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Influence of Gibberellic Acid on the Hemolymph Content of the Greater Wax Moth, *Galleria mellonella* (Lepidoptera: Pyralidae) Larvae

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ABSTRACT

The influence of the plant growth regulator gibberellic acid (GA_3) on the hemolymph contents of *Galleria mellonella* (Lepidoptera: Pyralidae) was examined. *G. mellonella* larvae were fed with a synthetic diet. The effects of GA_3 on the hemolymph content of these larvae were tested through a diet incorporation assay. Different concentrations (2, 5, 10, 50, 100, 200, 500 and 1000 mg/L) of GA_3 were added to the synthetic diet and protein, lipid, carbohydrate and glycogen levels in the hemolymph were evaluated for the GA_3 concentrations. The hemolymph protein level of the larvae increased significantly at 5, 10, 50 and 200 mg/L with respect to the control group. The lipid level of hemolymph fluctuated among the tested GA_3 concentrations. It was significantly reduced at 5 and 10 mg/L, but increased at 50 and 200 mg/L. GA_3 application also enhanced the carbohydrate level at 50, 100, 200 and 1000 mg/L and the glycogen content at 100 and 200 mg/L.

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Authors' Contribution
NEAG designed the research, reared the insects and wrote the manuscript. OO performed the experiments and analyzed the data.

Key words
Carbohydrate, *Galleria mellonella*, Gibberellic acid, Lipid, Protein.

INTRODUCTION

Galleria mellonella (L.) (Lepidoptera: Pyralidae), known as the greater wax moth or honeycomb moth, is a serious pest for the apiculture industry. Larvae of this moth species feed on the honeycomb, honey and wax found in bees nests (Jindra and Sehnal, 1989; Büyükgüzel and Kalender, 2009). The industry has traditionally relied on synthetic insecticides for the control of insect pests. However, because of the negative effect of this kind of insecticides on non-target organisms and on the environment in general, new environmentally safer alternative natural compounds are being encouraged (Hussein, 2005; Rehman et al., 2009; Ilyas et al., 2017). One alternative may be the use of plant growth regulators (PGRs) against pest species. Recently, many researchers have focused on the effects of various PGRs on herbivores (Abdellaoui *et al*., 2015). They showed that plant growth regulators may have a significant impact on the development, survival and reproduction of herbivores (Kaur and Rup, 2002; Tsagkarakis *et al*., 2012; Prado and Frank, 2013). As a consequence, some authors have even proposed the use of certain PGRs as successful chemosterilants against insect pests (Kaur and Rup, 2002; Paulson *et al*., 2005; Abdellaoui *et al*., 2013). The present study was undertaken to determine the influence of GA_3 on the hemolymph content of the greater wax moth, *G. mellonella*.

MATERIALS AND METHODS

A laboratory stock culture of *Galleria mellonella* has been maintained for several years on an artificial diet described by Bronskill (1961) and modified by Sak *et al*. (2006) at the Animal Physiology Research Laboratory, Ondokuz Mayıs Univesity. All insects were kept under continuously illuminated laboratory conditions, at a temperature of 25 ± 2 °C and a relative humidity level of 60 ± 5 %.

The effects of GA_3 on the hemolymph contents of *G. mellonella* were tested through diet incorporation assays. In the control experiment, the insects fed with a diet including honeycomb (200 g), bran (860 g), glycerol (300 ml), honey (150 ml) and distilled water (150 ml). GA_3 was dissolved in distilled water to prepare stock solution (1000 mg/L). Different GA_3 concentrations (2, 5, 10, 50, 100, 200, 500 and 1000 mg/L GA_3) were prepared from the stock solution and added to the diet of insects. 10-15 *G. mellonella* adults were placed into glass jars (500 ml) containing either a GA_3- supplemented or a GA_3- free diet. Hemolymph samples were collected with a glass microcapillary tube from late instar larvae of *G. mellonella*. The samples were transferred into microcentrifuge tubes

