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Effects of sublethal concentrations of propargite to white blood cells of common carp (*Cyprinus carpio*, Linnaeus, 1758).

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ABSTRACT

In this study, effects of propargite, an organosulphiric pesticide were investigated in carp's (*Cyprinus carpio*) white blood cells such as lymphocyte (LYM), neutrophile (NEU), monocyte (MON), basophile (BAS) and eosinophile (EOS). Fish were exposed to control (only distilled tap water), low (0.04125 ppm), medium (0.0825 ppm) and high (0.165 ppm) concentrations of propargite. As a result of study, percentage of BAS, NEU and MON cells were generally showed an increase on the 14th day compared to the control group, in case percentage of LYM and EOS cells were decreased on the 14th day according to the control group. In conclusion, changes observed among the white blood cell types could demonstrate the immunosupresive effect of propargite.

Introduction

In the present day, necessity to use of pesticides is increasing gradually according to the use of available resources more effectively and giving more emphasis to aquaculture activities due to the increment of World population and insufficient cultured areas. But it has been emerged that, these substances which were being used for a beneficial purpose such as supplying the mankind's needs were also adversely affecting the vital functions of other living organisms such by reaching to the non-targeted organisms in time and threatening the ecological balance. Therefore, researches on environmental pollution and its effects on ecosystem due to the pesticides have gained momentum in the last few years (Adhikari et al. 2004; Mgbenka et al. 2005; Patnaik and Patra, 2006; Sepici-Dinçel et al. 2007; Ramesh and Saravanan, 2008; Mikula et al. 2008; Fırat et al. 2011; Saravanan et al. 2011; Jayaprakash and Shettu, 2013).

Fish and other aquatic organisms are very sensitive to environmental changes due to their direct interaction with their environment and depending on this function; they are convenient indicators for observing the pollution that grows due to the pesticides (Chandrasekar and Jayabalan, 1993; Satyanarayan et al. 2004; Giron-perez et al. 2006; Ahmad, 2011).

Pesticides can contaminate the aquatic organisms by floods, several water leakages and erosions during their application to agricultural areas or their direct implementation on targeted organisms (Satyanarayan et al. 2004; Velisek et al. 2009a; Naveed et al. 2011).

An organosulphuric pesticide propargite's solution in water condition changes according to water pH. It has been reported that, half life of propargite in pH 5, 7 and 9 conditions changes between 120-702, 48-78 and 2-3 days, respectively (Xu, 2001). However, the risk of propargite's reaching to the aquaculture environments by infiltaring through the soil is inversely correlated with organic substance amount in soil. While the propargite reaches to the water, it has been reported that, highly toxic for aquatic organisms and its LC_{50} value for *Cyprinus carpio* was found to be 330 ppb for 48 hours (Turner, 2002). But there has been no study on carp conducted by the propargite out of this data. With this study, a widely used pesticide propargite and its sublethal effects on the white blood cell types of carp was investigated. Therefore, this study has

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been expected to give additional insight the lack of researches in this area.

Material and methods

Experiment fish *Cyprinus carpio* were obtained from Fisheries Productions Search, Aquaculture and Education Institute of Akdeniz in Beymelek, Antalya. Fish were kept in fiberglass tanks (80 L) for 30 days for adaptation to ambient conditions before the experiment. During the adaptation time, water in each tank was changed with still, aerated and heated tap water every two days and fish were fed with pine content carp feed once a day. Subsequently, fish were adapted to ambient conditions, taken for the length and weight measurements (average length: 14.25±0.06 cm, average weight: 43.75±0.37 gr). As for immediately after they were separated to 12 experiment aquariums (50 L) in each having 15 fish and a 3 triplicate experimental design was planned. Feeding was discontinued before 24 hours the experiment has started and fish were fed with the rate of 2 % of their body weight twice a day. The experiment was continued for 14 days and during this period fish were exposed to control (only tap water), low, medium and high concentrations of propargite (Sigma-Aldrich-Steinheim, Germany, 99.5 %) concentrations. Sublethal doses of propargite were determined according to Turner (2002), who has found LC_{50} value of propargite in *Cyprinus carpio* as 0.33 ppm. Accordingly, low (LC_{50}/8 0.04125 ppm), medium (LC_{50}/4 0.0825 ppm) and high (LC_{50}/2 0.165 ppm) concentrations of propargite were calculated. The main stock solution was prepared by dissolving in acetone. Concentrations that were used in the experiment were obtained by appropriate dilutions (APHA, AWWA, WEF, 1998). During the experiment fifty percent of water was changed every day and same amount of propargite solutions were added to water in aquariums. Experiment was carried out in a total of 3 times, including the 0 day (without any chemical treatment), 7 th and 14 th days of sampling. On the 0 day one fish was taken from each aquarium, in each case seven fish on the 7 th and 14 th days and white blood cell types were examined.

