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Research Article **Araştırma Makalesi**

Determination of Letal Concentrations (LC_{50}) of Cyfluthrın, Dimethoate Insecticides on *Gammarus pulex* (L., 1758)

Cyfluthrin, Dimethoate Böcek İlaçlarının *Gammarus pulex* (L., 1758) Üzerindeki Letal Konsantrasyonlarının (LC_{50}) Belirlenmesi

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Abstract: In this study, *Gammarus pulex* (L., 1758) individuals obtained from Tunceli Munzur Stream were exposed to different concentrations of two insecticides containing cyfluthrin and dimethoate active ingredients, and LC_{50} values were determined from acute toxicity tests. During the study, parameters such as water temperature, pH, and dissolved oxygen were constantly controlled to ensure that they did not change. In this study, experiments were carried out by placing 0.5 liters of water in 1-liter glass aquariums. 10 *G. pulex* individuals were used for each concentration group. To determine the LC_{50} value, the mobility of the living things was observed and recorded in 24-hour periods. *G. pulex*, which lost its motility, was removed from the aquarium and excluded from the study. The study was carried out in 3 replications and the LC_{50} value for dimethoate was determined as 170.51 ± 8.15 µg/l, while the LC_{50} value for cyfluthrin was determined as 0.800 ± 0.12 µg/l.

Keywords

- *Gammarus pulex*
- Cyfluthrin
- Dimethoate
- Pesticide
- Acute toxicity

Özet: Bu çalışmada Tunceli Munzur Akarsuyundan elde edilen *Gammarus pulex* (L., 1758) bireyleri cyfluthrin ve dimethoate etken maddelerini içeren iki insektisitin farklı konsantrasyonlarına maruz bırakılarak, akut toksisite testlerinden LC_{50} değerleri belirlenmiştir. Çalışma boyunca su sıcaklığı, pH, çözünmüş oksijen gibi parametreleri sürekli kontrol edilerek değişmemesi sağlanmıştır. Yapılan bu çalışmada deneyler 1 litrelik cam akvaryumlarda 0,5 litre su konularak gerçekleştirilmiştir. Her bir konsantrasyon grubu için 10 adet *G. pulex* bireyi kullanılmıştır. LC_{50} değerinin belirlenmesi için 24 saatlik periyotlarla canlıların hareketlilik durumları gözlemlenerek kaydedilmiştir. Hareketliliğini kaybetmiş *G. pulex*'ler akvaryum içerisinden alınıp çalışma dışı bırakılmıştır. Çalışma 3 tekrarlı yürütülmüş olup, dimethoate için LC_{50} değeri 170,51 ± 8,15 µg/l tespit edilirken, cyfluthrin için LC_{50} değeri 0,800 ± 0,12 ng/l olarak olarak belirlenmiştir.

Anahtar kelimeler

- *Gammarus pulex*
- Cyfluthrin
- Dimethoate
- Pestisit
- Akut toksisite

1. INTRODUCTION

All kinds of damage done by people to the environment they live in by unnatural means create environmental pollution. This environmental pollution, on the other hand, directly or indirectly affects all living things, especially humans, at least once in different stages of their lives from birth to death. Although it is in the hands of people to reduce or increase these effects, they are constantly increasing due to economic concerns. Pesticides used to establish large industrial facilities, to release waste into the environment, to mix with water, and to get more efficiency in agriculture are just a few examples of the factors that cause environmental pollution.

The effect of pollutants on the organism may differ according to abiotic factors (temperature, oxygen, pH, light, etc.) and factors such as height, weight, and sex of the vivid.

The transmission routes of pesticides to the aquatic environment are generally by mixing with wind rainwater, drainage waters, surface flows, and irrigation waters, spraying against aquatic organisms or plants living in water channels, mixing with sewage and sewage waters in residential areas, and the discharge of pesticide manufacturing residues. In addition, as a result of direct applications to water (for example, in mosquito control), pesticides are retained by aquatic plants or bottom mud (Atamanalp and Yanık 2001).

Pesticide residues in water accumulate in dissolved form or the form of transformation products, sediments, benthic invertebrates, aquatic plants, plankton, aquatic organisms, and fish (Sarıgül, 2007).

