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Effect of Sublethal Doses of Propargite (Acaricide) to Hematological Parameters of Common Carp (*Cyprinus carpio*, Linnaeus, 1758)

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Abstract: In this study, certain hematological parameters in the blood parameters of common carp (*Cyprinus carpio*) which exposed to different sublethal concentrations of propargite has been researched. Fish were exposed to control (only dechlorinated water), low (0.04125 mgL^{-1}), medium (0.0825 mgL^{-1}) and high (0.165 mgL^{-1}) doses of propargite concentrations for 14 days. On the 7th day of the experiment Red Blood Cell (RBC) value only in high dose, Hematocrit (Hct) ratio and Hemoglobin (Hb) values in all doses, Mean Corpuscalar Volume (MCV) and Mean Corpuscalar Hemoglobin (MCH) values in low and medium dosegroup were decreased while Mean Corpuscalar Hemoglobin Concentration (MCHC) value only in low dose was increased compared to the control ($p<0.05$). On the 14th day of the study, hematological parameters as RBC value, Hct ratio, Hb value and MCV value were decreased ($p<0.05$) in all dose groups compared to the control group while MCHC value was increased in all dose groups ($p<0.05$).

Key words: Propargite, *Cyprinus carpio*, hematology, pesticide, MCHC, MCH

INTRODUCTION

Now a days, at the beginning of the problems that are occurring in the world, the increasing population of the world and the necessity of meeting the needs of mankind. Human beings are unable to meet their food needs from time to time in order to sustain their lives and this point enters into artificial quests. On the other hand, factors such as urbanization, modernization and migration cause the addition of new ones to the needs of human beings. At this point, various artificial materials such as pesticides have been used in order to make use of the resources available in the most efficient way or to develop new techniques. However, these substances which are used to serve a beneficial purpose, have become a threat to the natural environment and become one of the main sources of environmental pollution (Tulgar, 2014).

Fish and other aquatic organisms are highly susceptible to environmental changes due to direct contact with water. Depending on these characteristics, they are excellent indicators for monitoring and measuring pesticide pollution (Chandrasekar and Jayabalan, 1993; Satyanarayan *et al*., 2004; Giron-Perez *et al*., 2006; Ahmad, 2011). Pesticides are contaminated directly into aquatic organisms by dermal contact, during the respiration through gills or as nutrients (Satyanarayan *et al*., 2004; Velisek *et al*., 2009; Naveed *et al*., 2011).

The propargite used in this study is an organosulfuric pesticide which has an acaricide and miticide effects and used in various fruit trees, vegetables and greenhouse origin ornamental plants (Pal and das Gupta, 1994; EPA., 2001; Xu, 2001; PAN., 2013; PMEP., 2013). It was first registered in the United States in 1969 and registered under the trade names Omite® and Comite® (Tulgar, 2014).

The solubility of propargite in water varies according to the pH of the water. 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, contamination of propargite from the soil to the aquatic environment is inversely proportional to the amount of organic matter of the soil. When propargite pesticide reaches water environment, it is highly toxic for aquatic organisms. The Lethal dose (LC_{50}) in carp (*Cyprinus carpio*) fish is 330 ppb for 48 h (Turner, 2012). But there is no more study on propargite in carp fish. In addition to this research, it is aimed to observe the hematological findings changes in sublethal doses of carp fish which has a widespread use.

MATERIALS 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 2 days and fishes were fed with pine content carp feed once a day. Subsequently, fishes were adapted to ambient conditions, taken for the lenght and weight measurements (average length: 14.25±0.06 cm, average weight: 43.75±0.37 g). As for immediately after they were seperated to 12 experiment aquariums (50 L) in each having 15 fish and a 3 triplicate experimental design was planned. Feding was discontinued before 24 h 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, fishes 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 (2012) who has found LC_{50} value of propargite *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 *et al*., 1998). During the experiment 50% 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), 7th and 14th days of sampling. On the 0 day one fish was taken from each aquarium in each case seven fish on the 7th and 14th days and hematological parameters 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 muscosa 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.

Hematocrit value calculated by microhematocrit method. Hematocrit tubes that, filled up with blood, keep waited in centrifuge for 5 min at 10500 g rpm. After these processes % hematocrit value was measured by using a scale (Blaxhall and Daisly, 1973).

Hemoglobin value calculated by cyanomethemoglobin method (Blaxhall and Daisly, 1973). 20 mL. Blood sample were taken and added into the Drapkin solution. Samples keep waited in this solution for incubation, right after solution has been readed. Results were calculated as gdL^{-1}. Mean erythrocyte Volume (MCV) was calculated by the following equation (Lewis *et al*., 2006):

$$MCV(FI): Hct \times 10 \,/\, RBC\left(10^{6} \mu L^{-1}\right)$$

Mean Corpuscular Hemoglobin (MCH) value determined by the following equation (Lewis *et al*., 2006):

$$MCH(pg): \left[Hb\left(gdL^{-1}\right) \times 10\right] / \, RBC\left(10^{6} / mm^{-1}\right)$$