Fish were anesthetized with MS 222 (150 mg/L) during blood sampling (Smith et al. 2007). Right rear portion of the anal fin was cleaned with alcohol to prevent the interference of mucosa to blood and in the shortest time blood samples were taken from the caudal vein with a 5 ml. plastic injection (Val et al. 1998). Blood samples were put into the potassium triethlene diamine tetra acetic acid (K_3EDTA) and gel serum tubes for blood analysis.

Primarily a little amount of blood was dropped on to the microscope slide and spread by the help of a lamel, afterwards allowed to stand at room temperature for drying. Later, slides were painted by May-Grünwald-Giemsa dye and washed with distilled water and dried again. After the completion of these operations slides were examined under a microscope at 1000x magnification with a help of immersion oil. During the examination period, 100 leukocyte cells were counted and percentage of leukocyte cell types such as lymphocyte, neutrophile, basophile, monocyte and eosinophil were determined (Esteban et al. 2000; Pavlidis et al. 2007).

Results

White blood cell types of fish groups, exposed to different concentrations of propargite during the study period have shown various changes. Accordingly, white blood cell types as percentage of LYM cells were significantly decreased, in the medium and high concentration groups compared to the control group (P < 0.05) on the 14th day (Figure 1). In this case, percentage of NEU increased in all propargite groups on the 7th and 14th days (P < 0.05) compared to the control group (Figure 2). The percentage of MON cells, increased significantly in all propargite groups in the 14 th day (P < 0.05) compared to the control group (Figure 3). However, percentage of BAS cells decreased significantly on the 7th day in low and high concentrations of propargite (P < 0.05) compared to the control, but at the same time, they also increased significantly on the 14th day (P < 0.05) in the low and high concentrations (Figure 4). The percentage of EOS cells decreased significantly on the 7th day in medium and high concentrations and on the 14th day in low and medium concentrations (Figure 5) compared to the control (P < 0.05).

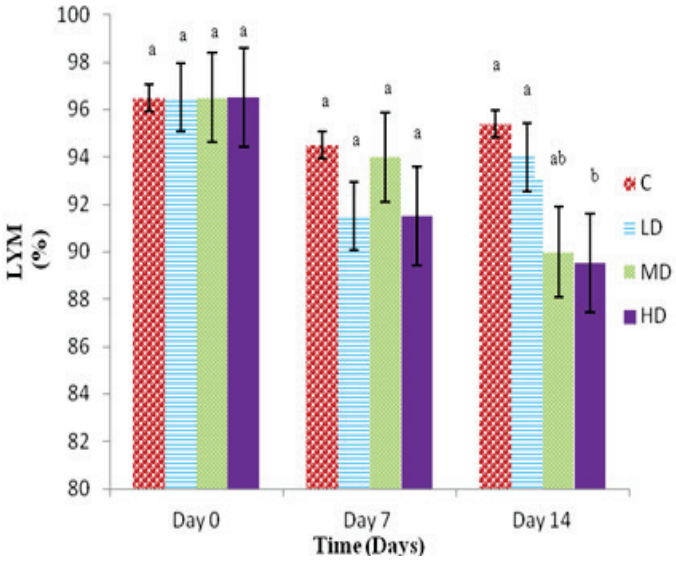

Figure 1. LYM values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mg L^{-1}, MD: 0.0825 mg L^{-1}, HD: 0.165 mg L^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important (P < 0.05).