As a result of unconscious and misuse of pesticides, negative effects on nature and human life occur. Unconscious and overused pesticides affect non-target organisms by being carried into rivers, lakes, and seas by winds, rainwater, and groundwater.

These negativities seen in aquatic creatures do not have the same effect on every living thing; It affects different events of living things such as nutrition, circulation, and reproduction and creates a stress effect on living things.

The organisms most affected by the pollution of the aquatic environment are the organisms living in that aquatic environment. These organisms living in polluted environments will either move away from this environment, adapt to this environment, or perish by dying. For this reason, living things choose the most suitable habitats for themselves. Such organisms are an indicator of their habitat, an indicator, or a biomarker. *Gammarus,* which is a clean water indicator, is one of the creatures that have both economic and aquatic indicator features (Demirsoy, 1998).

Many scientific studies have been conducted to examine the effects of pesticides on various aquatic organisms. In one of these studies, Felten et al (2007) investigated the effect of cadmium on physiological and behavioral responses of *Gammarus pulex*. Adam et al. (2009) applied propiconazole, tebuconazoline, 3-iodo-2-propynyl butyl carbamate (IPBC, fungicide), and cypermethrin to *G. pulex* as a single or a mixture to determine the toxicity of insecticides and fungicides used as wood preservatives. Vellinger et al (2013) studied the single and combined effects of cadmium and arsenate in *Gammarus.* Uğurlu et al. (2015) investigated the toxicological effects of thiamethoxane on *Gammarus kischineffensis*. Demirci (2018) evaluated the acute toxic effects of imidacloprid and acetamiprit on *G. kischineffensis.* In this study, it was aimed to determine the acute toxicity of dimethoate and cyfluthrin pesticides on *G. pulex,* which is a clean water indicator.

2. MATERIAL and METHOD

2.1. Material

2.1. Collection of *G. pulex*

The *G. pulex* individuals used in the study were collected from the side branches of Munzur Stream in Tunceli province with the help of a bottom scoop, the air was reinforced, and they were brought to the Munzur University Fisheries Faculty research laboratory with tanks (Figure 1).

G. pulex individuals were placed in 40x20x20 cm aquariums and adapted to laboratory conditions for 4 weeks. Airflow was provided with air motors for the oxygen requirement of the aquariums.

