Behavioral Differentiation Induced by Insecticide Lambda-Cyhalothrin in Mosquito Fish, *Gambusia affinis*

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A B S T R A C T

Test toxic material, one of most used pesticide, the Lambda cyhalothrin (*LCY*) (TEKVANDO 5EC) belongs to a group of chemicals called synthetic pyrethroids and pesticides and potential toxic pollutant contaminating aquatic ecosystems, was investigated in the present study for behavioral toxicology. Mosquito fish, *Gambusia affinis* (Baird & Girard, 1853) were selected for responses of Lambda cyhalothrin by Using to video-based movement analysis system. Selected 3 different doses 0.1, 0.5 and 0.75 ppm were used analyzed behavioral changes according to control group. The following behaviors were measured: total distance, travelled and average speed for treatment and control groups, maximum and average speed and time moving away from the dose applied point. This suggests that *LCY* contamination has the potential to significantly affect ($p<0.05$) the behavior of Mosquito fish, *G. affinis*.

Keywords: *Gambusia affinis*, behavioral toxicology, lambda cyhalothrin, toxic effect

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Insektisit Lambda Cyhalothrin Maruz Bırakılan Sivrisinek Balığı *Gambusia affinis*'te Davranış Değişimi

Öz: İnsektisitler, sucul ekosistemde insan kaynaklı toksik kirleticilerin başında gelmektedir. Bu çalışmada, bir isektisit olan lambda-cyhalothrin (*LCY*) 'in farklı dozlarının sivrisinek balığında toksik etkiye bağlı davranışsal etkileri aştırılmıştır. Sentetik piretroidler ve böcek ilaçları ve potansiyel toksik kirletici olarak sucul ekosistemlerin kirlenmesine neden başlıca kirleticiler arasındadır. Sivrisinek balığı, *Gambusia affinis* (Baird & Girard, 1853) video tabanlı hareket analizi sistemi kullanarak söz konusu pestisite verilen cevaplar ortaya çıkarılması için kullanmıştır. Seçilen 3 farklı doz 0,1, 0,5 ve 0,75 ppm ve kontrol grubuna göre yapılana analizler davranış değişikliklerin belirlenmesinde kullanılmıştır. Çalışmada araştırılan davranış parametreleri; toplam mesafe, hareket mesafesi ve ortalama hız, uygulanan noktaya göre hareket miktarı hem uygulama hem de kontrol grupları için, maksimum ve ortalama hız ve süredir. Bu çalışma, *LCY*'nin sivrisinek balığı, *G. affinis* davranışlarını önemli ölçüde etkileme potansiyeline ($p<0,05$) sahip olduğunu göstermektedir.

Anahtar kelimeler: *Gambusia affinis*, davranışsal toksikoloji, lambda cyhalothrin, toksik etki

Introduction

Today, pesticides are playing a very important role in modern agriculture. Because they reduce the total crop losses of pesticides is relatively reliable, durable and relatively complete control. Also pests with minimal costs and effort they have, increase crop yields by killing. However Pesticide are one of the strongest polluting the water environment (Murty 1986). Pesticide are considered as potent pollutants of the water environment with undesirable effects on non-target organisms such as fish and water animals (Guner 2009; Hoagland et al. 1993; Khan et al. 2012).

Fish are ideal sentinels and test organisms for behavioral assays of various stress factors and toxic chemicals (such as heavy metals and pesticide) exposure due to their ecological relevance in many natural systems (Fırat et al. 2011; Xin et al. 2015).

Many environmental stress affected fish behavior (Osten et al. 2005). Environmental stress may change depending on fish behavior. Therefore, a change in behavior and toxicological effects of environmental

risk assessment analysis is a useful tool for environmental risk assessment and analysis of toxicological impact (Rao et al. 2006). There are numerous technical and biological factors have made sublethal effects on fish behavior (Kane et al. 2004). It is used more often with extreme behavior. Neurotoxicology effect on the nervous system is used to identify the negative effects of chemical substances and characterized (Evangelista de Duffard and Duffard 1996).

Like other pyrethroids, *LCY* disrupts the nervous system in insects, causing paralysis and death. Pyrethroids insecticides bind to voltage-sensitive sodium channels and modify their gating kinetics, thereby disrupting nerve functions and producing acute neurotoxic effects in both insects and nontarget organisms (Choi and Soderlund 2006).