Discussion

All leukocyte types play an important role in the immune system of body (Murad and Houston, 1998). In this study, the percentage of LYM and EOS cells were found to be decreased, whereas the percentage of BAS, NEU and MON cells were increased.

In the present study, decreases in the percentage of LYM cells have shown similarity with the carp (*C. carpio*) that were exposed to Sencor 70 WG which is a trademark component of a herbicide metribuzin (Velisek et al. 2009b) and the rainbow trouts (*Oncorhynchus mykiss*) that were exposed to pentachlorophenol (Shelly et al. 2009).

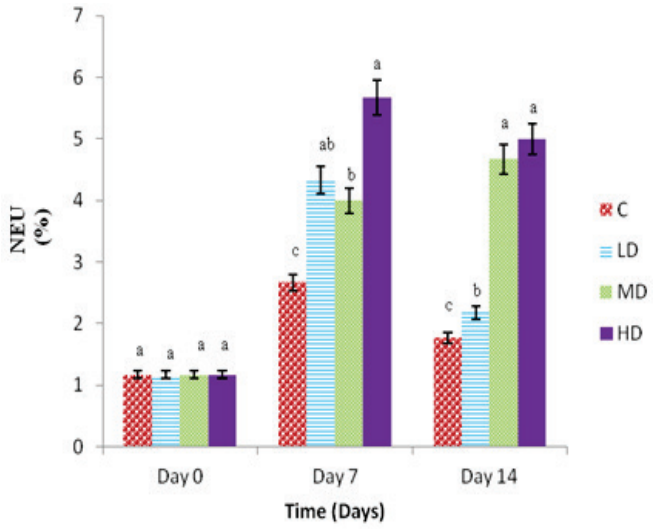

Figure 2. NEU values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mg L^{-1}, MD: 0.0825 mg L^{-1}, HD: 0.165 mg L^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($P < 0.05$).

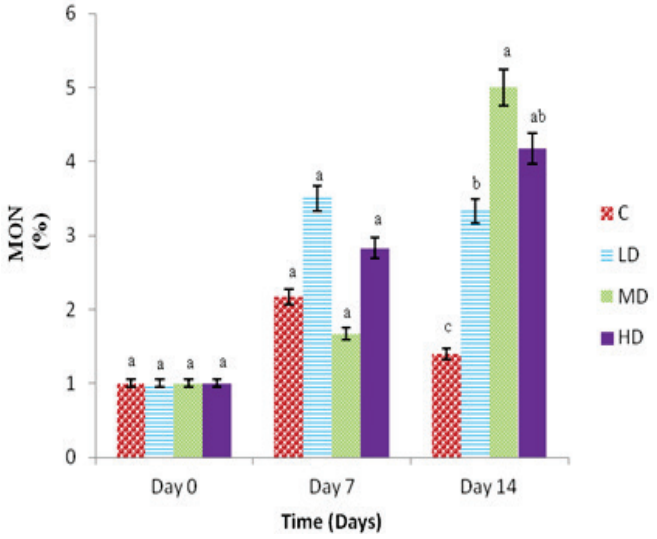

Figure 3. MON values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mg L^{-1}, MD: 0.0825 mg L^{-1}, HD: 0.165 mg L^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($P < 0.05$).

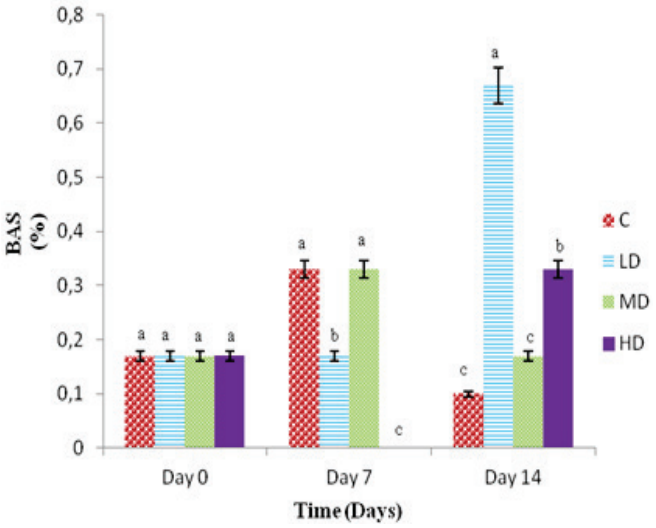

Figure 4. BAS values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mg L^{-1}, MD: 0.0825 mg L^{-1}, HD: 0.165 mg L^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($P < 0.05$).