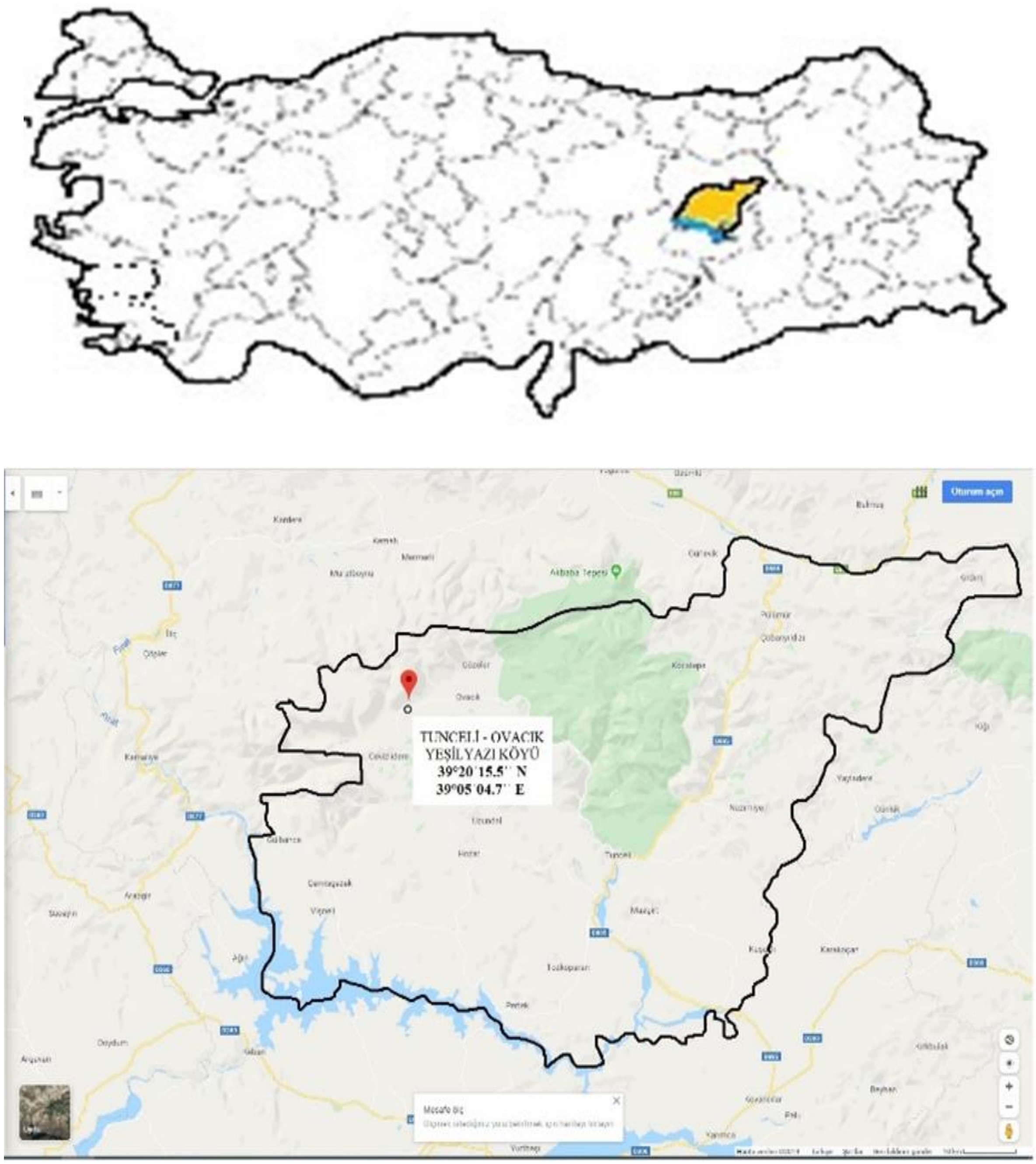

Figure 1. The area where the experimental organism (*G.pulex*) was collected

2.2 Adaptation of *G. pulex* to the Laboratory

For the adaptation of *G. pulex* to laboratory conditions, suitable environments for natural habitats have been prepared. For this purpose, sediment taken from the natural habitat of *G. pulex* was washed with pure water and placed in stock aquariums. Again, water brought from the natural environment of *G. pulex* was added to them. The stock aquariums were supplemented with oxygen using an air motor. In ambient lighting, a photoperiod of 12 hours dark and 12 hours light was used. With the thermostatic air conditioner, the ambient temperature where the aquariums are left is fixed at 18 ^{0}C. After the adaptation medium was prepared, *G. pulexs* collected from the Munzur Stream were placed in stock aquariums. *G. pulex* is left to adapt to laboratory conditions. 70% of the water in the stock aquariums was renewed once a week. For the feeding of *G. pulex*, shrub willow tree leaves were collected and left to rot.

2.3. Range Experiment

Before the study, a range determination study was performed to determine dimethoate and cyfluthrin concentrations. In the spacing tests, 10 *G. pulex* individuals were placed in each of the aquariums.

Range determination experiments were performed for each pesticide. After the range determination experiments, the concentration ranges of dimethoate and cyfluthrin pesticides were determined to be applied in the LC_{50} experiments.

During the first application, abnormal movements such as fast and reverse swimming were observed in living things. On the 3rd and 4th days of the application, limitation of movement was observed.

2.4. Experiment Design

Glass aquariums with a volume of 1 liter were used for the experiments in the study and 10 *G. pulex* were placed in each aquarium. According to the results of range determination experiments, the trial design for dimethoate pesticide (K (0,0), D1 (25µg/l), D2 (50 µg/l), D3 (100µg/l), D4 (200 µg/l) and D5 (400 µg/l)) concentrations were determined (Table 1).

For Cyfluthrin pesticide, the trial design (K (0.0), C1 (0.2 µg/l), C2 (0.4 µg/l), C3 (0.8 µg/l) and C4 (1.6 ng) /l) concentrations were determined (Table 2).

LC_{50} experiments were carried out statically over a period of 96 hours. In each experiment, dead individuals were counted and removed from the aquarium in 24-hour periods. No feeding was done to the animals during the experiment. In the experimental application, water taken from the environment where *G. pulex* was collected was used. All experimental studies were applied in 3 replications.

