1</sup>; in *Rhamdia quelen* the LC<sub>50</sub> value was 5.3 mg L<sup>-1</sup> indicating the high resistance of this fish to tebuconazole<sup>[65]</sup>. Sancho *et al.*<sup>[67]</sup> reported that tebuconazole, a triazole fungicide, showed LC<sub>50</sub> values to zebrafish ranging from 23.0 mg L<sup>-1</sup> (24 h) to 19.7 mg L<sup>-1</sup> (48–96 h). The 96 h LC<sub>50</sub> for tebuconazole on zebrafish was found to be 26.8 mg L<sup>-1</sup><sup>[15]</sup>. Similarly, Tomlin<sup>[68]</sup> reported that *D. rerio* is less sensitive to tebuconazole than *O. mykiss* (4.4 mg L<sup>-1</sup>) or (5.7 mg L<sup>-1</sup>) and *Lepomis macrochirus*. According to the LC<sub>50</sub> values obtained from previous results, tebuconazole is also less toxic to *D. rerio* rather than other fungicides as tricyclazole<sup>[26]</sup>. In the present study the changes in the LC<sub>50</sub> value among different species may be due to the physicochemical parameters of the water, size, sex and health condition of the fish.

In the physiology of the aquatic organism, blood being the medium of intercellular and intracellular transport indicates the physiological state of the animal<sup>[6]</sup>. In aquatic organisms such as freshwater fish the water influx and ion efflux to their environments are compensated by passive fluxes of ions by taking from the environment through branchial epithelium<sup>[69-71]</sup>. In general, the inorganic ions are important in carrying out of cellular metabolism<sup>[72]</sup>. Sodium is the chief cation and plays a significant role in osmoregulation and acid base balance of organisms<sup>[73, 74]</sup>. Due to active movement across gill and its concentration in the external medium, sodium ion is widely used to assess the stress condition<sup>[75]</sup>. Likewise, potassium is the main extracellular cation and performs many physiological functions such as acid base balance, osmotic pressure, nerve and muscle functions<sup>[76-79]</sup>. Chloride ion is one of the major anions and plays a major role in osmotic pressure regulation<sup>[77]</sup>. The exchange of chloride ions across the gill epithelium for bicarbonate ions helps to regulate acid-base balance<sup>[79]</sup>. Any imbalance in the levels of these ions in animals will lead to impairment of various physiological activities<sup>[80]</sup>.

In the present study, plasma sodium and potassium ions found to be decreased in tebuconazole treated fish after 96 h exposure. However, plasma chloride level increased. In sublethal treatment plasma sodium (except 7<sup>th</sup> day) found to be decreased. Potassium ion was increased throughout the exposure period (21 days). However, a biphasic response was observed in plasma chloride level. Kabeer Ahamed Sahib *et al.*<sup>[81]</sup> reported a decrease in Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> ions in tissue of fish exposed to malathion. The decline of sodium and chloride ions in atrazine exposed fish *Prochilodus lineatus* may be due to the decline in plasma osmolality<sup>[1]</sup>. The decreased level of sodium and chloride ions in propiconazole treated fish *Labeo rohita* might have caused due to lesser intake of these ions into the body or efflux of the same to the exterior due to the lipophilic nature of the pesticide<sup>[40]</sup>.

In freshwater organisms the gill plays an important role in the regulation of the osmotic and ionic balance<sup>[6, 38]</sup>. Furthermore, the blood of the fish is hypertonic to the external environment which may lead to high rate of diffusive ion loss and osmotic water gain through the gills<sup>[82]</sup>. However, the gill tissue is highly vulnerable to aquatic pollutants. These pollutants may alter the permeability of gill membrane resulting alterations in ion regulation<sup>[39, 83]</sup>. In freshwater

fish, the gills are the principal organs for ion uptake, respiration, acid–base equilibrium and nitrogen excretion<sup>[1]</sup>. They are the first organs to contact and respond to environmental contaminants. In general, aquatic pollutants may cause structural damage in the gill which may result in disturbances in the osmoregulatory function of aquatic organisms. In fish, lipophilic materials such as pesticides are taken up by the gills<sup>[84]</sup>. These lipophilic materials may accumulate in the gill membrane and affect the membrane permeability<sup>[85]</sup>. Further, osmoregulatory failure may also be a reason for decreased levels of major plasma electrolytes.

In the present investigation the decreased level of sodium, potassium and chloride ions in fish treated with tebuconazole fungicide might have resulted from the accumulation of tebuconazole on the gill surface either damage or alter the membrane permeability. In general, tebuconazole are highly lipophilic in nature. The hydrophobic interactions between proteins and lipids may change the protein configurations affecting the transport rates and enzyme activities<sup>[6]</sup>. The alterations in the plasma electrolyte level in *Sarotherodon melanotheron* exposed to pesticides indicate the loss of renal function<sup>[86]</sup>. In addition, mitochondria-rich cells (MRCs) in the gill epithelium play a major role in uptake of major ions and toxicants such as pesticides and metals may affect the MRC density and function<sup>[87, 88]</sup>. The decline in the plasma osmolality may be another possible reason for the observed decrease in plasma electrolyte levels. In this line, Muralidharan<sup>[74]</sup> reported that the decrease in chloride ion in fenthion treated fish might have resulted from leakage of Cl<sup>-</sup> from tissues to surrounding water due to pesticide stress. Inhibition of the Na<sup>+</sup>/K<sup>+</sup> ATPase activity due to pesticide stress may be another possible reason for the decrease in plasma potassium level<sup>[6]</sup>.