Mean Corpuscular Hemoglobin Concentration (MCHC) determined by the following equation (Lewis *et al*., 2006):

$$MCHC\left(g^{-1}\right): \left[Hb\left(gdL^{-1}\right) \times 10\right] / \, Hct$$

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

RESULTS AND DISCUSSION

Hematological values of fish groups, exposed to different concentrations of propargite during the study period have shown various changes. Accordingly, RBC value only in high dose was significantly decreased compared to the control ($p<0.05$) on the 7th day and on the 14th day significantly decreased compared to the control ($p<0.05$) in all dose groups (Fig. 1). Hb values and Hct ratio in all dose groups were significantly decreased compared to the control ($p<0.05$) on the 7 and 14th days (Fig. 2 and 3). MCV value in low and medium doses significantly decreased compared to the control ($p<0.05$) on the 7th day and on the 14th day in all dose groups decreased significantly compared to the control ($p<0.05$)

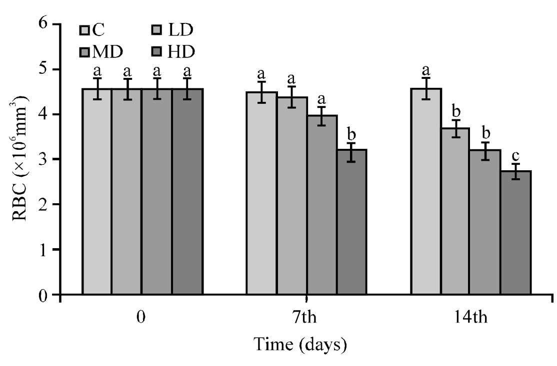

Fig. 1: RBC values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mg L^{-1}, MD: 0.0825 mg L^{-1}, HD: 0.165 mgL^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($p<0.05$)

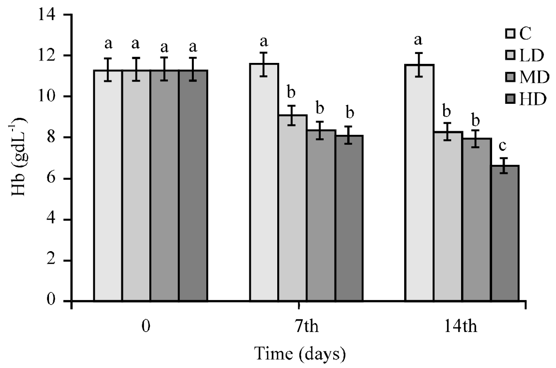

Fig. 2: Hb values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mgL^{-1}, MD: 0.0825 mgL^{-1}, HD: 0.165 mgL^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($p<0.05$)

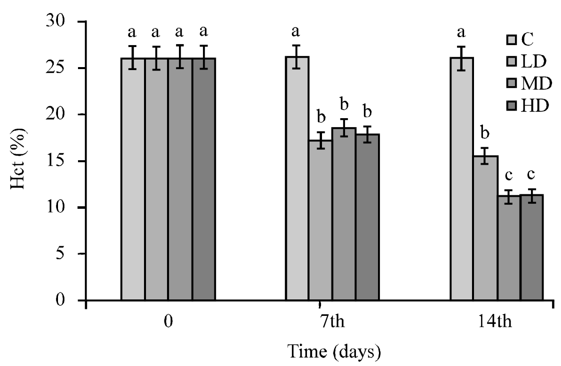

Fig. 3: Hct values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mgL^{-1}, MD: 0.0825 mgL^{-1}, HD: 0.165 mgL^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($p<0.05$)

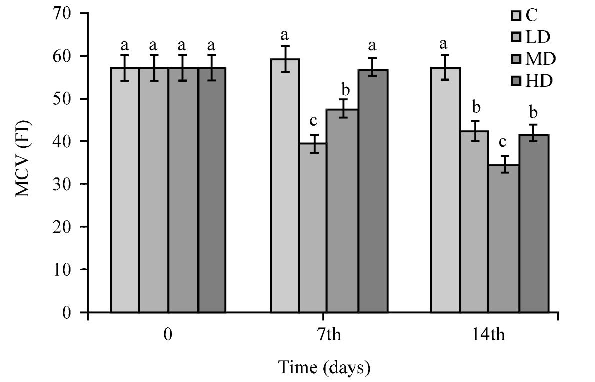

Fig. 4: MCV values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mgL^{-1}, MD: 0.0825 mgL^{-1}, HD: 0.165 mgL^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($p<0.05$)

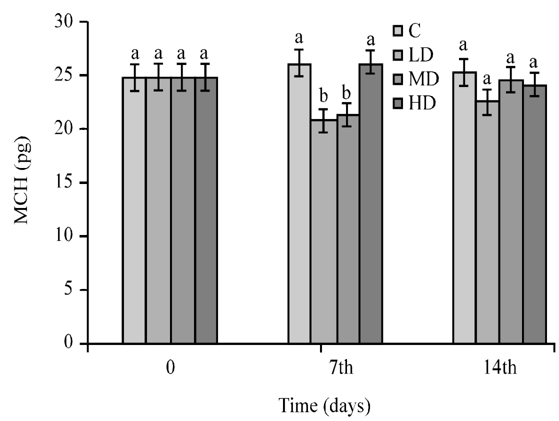

Fig. 5: MCH values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mgL^{-1}, MD: 0.0825 mgL^{-1}, HD: 0.165 mgL^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($p<0.05$)

(Fig. 4). MCH value in low and medium dose groups were significantly decreased on the 7th day (Fig. 5). MCHC value only in low dose significantly increased compared to the control ($p<0.05$) on 7th day and on the 14th day was significantly increased in all dose groups compared to the control ($p<0.05$) (Fig. 6).