Sublethal exposure to chemicals often causes changes in swimming behavior, reflecting alterations in sensory and motor systems. Swimming behavior also integrates functions of the nervous system (Brewer et al. 1999). Changing behavior of fish is varied. This kind of change in behavioral patterns, such as color changes, skin, hyperactivity, loss of balance, speed swimming, increased surface activity, opercula activities improved rate, significantly in fish (*Clarias batrachus*) were observed (Kumar et al. 2011).

The present test animal, *Gambusia affinis* (Baird and Girard, 1853) is a member of Poeciliinae subfamily of Poeciliidae (Poeciliids) and inhabits standing to slow-flowing water but are most common in vegetated ponds and lakes and quiet pools of streams (Muranli and Guner 2011). *G. affinis* feeds on zooplankton, small insects and detritus and are used as live food for carnivorous aquarium fishes. *LCY* runoff from agricultural fields killed natural fish populations in great amount (Gu et al. 2007) in masses in various parts of the world also, in addition, recent research has shown that exposure to lambda cyhalothrin, even at sublethal doses, induces behavioral and biochemical changes in fish (Guet al. 2007; Guner 2012; Muranli and Guner 2011).

Since *G. affinis* was a widespread organism and was used for biological control of mosquito (Muranli and Guner 2011) and to demonstrate effects on non-target organisms' behavior changes, this study was undertaken to evaluate behavioral effects of lambda cyhalothrin on *G. affinis* in controlled laboratory environment.

Materials and Methods

Lambda-cyhalothrin (mixture 1:1 of S and R isomers); molecular Formula $C_{23}H_{19}ClF_3NO_3$ (CAS no: 91465-08-6), emulsion concentration is 50 mg/l. Commercial grade lambda cyhalothrin (Tekvando 5EC) (proved by Sefa Tarım A.Ş, Turkey) was used in this study. The 96-h LC_{50} value for mosquito fish was estimated as 1.107 µg/l (Guner 2009).

Fish employed

Mosquito fish (*G. affinis*) were obtained from Güllapoğlu Lake, Edirne Turkey in October 2012 and held in flowing dechlorinated (active carbon filtered) Edirne city tap water. They were transferred to glass aquarium (50x50x100 cm, 100 L) in the aquarium room and maintained at 20 ºC, 14:10 light: dark regime. This arrangement was left undisturbed for seven days, during which animals became accustomed to room conditions. Dechlorinated aquarium water was aerated by air pump for over 24 h. Male and female adult fish were maintained in dechlorinated tap water at 20 °C under a 14L:10D photoperiod, with a diet of commercial fish flakes (Tetra Min Pul). Treatment (3 fish per doses) and control groups consist of 3 fishes. The fish used were adult both females and males between 20 and 30 mm total length (about <1 g). They were transferred to 1 liter polypropylene (r: 170 mm, depth: 80 mm) container for tests. Water parameters during the experiment remained constant as follows: dissolved oxygen 11.83±2.511 mg/l, temperature 20 ±2 °C, pH 8.19± 0.92, and conductivity 632.198±9.98 µhos.

Experimental system hardware and software

The video output from the camera was fed to a computer running an animal movement tracking software (Any-Maze Version 4.69). After 5 min of acclimation, the movement of each fish in aqua was recorded with the video USB camera (SN9C105 model 640 x 480 pixels) for 5 minutes.

Apparatus

LCY applied (doses 0.1, 0.5 and 0.75 ppm) on center point of apparatus (Figure 1) by ependoft multi pipette. And also figure 2 shows dose applied in the apparatus. 3 fish was tested all doses groups (0.1, 0.5 and 0.75 ppm) and control group. When calculating the distance of the animal from this point, the system will use the distance from the part of the animal closest to the point.

