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The Acute Effect of Malathion on Acetylcholinesterase Activity in *Gammarus pulex* (Freshwater Amphipoda)

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Research Article

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Abstract

In this study was investigated the acute (24 and 48 h) effect of sublethal concentrations of malathion, organophosphates insecticide, on the AChE activity of *Gammarus pulex*.

For this purpose, two sublethal concentrations of malathion (0.1 and 0.2 mg l^{-1}) were applied to *G. pulex* for 24 and 48 h. After 24 and 48 hours of malathion exposure, *G. pulex* samples were taken. In the samples taken, AChE enzyme activity and protein level were determined and specific AChE enzyme activity was calculated.

Sublethal concentrations of malathion caused time-dependent increased inhibition of AChE activity in *G. pulex*. In the group exposed to 0.1 mg l^{-1} malathion concentration, inhibition of AChE was detected as 50% at 24 h and 74% at 48 h, compared to control. Similarly, in the group exposed to 0.2 mg l^{-1} malathion concentration, 60% and 68% AChE inhibition at 24 and 48 h were observed, respectively, compared to the control.

As a result, acute exposure of *G. pulex* to malathion for 24 and 48 h caused in the high rate inhibition of the AChE activity. Further, the results show that up to 74% AChE inhibition levels in *G.pulex* do not cause acute death, and measurement of AChE activity in *G. pulex* will be the biomarker of acute malathion exposure and effects.

Keywords: Acetylcholinesterase activity, *Gammarus pulex*, malathion, organophosphates

Malathionun *Gammarus pulex* (Tatlı Su Amphipodu)'te Asetilkolinesteraz Aktivitesi Üzerine Akut Etkisi

Özet

Bu çalışmada, organofosfat insektisit malathionun subletal konsantrasyonlarının *Gammarus pulex*'in AChE aktivitesi üzerine akut (24 ve 48 saat) etkisi incelenmiştir. Bu amaçla, *G. pulex*'e malathionun iki farklı sublethal konsantrasyonu (0.1 ve 0.2 mg l^{-1}) 24 ve 48 saat boyunca uygulandı. 24 ve 48 saat malathion maruziyetinin sonunda *G. pulex* örnekleri alındı. Alınan örneklerde AChE enzim aktivitesi ve protein düzeyi belirlenerek, spesifik AChE enzim aktivitesi hesaplandı.

Malathionunun sublethal konsantrasyonları *G. pulex*'te AChE aktivitesininin zamana bağlı artan inhibisyonuna neden oldu. 0.1 mg l^{-1} malathion konsantrasyonuna maruz kalan grupta, AChE'nin inhibisyonu, kontrol ile karşılaştırıldığında, 24 saatte %50 ve 48 saatte %74 olarak tespit edildi. Benzer şekilde, 0.2 mg l^{-1} malathion konsantrasyonuna maruz kalan grupta da kontrol ile karşılaştırıldığında sırasıyla 24 ve 48 saatte % 60 ve % 68 AChE inhibisyonu gözlendi.

Sonuç olarak, *G. pulex*'in malathiona 24 ve 48 saat boyunca maruz kalması AChE aktivitesinin yüksek oranda inhibe edilmesine neden olmuştur. Ayrıca, sonuçlar *G.pulex*'teki % 74'e kadar AChE inhibisyon seviyelerinin akut ölüme neden olmadığını ve *G. pulex*'teki AChE aktivitesinin ölçümünün akut malathion maruziyetinin ve etkilerinin biyobelirteci olacağını göstermektedir.

Anahtar kelimeler: Asetilkolinesteraz aktivitesi, *Gammarus pulex*, malathion, organofosfatlar.

INTRODUCTION

Over the centuries pesticides have been used in agriculture to combat unwanted pests and to improve food production by controlling disease vectors (Hamed, 2015). However, the residues formed as a result of the random use of pesticides in agricultural areas cause water, soil, and air pollution, disrupting the balance of the ecological system (Oruç and Üner, 1999). Organophosphate (OP) compounds, which are among the common pesticides, are widely used in medicine, industry, and agriculture (Fahmy, 2012; Hamed, 2015). AChE activity is a highly specific biomarker for measuring

insecticide pollution with OP (Kirby et al., 2000; De laTorre et al., 2002; Feng et al., 2008). Organophosphates (OPs) all have an acute toxic effect and are designed to inhibit AChE and thus blocking cholinergic nerve transmission (Anderson and Lydy, 2002). The inhibition occurs by inhibiting the natural catabolism of the neurotransmitter as a result of the covalent linkage between the active site of the enzyme and the OP insecticide (Barr and Needham, 2002). Inhibition of this enzyme on the organism results in the accumulation of ACh in cholinergic synapses and the continuous and excessive stimulation of nerve and muscle fibres, thus causing paralysis and ultimate death in the organism (Kirby et al., 2000; Forget et al., 2003; Pala and Serdar, 2018). Malathion, a widely used OP pesticide, is a common contaminant in aquatic ecosystems. High malathion concentrations such as 0.6 mg l^{-1} in natural wetlands were detected (Giri et al., 2012; Mişe Yonar, 2013). OP insecticides may inadvertently contaminate the surface water as a result of the deliberate application or unintentional air spray, basin drainage, or accidental spill and drift during the control of biting insects. Aquatic invertebrates in surface waters may in this way be exposed to insecticide levels which range from sublethal to acute lethal (Day and Scott, 1990).

