RESEARCH ARTICLE   **ARAŞTIRMA MAKALESİ**

Acute toxicity of the synthetic pyrethroid alpha-cypermethrin on the tadpoles of variable green toad, *Bufotes variabilis* (Amphibia:Anura)

Sentetik piretroit alfa-sipermetrinin değişken desenli gece kurbağası, *Bufotes variabilis* iribaşlarındaki akut toksisitesi (Amphibia: Anura)

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Abstract: In this study, acute toxic effects of alpha-cypermethrin were investigated in the tadpoles of *Bufotes variabilis* (Pallas, 1769). Tadpoles at Gosner stage 19 were exposed to 0, 0.1, 5 and 10 µgl-1 of alpha-cypermethrin concentrations for 96 hours. Mortality, the changes in some morphological measurements (snout-vent length, body width, tail length, and wet weight), morphological anomalies and behavioral changes were evaluated at the end of the experiment. The 96 hours LC50 value was calculated as 15.62 µgl-1. Such morphological changes as axial anomaly, visceral edema, deformation of the mouth, and tail deformation were observed in 83% and 95% of the tadpoles in the groups treated with 5 and 10 µgl-1 of alpha-cypermethrin. Behavioral changes such as shortening of the swimming distance and immobility were recorded in all tadpoles exposed to alpha-cypermethrin. When all findings were evaluated totally, it was concluded that alpha-cypermethrin had negative effects on the development of the tadpoles of *B. variabilis*

Keywords: Bufotes variabilis, tadpole, pyrethroid, alpha-cypermethrin, anomalies

Öz: Bu çalışmada, *Bufotes variabilis* (Pallas, 1769) iribaşları üzerinde alfa-sipermetrinin akut toksik etkileri araştırıldı. Gosner 19. evredeki iribaşlar, 96 saat süreyle 0; 0,1; 5 ve 10 µgl-1 konsantrasyonlarda alfa-sipermetrine maruz bırakıldı. Çalışma sonunda; mortalite, bazı morfolojik ölçümlerdeki değişiklikler (burun-anüs açıklığı arası uzunluğu, vücut genişliği, kuyruk uzunluğu ve ıslak ağırlık), morfolojik anomaliler ve davranışsal değişiklikler değerlendirildi. 96 saatlik LC50 değeri 15,62 µgl-1 olarak hesaplandı. 5 ve 10 µgl-1 alfa-sipermetrin uygulaması yapılan iribaşların, %83 ve %95'inde aksiyal anomali, viseral ödem, ağız deformasyonu ve kuyruk deformasyonu gibi bazı morfolojik değişiklikler gözlendi. Alfa-sipermetrin uygulaması yapılan tüm iribaşlarda yüzme mesafesinde kısalma ve dengesizlik gibi davranışsal değişiklikler kaydedildi. Tüm bulgular birlikte değerlendirildiğinde, alfa-sipermetrinin, *B. variabilis* iribaşlarının gelişimi üzerine olumsuz etkileri olduğu sonucuna ulaşıldı.

Anahtar kelimeler: Bufotes variabilis, iribaş, piretroit, alfa-sipermetrin, anomaliler

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INTRODUCTION

The use of pesticides in Turkey started with DDT (dichloro-diphenyl-trichloroethane) in the 1960s and increased in time, and its amount has reached 30 thousand tons per year (Durmuşoğlu et al., 2010). In Turkey, 47% of the pesticides used are insecticides (Turabi, 2007). Because these chemicals (organochlorinated and organophosphorous) ultimately remained inadequate in pest control, first pyrethroids and then neonicotinoids were produced as alternatives (Kovganko and Kashkan, 2004). Pyrethroids are structural derivatives of the natural pyrethrins obtained from *Chrysanthemum cinerariaefolium* (Casida, 1980). The pyrethroids with the same effect on the sodium channels in the nervous system are divided into two as Types I and II. However, those in Type II, in which alpha-cypermethrin is also included, keep these channels open for a longer period (Kakko, 2004). Alpha-

cypermethrin is effective when it is taken by contact and orally. As a result of intoxication, conduction of nerve impulses is inhibited and a fast death takes place. Alpha-cypermethrin is used in pest control in animal husbandry (in ectoparasite control), agriculture, and public health. It is one of the four cis isomers of cypermethrin and about 3-4 times more toxic than (IPCS, 1992; WHO, 2006). Due to the effect of alpha-cypermethrin on the sodium channels, its neurotoxic effects on mammals have been investigated in the majority of the studies carried out so far (Luty et al., 1998; Luty et al., 2000; Yu-Tao et al., 2009). Some fish species have been used to investigate toxicological effects of alpha-cypermethrin (Yılmaz et al., 2004; Sarıkaya, 2009). Rather few studies on the toxicity of alpha-cypermethrin in amphibians have been encountered (Greulich and Pflugmacher, 2003; Yu et al., 2013). In addition, the

literature contains no study in which the toxic effects of alpha-cypermethrin on the tadpoles of *B. variabilis* are investigated.

