Original article (Orijinal araştırma)

Influence of dietary indole-3-acetic acid on phenoloxidase and hemolytic activities in *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae) and *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) in a host-parasitoid system[1]

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Indole-3 asetik asit'in *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae) ve *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae)'nın konukçu-parazitoit sisteminde fenoloksidaz ve hemolitik aktivitelerine beslenme yoluyla etkileri

Abstract

The continuity of food demand has caused modern agriculture to be heavily dependent on chemical inputs. Plant growth regulators (PGRs) are naturally occurring or synthetic compounds that have the potential to control pest insects through their chemosterilant activity. Along with pests, non-target organisms such as parasitoids in agroecological systems are likely to be influenced by indirect contact via their hosts or direct contact with hosts and plants at the tritrophic level. This study demonstrated the dietary effects of PGR indole-3-acetic acid (IAA) on hemolytic activity and phenoloxidase activity in stored product pest *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) and parasitoid *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae). The significant increase in hemolytic activity in *G. mellonella* and *P. turionellae* hemolymph were observed in 500, 5,000 and 10,000 ppm IAA-treated groups. Phenoloxidase activity in *G. mellonella* hemolymph significantly decreased with all IAA doses compared to control, however, the reductions in phenoloxidase activity in *P. turionellae* hemolymph were significant between 500 and 10,000 ppm. Since IAA interacts with both host and parasitoid immunity, it should be used with caution in agricultural areas with high host-parasitoid population.

Keywords: *Galleria mellonella*, hemolytic activity, Indole-3-acetic acid, phenoloxidase activity, *Pimpla turionellae*

Öz

Gıda ihtiyacının sürekliliği, modern tarımın büyük ölçüde kimyasal girdilere bağımlı hale gelmesine neden olmuştur. Bitki büyüme düzenleyicileri, kemosterilant aktiviteye sahip doğal olarak oluşan veya sentetik bitki kaynaklı kimyasallardır ve zararlı böcekleri baskılama potansiyeline sahiptir. Zararlılarla birlikte, agroekolojik sistemlerdeki parazitoitler gibi hedef olmayan organizmaların, konukçuları aracılığıyla dolaylı temastan veya konukçu ve bitkilerle tritrofik etkileşim yoluyla doğrudan temastan etkilenmesi muhtemeldir. Bu çalışma bitki büyüme düzenleyicisi indol-3-asetik asidin (IAA) depolanmış ürün zararlısı *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) ve parazitoit *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae)'nın hemolitik aktivitesi ve fenoloksidaz aktivitesi üzerindeki besinsel etkilerini göstermektedir. *Galleria mellonella* ve *P. turionellae* hemolenfinin hemolitik aktivitelerinde istatistiksel olarak önemli artışlar 500, 5000 ve 10000 ppm IAA dozları tatbik edilen gruplarda gözlenmiştir. *Galleria mellonella* hemolenfinin fenoloksidaz aktivitesi, kontrole kıyasla tüm IAA uygulanan dozlarda önemli ölçüde azalırken, *P. turionellae* hemolenf fenoloksidaz aktivitesindeki azalmalar 500 ve 10000 ppm arasında anlamlı bulundu. IAA, hem konukçu hem de parazitoit bağışıklığı ile etkileşime girdiğinden, konukçu-parazitoit popülasyonu yüksek olan tarım alanlarında dikkatle kullanılmalıdır.

Anahtar sözcükler: *Galleria mellonella*, hemolitik aktivite, Indole-3-asetik asit, fenoloksidaz aktivite, *Pimpla turionellae*