Zones

Figure 2 shows the area(s) in the apparatus which correspond to the zones determined. Four different zones were defined. The system considered an animal to be in a particular zone whenever its center point is within this zone (Figure 2).
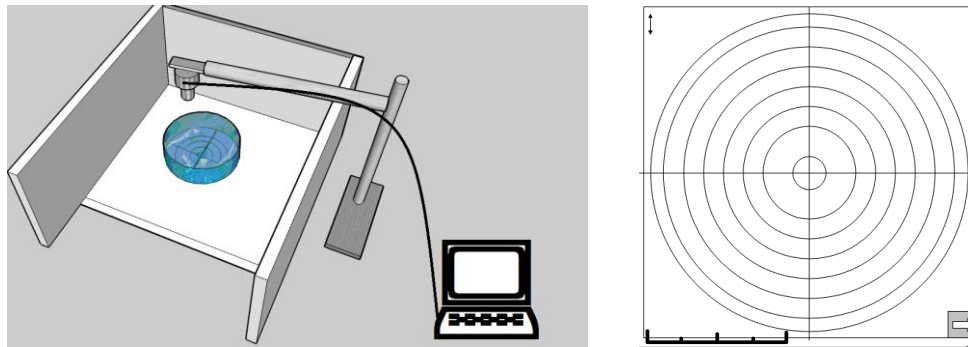
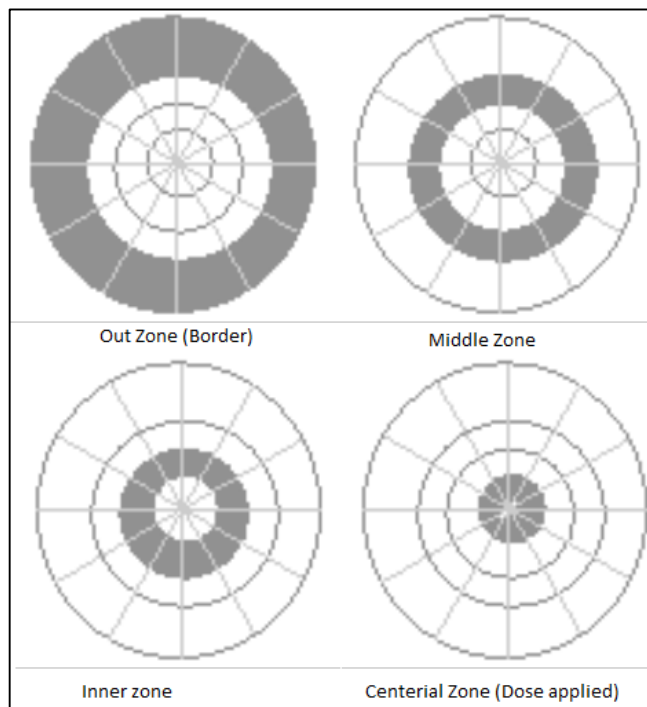**Figure 1.** Toxic test system and apparatus map (Guner 2013).

Figure 2. Positions of the zones in the toxic test apparatus.

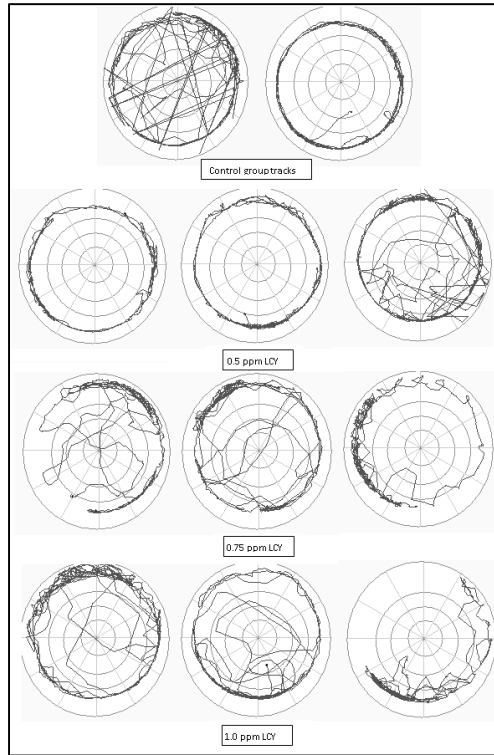## Results

After 5 min of acclimation, the movement of each fish in aqua was recorded and analyzed. Some fish behavior mean and standard deviation of the parameters analyzed are listed below the table (Table 1) and graphs to control exposures and doses (Figure 3, Figure 4, Figure 5 and Figure 6). Traces path taken during the experiments during the experimental period of the fish were shown Figure 3.