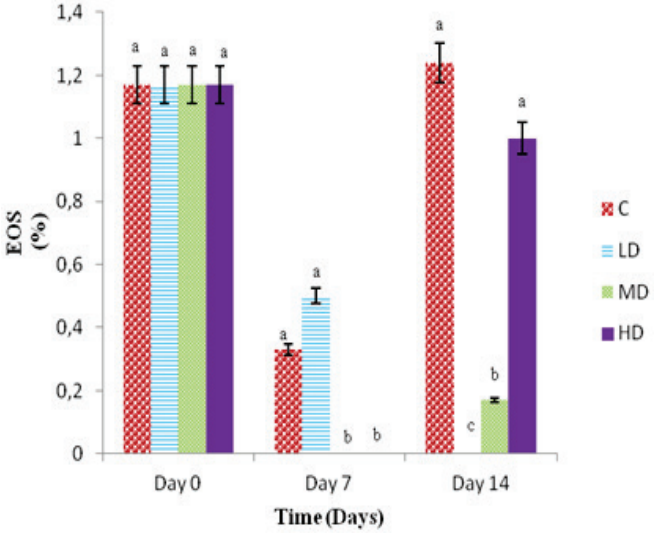

Figure 5. EOS values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mg L^{-1}, MD: 0.0825 mg L^{-1}, HD: 0.165 mg L^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($P < 0.05$).

However, decreases in the percentage of LYM and increases in the percentage of NEU were similar with the results of the studies that were carried out with carp (*C. carpio*): fish which were exposed to a glyphosate base herbicide, Roundup (Gholami-Sevedkolaei et al. 2013) and an organophosphorous insecticide: phosalone (Kaya et al. 2014). Also in a study that was performed with African cat fish (*Clarias gariepinus*) exposed to 4-nonylphenol, which is a substance used in the compound of various pesticides, the percentage of LYM cells were found to be decreasing whereas the percentage of NEU cells were increasing ($p<0.05$) compared to the control (Mekkawy et al. 2011). On the other hand, increases in the percentage of MON cells in this study, have shown familiarity with the results in *C. carpio* fish which were exposed to atrazine and metribuzin (Svobodova and Pecena, 1988; Velisek et al. 2009b). In addition, the percentage of the BAS cell increase in the study, has shown similarity with the research carried out with *Oreochromis niloticus* that were exposed to thiamethoxama (Barnali and Susanta, 2011). Decreases in EOS cells, bear close qualifications with two study results, at first *Clarias batrachus* that were exposed to lindane (Thakur and Pandey, 1990) and secondly *C. gariepinus* that were exposed to 4-nonylphenol, a substance which used in the compound of some pesticides (Mekkamy et al. 2011).

It has been reported that, changes in the white blood cell types occured depending on pesticide related stress. This condition could increase the sensitivy against diseases by weakening the specific and non-specific immune system (Rehulka and Minarik, 2004; Wedemeyer, 1997) and these

changes were characterized with decreasing lymphocyte rate and increasing neutrophil rate (Modesto and Martinez, 2010; Witeska, 2005). Generally, all stress factors can cause several changes in the percentage of white blood cells and all leukocyte types play a significant role in the immune system body (Murad and Houston, 1998; Barnali and Susanta, 2011). Also, in several studies it has been reported that, pesticides such as chlorpyrifos (Harford et al. 2005), diazinon (Ivan et al. 2007) and phosalone (Kaya et al. 2014) had an immunosuppressive effect on fish.

White blood cell types of common carp groups that were exposed to different concentrations of propargite have shown various changes and these results can demonstrate the immunosupresive effect of propargite.

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