Introduction

The term chemical control in agricultural systems covers a wide range of substances including herbicides, insecticides, rodenticides, fungicides, plant growth regulators and others. The indiscriminate uses of chemical compounds against insect pests possess hazards to non-target organisms due to the accumulation of chemicals in nature and their toxic effects on the environment (Brzozowski & Mazourek, 2018). The repeated applications of insecticides lead to the transmission of genes encoding rapid biochemical detoxification processes on the succeeding generations. Eventually, the agricultural pest populations can become totally immune and resistant, and not controlled effectively. The problems caused by chemical control increase the importance of biological control. Among the agents used in biological control, parasitoids are perhaps the most suitable, least risky and most effective (Wang et al., 2019). However, during chemical control, these non-target beneficial insect species with parasitoid character are also directly or indirectly exposed to various chemicals. Concerns about chemicals in the natural environment and their possible ecotoxic risks have focused more on pesticides. In addition to pesticides, other chemicals to which parasitoids are indirectly and / or directly exposed are plant growth regulators (PGRs) (Uçkan et al., 2008, 2011a, b). Many authors have reported that PGRs have the potential to diminish pest insect populations by influencing reproduction, development, fecundity and egg viability (Er & Keskin, 2016; Zhao et al., 2017), to effect biochemical and histological parameters (Abdellaoui et al., 2013), and induce oxidative stress (Altuntaş, 2015). Indole-3-acetic acid (IAA) is such a commercial PGR and also a common endogenous auxin class synthesized from an amino acid tryptophan (Ahmad et al., 2005). IAA is also known as a universal signal molecule existing in a large range of organisms such as bacteria, fungus, plants and animals, including mammals (Lins et al., 2006; Kaya et al., 2021b). The various commercial and exogenous uses of IAA in various crops and its physiological impacts on plants can negatively affect plant-insect interactions (Prado & Frank, 2013). These compounds are used not only to altered agronomic characteristics in plant growth processes, but also as chemosterilants for harmful insects (Abdellaoui et al., 2013; Özyılmaz et al., 2019). Despite their widespread use in agriculture, limited numbers of studies are included in literature and still more work needs to be done concerning their side-effects on non-target parasitoids, pest insects and their interactions in tritrophic contexts (Prado & Frank, 2013). Detrimental effects of IAA on insect survival, metabolism and reproduction, life table and some biochemical parameters have been particularly demonstrated in different agricultural pests (Uçkan et al., 2011a, 2014, 2015). IAA has shown to reduce longevity and increase larval development time of the biocontrol agent endoparasitoid *Apanteles galleriae* Wilkinson, 1932 (Hymenoptera: Braconidae) reared on its host the lesser wax moth *Achroia grisella* (Fabricius, 1794) (Lepidoptera: Pyralidae) (Uçkan et al., 2011a). Dose-responsive influences of IAA on total protein, lipid, and glycogen levels on *A. galleria* were also demonstrated (Uçkan et al., 2014). Adverse effects of plant growth regulators on life table parameters and biochemical markers in a host-parasitoid interaction suggest that PGRs have the potential to influence the functioning of immune system in pest insects and their parasitoids. In addition, recent studies showed elevated total hemocyte counts in *A. grisella* (Çelik et al., 2017) and *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) (Kaya et al., 2021b) due to IAA application interrelated with the increased granulocyte incidences, but no changes in the parasitoid *Pimpla turionellae* L., 1758 (Hymenoptera: Ichneumonidae) hemocyte counts that are the main components of the insect cellular immunity (Kaya et al., 2021b).

Insect innate immune system relies on humoral and cellular components that function interactively in order to provide a response for pathogen infections and foreign materials such as parasitoid eggs (Strand, 2008). Cellular components consist of hemocytes that are analogous of macrophages in vertebrates having multiple functions such as nodulation, encapsulation, and phagocytosis (Wojda, 2017). While humoral immunity entails enzymatic cascades, which orchestrate coagulation and melanization reactions (Gillespie et al., 1997), synthesis of antimicrobial peptides, intermediates of reactive oxygen and nitrogen, and lipid peroxidation (Altuntaş et al., 2021). Among them one important enzyme in insect