A rapid change in ecological systems is observed as a result of the human activities which have intensified in the recent years. Especially the pesticides used in agricultural activities damage natural habitats and the chemical pollution occurring under the anthropogenic impact is observed. The effects of pesticides on organisms evidently appear in aquatic ecosystems (Ginebrada et al., 2014). When risk assessment is performed, it is seen that amphibians are more threatened than other aquatic vertebrates (Tyler, 1998). The use of shallow and seasonal puddles generally nearby agricultural lands for spawning by amphibians is the most important reason why this risk is greater (Collins and Storfer, 2003). Furthermore, the small bodies, sensitive and permeable skin and limited activity capacities of the amphibians which spend their embryonic and larval periods completely in water also increase their sensitivity to the pollutants in the environment (Wells, 2007). The specimens of *B. variabilis* are distributed in the Anatolian part of Turkey (Stöck et al., 2006). According to the IUCN (2015) criteria, they are in the "Data Deficient" category.

This study aims to determine the morphological and behavioral changes likely to occur after the administration of alpha-cypermethrin to the tadpoles of *B. variabilis* at stage 19 for 96 hours and to calculate the lethal concentration (LC_{10}, LC_{50}, and LC_{90}) values.

MATERIAL AND METHODS

The tadpoles of *B. variabilis* were obtained under laboratory conditions from the adult individuals collected in amplexus from nature. The adult frog samples were collected at Çanakkale, Turkey (35 S 452284 E, 4444377 N). After the fertilisation and the fertilised eggs were divided into four equal parts. The tadpoles were housed in glass aquaria with dimensions of 350×350×250 mm and filled with rested tap water at a depth of 100 mm. *B. variabilis* tadpoles at stage 19 were used in the experiment (Gosner, 1960). Each application group was composed of 20 randomly selected tadpoles. The study was performed with three replications. At the early developmental stages, the tadpoles were fed first on plant-based fish feed and then on boiled spinach and lettuce. Feeding the tadpoles had been continued until 24 hours before the application. The tadpoles were not fed during the application for not to change the environmental conditions (OECD, 2008). During the experiment, temperature (ºC), pH and dissolved oxygen (mgl-1) values were measured by using an Orion ecological kit. The tadpoles were housed under 14:10 photoperiod. The nutrient wastes, feces and dead tadpoles in the aquaria were removed in daily periods. Alpha-cypermethrin (CAS NO. 67375-30-8) HEKTAŞ with 97% purity was used as a toxic substance in the study. For the stock solution, alpha-cypermethrin was dissolved in acetone and prepared in fresh form. The stock solution was diluted to obtain the application concentrations (0.1, 5, and 10 µgl-1). The snout-vent length (mm), tail length (mm), body width (mm) and wet weight (g) values of the tadpoles were measured both before and after the application. The wet weight (WW) values were measured by means of a scale sensitive to 0.0001 g. A digital compass sensitive to 0.01 mm was used to measure snout-vent length (SVL), tail length (TL), and body width (BW). Moreover, the behavioral changes seen such as swimming distance, immobility, balance and reaction to a stimulus in the tadpoles during the application were recorded. For these purpose we selected ten tadpoles from each control and treatment group randomly. After stimulation, the swimming distance of the tadpoles per group (average distance = 5 cm) was recorded with a cronometer. Then these records were analyzed. Following the exposure of alpha-cypermethrin to the tadpoles of *B. variabilis* for 96 hours, the Probit analysis was used to calculate the LC_{10}, LC_{50}, and LC_{90} values. The lethal concentration values were calculated at 95% confidence interval. In addition, a one-way analysis of variance (ANOVA) was used to compare the morphological measurements among the groups.

RESULTS

The mean temperature (ºC), pH and dissolved oxygen (mg/l) values measured after the exposure of the tadpoles of *B. variabilis* to alpha-cypermethrin for 96 hours are presented in Table 1.