Gammarids are commonly used organisms for risk assessment of freshwater quality criteria (Rinderhagen et al., 2000; Serdar et al., 2018). Generally, they are found in the source parts of rivers and are an important food source for fish, birds, and amphibians (MacNeil et al., 2002), and leaf litter plays an important role in the breakdown process (Forrow and Maltby, 2000). As a result, they are active throughout the entire food chain. Therefore, these species are often preferred as test organisms because they can be sampled by collecting throughout the year and they can be easily identified, controlled, and maintained in the laboratory conditions (Xuereb et al., 2009; Uğurlu et al., 2015). Moreover, the limitations of mammalian studies in the field of environmental toxicology have led to an increasing interest of many amphipod invertebrates, such as *Gammarus* (Demirci, 2018). The Gammarides are reported to be among the most susceptible species to cholinesterase (ChE) compounds (Kuhn and Streit, 1994; Xuereb et al., 2007; Xuereb et al., 2009). Because, in the study as a test organism, we selected *Gammarus pulex*, one of the most common freshwater benthic macro-invertebrates in Europe.

Investigations have been performed on Gammarus species for the toxic effects of various pesticides (Cold and Forbes, 2004; Demirci, 2018; Serdar, 2019; Pala, 2019). But, there is no report found, for the acute effect of malathion on AChE of *G. pulex*.

This study aimed to determine the acute effect of sublethal malathion concentrations on the AChE activity in *G. pulex* as a non-target aquatic organism, to contribute toxicity database of malathion in Turkey.

MATERIALS and METHODS

Chemicals

The insecticide used in this study was the commercial-grade formulation of malathion 65% Malathion EM (active ingredient malathion, 650 g l^{-1}) was provided by Koruma companies-Agrochemicals, İzmit, Turkey. 1 ml of malathion was taken and dissolved in distilled water thus a 1 l stock solution was prepared. Sublethal concentrations of calculated malathion were taken from this stock solution. The chemicals used in all other stages of the study were purchased from Sigma–Aldrich Chemical (St Louis, MO, USA).

Test organisms

G. pulex, a freshwater amphipod, was used as the test organism in this study organisms were collected with dip nets from the river's origins the Munzur River in Tunceli, Turkey. The region where *G. pulex* individuals collect was close to the river source, lacking from observable industrial and domestic disturbances and the agricultural activity was negligible level. Also, Gueltekin et al. (2017) in the water quality study based on their biotic index on the Munzur River reported that the river is an indicator of high ecological water quality and maybe the reference river. Therefore, non-contaminated populations were used as test organisms. The organisms were placed in plastic bottles filled with river water, with airflow, and transferred to the laboratory. In the region of the river where *G. pulex* individuals were collected, river water was taken together with the living organisms, for use during the adaptation period. Organisms were placed in aquariums with this water. And throughout the adaptation, 30% of the aquarium water was replaced once in three days chlorine-free water. And organisms were adapted to the stock aquariums at 18 ± 0.5° C and 12/12h light/dark photoperiod

surroundings for one month under controlled climatic conditions and were fed with rotten willow leaves during adaptation.

Experimental setup and exposure

The sublethal concentrations of malathion (0.1 and 0.2 mg l^{-1}; approximately 1/16 and 1/8 of LC_{50} value) were selected based on a study by Şahinkuşu (2018), who reported that the LC_{50} value (96 h) of malathion for *G. pulex* was 1.58 ± 0.56 mg l^{-1}. Acute toxicity tests were performed based on the modified OECD (OECD 202, 2004) standard procedure. In the experiment, 1 L glass aquarium with a ventilated and containing 0.5 L water were used. Pesticide stock solution was prepared by dissolving in distilled water. Three different experimental groups were formed, one being the control group. Organisms were added to the aquariums as 10 individuals in each group. *G. pulex* individuals used in the experiment were selected from similar size (W= 0.0348 ± 0.0012 g and L= 11.17 ± 0.73 mm) and healthy individuals. Organisms were exposed to 0.1 mg l^{-1} and 0.2 mg l^{-1} concentrations of malathion for 48 h and the experiment was performed in triplicate for all concentrations. The organisms didn't feed in the experiment and other experimental conditions (light, temperature, etc.) were maintained as mentioned above.