immunity is phenoloxidase (PO) that is responsible for the conversion of phenols to quinones which subsequently turn into melanin form against pathogens and parasitoid eggs (Eleftherianos & Revenis, 2011). In the process melanogenesis both intermediary products such as superoxide, quinones, diphenols, hydrogen peroxide and PO have unique roles in humoral immunity (Santoyo & Aguilar, 2011). Several studies have demonstrated that cellular immunity of insects and the survival ability under stress conditions are susceptible to different environmental factors such as botanical sourced xenobiotics (Zibaee & Bandani, 2010; James & Xu, 2012; Altuntaş et al., 2021). However, our knowledge on the effects of plant-derived compounds concerning the PO enzyme, which is an important component of both cellular and humoral immunity, is mostly dependent on plant extracts or essential oils. Zibaee & Bandani (2010) demonstrated the negative effect of *Artemisia annua* L. (Asterales: Asteraceae) extract on nodule formation and PO activity in *Eurygaster integriceps* Puton, 1881 (Hemiptera: Scutelleridae) (Zibaee & Bandani, 2010) whereas Kaya et al. (2021b) showed an increase in PO activity in *G. mellonella* due to injection with *Helichrysum arenarium* L. (Asterales: Asteraceae) extract. A dose-dependent increase was also observed in PO enzyme activity in non-mulberry silkworm *Antheraea assamensis* Helfer, 1837 (Lepidoptera: Saturniidae) with topical application of essential oils obtained from *Ocimum sanctum* L. (Lamiales: Lamiaceae), *Ocimum gratissimum* L. (Lamiales: Lamiaceae) and *Ageratum conyzoides* L. (Asterales: Asteraceae). However, we could not find any report demonstrating the effects of a plant growth regulator IAA on PO activity in *G. mellonella* and its parasitoid *P. turionellae*. Another parameter thought to be related to humoral immunity is hemolytic activity which also represents the common initial toxicity assessment (Greco et al., 2020). Several studies demonstrated the hemolytic activity in hemolymph in many invertebrate groups including *G. mellonella* (Phipps et al., 1989). Also, fluctuations of hemolytic activity paralleled the pattern of antimicrobial activity (Phipps et al., 1989) that is one of the main characteristics of insect immunity. Nevertheless, to our knowledge, there are no reports on the effects of plant growth regulators on hemolytic activity in insect hemolymph.

Galleria mellonella is a ubiquitous pest of honeycombs and honey bee colonies and also used as a powerful model organism to test the ecotoxicological, immune and physiological effects of environmental pollutants (Altuntaş et al., 2021). Endoparasitoid *P. turionellae* uses many agricultural pests that feed on plants for oviposition and it is probable that its larvae developing inside the host may be exposed to agricultural chemicals that accumulates in hosts and adult parasitoids by feeding on honey and fruit nectar. This kind of tritrophic interaction between plants, pest insects and their natural enemies are an integral part of agricultural ecosystems. Understanding the tritrophic interactions is essential for monitoring the roles of parasitoid insects in biological control and IPM programs. IAA is a universal signal molecule and also a widely used plant growth regulator in agricultural systems. Therefore, it is most likely that parasitoid *P. turionellae* may be exposed to IAA via host insects or plants. To further clarify the immune modulator effects of IAA, we studied PO and hemolytic activities in insect hemolymph as indicators of immune function in the model host-parasitoid system of *G. mellonella-P. turionellae*.

Materials and Methods

Insects

Model organism *G. mellonella* were reared at 25 ± 5ºC, 60 ± 5% RH and 12:12 h L:D photoperiod conditions at Kocaeli University Animal Physiology Laboratory. *Galleria mellonella* larva were fed with artificial Bronskill (1961) diet. Parasitoid *P. turionellae* cultures were reared under the same laboratory conditions with their host *G. mellonella*. Individuals of the parasitoids were cultured by parasitizing *G. mellonella* pupae. Obtained *P. turionellae* adults were fed with a 30% honey solution diluted with water daily and kept in wire cages of 20 × 20 × 20 cm. Pupal hemolymph of *G. mellonella* was also provided to parasitoid adults twice a week for obtaining protein by hemolymph feeding.

Experimental groups

IAA (Merck 10 g, Darmstadt, Germany) doses (0, 50, 500, 1,000, 5,000 and 10,000 ppm) were selected according to Kaya et al. (2021b) and used in all experimental analyses. Newly hatched *G. mellonella* larvae were fed with 10 g artificial diet including different doses of IAA. Control experiments included *G. mellonella* larvae fed with diet containing distilled water instead of IAA. Larval feeding process was repeated until the host insects reached last instars. For each control and experimental assay 10 last instar larvae were used in three replicates (n = 30). To determine the influence of IAA on parasitoid hemolytic and phenoloxidase activity last instar IAA-fed host larvae were pupated and provided to parasitoid *P. turionellae* as host for oviposition in three replicates. Parasitization was conducted on day 2 of the IAA-fed *G. mellonella* pupae by exposing one individual pupa to an 15-20-d-old *P. turionellae* female previously determined to lay the highest number of eggs in this period (Şeker & Yanıkoğlu, 1999).

