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Investigatıon of *In Vitro* Cytotoxic Effects of Flumethrin by Using Brine Shrimp Lethality Assay

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Research Article

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Abstract

Flumethrin is a synthetic insecticide, a type II pyrethroid derivative group derived structurally from naturally occurring pyrethrins in some plants. It is widely used in veterinary medicine, public health and agricultural struggle. It is commonly used in cattle and sheep for the treatment and control of infestations due to biting flies, sucking and stinging lice, ticks, scabies and other external parasites. It is used in the treatment of scabies infections in dogs. It is also frequently used in the control of Varroa disease in beehives. According to the World Health Organization (WHO) classification, flumethrin has class II toxicity (moderately hazardous). The acute oral LC_{50} value of flumethrin was 41-3849 mg/kg in female Wistar mice. Flumethrin is toxic to fish and aquatic animals. It has been reported that flumethrin is also toxic to honey bees, causing behavioral disorders and nerve damage, shortening hive performance and life span.

Brine-shrimp (*Artemia salina*) Letalite assay is one of the toxicity tests used to determine LC_{50} level. *Artemia salina* larvae are widely used in the determination of cytotoxicity of samples which biological activities are investigated. The lethal effect of toxic substances on *Artemia salina* larvae in vivo allows the use of the quick and simple method "Brine Shrimp **Letalite** Test". Information is limited on what adverse effects of acute or low-dose repeated exposure to flumetrine can cause. In this study, the cytotoxic effect of flumethrin was investigated by Brine Shrimp Letalite assay. 9 different concentrations of flumethrin (0.005, 0.01, 0.025, 0.05, 0.1, 0.5, 0.75, 1 and 2.5 µg/L) were used in the experiment. At the end of the study, 50% lethal concentration of flumethrin to *Artemia salina* larvae was determined to be 0.67 µg/L. The LC_{50} values of flumethrin below 100 ppm are found to be within the toxic limits in terms of upper and lower safety limits.

Keywords: Flumethrin; Brine Shrimp Lethality Assay; *Artemia Salina;* Cytotoxic Effect

Introductıon

The use of intensive and uncontrolled drugs in the protection against agricultural pests causes environmental pollution, deterioration of the natural balance and resistance to these compounds in a short time [1]. The rapid increase in the world population brought about the increase in the need for agricultural products. With the spread of intensive and irrigateds agriculture, plant diseases and pests have become epidemics. Although the discovery of pesticides of organic origin has been a glimmer of hope, the remnants of the environment have directed humanity to new searches again [2]. Pyrethroids are still very important drugs because of their safety and wide usage. Many insecticides currently available for use are more toxic in insect than in mammals. In many cases, this is closely related to the component's

selective effect on target structures. Pyrethroids are synthetic insecticides based on the structure of pyrethrins containing six insecticidal components of the natural insecticide pyrethrum. Since the 1970s, pyrethroids have been included in the insecticide group (25%), which is widely used in agricultural and environmental health in the world. These compounds have a spreading effect with nerve palsy. The mechanism of action is similar to DDT [3,4].

Flumethrin is a synthetic insecticide, a type II pyrethroid derivative group derived structurally from naturally occurring pyrethrins in some plants. Synthetic pyrethroids are nervous system poison, which shows toxic effects on sodium channels of target arthropod and affects axons on the peripheral and central nervous system. It is widely used in veterinary medicine, public health and agricultural struggle. It is commonly used in cattle and sheep for the treatment and control of infestations related to biting flies, sucking and insect lice, tick, scabies and other external parasites [5-7]. It is used in the treatment of scabies infections in dogs. It is also frequently used in the control of Varroa disease in beehives. It is effective on both adult and larvae of susceptible parasites. The drug is resistant to destructive enzymatic effects and environmental conditions in susceptible parasites and has a long-lasting and protective activity. Although they are safer than some other pesticide groups, they are known to cause long-term or high-dose poisoning [8-12]. According to the World Health Organization (WHO) classification, flumethrin has toxicity class II (moderately hazardous) [13]. The acute oral LD50 value of flumethrin is 41-3849 mg/kg in female Wistar mice [14]. Flumethrin is toxic to fish and aquatic animals [15]. It has been reported that flumethrin is also poisonous in honey bees, causing behavioral disorders and nerve damage in the bees, shortening hive performance and life span [16]. In our country, pyrethroids containing different active substances are used in various ways under various trade names.

