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Accumulation of sublethal doses of popargite (Acaricide) in muscle tissues of carp (*Cyprinus carpio*, Linnaeus, 1758)

Arınç Tulgar
Food Processing Program, Lapseki Vocational School, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

Abstract
In this study, an organosulfide pesticide propargite was examined in the carp (*Cyprinus carpio*, Linnaeus, 1758) fish muscle tissue. For this purpose, fish were exposed to control (rested tap water), low (0,04125 mg L^{-1}), medium (0,0825 mg L^{-1}) and high doses (0,165 mg L^{-1}) of propargite concentrations for 14 days. While the experiment was carried out with 3 replications, sample fishes were taken on days 0, 7 and 14.

Keywords: pesticide, propargite, *Cyprinus carpio*, accumulation

Introduction
Nowadays, various auxiliary substances such as pesticides are used to meet the needs of the growing world population and to make the most efficient use of the resources available. Although these substances are used to serve a useful purpose, they have reached the aquatic environments through rain and flood waters, sewage wastes, erosion and soil leakage, causing the non-target organisms to be affected. Therefore, they have negative side effects in various activities such as growth, reproduction and nutrition. Also, they can even reach the food chain and reach people in increasing concentrations (Adhikari *et al*., 2004; Ramesh and Saravanan, 2008) [1, 13]. This is because fish and other aquatic organisms are susceptible to environmental changes due to their direct contact with water. Even in this respect, they are excellent indicators in the measurement of pollution (Ahmad, 2011; Chandrasekar and Jayabalan, 1993; Giron-Perez *et al*, 2006; Satyanarayan *et al*; 2004; Tulgar and Çelik, 2015) [2, 3, 7, 15, 17]. Propargite pesticide used in this study is a group of organosulfide pesticides and is an acaricide and mycit, used in ornamental plants with various fruit trees, vegetables and greenhouse origin (EPA, 2001; Funk, 2013; Pal and Das Gupta, 1994; PAN, 2013; PMEP, 2013; Xu, 2001) [4, 6, 10, 11, 12]. It was first registered in the United States in 1969 and produced by Uniroyal Chemical Company and registered under the trade names Omite® and Comite® (Tulgar, 2014) [16]. Propargite is a highly toxic pesticide and its half-life is inversely proportional to the pH of the water (Xu, 2001) [21]. Propargite's lethal dose (LC_{50}) for C*yprinus carpio* was reported as 330 ppb for 48 hours (Turner, 2002) [18]. However, there is not much resources on propargite and carp fish, and this study is thought to contribute greatly to the literature.

Material and Method
Carp species (*Cyprinus carpio*) were used in the study. Fishes were brought from Mediterranean Fisheries Research, Production and Training Institute, Beymelek Facilities (Antalya) and stored in fiberglass tanks (80 L.) for 30 days for adaptation. At the end of the adaptation period, 180 fish were selected and their length (mean length: 14.25 ± 0.06 cm) and weight (mean weight: 43.75 ± 0.37 gr.) were measured. Fishes were divided into 12 aquariums (50 l.) each with 15 pieces and the experimental design was created. Feeding was stopped 1 day prior to the start of the experiment and 2% of the body weight was given to the fish twice a day during the experiment. The experiment was carried out for 14 days and during this time, fishes were exposed to control (C: rested tap water), low dose (LD: 0.04125 mg L^{-1}), medium dose (MD: 0.0825 mg L^{-1}) and high dose (HD: 0.165 mg L^{-1}) of propargite concentrations. The literature was used to determine the concentration (Turner, 2002) [18]. In the experiment, sampling was done 3 times, on day 0 (without any chemical application), on day 7 and 14. On day 0, one fish was taken from each aquarium, and seven fish were taken on the 7th and 14th days. Muscle tissue was separated from the fish by sterile dissection instruments and used in pesticide residue analysis. Analysis of pesticide residues was carried out in the literatüre (FDA, 1994) [5]. Fish samples of different dosage applications kept in -18º C in locked refrigerator bags were transferred to the laboratory in ice storage box and they were kept at 4º C for 1 day to dissolve. The sample groups removed from the refrigerator respectively were minced after the muscle part of each fish have taken. Minced meats were put in plastic containers. After all of the sample groups were prepared in this way, 25 grams of each of the containers were sampled and weighed in the sensitive scale, then they were placed in volumetric flasks (250 ml). 2 grams of sodium sulfate and 150 milliliters of petroleum benzene were added on the samples. After these processes, the volumetric flasks were shaken and their mouths closed. They were then allowed to stand overnight at room temperature in a light-free environment. Samples kept overnight were placed in empty volumetric flasks (250 ml). The samples were then filtered through the blotter paper and the liquid portions were taken. The liquid samples were then evaporated in the evaporator set to a temperature of 30-35º C. The mouth of the balloons removed from the evaporator is covered with a lid. After all of these procedures were completed, a syringe (25 ml) was prepared for each volumetric flask and fluorosil cartridges were mounted to the ends. Immediately after the sample number of blank volumetric flasks (250 ml) were prepared. The closed volumetric flasks were opened and each was placed in 5 milliliters of acetone and the volumetric flasks