Sampling hemolymph

Ten μl of hemolymph from IAA-fed *G. mellonella* last instar larvae was sampled from the first abdominal proleg. Each larva was pierced on the abdominal proleg with a sterile microneedle and the drawn hemolymph was collected with the aid of a scaled microcapillary tube (Sigma-Aldrich, St Louis, MO, USA). In terms of *P. turionellae*, larvae collected from parasitized host pupae 8 days post-parasitization that is large enough to obtain clear hemolymph (Kaya et al., 2021b) and hemolymph collected with a scaled microcapillary tube. Hemolymph collected from 10 individuals were pooled in an ice-cold 1.5 ml eppendorf tube containing 1 μg N-phenlythiourea (Sigma) and stored at -80 ºC for hemolytic activity.

In parallel set of experiments, 100 μl hemolymph obtained by the same method from 10 individual IAA-fed last instars and *P. turionellae* larval hemolymph dissected from parasitized host pupae were transferred to ice-cold 1.5 ml eppendorf tubes containing 1 μg N-phenlythiourea (Sigma) and used immediately for phenoloxidase enzyme activities without freezing in three replicates.

Assaying hemolytic activity

The rat has been sedated with xylene from the femur head and its cardiac environment was sterilized with alcohol and 3 ml of blood was taken from the heart by intracardiac puncture method using a 21 gauge cannula. The blood was then mixed with sterile Alsever solution at a ratio of 1:5 in a 30-ml Falcon tube and stored at +4ºC (Guzman et al., 1993) (Kocaeli University ethics committee decision no: KOÜ HADYEK 8/2-2013).

Cells were subsequently centrifuged in a salt solution (NaCl 0.15 M) and TBS-Ca (Tris buffered saline: 50 mM Tris-HCl, 100 mM NaCl, pH 7.5 with 10 mM $CaCl_2$) twice at 800 g and 10°C for 10 min and then washed. Finally, erythrocytes were resuspended in 8% (v/v) TBS-Ca. Hemolytic activity was measured by taking 850 μl of TBS-Ca, 100 μl of 8% rat erythrocytes and 50 μl of hemolymph sample. This mixture was incubated at 25°C for 30 min and microfuge for 30 s. The resulting hemoglobin was measured as an end point in the ELISA plate reader at a wavelength of 540 nm. TBS-Ca buffer was used as control. Full hemolysis was achieved by mixing 900 μl of ammonium chloride (0.15 M) with 100 μl of rat erythrocytes (8%). The resulting result gave the percentage of hemolysis (Guzman et al., 1993).

Assaying phenoloxidase activity

Hemolymph from 10 final instars *G. mellonella* larvae and 5 parasitoid larvae was collected for every independent experiment. Eight μl of pooled hemolymph was transferred into 800 of PBS. 100 μl hemolymph PBS mixture from both control and experimental groups was added into a 96-well microplate. 100 μl of 20 mM dihydroxy-L-phenylalanine (L-DOPA, Sigma-Aldrich) dissolved in phosphate buffer solution was added on the hemolymph samples and incubated for 30 min at 25°C. After the incubation, the microplate was read in ELISA plate reader at 490 nm absorbance at intervals of 1 min from 0 to 30 min. PBS was used instead of hemolymph in controls. The data obtained for each subject was determined as U/mg protein. The protein concentration was assayed using the method of Bradford (1976).

Statistical analyses

The obtained experimental results were compared with one-way ANOVA in the SPSS statistical program (SPSS, 2018). Differences between means were evaluated with Tukey HSD. Percentage data was normalized by arcsine transformation before analyses. The results were statistically significant at the $P < 0.05$.

Results

Effects of IAA on hemolytic activities in *G. mellonella* and *P. turionellae* hemolymph

Results obtained from the hemolytic activity assays of *G. mellonella* hemolymph demonstrate that the mean percentage of hemolytic activity was 22 ± 1.6 in control groups. An increase in hemolytic activity was observed in all IAA-treated groups. However, the elevations were only significant in 500, 5,000 and 10,000 ppm compared to control (F = 92.0; df = 5,24; p = 0.00) (Figure 1). In addition, comparisons between doses revealed that 5,000 ppm was more effective than any other dose.
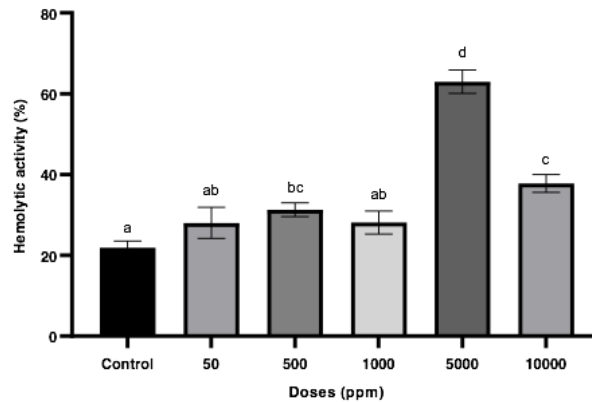

Figure 1. IAA-induced effects on hemolytic activity in *G. mellonella* larval hemolymph. Means with the same letters are not significantly different (one-way ANOVA followed Tukey's HSD, P < 0.05).

