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Effect of Cadmium and Lead on Total Hemocyte Count of *Achroia grisella* Fabr. (Lepidoptera: Pyralidae)

Kadmiyum ve Kurşunun Achroia grisella Fabr. (Lepidoptera: Pyralidae)'nın Toplam Hemosit Sayısına Etkisi

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Abstract
The effect of cadmium (Cd) and lead (Pb) on total hemocyte count (THC) of *Achroia grisella* was examined under laboratory conditions. Larvae were reared on an artificial diet medium contaminated with three different concentrations (50, 100 and 200 mg/kg) of Cd and Pb. In the control group the insects fed a heavy metal free diet. All of the insects were kept at 25 ± 2 °C temperature, 60 ± 5% relative humidity and 16L:8D photoperiod conditions.The obtained data showed that the total hemocyte count of larvae exposed to Cd resulted in a significant decrease at all concentrations, whereas total hemocyte count of Pb-treated groups decreased only at the highest concentrations of Pb.

Keywords: Achroia Grisella, Hemolymph, Total Hemocyte Count

Öz
Kadmiyum (Cd) ve kurşunun (Pb) Achroia grisella'nın toplam hemosit sayısı üzerindeki etkisi laboratuar koşullarında araştırılmıştır. Larvalar üç farklı konsantrasyonda (50, 100 ve 200 mg/kg) Cd ve Pb ile kontamine olmuş yapay besin ortamında yetiştirilmiştir. Kontrol grubunda böcekler ağır metal içermeyen besin ile beslenmiştir. Tüm böcekler 25 ± 2 °C sıcaklık, 60 ± 5% bağıl nem (RH) ve 16A:8K fotoperiyot koşullarında tutulmuştur. Elde edilen bulgular kadmiyuma maruz kalan larvaların toplam hemosit sayısının tüm konsantrasyonlarda azaldığını, kurşuna maruz kalanlarda ise toplam hemosit sayısının sadece en yüksek konsantrasyonda azaldığını göstermiştir.

Anahtar kelimeler: *Achroia Grisella, Hemolenf, Toplam Hemosit Sayısı*

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1. Introduction

Heavy metal pollution in air and water has been associated with various negative effects on insects (Borowska et al., 2004; El-Sheikh et al., 2010; Suganya et al., 2016). Borowska et al. (2004) showed that rearing *Musca domestica* (Diptera: Muscidae) larvae on an artificial media contaminated with copper (Cu), zinc (Zn), lead (Pb) and cadmium (Cd) resulted in their accumulation in the body and this accumulation disturbed the larval development and the survival rate of larvae and pupae. Suganya et al. (2016) studied the toxicity and antioxidant enzymes activity of *Spodoptera litura* (Lepidoptera: Noctuidae) larvae against heavy metal application. They observed that Cd and Pb metals are toxic on *S. litura* larvae and cause strong oxidative stres. In another study El-Sheikh et al. (2010) proved that the larval mortality percent of *Culex pipiens* L. (Diptera: Culicidae) larvae increased as the concentration of heavy metals namely; Cd, Cu, Pb and mercury (Hg) increased. In addition, Bischof (1995) reported that different types of metals significantly affects the biochemical composition of parasitized *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae.

Insect hemocytes are similar to vertebrate leukocytes and the efficiency of the immune system in insects is correlated with the number of hemocytes and their function (Berger and Slavičková, 2008; Borowska and Pyza, 2011). Their primary functions are coagulation, phagocytosis, encapsulation, detoxification, and storage and distribution of nutritive materials (Sanjayan et al., 1996; Eslin and Prévost, 1998; Siddiqui and Al-Khalifa, 2014). Previous studies showed that heavy metals and insecticides have a clear effect on hemocyte profile of insects (Borowska and Pyza, 2011, Kurt and Kayış, 2015).