were shaken well and then placed in syringes with fluorosyl cartridges. The liquid in the syringe is filtered into newly prepared volumetric flasks and this was repeated for each sample. Then, 3 long flasks were taken and the petroleum benzene and petroleum ether solutions were prepared to be 94/6, 85/15 and 50/50 respectively. 5 ml. were drawn with the help of different pipettes from these flasks and they were filtered into 250 ml. volumetric flasks which were containing acetone. Finally, the liquid in the volumetric flasks were evaporated in the evaporator set at 30-35º C, respectively. Immediately after, 5 ml. of acetone was added to the volumetric flasks. On the other hand, two vial tubes were prepared for each sample and 800 µl A1 mobile phase was added to them. Then, 200 µl of each specimen was added to these vial tubes and complete to 1000 µl. The covers of the vials were closed and this was repeated for each sample. The vials were read in liquid chromatography / mass-mass spectrometer.

Two-way variance analysis (ANOVA) was applied to compare the effects of dose groups and experiment times. Statistical analysis calculated according to SPSS 17 package programme. Differences between the groups determined as $p<0.05$ (Logan, 2010) [9].

Results and Discussion

In the study, it was determined that the accumulation of propargite in fish tissue changed according to time and concentrations and increased propargite accumulation ($p<0.05$) with increasing dose (Figure 1).

In one study, the eel species (*Anguilla anguilla*) were exposed to a sublethal dose of 0.04 ppm of fenitrothion. At the end of the study, it was observed that the fenitrothion accumulations in the muscle tissue reached 1.113, 0.870, 1.480, 1.937, 2.538 and 2.523 μgg^{-1} respectively after 2, 8, 24, 48, 56 and 72 hours. In this study, the increase in pesticide accumulation with dose increase is similar to our study (Sancho *et al*., 1998). In another study, it has been reported that increased accumulation of pesticides in the mosquito fish (*Gambusia affinis*) tissues exposed to 0.25 μgL^{-1} dose of endosulfan sulphate for 1, 2, 3, 4 and 5 weeks has increased in time (Hoang *et al*., 2011) [8]. The findings of the study are similar to the results of our study. In another study, it has been reported that in fish (*Cyprinus carpio*) exposed to sublethal doses of chlorpyrifos 1.16, 11.6 and 116 μgL^{-1} for 40 days, pesticide deposits in spleen and in primary kidney were found to be 0.35, 1.59, 3.38 mg and 0.53, 1.31 and 1.91 mg kg^{-1}, respectively (Wang *et al*., 2013) [19]. Pesticide accumulations in different tissues were found to increase with dose increase in a similar way as in our study. It was reported that the accumulation rates of pesticides in fish tissue are closely related to the environment, length, weight, age, and fat content of the fish and it may also be related to pollutants (Wang *et al*., 2012) [19]. According to this study, it is considered that it is a normal result that pesticide residue accumulations in fish tissue obtained in this study and similar studies are quite different amounts.

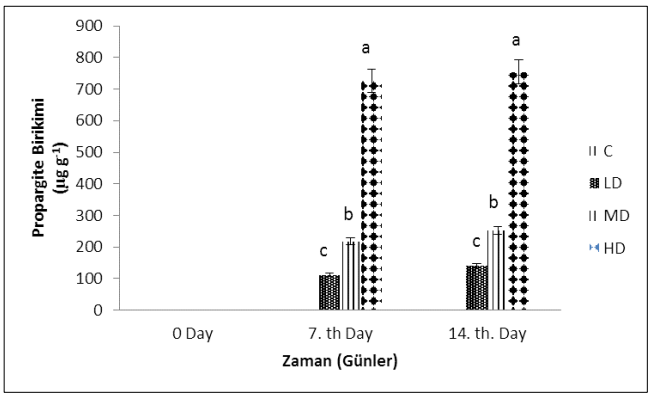

Fig 1: Propargite values of carp (*C. Carpio*) tissues exposed to various propargite concentrations (C: 0, LD: 0.04125 mg L^{-1}, MD: 0.0825 mg L^{-1}, HD: 0.165 MG L^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($p<0.05$).

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