The hemolytic activity obtained from untreated hemolymph samples of *P. turionellae* displayed 13 ± 3.3. Treatment of different concentrations of IAA resulted in a remarkable increase in hemolytic activity in *P. turionellae* hemolymph (Figure 2). The increase in 500, 5,000 and 10,000 ppm was statistically significant (F = 86.5; df = 5,9; p = 0.00). Maximum hemolytic activity was observed at 5,000 ppm.
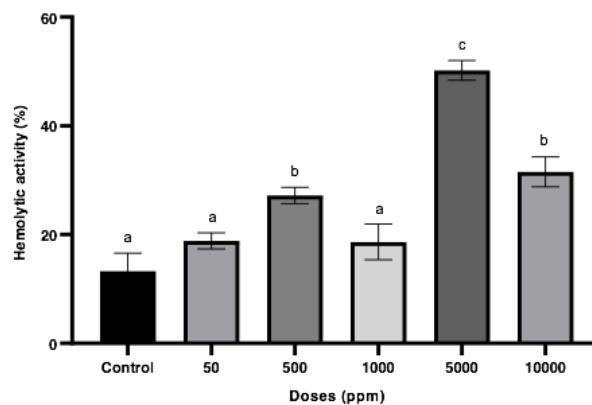

Figure 2. IAA-induced changes on hemolytic activity in *P. turionellae* larval hemolymph. Columns with the same letters do not differ significantly (one-way ANOVA followed Tukey's HSD, P < 0.05).

Effects of IAA on phenoloxidase activity in *G. mellonella* and *P. turionellae* hemolymph

The effects of IAA treatment on phenoloxidase enzyme activity in *G. mellonella* hemolymph is shown in Figure 3. Phenoloxidase activity in hemolymph significantly decreased in all IAA-treated doses compared to control (F = 224; df = 5,24; p = 0.00). Among the experimental groups, the lowest phenoloxidase activity was 0.223 u/mg protein at 1,000 ppm (Figure 3).
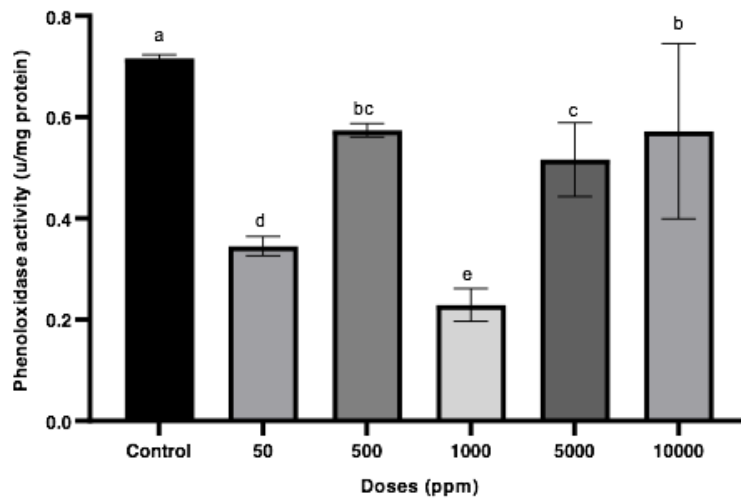

Figure 3. IAA-induced changes on phenoloxidase activity in *G. mellonella* larval hemolymph. Columns with the same letters do not differ significantly (one-way ANOVA followed Tukey's HSD, P < 0.05).