In contrast to decrease of plasma electrolyte level, an elevation in the major cation and anion has been observed in *Tilapia mossambicus* exposed to atrazine which may be due to their consequent reduction in the tissues of fish<sup>[89]</sup>. Das and Mukherjee<sup>[90]</sup> reported that the significant increase of Na<sup>+</sup> and Mg<sup>2+</sup> ions in cypermethrin treated fish is possibly due to injuries caused by the pesticide in the kidney, liver and muscle. Elevation in sodium ion was observed in *Cyprinus carpio* chronically exposed to fenthion<sup>[74]</sup>. The elevation of serum sodium and potassium ions in *Cyprinus carpio* exposed to butachlor and oxadiazon may be due to toxic effect of these pesticides on gill structure<sup>[91]</sup>. They also reported that the increase in potassium ion might have resulted from transfer of these ions from other tissues to blood. The significant increase in chloride and potassium ions in diazinon treated fish *Rutilus rutilus* could be due to diazinon-mediated changes in the gill structure and/or elevation of cortisol level due to diazinon toxicity may enhance the gill permeability<sup>[92]</sup>. The elevation of blood electrolyte level in *Heterobranchus bidorsalis* exposed to cypermethrin indicates gill and kidney damage, which may have affected the osmoregulatory process<sup>[93]</sup>. The fluctuations in the electrolyte levels can be compromised with stress due to the toxicant effect on the fish physiology<sup>[94]</sup>.

In the present investigation the significant increase in plasma potassium level in sublethal treatment indicate might their consequent reduction in the tissues due to imbalances in the osmoregulation process. During stress condition the loss of water from the circulation may lead to a rise in major ions<sup>[95]</sup>. In general, aquatic pollutants evoked either increase or decrease in the concentration of major cations and anions as well as chloride and inorganic phosphate anions<sup>[77]</sup>.

Furthermore, alterations in plasma electrolyte level may also be caused by the impact of xenobiotics on ion regulating organs [96] or the endocrine system [97] or active transport processes [98]. The increase in sodium and magnesium ions in pyrethroid treated fish *Rhamdia quelen* may be due to structural damage or lesions in the kidney, liver and muscle which were aggravated due to the stress associated with the subsequent increase in cortisol levels [99, 100]. In general, fungicides may easily penetrate into the blood of fish through gill epithelium and exert toxic effects [16].

In freshwater organisms, the enzyme adenosine triphosphatase (ATPase) is present in the basolateral membrane of gill epithelial cells [101] and plays a major role in regulation of cellular functions [3]. ATPase activity is widely used in toxicological assay as an indicator of cellular activity [102, 103] or indicator of pollution stress in aquatic animals due to its sensitive to pesticides [104]. Na<sup>+</sup>/K<sup>+</sup>-ATPase plays a vital role in regulation of trans membrane transportation of ions and the activity of this enzyme are influenced by aquatic pollutants [50, 105]. This enzyme is highly responsible for the maintenance of the Na<sup>+</sup> and K<sup>+</sup> ionic gradients across the cell membrane [106]. The alterations of this enzyme activity are widely used in pollution monitoring method or a sensitive indicator of environmental toxicity [3].

Environmental toxicants can alter the activity of this enzyme by direct interaction or through the disruption of energy producing metabolic pathways [107]. A change in the ATP content under stress condition may cause damage to the energy supply and ion balance, as well as alteration of ATPase [108]. In the present study, gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was found to be increased in both the concentrations. A significant increase in gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity has been reported in fish exposed to pyrethroids [109]. Likewise, Na<sup>+</sup>/K<sup>+</sup>-ATPase activity was elevated in gill and muscle tissues of *Anguilla anguilla* exposed to thiobencarb [110]. In *Clarias batrachus*, low doses of deltamethrin enhanced gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activities than the higher doses [51].

In the present study the observed elevation in gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity during acute and sublethal treatments may be due to an adaptive mechanism to support the increased ion uptake or a secondary stress response to tebuconazole toxicity. In the present study tebuconazole at acute and sublethal concentration provoked cellular activity in *Cirrhinus mrigala*. Tebuconazole has the potential to induce severe morphological, ultrastructural, and functional alterations in gills of zebrafish at environmentally realistic concentrations [16]. These structural may result in the impairment of the osmoregulatory and respiratory functions of the gills. In contrast to our findings, a significant inhibition of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity has been reported in fish *C. punctatus* exposed to monocrotophos [50], in *A. anguilla* exposed to herbicide thiobencarb [110], in *Cyprinus carpio* exposed to cypermethrin [6] and in zebrafish upon exposure to tebuconazole [16]. Pesticide alters Na<sup>+</sup>/K<sup>+</sup>-ATPase activity by direct interaction with the enzyme and lipophilic metabolites by causing cellular necrosis [111].

## 5. Conclusion

From the findings of the present study we suggested that tebuconazole at acute and sublethal concentrations alter the plasma electrolyte levels and gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity. Due to its high lipophilic nature tebuconazole may accumulate in the gill region and induced structural alterations which in turn lead to osmoregulatory failure. Hence,

alterations of these parameters can be effectively used as biomarkers for monitoring of tebuconazole in the aquatic environment.

## 6. References

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