AChE activity assay

Samples were taken from all the aquariums at the 24 and 48 hours of study to determine the AChE activity. The taken samples were weighed and homogenized with a Teflon-glass homogenizer in a pH 7.4 0.05 M sodium-phosphate buffer, containing 0.25 M sucrose, to obtain 1/10 whole homogenate. It was then centrifuged at 3500 rpm for 15 minutes at 4 °C. Supernatants obtained after centrifugation was used as an enzyme source.

AChE enzyme activity of *G. pulex* determined according to the method of Elman et al. (1961). For the AChE activity determine, 200 µl of supernatant was taken in a cuvette and 2.55 ml 0.1 M phosphate buffer (pH 8.0), 50 µl etopropazine, 100 µl of DTNB [5,5-dithio-bis(2-nitrobenzoic acid)], were add and it was incubated at 30℃ for 5 minutes then 20 µl of ACh iodide were added to it. The change in absorbance at 412 nm was measured for 5 min at on UV–Vis Spectrophotometer.

Protein concentrations were measured according to Lowry et al. (1951) and were used to calculate specific enzyme activity

Statistical analysis

Statistical analysis of the data obtained at the trial was made using the SPSS 24.0 statistical program. The data obtained were tested by one-way analysis of variance (Oneway-ANOVA) (Cimen, 2015). Results were considered statistically significant at $p<0.05$.

RESULTS and DISCUSSION

The changes of the AChE activity in the control group and groups exposed to sublethal concentrations of malathion was given in Figure1. The AChE activity of the control group non-exposed to malathion was 0.50 ± 0.04 U/mg protein at 24 hours, 0.47 ± 0.03 U/mg protein at 48 hours. The enzyme activity of the malathion-exposed groups was lower than that of the control group 24 and 48 hours after exposure, and the difference between them was statistically significant ($p<0.05$).

The AChE activity of the groups exposed to 0.1 mg l^{-1} and 0.2 mg l^{-1} of malathion concentrations for 24 hours was 0.25 ± 0.02 U/mg protein and 0.20 ± 0.01 U/mg protein, respectively. It also had a 50% and 60% inhibition rate compared to the control, respectively (Figure2).

The activity AChE in *G.pulex* after exposure to 0.1 mg l^{-1} malathion for 48 hours was 0.12 ± 0.01 U/mg protein, while after 0.2 mg l^{-1} malathion-exposed for 48 hours was 0.15 ± 0.04 U/mg protein. Besides, the enzyme activity was observed significantly an inhibition rate of 74% and 68% compared to the control group, respectively (Figure2).

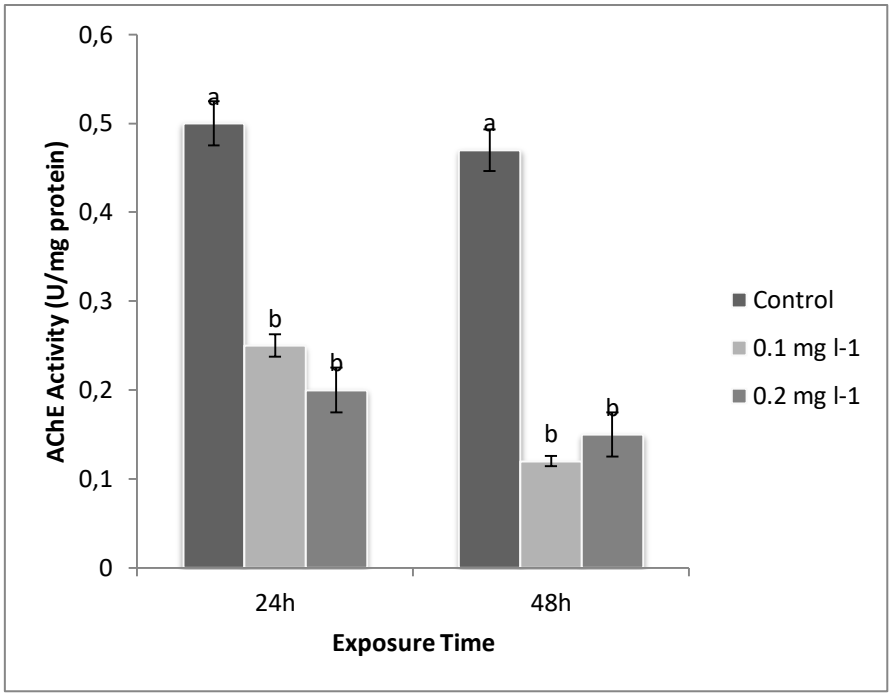

Figure1. Changes in the AChE activity of groups in different exposure time (24 and 48h)
*a.b, The difference between the values indicated by is statistically significant ($p<0.05$)

Aquatic invertebrates are more susceptible to OP insecticides than vertebrate animals (Giesy et al., 1999; Hyne and Maher, 2003) and the inhibition of AChE activity had been measured in some aquatic invertebrate species (Kozlovskaya et al., 1993; Moulton et al., 1996; McLoughlin et al., 2000; Varó et al., 2002; Barata et al., 2004; Kristoff et al., 2006; Gauthier et al., 2016).