Phenoloxidase activity in *P. turionellae* hemolymph treated with different doses of IAA is shown in Figure 4. Phenoloxidase activity in the hemolymph of parasitoid *P. turionellae* decreased with IAA doses more than 50 ppm. However, the reductions were only statistically significant in 500 and 10,000 ppm compared to control (F = 25.0; df = 5,9; p = 0.00). The lowest phenoloxidase activity was determined as 0.04 u/mg at 500 ppm IAA treatment.
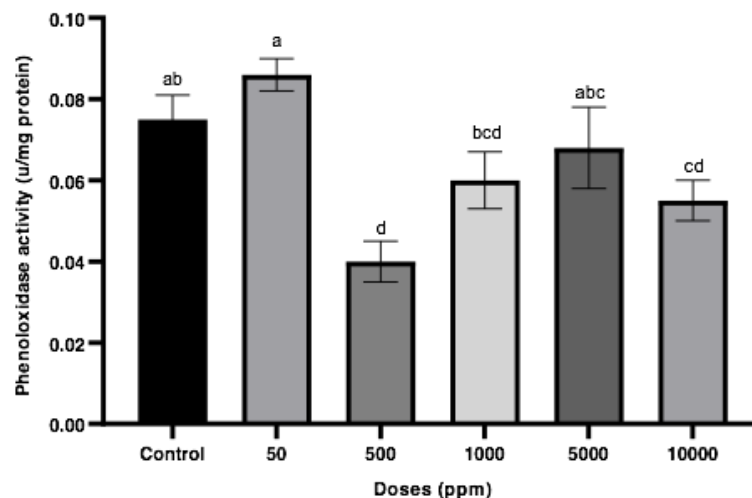

Figure 4. IAA-induced changes on phenoloxidase activity in *P. turionellae* larval hemolymph. Columns with the same letters do not differ significantly (one-way ANOVA followed Tukey's HSD, P < 0.05).

Discussion

Recent studies have focused on providing new strategies that can be an alternative to intensive insecticide use in the effective suppression of agricultural pests in agroecological systems. Literature in the last four decades (Ahmad et al., 2003; Ghoneim, 2018) demonstrated that plant growth regulators can be used as an alternative of chemical insecticides that have detrimental effects on human health, environment and non-target organisms. Plant growth regulators are now considered as candidate plant-derived bioinsecticides that have potential for control of pest insects by affecting reproductive potential, hormonal balance, food metabolism and other physiological processes (Mu et al., 2003; Abdellaoui et al., 2013; Özyılmaz, 2019; Kaya et al., 2021a). Along with pest insects, other non-target organisms, such as parasitoids, are likely to be influenced by indirect contact via their hosts or direct contact with plants (Zhao et al., 2017; Kaya et al., 2021a). The present study focused on indirect effects of IAA on hemolytic and phenoloxidase activity in *G. mellonella* and its parasitoid *P. turionellae* developed inside IAA ingested host model.

Data from our experiments showed that nutrient-mediated IAA application caused a dose-dependent increase in hemolytic activity in *G. mellonella* hemolymph and the elevation was significant at 500, 5,000 and 10,000 ppm compared to the control group. Although an increase was observed at 5,000 ppm, there was a sharp decrease in the hemolytic activity in the larval *G. mellonella* at 10,000 ppm. It was discussed in a previous study that high doses of a common plant growth regulator GA_3 might exceed the ability of cellular defense system to response and the toxicological influences of PGRs are variable depending on concentrations and physiological repair mechanisms (Altuntaş, 2015). In combination with previous studies, we can conclude that reduction of this immune response at 10,000 ppm may be associated with a physiological adaptation to compensate for IAA-induced stress. Similar results were obtained in the hemolytic activity in *P. turionellae* final instar larval hemolymph exposed indirectly to IAA via its host on rat erythrocytes. In a previous study it was demonstrated that hemolytic activity in arthropod hemolymph was increased by microbial infection or parasitization associated with insect immunity by lysing cells of invading organisms (Wang et al., 2015). IAA-induced elevated hemolytic activity in *G. mellonella* and *P. turionellae* in our study demonstrates that both the host and the parasitoid contain hemolysin to perform immune function and the expression of hemolytic genes might be affected by IAA. However, it is unlikely that the increased hemolytic activity in both the host and the parasitoid was part of the PO system because, despite the elevated hemolytic activity, we observed reduced PO activity in *G. mellonella* and *P. turionellae* hemolymph. These results indicate that the hemolytic activity is not associated with hemocytes of both insects and further studies are necessary to elucidate the specific hemolysis mechanisms. The cell-free hemolytic activity in *G. mellonella* hemolymph was detected in a previous study and the hemolysin protein of *G. mellonella* was shown to be larger than hemolysins of other invertebrates (Phipps et al., 1989). In the same study conducted on erythrocytes taken from three experimental animals (rabbit, guinea pig and sheep), it was determined that hemolytic activity increased if the host hemolymph was infected with a bacterial species, *Pseudomonas aeruginosa* S. (Pseudomonadales: Pseudomonadaceae) (Phipps et al., 1989). Sasaki et al. (2010) found that the hemolymph of the mosquito *Armigeres subalbatus* (Coquillett, 1898) (Diptera: Culicidae) induced hemolytic activity against human red blood cells and lectin was responsible for the hemolysis of the cells. Even though hemolysis represents one of the most commonly performed toxicity tests (Greco et al., 2020), we could not find any report on hemolytic activity of a plant growth regulator on an agricultural pest and their parasitoids. However, a few studies have demonstrated the adverse effects of IAA on life history traits, immune functions and antioxidant systems of the endoparasitoid *P. turionellae* (Uçkan et al., 2008, 2011a; Kaya et al., 2021b). In addition to the existing studies the present study demonstrates the elevated levels of hemolytic activity in the host depending on IAA application that will damage the parasitoid develop inside the host. These adverse effects will reduce the effectiveness of parasitoids in sustainable agriculture by disrupting the host-parasitoid relationship.