Daha fazla göster
Daha az göster

Artemia is an important test organism used for ecotoxicity tests [17]. The lethal effect of toxic substances on *Artemia* salina larvae allows the use of the quick and simple method "Brine Shrimp Lethality Test [18,19]. This test was developed by Michael, et al. [20] and was adopted as a convenient method for the potential toxic effects of many chemicals or plant extracts [21,22]. The main advantages of using A. salina as a material in toxicity tests are: (1) a rapid method (the time from cyst to larvae is 28-72 hours), (2) the cost is inexpensive, (3) the larvae are homogeneous, (4) can be used year-round without culture [17,23], (5) have knowledge about the biology and ecology, (6) easy to manipulate and maintain in laboratory conditions (7) can be produced easily even in a small environment and well-plate, and (8) highly adapt to various test conditions [17,24]. In addition, the results obtained with the modified form of this test with *Artemia salina* larvae are compared with the results obtained in toxicity tests with mammalian cell cultures [25]. In studies conducted to determine whether there is a correlation between some aquatic toxicity tests using *Artemia salina* and toxicity tests using rodents (mouse or rat), there was generally a good correlation between the results. Similarly, these chemicals have been observed to test their oral acute toxicity potential and give slightly better results than rodent tests compared to acute doses for humans [26,27].

Information is limited about what undesirable effects acute or low-dose repeated exposure to flumetrine can cause. The aim of this study was to investigate the cytotoxic profile of flumethrin. Due to insufficient experimental studies on the subject, the data to be obtained contribute to science.

Materials and Methods

In this study, A. salina cysts of Agua tech brand were used as material. The toxicity test of flumethrin against *A. salina* was carried out according to the protocol proposed by Paredes, et al. [28]. *A. salina* eggs were incubated in a 500 ml glass aquarium containing salty water (3.6 g / 100 mL) with plenty of oxygen. Experiments were performed for 24 hours at 28°C, pH 8-8.5 for 16/8 light / dark period. A 60 W light bulb was used for lighting and hatching. After 48 hours of incubation, the mature Artemia larvae were hatched and collected with the aid of a pastor pipette and placed into test tubes containing 4.5 ml of sea water (10 larvae per tube). 9 different concentrations of flumethrin (0.005, 0.01, 0.025, 0.05, 0.1, 0.5, 0.75, 1 and 2.5 µg / L) were used in the experiments. Serial dilutions of DMSO at decreasing concentrations were performed to determine whether DMSO used as the solvent affected the toxicity results. Saline was used as negative control. After 24 hours of treatment with flumethrin, the dead and alive larvae were counted and recorded under a stereo microscope. The experiments were carried out in three replicates. At the end of the period, mobile and immobile Artemia samples were counted at each concentration of substance. Results were evaluated by SPSS 16.0 statistical package Probit analysis and 95% confidence limits were calculated with LC_{50} values.

Results

As a result of the evaluation of Flumethrin data by Probit analysis; LC_{50} values were not reached at concentrations of 0.005, 0.01, 0.025, 0.05, 0.1 µg/L. However, Flumethrin was found to have a cytotoxic effect close to LC_{50} after 0.5 µg/L. The concentration (LC_{50}) at which Flumethrin kills 50% of A. salina larvae was determined to be 0.67 µg/L. DMSO used as

solvent showed no toxic effects on A. salina larvae at specified concentrations. Figure 1 shows data based on exposure of Artemia salina to flumethrin in different concentrations.