Table 1. Experiment design and concentrations determined for dimethoate

Recurrences	Groups (µg/l dimethoate)					
	K	**D1**	**D2**	**D3**	**D4**	**D5**
I	0,0	25,0	50,0	100,0	200,0	400,0
II	0,0	25,0	50,0	100,0	200,0	400,0
III	0,0	25,0	50,0	100,0	200,0	400,0

Table 2. Experiment design and concentrations determined for Cyfluthrin

Recurrences	Groups (µg/l cyfluthrin)				
	K	**C1**	**C2**	**C3**	**C4**
I	0,0	0,2	0,4	0,8	1,6
II	0,0	0,2	0,4	0,8	1,6
III	0,0	0,2	0,4	0,8	1,6

2.5. Determining the LC_{50} Value

To determine the LC_{50} value, experimental groups in which different dimethoate and cyfluthrin concentrations were applied separately were formed together with the control group. For each group, 10 live were used. 96 hours after administration of dimethoate and cyfluthrin, viable and deceased individuals were counted. LC_{50} value was determined by using SPSS 24.0 statistical package program Probit Analysis.

In all experimental stages of the study, 0.5 liters of dechlorinated water taken from the natural environment of the creatures were used in 1-liter glass aquariums.

For each concentration, 10 *G. pulex* were placed in these aquariums. To determine the LC_{50} value, the mobility of the living things was observed and recorded in 24-hour periods.

G. pulex, which lost its mobility, was removed from the aquarium and excluded from the study.

3. RESULTS

3.1. Acute Toxicity (LC_{50}) Values

3.1.1. LC_{50} Value of Dimethoate Insecticide

In the study, the LC_{50} value of dimethoate insecticide on *G. pulex* was determined as 3 repetitions and the average values are given in Table 3. The mean LC_{50} value of the dimethoate pesticide was found to be 170.51±8.15 µg/l, the lower band level average value was 119.89±7.9 µg/l, and the upper band level average value was 228.53±9.0 µg/l. (Table 3).

Table 3. LC_{50} values of *G. pulex* exposed to dimethoate insecticide

	LC_{50} Value		
	LC_{50} (µg/l)	**Lower Level (µg/l)**	**High level (µg/l)**
I. Recurrence	178.69	127.80	237.59
II. Recurrence	162.37	111.96	219.51
III. Recurrence	170.50	119.90	228.48
Average	170.51 ± 8.15	119.89 ± 7.9	228.53 ± 9.0

Table 4. Mortality rates after 96 hours in *G. pulex* exposed to dimethoate insecticide

	Dimethoate concentrations applied to the experimental groups (µg/l)	**Number of *G. pulex* used in trial**	**Number of *G. pulex* died during 96 Hours**	**% Death**
I. Recurrence	0 (K)	10	0	0
	25 (D1)	10	1	10
	50 (D2)	10	2	20
	100 (D3)	10	4	40
	200 (D4)	10	6	60
	400 (D5)	10	9	90
II. Recurrence	0 (K)	10	0	0
	25 (D1)	10	1	10
	50 (D2)	10	2	20
	100 (D3)	10	4	40
	200 (D4)	10	8	80
	400 (D5)	10	9	90
III. Recurrence	0 (K)	10	0	0
	25 (D1)	10	1	10
	50 (D2)	10	2	20
	100 (D3)	10	4	40
	200 (D4)	10	7	70
	400 (D5)	10	9	90

In the study, mortality rates of all groups (K, D1, D2, D3, D4 and D5) were determined in *G. pulex* individuals exposed to dimethoate insecticide within 96 hours. (Table 4).

G. pulex individuals have a 10% death rate in all 3 repetitions in the D1 group, a 20% mortality rate in each relapse for the D2 group, a 40% mortality rate in each replication for the D3 group, and 60% in the I. Replica for the D4 group. 80% in recurrence and III. It was determined that the highest mortality rate was 70% in the recurrence and 90% in the D5 group (Table 4).

3.1.2. LC_{50} Value of Cyfluthrin Insecticide

In this study, the LC_{50} value of cyfluthrin on *G. pulex* was determined in 3 repetitions and the average values are given in Table 5. The mean LC_{50} value of the Cyfluthrin insecticide was found to be 0.800 ± 0.12 µg/l, the lower band level average value was 0.570 ± 0.12 µg/l, and the upper band level average value was 1.059 ± 0.13 µg/l (Table 5).