In insects, the phenoloxidase cascade is important for the melanization process (Pech & Strand, 2000) and also an important indicator of immune stimulation. Numerous studies reported the effects of plant growth regulators on cellular immunity of various insect pests (Çelik et al., 2017; Kaya et al., 2021b). However, to our knowledge none of these studies discussed the effects of IAA on phenoloxidase activity in an insect pest and its parasitoid. The obtained data in this study demonstrates that IAA decreased the phenoloxidase activity in both the parasitoid *P. turionellae* and host *G. mellonella*. At the lowest dose of 50 ppm IAA caused nearly 50% reduction of PO activity in *G. mellonella* hemolymph compared to control, however, at higher doses the reductions were more moderate except for 1,000 ppm. Our results are consistent with a previous study demonstrating the sharp reduction in melanization response of *G. mellonella* due to 50 ppm IAA application (Kaya et al., 2021b). In the same study IAA-related elevations of total hemocyte counts was found to be quite remarkable at 50 ppm compared to other doses. Since hemocytes are the predominant source of PO activity in insects, the increase in hemocyte count at 50 ppm in the previous study (Kaya et al., 2021b) seems to be the reason for the increase in PO activity in the current study at the same dose of IAA. Although, IAA was strongly immunosuppressive at 50 ppm on the host insect it had an opposite effect on the parasitoid PO activity at 50 ppm. We suggest a hormetic-like effect triggered by low dose stimulation of toxic materials on the parasitoid *P. turionellae* (Kefford et al., 2008). Low dose stimulation effect of IAA seems advantageous for successful parasitization, however, this is not the case at higher doses. In a previous work it was determined that insect growth regulators buprofezin and pyriproxyfen reduced the phenoloxidase activity in *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) (Nasr et al., 2010). Another report demonstrated the oxidative stress induced by IAA on *G. mellonella* and the parasitoid *P. turionellae* larvae (Özyılmaz et al., 2019). It is obvious that along with pest insects, non-target biological control agents are likely to be affected indirectly from IAA by trophic alteration.

Phenoloxidases are expressed in insects as inactive proPOs and are converted to active PO when required (Santoyo & Aguilar, 2011). PO activation is triggered by a serine protease cascade that is highly dependent on Ca^{2+} concentration or pH, which is somewhat analogous to the coagulation pathway and complement system in human plasma (An et al., 2013). In a previous study, it was demonstrated that IAA can strongly inhibit human blood coagulation with antithrombotic and antiplatelet activities with reduced intracellular Ca^{2+} (Lee et al., 2016). This study showing the IAA-inhibiting effects of coagulation pathway is in line with our study. It is possible that IAA suppresses PO activity in insects by inhibiting the serine proteases and coagulation pathway that is analogous to human plasma. Non-target effects of IAA on developmental biology such as parasitism abilities and rates, emergence rates, immune parameters, and antioxidant defense system were discussed before (Uçkan et al., 2011a, 2014, 2015; Çelik et al., 2017; Zhao et al., 2017; Özyılmaz et al., 2019). These adverse effects on parasitoid insects may be the result of suppressed phenoloxidase activity that is one of the main characters of humoral immunity. Combined with the previous studies demonstrating the adverse effects we can conclude that IAA induced changes on hemolytic and phenoloxidase activities in host and parasitoid insects could be a potential risk on host-parasitoid interactions in biologic control programs.

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