In this study, inhibition in the AChE activity was observed in both malathion concentration exposure (0.1 and 0.2 mg l^{-1}). The highest inhibition (74%) was detected in organisms exposed to 0.2 mg l^{-1} malathion for 24 hours (Figure 2). The results of this study related to being consistent with the findings of previous studies in Gammarids exposed to other OPs. For example; Fenitrotion and parathion-methyl concentrations have been reported to lead to significant reductions in AChE activity in three different Gammarus species (Kuhn and Streit, 1994). In *G. pulex*, strong AChE inhibition (94%) was observed after exposure to OP insecticide chlorpyrifos at a concentration of 2.86 nM for 96 hours (Xuereb et al., 2007). Similarly, Xuereb et al. (2009) reported that chlorpyrifos plays a role in inhibiting AChE activity when *G. fossarum* is exposed to chlorpyrifos for a short time (96 hours). Also, exposure to diazinon oxon for 24-hours has been reported to cause an almost complete inhibition (92%) in the AChE activity of *G. pulex* (Elwahaishi et al., 2019). Pala (2019), was reported that inhibition in AChE activity of *G. pulex*, which was exposed to OP glyphosate-based herbicide (GBH) concentrations (10, 20, 40 μg l^{-1}) for 24 and 96 hours, was observed, and the inhibition range to be min 23% max 53%.

Furthermore, the results of this study showed that the AChE activity was affected step by step at 24 and 48 hours exposure to malathion concentrations and the inhibition rate increased with time (Figure 2). This result is similar to the findings obtained by Xuereb et al. (2009). They reported that after exposure to 28 nM concentration of chlorpyrifos for 24, 48, and 96 h, in the AChE enzyme activity of *G. fossarum*, 19%, 33%, and 70% inhibition was observed, respectively. Similarly, Pala (2019) was revealed that AChE inhibition increased in *G. pulex* depending on GBH concentration and exposure time.

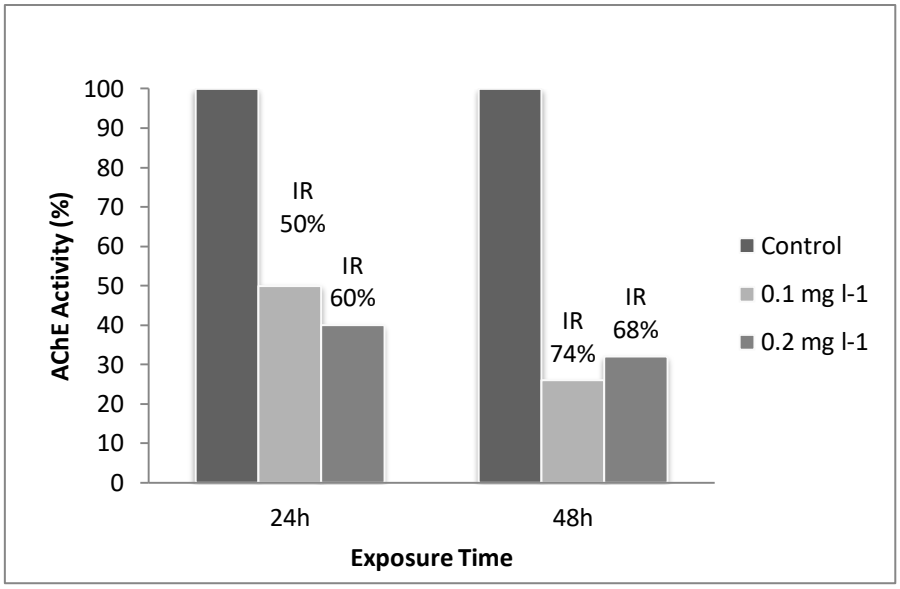

Figure 2. The AChE activity inhibition rates (IR) of groups in different exposure time (24 and 48 h) (%)

CONCLUSION

Sublethal concentrations of OP insecticide malathion caused in time-dependent increasing inhibition of AChE activity in *G. pulex*. Also, the results showed that up to 75% AChE inhibition levels in *G. pulex* did not lead to death. Thus, it is suggested that AChE in *G. pulex* can be effectively used as a biological marker of organophosphate pesticide toxicity.

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