Achroia grisella (L.) (Lepidoptera: Pyralidae) is a ubiquitous pest species of honey bee colonies globally. Larval stages of this moth species feed on wax, polen and honey, thereby they cause heavy economic damage in apiculture industry. Due to this reason, many researchers have focused on its biology, behaviour and control. *A. grisella* is also used as a model organism and system for insect physiology, genomic and proteomic investigations (Ellis et al., 2013; Mahgoup et al., 2015; Gleason et al., 2016; Çelik et al., 2017). The aim of this study was to determine the effect of Cd and Pb on total hemocyte number of *A. grisella*.

2. Material and Method

A laboratory stock culture of *A. grisella* has been maintained for several years on an artificial diet of honeycomb (200 g), bran (860 g), glycerol (300 ml), honey (150 ml) and distilled water (150 ml) at the Animal Physiology Research Laboratory, Ondokuz Mayıs university.

The effect of heavy metals on Total Hemocyte Count (THC) of *A. grisella* was tested through diet incorporation assay. *A. grisella* larvae were fed on an artificial diet contaminated with three different concentrations (50, 100 and 200 mg/kg) of Cd or Pb throughout all larval stages. In the control group the insects fed a heavy metal free diet. All of the insects were kept at 25 ± 2 °C temperature, 60 ± 5% relative humidity (RH) and 16L:8D photoperiod conditions. For analysis of THC of *A. grisella*, last instar larvae were pierced on the first hind leg with a sterile needle. Five µL of hemolymph was spread on a glass slide and allowed to air dry for 20-30 minutes to facilitate the adhesion of hemocytes to the glass. Cells were fixed in methanol:acetic acid (3:1) for 10 minutes. The slides were stained with Giemsa for 10 minutes and they were rapidly washed with distilled water. After air drying the slides were treated with xylene and then mounted in Entellan. THC were assayed under a Zeiss Primo Star microscobe. Ten larvae were evaluated for each experimental and control group. Direct microscobic somatic cell counting (DMSCC) method was used to determine THC of *A. grisella* larvae (Fitts and Laird, 2004; Kul, 2013). Twenty randomly selected area was used to count hemocytes for each slide. THC were obtained by multiplying the mean cell numbers by the microscope factor obtained by calculating the microscope sight field. Data in graphs were represented as mean ± standard error of the mean values. Statistical differences between the treated and control groups were determined by non-parametric Kruskal–Wallis H-test followed by Mann-Whitney U-test using SPSS 21.0. Differences were consider significant at $p \leq 0.05$.

3. Results

The effect of cadmium (Cd) on THC of *A. grisella* is presented in Figure 1. The results showed that treatment with Cd influenced the THC of *A. grisella*. The mean number of THC of Cd-treated larvae declined significantly at all tested concentrations compared to control ($p \leq 0.05$) (Figure 1). However, THC did not indicate a

considerable change among different concentrations ($p > 0.05$).

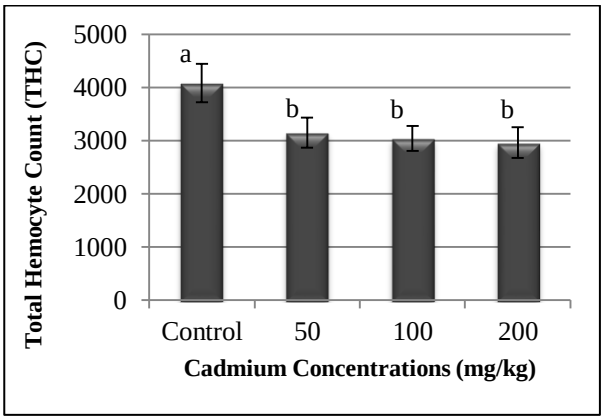

Figure 1. Effect of different concentrations of cadmium (Cd) on total hemocytes count of *A. grisella*. Different letters denote significant differences ($p \leq 0.05$).

Our results showed that THC of the lead (Pb)-treated groups also showed a tendency to decrease compared with the control (Figure 2). This decrease was only significant at 200 mg/kg compared to the control ($p \leq 0.05$).