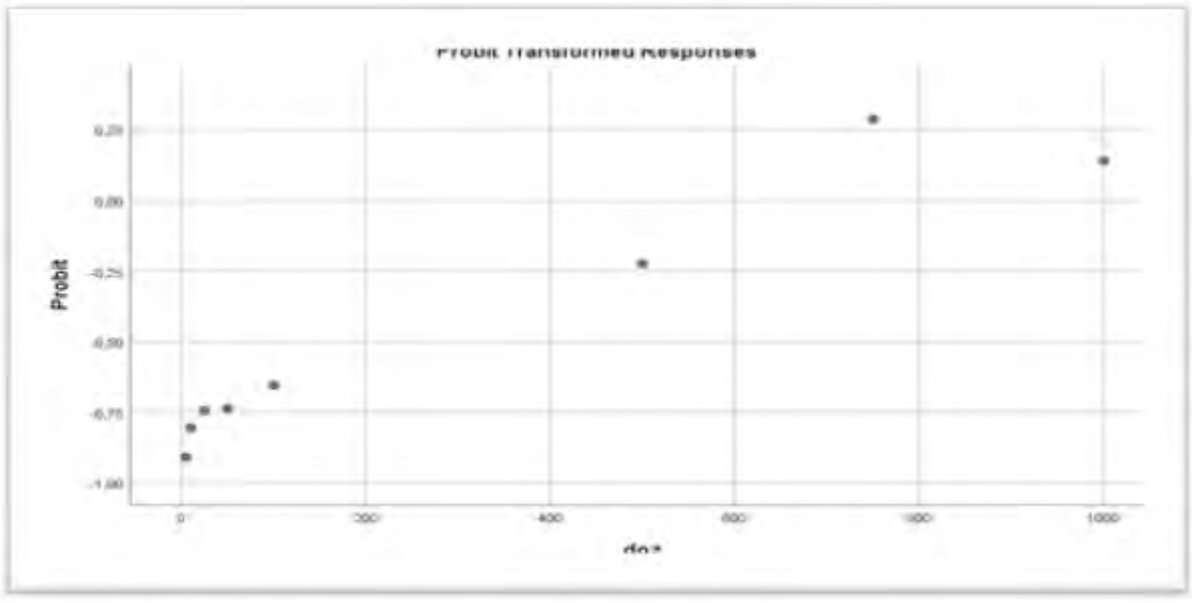

Figure 1: Data base on exposure of Artemia salina to flumethrin in different concentrations.

Discussion

Pyrethroids are a widely used insecticide class applied inagricultural, turf grass, commercial, and residential settings to control a broad range of insect pests. Application ofsynthetic pyrethroids is increasing as they are used to replace other insecticides, such as organo-phosphates and carbamates, in residential pest control andin mosquito abatement programs [29,30]. Pyrethroids may enter aquatic ecosystems via spraydrift, run-off, and wastewater treatment plant effluent [29]. Pyrethroids offer low toxicity to human applicators and non-target mammals and birds, they are highly toxic to invertebrates and also fishes [31]. Saltwater arthropods are more susceptible to pyrethroidchemicals than freshwater arthropods [32].

In the Brine Shrimp Letalite Test, the concentration of chemicals leading to the death of half of the A. salina larvae (LC_{50}) was accepted as the active concentration. Raw chemicals with LC_{50} values below 100 ppm are highly toxic; Chemicals with LC_{50} values between 100 ppm and 500 ppm are considered to be toxic, LC_{50} values between 500 ppm and 1000 ppm are considered weak toxic and chemicals with LC_{50} values above 1000 ppm are considered non-toxic. According to the data obtained in this study, According to the data obtained in this study, flumethrin was found to be within toxic limits in terms of LC_{50} values below 100 ppm.

There isn't enough study examining the toxicity of pyretroids on artemia salina. Liu, et al. [33] examined the acute toxicity of preterm pesticide to Artemia and showed that the sensitivity of Artemia to different pyrethroids was very different. LC_{50} concentrations against Artemia were found to be 4.68, 14.82, 18.12, 38.21,> 100,> 100 and> 100 mg / L for permethrin, chlorenthrin, imiprothrin, tetramethrin, bifenthrin, lambadacyhalothrin, deltamethrin, respectively. Therefore, the use of more toxic permethrin should be avoided in saline environments containing Artemia and suggested the use of less toxic pesticides such as bifentrin, beta-cyhalothrin and deltamethrin.

Nafisa, et al. [34-36] evaluated the acute toxicity (LC_{50}) of pyrethroid pesticide (fenvalerate) on Artemia (brine shrimp). The value the LC_{50} of fenvalerate was found to be 0.18 ppm for Artemia.
>100和>100 mg·L $^{-1}$ 。

Conclusıon

The results of this study showed that the cytotoxic effect on *A. salina* larvae exposed to flumethrin in vitro in 24 hours was observed after 0.5 µg/L concentration and no cytotoxic effect was found in other concentration ranges (0.005, 0.01, 0.025, 0.05, 0.1 µg/L). LC_{50} values of flumethrin below 100 ppm have been found to be within very toxic limits. Flumethrin appear to be highly lethal at very low

concentration. High sensitivity is alarming as it may have implications on natural resources.

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