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containing 1-phenyl-2-thiourea and then centrifuged at 10.000 g for 5 min at 4 °C before analysis. To determine the effect of GA_3 on the hemolymph protein content of *G. mellonella*, a 1 µl hemolymph sample was transferred into a new microcentrifuge tube and 250 µl phosphate buffered saline (pH 7.4) was added to the sample. Each sample was centrifuged at 3500 rpm for 15 min at 4 °C. After centrifugation, 100 µl supernatant was taken and the protein content was quantified according to Lowry *et al*. (1951) method using a folin-phenol reagent. Absorbance at 695 nm was read and compared to bovine serum albumin standards.

Lipid, carbohydrate and glycogen levels of the hemolymph were measured using a series of biochemical tests previously used by different researchers on insects (Olson *et al*., 2000; Fadamiro and Heimpel, 2001; Lee *et al*., 2004). One µl hemolymph sample was placed into a microcentrifuge tube with 50 µl 2% sodium sulphate solution and 450 µl chloroform-methanol (1:2) was added. The tube was then placed in a centrifuge and spun at 14 000 g for 2 min. After centrifuging, 200 µl of supernatant was transferred into a glass test tube (10 × 50 mm) for the lipid assay. Another 200 µl was transferred to a similar glass tube for the carbohydrate assay. The precipitate resulting from the centrifugation was used for the glycogen analysis. All tubes were heated at 90 °C until all of the solution had evaporated from the lipid and glycogen tubes and approximately 50 µL of solution remained in the carbohydrate tubes. For the lipid assay, 40 µl of sulphuric acid was added and heated at 90 ºC for 2 min. Then 960 µl of vanillin-phosphoric acid reagent was added (van Handel, 1985b), mixed and kept at room temperature for 30 min. Absorbance was measured at 525 nm with a spectrophotometer and compared with lipid standards using corn oil.

For the carbohydrate assay, 950 µl of anthrone reagent was added to the second tube of supernatant, the mixture reacted for 10 min at 90 ºC (van Handel, 1985a) and then cooled. Absorbance was measured at 625 nm and compared with glucose standards. For the glycogen assay, we added 1 ml of anthrone reagent to the precipitate, vortexed the resulting solution and heated it at 90 ºC for 15 min. Absorbance was measured at 625 nm and compared with glucose standards.

Statistical analysis

All the bioassays were repeated three times and the results were represented as means ± standard errors (SE). Means were compared using one-way analysis of variance (ANOVA) and the Tukey-HSD test. SPSS software for Windows version 20 was used to perform statistical analysis. Results were considered statistically significant when $P \leq 0.05$.

RESULTS

Data on the hemolymph protein content of *G. mellonella* after treatment with different concentrations of GA_3 are shown in Figure 1A. GA_3 treatment generally resulted in an increase in the hemolymph protein content of larvae. This increase was significantly greater at 5, 10, 50 and 200 mg/L compared to the control ($F=10.017$, $df= 8, 171$ $P= 0.000$) (Fig. 1A).

Our results showed that lipid content of hemolymph fluctuated among GA_3-treated groups with a significant decline at 5 and 10 mg/L and a considerable increase at 50 and 200 mg/L, compared with untreated *G. mellonella* larvae ($F=22.465$, $df= 8, 171$ $P= 0.000$) (Fig. 1B).

As shown in Figure 1C, low concentration (< 50 mg/L) GA_3 applications did not make any significant change in carbohydrate content of hemolymph compared to the control. However, carbohydrate level significantly increased at higher concentration GA_3 applications (≥ 50 mg/L), except for 500 mg/ml, compared to the untreated group ($F=22.319$, $df= 8, 167$ $P= 0.000$).

The results of this study also indicated that the glycogen content of hemolymph showed some fluctuations between the control and some GA_3-treated groups (Fig. 1D). We determined a significant increase only at 100 and 200 mg/L with respect to the control ($F=25.020$, $df= 8, 151$ $P= 0.000$).