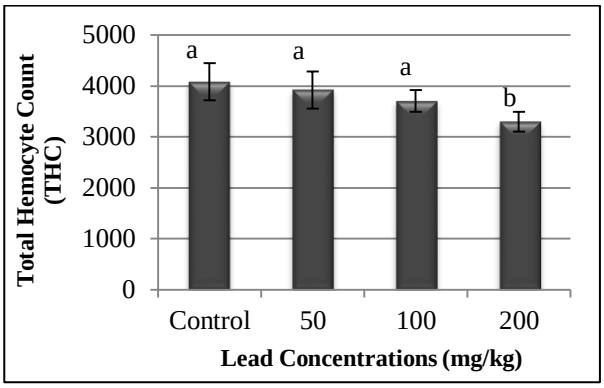

Figure 2. Effect of different concentrations of lead (Pb) on total hemocytes count of *A. grisella*. Different letters denote significant differences ($p \leq 0.05$).

4. Discussion

Invertebrates are good models to study toxicity of heavy metals and are useful bioindicators of contamination of the environment (Borowska and Pyza, 2011). Previous studies showed that toxicity of heavy metals on immune cells are related to the species, dose, the method of application, developmental stage of animals and animal's sensitivity (Kazimírová and Slovák, 1996; El-Sheikh et al., 2010; Suganya et al., 2016). Here, we tested the heavy metal toxicity at cellular levels and examined the lesser wax moth's hemocytes as an element of its immune system.

Our results showed that THC of *A. grisella* larvae significantly decreased at all treated Cd concentrations. The mean THC of Pb- treated groups also decreased but the differences were significant at only Pb 200 mg/1000 mg diet, as compared to control. These results are in agreement with those obtained by Borowska et al. (2004) where they reported that total number of circulating hemocytes and their adhesion ability significantly decreased with heavy metal application in *M. domestica*. These changes, interestingly, are similar to those produced by some of the insecticides and botanicals. For example, Sharma et al. (2003) observed a dose-dependent reduction in THC in the last instar larvae of *S. litura* after 48 h of oral treatment of Neem gold. Likely, Kurt and Kayış (2015) proved that deltamethrin treatment caused a decrease in the THC of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae at 24, 48 and 72 h. In contrast to our results, Çelik et al. (2017) found that indole-3-acetic acid application increased the THC in *A. grisella* larvae at all tested doses (2-1000 ppm). Likewise, Altuntaş et al. (2012) determined that gibberellic acid increased the THC in *G. mellonella* larvae.

There are some reports dealing with effects of heavy metals on endocrine system in insects. Endocrine system regulates the hemocyte populations and differentiations in insects (Prasada Rao et al., 1984; Ahmad and Khan, 1988; Sezer and Özalp, 2015). For instance, Sendi and Salehi (2010) showed that treatment with juvenile hormon analogue methoprene significantly decreased the THC in *Papilio domeleus* (Lepidoptera: Papilionidae). Similarly, Ahmad (1995) revealed that B-beta ecdysone and makisterone A (a phytoecdysone) treatment caused a decrease in THC of fifth instar nymphs of *Dysdercus cingulatus* (Hemiptera: Pyrrhocoridae). In addition, İzzetoglu and Karaçalı (2003) noted a significant adverse influence of 20- hydroxydysone on hemocytes of *G. mellonella* in vitro conditions. Sezer and Özalp (2015) also observed that pyriproxyfen treatment caused reduction in total hemocyte count of *G. mellonella*. Banakou and Dailianis (2010) reported that lipid peroxidation and DNA damage in Cd-treated hemocytes of molluscs ultimately contribute to cell death. These previous studies may help us to explain, at least, in part, the reason for decreasing hemocytes numbers in hemolymph of *A. grisella* with heavy metal application.

However, to complete our knowledge about heavy metals on the immune system of *A. grisella* more detailed studies are required.

This study was presented as a poster presentation at Ecology 2018 International Symposium held in Kastamonu and the abstract was published in the congress book.

Kaynaklar

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