**Table 1.** Some fish behavior parameters mean and standard deviation.

Treatment		Control	*LCY* 0.5 ppm	*LCY* 0.75 ppm
	N	10	5	5
Average speed (m/s)		0.023±0.0051	0.0230±0.0115	0.0274±0.0062
Maximum speed (m/s)		0.637±0.1903	0.623±0.0883	0.680±0.1234
Total distance travelled by the animal's head (m)		6.549±1.2488	6.399±4.3405	6.773±2.3667
Average distance from the dose point (m)		0.098±0.0070	0.103±0.0084	0.098±0.0023
Time moving away from the dose point (s)		22.08±6.45	20.16±13.80	26.54±4.23

Figure 3. The track plots of the animals during tests.
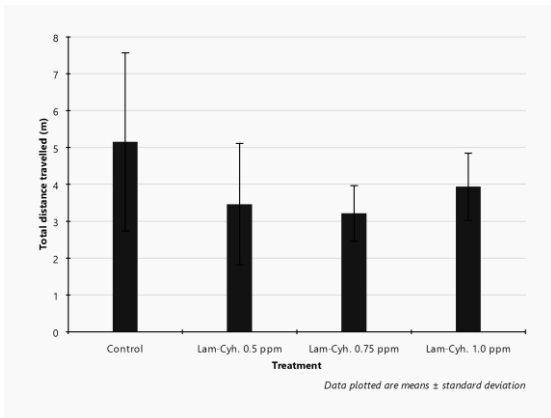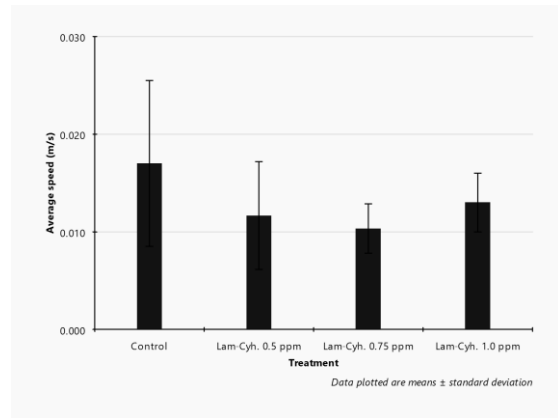
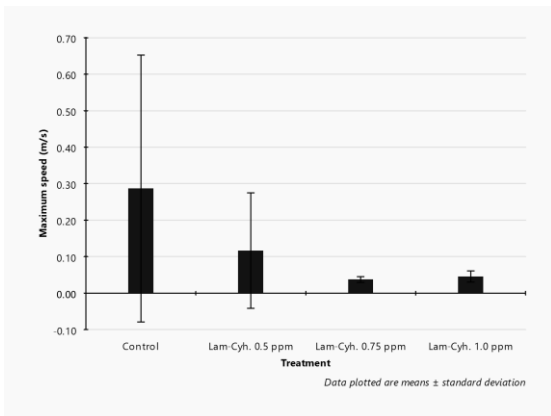(a)   (b)
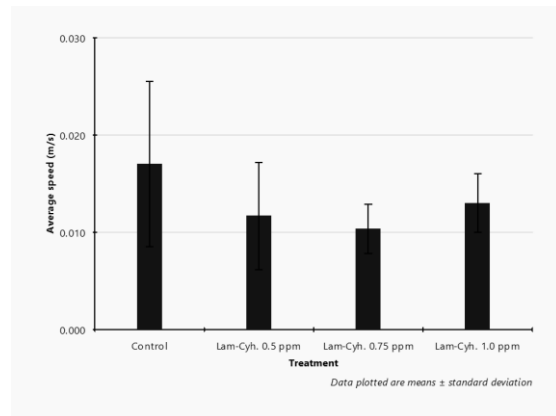
Figure 4. a) Total distance travelled and b) average speed for treatment and control group.

(a)   (b)

Figure 5. a) Maximum and b) average speeds for test groups.

The results obtained from the experiments are summarized below:

• Swimming behavior of the fish was affected with different doses of Lambda-cyhalothrin (0.1, 0.5 and 0.75 ppm).

• The comparison of treatment and dose groups showed that swimming speeds in dose groups were slower (*p*<0.05).

• Depending on the amount of pesticide avoidance behavior of fish was determined that the point of application.

• When the test pesticide was applied in the test box, the fish has chosen the external regions of the boxes possibly for protection (see on figure 3).