Table 1. The mean temperature, pH and dissolved oxygen values of each concentration in the acute toxicity tests of alpha-cypermethrin on *B. variabilis*

Alpha-cypermethrin Concentrations (μgl^{-1})	Temperature (ºC)	Ph	Dissolved Oxygen (mgl^{-1})
0 (Control)	21.2	7.9	8.4
0 (Acetone-control)	21.0	7.7	8.0
0.1	20.8	7.7	8.1
5	21.4	8.0	7.9
10	20.9	7.7	8.0

At the end of the application, the highest mortality rate (40%) was detected in the group treated with 10 µgl-1 of alpha-cypermethrin. As a result of exposure to alpha-cypermethrin for 96 hours, the LC50 in the tadpoles of *B. variabilis* was calculated as 15.62 µgl-1 (Table 2).

Table 2. Lethal concentration values in the tadpoles of *B. variabilis* exposed to alpha-cypermethrin

Species	Gosner Stage	Lethal concentrations (μgl^{-1}) 95% CI		
		96 hours		
		LC_{10}	LC_{50}	LC_{90}
B. variabilis	19	2.74 (1.69-3.75)	15.62 (12.73-17.62)	89.02 (52.25-120.50)

Some morphological measurements (SVL, BW, TL, and WW) at the beginning of the experiment is presented in Figure 1, and at the end of the experiment is presented in Figure 2.
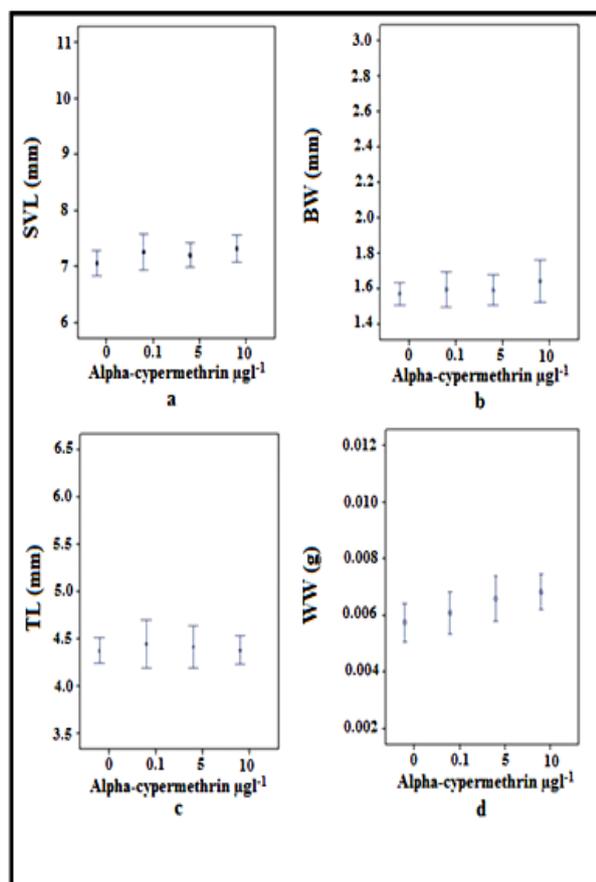
Figure 1. Morphological measurements (At the beginning of the experiment), a. SVL (snout-vent length) (mm), b. BW (body width) (mm), c. TL (tail length) (mm), and d. WW (wet weight) (g)
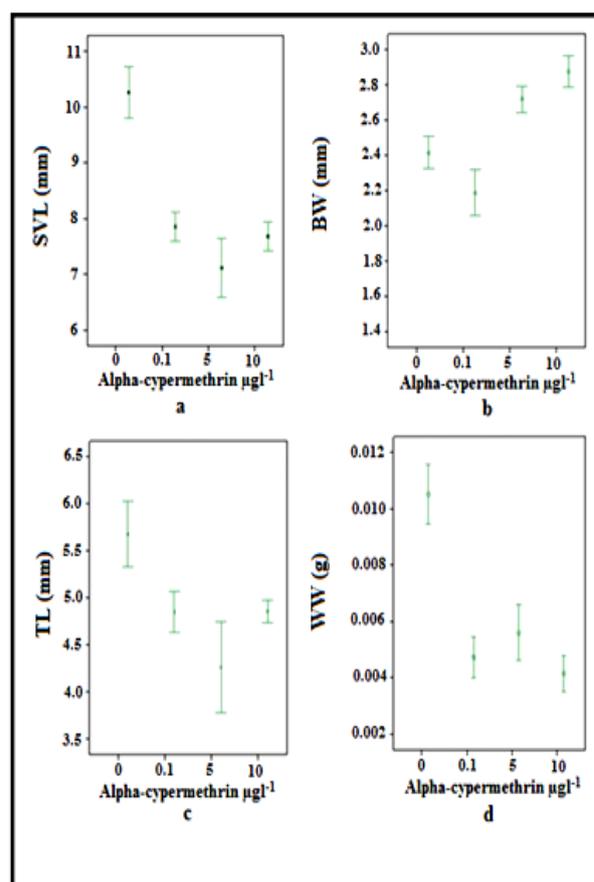