DISCUSSION

In this study we examined the influence of GA_3 on the hemolymph content of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), larvae. Incorporation of GA_3 into the diet generally resulted in an increase in the hemolymph protein content of larvae. Similarly, Uçkan et al. (2014) showed that indol-3 acetic acid (IAA) treatment yielded a significant increase in hemolymph protein levels of *Achoria grisella*. However, the effects of PGRs on insects can vary. For example, Abdellaoui et al. (2013), showed that the GA_3 caused a significant reduction in the hemolymph protein content of fifth instar larvae of *Locusta migratoria migratoria,* when applied topically or by forced ingestion. In another study, the same authors also reported a significant negative effect of GA_3 on ovarian protein content in *L. migratoria* females (Abdellaoui et al., 2015).

The results of present investigation indicated that GA_3 treatment made some fluctuations in lipid content of the hemolymph. However, a contradictory effect was reported for the same moth species by Uçkan et al. (2011). They showed that GA_3 treatment had the effect of lowering the hemolymph lipid content of *G. mellonella.* This could

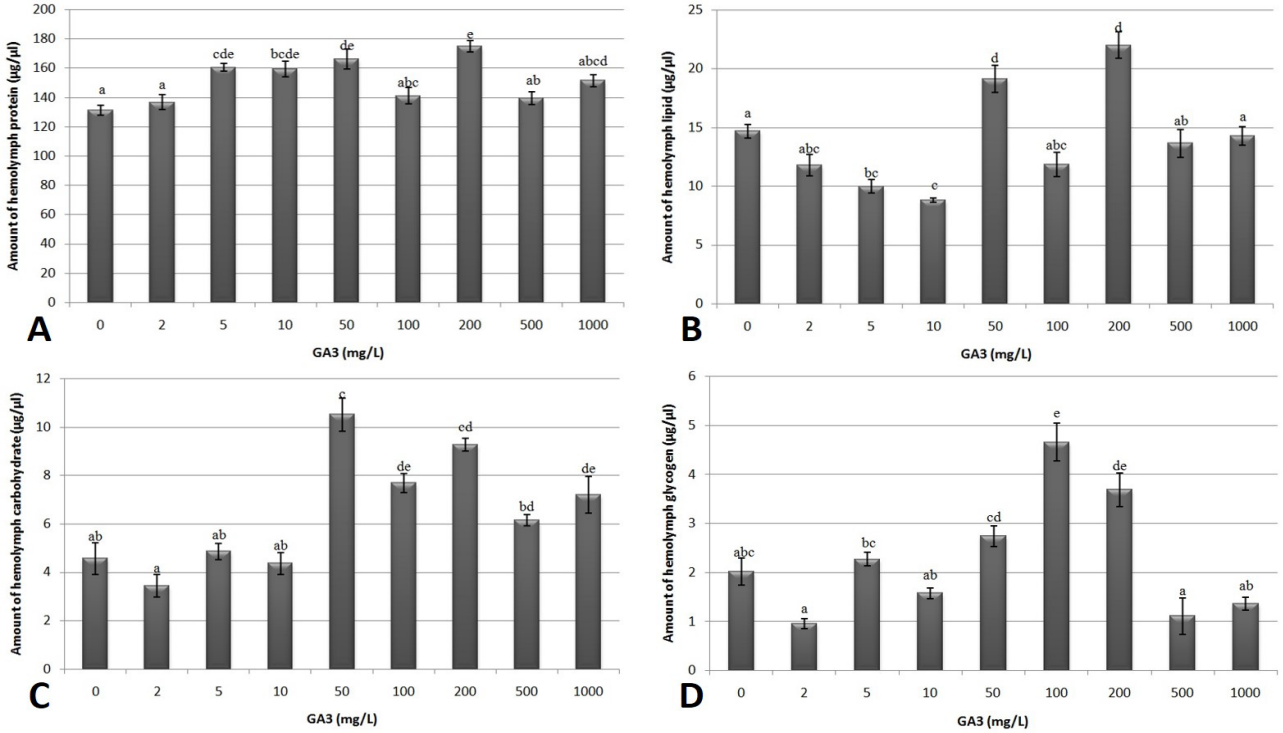

Fig. 1. Effects of different GA3 concentrations on the hemolymph protein (A), lipid (B), carbohydrate (C) and glycogen (D) levels of *Galleria mellonella*. Means and standard errors given, letters denote significant difference based on Tukey-HSD test (P≤0.05).