Table 5. LC_{50} values of *G. pulex* exposed to Cyfluthrin insecticide

	LC_{50} Value		
	LC_{50} (µg/l)	**Lower Level (µg/l)**	**High Level (µg/l)**
I. Recurrence	0,714	0,486	0,965
II. Recurrence	0,752	0,525	1,005
III. Recurrence	0,935	0,700	1,207
Average	0,800±0,12	0,570±0,12	1,059±0,13

In the study, mortality rates of all groups (K, C1, C2, C3 and C4) were determined within 96 hours of *G. pulex* individuals exposed to cyfluthrin insecticide. (Table6). *G. pulex* individuals C1 group death at a rate of 10% in each 3 replication, C2 group 30% in I. Replica, II. 40% in recurrence, III. 20 mortality rate in recurrence, 80% in I. recurrence in C3 group, II. 60% in recurrence and III. 50% mortality rate in recurrence, I., and II. for the C4 group. It was determined that the mortality rate was 90% in recurrences and 80% in III recurrences (Table 6).

Table 6. Mortality rates after 96 hours in *G. pulex* exposed to Cyfluthrin insecticide

Cyfluthrin concentrations (µg/l) applied to the experimental groups		**Number of *G. pulex* used in trial**	**Number of *G. pulex* died during 96 Hours**	**% Death**
I. Recurrence	0 (K)	10	0	0
	0,2 (C1)	10	1	10
	0,4 (C2)	10	3	30
	0,8 (C3)	10	8	80
	1,6 (C4)	10	9	90
II. Recurrence	0 (K)	10	0	0
	0,2 (C1)	10	1	10
	0,4 (C2)	10	4	40
	0,8 (C3)	10	6	60
	1,6 (C4)	10	9	90
III. Recurrence	0 (K)	10	0	0
	0,2 (C1)	10	1	10
	0,4 (C2)	10	2	20
	0,8 (C3)	10	5	50
	1,6 (C4)	10	8	80

4. DISCUSSION

In recent years, it has been revealed in scientific studies that pesticides or insecticides used to increase productivity in agriculture and animal husbandry harm both terrestrial and aquatic organisms, which are out of their intended use and are not targeted, even in the smallest amounts. For this purpose, the acute toxicity of dimethoate and cyfluthrin insecticides used as pesticides in agriculture *on G. pulex* was investigated.

As a result of the research, for dimethoate; The mean LC_{50} value was 170.51±8.15 µg/l, the lower band level mean value was 119.89±7.9 µg/l, and the upper band level average value was 228.53±9.0 µg/l, while cyfluthrin For LC_{50} mean value 0.800 ± 0.12 µg/l, lower band level mean value 0.570 ± 0.12 µg/l, upper band level mean value 1.059 ± 0.13 µg/l.

As can be seen from the determined values, it was observed that both insecticides were effective on *G. pulex*. It was determined that even very low concentrations of Cyfluthrin insecticide were effective on *G. pulex*.

Many researchers have examined the effects of pesticides on other non-target aquatic organisms. Among these researchers, Köprücü and Aydın (2004) determined that deltamethrin pesticide; Aydın and

Köprücü (2005), diazinon pesticide; Aydin et al. (2005) determined the acute toxicity of cypermethrin pesticide on the embryo and larvae of *Cyprinus carpio*. In another study, Ural and Şimşek (2006) investigated the acute toxicity of dichlorvos pesticide on Silurus glanis offspring. Serdar (2021) calculated the LC_{50} value of Cyfluthrin pesticide in zebra mussels as 553.22 ± 27.3 µg/L in his study. In a different study, Yüksel et al. (2020) calculated the LC*50* value of the *G. pulex* they exposed to malathion pesticide as 1.03 ± 0.07 mg/L in their study. It has been determined that the findings obtained as a result of these studies on various fish species and the findings of this study show a complete similarity in terms of dying or adversely affecting the life of the living things even at low concentrations.