Figure 2. Morphological measurements (At the end of the experiment), a. SVL (snout-vent length) (mm), b. BW (body width) (mm), c. TL (tail length) (mm), and d. WW (wet weight) (g)

At the end of the experiment, SVL, BW, TL, and WW parameters was found to be significantly different between control and exposure groups (P<0.05). SVL, BW, TL, and WW values of the groups and the differences among the groups after the administration of alpha-cypermethrin are presented in Table 3.

The behavioral changes supposed to have resulted from the sublethal effects of alpha-cypermethrin at the end of the study are presented in Table 4.

The behavioral changes concerned were observed to have become evident at the increasing application concentrations

Table 3. The mean values of some morphological measurements

Alpha-cypermethrin concentrations (µgl⁻¹) (n=20)	SVL (mm)	BW (mm)	TL (mm)	WW (g)
0 (control)	10.31±0.22	2.42±0.04	5.69±0.74	0.010±0.002
0 (Acetone-control)	10.35±0.19	2.30±0.05	5.75±0.58	0.012±0.001
0.1	7.90±0.11[a]	2.19±0.05[a]	4.87±0.30[a]	0.004±0.001[a]
5	7.17±0.23[a]	2.72±0.03[b]	4.27±0.67[a]	0.005±0.001[a]
10	7.73±0.11[a]	2.88±0.04[b]	4.87±0.16[a]	0.004±0.001[a]

*Groups labeled with different letters are significantly different (P<0.05)

Table 4. The behavioral changes observed in the tadpoles depending on pesticide exposure.

Alpha-cypermethrin Concentrations (µgl^{-1}) (n=20)	Behavioral Changes
0 (control)	No change was recorded
0 (Acetone-control)	No change was recorded
0.1	Shortening of the swimming distance and immobility
5	Shortening of the swimming distance, immobility, and subsidence
10	Shortening of the swimming distance, immobility, a late reaction to a stimulus, and subsidence

No significant morphological anomaly was encountered in the tadpoles of the control groups. However, obvious morphological anomalies were observed in 60%, 83%, and 95% of the tadpoles treated with 0.1, 5, and 10 µgl-1 of alpha-cypermethrin, respectively. The visceral edema in the bodies of the tadpoles was observed to have become evident at the increasing doses of alpha-cypermethrin (Figure 3).
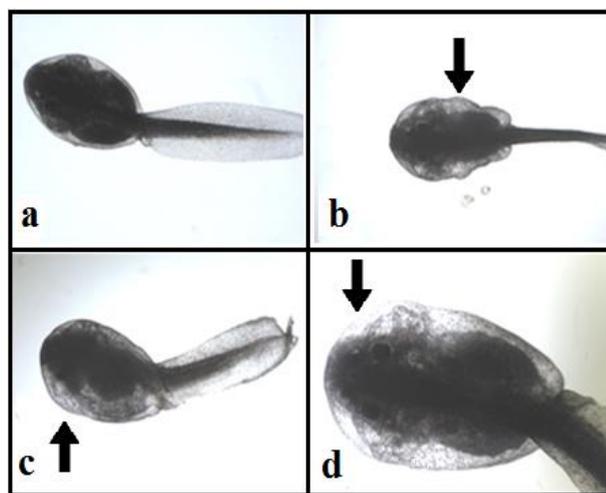

Figure 3. Overall appearances of the tadpoles at the end of the study a. 0 µgl-1 (control), b. 0.1 µgl-1, c. 5 µgl-1, and d. 10 µgl-1 of alpha-cypermethrin (visceral edema is shown with arrows)