Discussion

During the experiments, no mortality was observed in both of control and exposure groups. Although, under laboratory conditions of constant toxicant concentrations, lambda-cyhalothrin was highly toxic for fish (96-hour LC50 value of *LCY* was 1.107 μg/l) and aquatic invertebrates (Caliani et al. 2009; Guner 2009).

Fish are ideal experimental objects for behavioral assays of various stressors and toxic chemical exposure due to their: (I) constant, direct contact with the aquatic environment where chemical exposure occurs over the entire body surface; (II) ecological relevance in many natural systems (III) ease of culture; (IV) ability to come into reproductive readiness and (V) long history of use in behavioral toxicology. Alterations in fish behavior, particularly in non-migratory species, are also important indices for ecosystem assessment (Kaneet al. 2004). This study examined different parameters in fish behavior. In a short time (5 minutes) were observed behavioral changes due to the effect of LYC exposure. Fish behavior changes were observed in a similar study. The fish in toxic media (insecticide, malathion 50% EC) exhibited irregular, random, circular swimming movements, hyperexcitability, loss of equilibrium, and sinking to the bottom (Patil and David 2010).

It was reported that lambda-cyhalothrin were determined at different tissues and organs of fish such as blood, liver, brain, spinal cord, sciatic nerve, vas deferens, anococcygeus and myenteric plexus tissue. And also, plasma, liver, hypothalamus, cerebellum, medulla oblongata, frontal cortex, striatum, hippocampus, midbrain, spinal cord, vas deferens, anococcygeus, myenteric plexus and sciatic nerve concentrations of lambda-cyhalothrin were determined by HPLC (Anadon et al. 2006). Lambda-cyhalothrin in different fish tissues may have lead to behavior change.

LCY directly affects the nervous system. The histological studies indicate that LCT doses induced both axon or myelin degeneration, myelin sheath separation and vacuolization in nerve fibers. The results suggest that LCT had a neurotoxic potential (Askin et al. 2010).

Image analysis can be a useful tool for evaluating the effectiveness of flatfish behavior and welfare work. Accordingly, with the night life of the motor activity of the species in mind that a single population, were recorded by digital video for three nights. Subsequent image analysis was performed by sequential extraction of a frame image. Result show that s digital video-based index can be a highly reliable and accurate method for objectively measuring activity levels in sole with a low consumption of labor and time (Duarte et al. 2009). In this study, video-based digital image analysis hardware and software has been used. The system compares the results with different exposure dose and control group (see Figure 2, Figure 3, Figure 4, Figure 5 and Table 1).

Sublethal exposure to chemicals often causes changes in swimming behavior, reflecting alterations in sensory and motor systems. Swimming behavior also integrates functions of the nervous system. Rarely are the connections between physiology and behavior made. Although behavior is often suggested as a sensitive, early indicator of toxicity, behavioral toxicology has not been used to its full potential because conventional methods of behavioral assessment have relied on manual techniques, which are often time-consuming and difficult to quantify. This has severely limited the application and utility of behavioral procedures. Swimming behavior is particularly amenable to computerized assessment and automated monitoring. Locomotory responses are sensitive to toxicants and can be easily measured (Breweret al. 1999). Locomotory responses in this study, particularly Average speed and Maximum speed parameter shows the difference between the control and exposure groups.

This behavioral exposure, tracking, and analysis system can be applied to quantify differences in behavior associated with exposure to organic pollutants, heavy metals, pesticides, harmful algal bloom toxins and physical effects such as thermal pollution, radiation, vibration (sound). This behavioral exposure, tracking, and analysis automation system is made easily available for different organisms. In addition, the system has some advantages as a low cost, great benefits, speed, and accuracy with a large data set. Behavioral alterations, as identified by this movement tracking hardware and software system, also provides a potential tool for the quantification of differences in reproductive

behaviors, predator-prey interactions, behavioral changes across environmental gradients, and differences in swimming behavior between species. Analysis of behavioral responses to a variety of stimuli can provide quantitative measures of neural and mechanical disruption, reflecting biochemical and physiological alterations (Xie et al. 2015).

In this study, three different doses chosen for *LCY* 0.1, 0.5 and 0.75 ppm were applied to the fish. Exposure depends on the behavior of analyzes conducted and control groups were used to determine the changes. This study depend *LCY* pollution mosquito fish; *G. affinis* shows that it has the potential to significantly influence behavior.

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