Güner (2020), acute toxicity of cyhalofop butyl (LC_{50}) study on *Gambusia holbrooki*. The acute toxicity (LC_{50}) of this herb (Chillinger 200 EC 200, cyhalofob butyl), which is used extensively in the Thrace region, including Cyhalofob butyl, has been investigated. The acute toxicity (LC_{50} value) of this herbicide was investigated in common mosquito fish (*Gambusia holbrooki*) in the Thrace Region. The Lethal Dose 50 experiment was performed in 3 replicates in static test runs (water temperature 27.70 ±0.56 □C, water pH 8.88± 0.37, and conductivity 718.25±21.113 µhos). The experimental results obtained for the Chillinger 200 ec during the experiments were evaluated with the Trimmed Spearman-Karber method.

Serdar et al (2019), evaluation of the acute toxic effect of cadmium on *Gammarus pulex* (freshwater amphipoda) at different temperatures. As a result of the study, it was aimed to determine the change of LC_{50} values of Cd in *G. pulex* at 10, 14, and 18° C. 96 hours were determined at different temperatures of 10, 14, and 18 °C. LC 50 values were obtained by probit analysis; 51.79 ± 1.2 µg L -1 for 10°C, 47.67 ± 0.6 µg L -1 for 14°C, and 33.93 ± 0.6 µg L -1 for 18°C. It was determined that the values decreased depending on the temperature increase of LC*50*.

Serdar (2019), investigated the effect of dimethoate pesticides on some biochemical biomarkers in *Gammarus pulex*. The acute toxicity value (LC*50*) of dimethoate pesticide in *G. pulex* was determined. Superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx), and catalase (CAT) activities and malondialdehyde (MDA), glutathione (GSH) levels of *G. pulex* organism exposed to sublethal concentrations were investigated. Analyzed by ELISA for 24 and 96 hours. In conclusion, this study demonstrated the abilities of dimethoate pesticides to induce oxidative stress. The results showed MDA, GSH levels, SOD, CAT, GPx, and GST activities. *G.pulex* has stated that it can be used as an effective biomarker.

Cold and Forbes (2004) investigated the effects of short-term pyrethroid pesticide applications on the survival and proliferation of *G. pulex*. As a result, they determined that the exposure concentrations of the widely used pesticide esfenvalerate significantly affect the survival and reproduction of *G. pulex*.

Lukancic et al (2009), Physiological responses of two freshwater shellfish, *Asellus aquaticus L*. and *G. fossarum*, after exposure to two pesticides were measured. Both species responded to short-term exposure with elevated Respiratory (R) levels or lower levels of Electron Transfer System (ETS) activity. In both test types, it showed an effect for 1 hour at a concentration of 10 mg/L. Laboratory tests of both test types prove that *G. fossil* is more sensitive to short-term pesticide exposure than *A. aquaticus*. In this study, *G. pulex*'ler individuals were affected as a result of short-term exposure to pesticides. Studies show similarity in this aspect.

5. CONCLUSION

Pesticides used in agriculture contaminate the waters, which are vital for life, by mixing with the waters in various ways. The pesticides that fish and other aquatic organisms take into their bodies affect the natural balance by making a negative impact on human health and the food pyramid as a result of the consumption of fish by humans, birds, and other creatures that consume fish.

To minimize these damages, pesticide use should be controlled and farmers should be educated by authorized persons. Less toxic pesticides should be preferred, access to water sources should be prevented while spraying, pesticide containers used should not be washed in water sources, and used tools and containers should be destroyed and not released into the environment. Samples should be taken frequently from water sources and evaluations should be made.

More detailed research and scientific studies should be carried out on the damage caused by pesticides to nature and humans. Considering the importance of water for humans and other living things, it is necessary to investigate the effects and harms in these areas and to take necessary precautions.

In addition, it is obvious that new studies are needed to investigate the biological and environmentalist alternative removal methods of the determined harmful effect, to reduce the toxic effect on living things, and according to the conditions of the developing world.

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CONFLICT OF INTEREST

I declare that there are no financial interests or personal relationships that could affect this work

AUTHOR CONTRIBUTION

A.A; performing the experiment, article writing, R.A; inspection, O.S; data analysis

ETHICAL STATEMENTS

Ethical approval is not required as the creatures used in this study are invertebrates.

DATA AVAILABILITY STATEMENT

Data used in this study are available from the corresponding author upon reasonable request.

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