In the present study, decreases in the RBC count, Hb and Hct values have shown similarity with the carps (*Cyprinus carpio*) that were exposed to Endosulfan (Jenkins *et al*., 2003), Lindane (Saravanan *et al*., 2011a, b), Roundup® (Gholami-Seyedkolaei *et al*., 2013) and the *Labeo rohita* which was exposed to cypermethrin and carbofuran (Adhikari *et al*., 2004) and also *Rutilus frisii* Kutum (Shamoushaki *et al*., 2012), *Clarias gariepinus* (Adedeji *et al*., 2009) and *Silurus glanis* juveniles (Koprucu *et al*., 2006) that were exposed to diazinon. On the other hand, decreases in the RBC count also shows parallel results with the *Anabas testudineus* fishes exposed to monocrotophos (Santhakumar *et al*., 1999), *Clarias albopunctatus* fishes exposed to actellic (Mgbenka *et al*., 2005), *Mesopotamichthys sharpeyi* fishes exposed to paraquat (Safaieh *et al*., 2012) and *Anguilla anguilla* fishes exposed to molinate (Sancho *et al*., 2000).

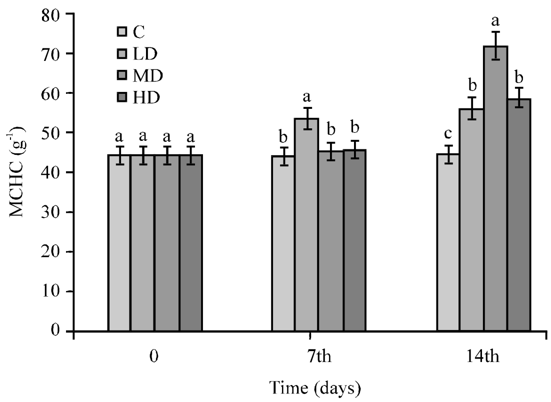

Fig. 6: MCHC values of carp (*C. carpio*) exposed to various propargite concentrations (C: 0, LD: 0.04125 mgL^{-1}, MD: 0.0825 mgL^{-1}, HD: 0.165 mgL^{-1}). Variations between the average concentrations were shown with different small letters in the same parameter and time is important ($p<0.05$)

Reductions in the RBC, Hb and Hct values can be attributed to the anemic condition due to the inhibition of events such as erythropoiesis and hemosynthesis, dysfunction in osmoregulation and an increased erythrocyte destruction rate in the hematopoietic organs (Jenkins *et al*., 2003; Ramesh and Saravanan, 2008; Ramesh *et al*., 2009; Adhikari *et al*., 2004; Sepici-Dincel *et al*., 2007; Kumar *et al*., 2011). The decrease in hemoglobin and blood iron seen in this study shows that hemoglobin production may decrease due to iron deficiency in fish and as a result anemia may occur (Jenkins *et al*., 2003). The lower Hb level is also explained by the deterioration of the iron expression mechanism (Chandrasekar and Jayabalan, 1993; Nandan and Nimila, 2012).

In the present study, decreases in the MCV value have shown similarity with the carp (*Cyprinus carpio*) exposed to lindane (Saravanan *et al*., 2011a, b) *Rutilus frisii* Kutum males (Shamoushaki *et al*., 2012) and *Silurus glanis* Juveniles (Koprucu *et al*., 2006) which were exposed to diazinon and also *Clarias albopunctatus* Juveniles exposed to actellic (Mgbenka *et al*., 2005) and *Clarias batrachus* exposed to Sevin® (Patnaik and Patra, 2006). On the other hand, increases in the MCHC value have shown parallel results with *Cyprinus carpio* that was exposed to endosulfan (Chandrasekar and Jayabalan, 1993) *Rhamdia quelen* exposed to cypermethrin (Borges *et al*., 2007) and *Oreochromis niloticus* exposed to Chlorpyrifos (Giron-Perez *et al*., 2006).

CONCLUSION

Changes in MCHC value are primarily related to RBC count, Hb and Hct values (Ahmad, 2011). Reduction of red blood cells due to the toxic effect of pesticides and their hypochromic microcytic anemia may be associated with a decrease in the MCV value (Atamanalp and Yanyk, 2003). Also, the decrease in the MCV value can be attributed to the high rate of immature blood cells in the circulatory system (Saravanan *et al*., 2011a, b). The increase in MCHC value may be associated with a decrease in Hct value due to hemolysis (Chandrasekar and Jayabalan, 1993). However, the increased MCHC value may be attributed to hereditary spherocytosis which is a congenital disorder.

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