indicate that PGRs influence the biochemical composition of insects differently depending on a number of factors. In another study, Uçkan et al. (2014) also reported that exposure to indol-3 acetic acid reduced the total lipid levels of *A. grisella* at 5, 10, 200, 1000 ppm.

Our data demonstrated that hemolymph carbohydrate level was significantly increased at 50, 100, 200 and 1000 mg/L with respect to the control. On the contrary, Rup *et al*. (1998) showed that GA_3 treatment significantly reduced the carbohydrate content of *Zaprionus paravittiger* larvae at 1000 and 2000 ppm. Similarly, Rup *et al*. (2000) reported that GA_3, alarB-9, indole-3-butyric acid and chlorogenic acid decreased the carbohydrate content of second instar *Lipaphis erysimi* (Kalt.) (Homoptera: Aphididae) nymphs. Altuntaş (2015) also observed that ethepton treatment reduced the glucose amount in the hemolymph of last instars of *G. mellonella*.

We observed that there was also a significant increase in hemolymph glycogen content of larvae at 100 and 200 mg/L with respect to the control. These findings corresponded to the results obtained by Blattattacharya *et al*. (2011). The authors reported an increase in the glycogen content of the silkworm, *Bombyx mori,* when treated with indole-3-butyric acid (IBA) and indole-3-pyruvic acid (IPA). Similarly, an increase in the glycogen content of the fat body following the application of benzyl-6-aminopurine (BAP) and IAA has been reported in *B. mori* (Hugar and Kaliwal., 1998). In contrast, Rup *et al*. (1998) reported a significant negative effect of GA_3 on the glycogen level of *Z. paravittiger* larvae.

Interestingly, GA_3 treatment did not cause any significant difference in the hemolymph composition of larvae at 500 and 1000 mg/L with respect to the control. Only at 1000 mg/L carbohydrate content of hemolymph increased considerably. Abdellaoui *et al*. (2009a) showed that GA_3 significantly reduced the food consumption by *Spodoptera littoralis* and *L. migratoria migratoria* larvae. The same authors also showed that GA_3 affect the gravimetric index of *L. migratoria* fifth instar nymphs (Abdellaoui *et al*., 2009b). Since GA_3 derived from mevalonic acid similarly to a juvenile hormone (JH), Uçkan *et al*. (2011) also suggested that much of the ingested GA_3 may have been degraded or digested by similar esterases and hydrolases that target JHs and other terpenoids in the midgut of *G. mellonella*.

Thus, despite the insects show differences in sensitivity according to the method of treatment used and the concentration tested, our finding that there was a

statistically insignificant difference between the control and high concentration treatments might also be related to any of the reasons reported in these previous studies.

CONCLUSIONS

The results of this study reveal that GA_3 treatment influences the hemolymph composition of the greater wax moth, *G. mellonella*. Most of the authors explain this phenomenon in terms of the hormonal changes of the metabolic processes, because of the similar biochemical configurations of GA_3 and JH. Our findings are not conclusive as they agree with the observed data on different insects for various PGRs but disagree with others. Therefore, it may be concluded that there could be different toxicity sensitivies between different insects and PGRs. We recommend that further experimental studies should be carried out in future to determine the effect of GA_3 on the life history parameters of the greater wax moth *G. mellonella*.

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Statement of conflict of interest

Authors declare that they do not have any conflict of interest regarding this study.

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