It was determined that axial deformations had become evident and the tail had been deformed in the tadpoles treated with 10 µgl-1 of alpha-cypermethrin (Figure 4).
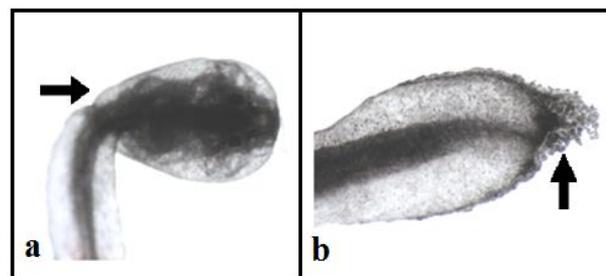

Figure 4. The morphological changes in the tadpoles treated with 10 µgl-1 of alpha-cypermethrin a. Axial anomaly (shown with an arrow), b. Tail deformation (shown with an arrow)

Anomalies were detected at the mouth of the digestive tract (Figure 5) and in the eyes of the tadpoles treated with 5 and 10 µgl-1 of alpha-cypermethrin.
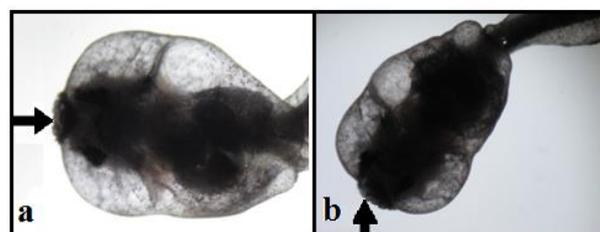

Figure 5. The mouth of the digestive tract in the tadpoles treated with a. 5 µgl-1, and b. 10 µgl-1 of alpha-cypermethrin (shown with an arrow)

DISCUSSION

As amphibians are living organisms which spend their larval periods in water, they can be seriously affected by the chemical pollutants in the environment. Morphological anomalies (visceral edema, axial anomaly, and tail deformation), a delay in the duration of larval development and an increase in the mortality rate can be observed in these living organisms, which are exposed to chemical pollution. Studies in which the effects of Type II pyrethroids on aquatic living organisms were investigated have been encountered in the literature (Bradbury and Coats, 1989; Golow and Godzi, 1994; Polat et al., 2002; Saha and Khaviraj, 2008). Synthetic pyrethroids are reported to be rather toxic for aquatic arthropods and fish (Solomon, et al., 2001). In a study carried out with the larvae of Rana arvalis, exposure of the larvae at different stages to alpha-cypermethrin was reported to have led to negative cases such as a reduction in the breeding success and a delay in the duration of metamorphosis (Greulich and Pflugmacher, 2003). Findings from the literature which were similar to those of the research concerned were determined in our study as well. Additionally, alpha-cypermethrin exposure at increasing concentrations was found to have caused morphological and behavioral changes (shortening of the swimming distance, immobility, a late reaction to a stimulus), and subsidence and an increase in the number of mortalities.

The LC$_{50}$ value calculated after the exposure to alpha-cypermethrin for 96 hours is reported to be 6.9 µgl-1 in the embryos of *Xenopus laevis* (Yu et al., 2013). Following the

exposure to cypermethrin-one of the B Type II pyrethroids for 96 hours, the LC_{50} values were calculated to be 0.4 µgl-1 in Scardininus erythrophthalmus, 1.2 µg/l in Phoxinus phoxinus, and 2.8 µgl-1 in Oncorhynchus mykiss, respectively (Bradbury and Coats, 1989). The value concerned was 6.5 µgl-1 in Rana temporaria and 9 µg/l in toads (Paulov, 1990). The LC_{50} value of alpha-cypermethrin is 5.99 µgl-1 in the Oreochromis niloticus at 96 hours (Sarıkaya, 2009), whereas LC_{50} is 9.43 µgl-1 in *Poecilia reticulata* at 96 hours, 20 µgl-1 in *Barbonymus*

gonionotus, and 4.5 µgl-1 in *Cyprinus carpio carpio*, respectively (Grayson et al., 1990; Yılmaz et al., 2004). In this study, LC_{50} was calculated as 15.62 µgl-1 for the tadpoles of *B. variabilis* following alpha-cypermethrin exposure for 96 hours. The toxic tolerance of the tadpoles of *B. variabilis* to alpha-cypermethrin is thought to be higher than that of the other species mentioned. The fact that this detected value was first calculated for the species concerned is thought to contribute to the literature.

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