SHORT COMMUNICATION

Indole-3-Acetic Acid induced oxidative stress in model host Galleria mellonella L. (Lepidoptera: Pyralidae) and its endoparasitoid Pimpla turionellae (L.) (Hymenoptera: Ichneumonidae)

D Özyılmaz¹, R Özbekeit,³ H Altuntaş², F Uçkan¹

¹Department of Biology, Faculty of Science and Literature, Kocaei University, Kocaei, 41380, Turkey
²Department of Biology, Faculty of Science, Eskeşehir Technical University, Eskeşehir, 26470, Turkey
³Current Institution: Fraunhofer Institute for Molecular Biology and Applied Ecology, Department of Bioresources, Giessen, 35394, Germany

Abstract

Investigation of the antioxidant and oxidative effects of dietary indole-3-acetic acid (IAA), a plant growth regulator, on pest Galleria mellonella L. (Lepidoptera: Pyralidae) and its endoparasitoid Pimpla turionellae (L.) (Hymenoptera: Ichneumonidae) was aimed in this study. Different doses of dietary IAA (50-10,000 ppm) caused an increase in lipid peroxidation in the hemolymph of the host, G. mellonella (L.) and its endoparasitoid P. turionellae (L.). When compared to the control, higher doses of dietary IAA decreased CAT, SOD and GST enzymes’ activities in G. mellonella. At higher IAA doses, the activity of SOD enzyme in the hemolymph of P. turionellae significantly decreased while CAT enzyme activity showed no significant change when compared to the control. Additionally, GST activity in the endoparasitoid larval hemolymph significantly increased at 500 and 1000 ppm IAA doses. These findings indicate that incorporating IAA in the diet of model host G. mellonella larvae leads to oxidative stress and, also negatively affects the survivability of both the host and its endoparasitoid.

Key Words: Pimpla turionellae; Galleria mellonella; indole-3-acetic acid; host-parasitoid interaction; oxidative stress

Introduction

Auxins are plant growth regulators (PGRs) that are involved in many developmental processes, including cell division and enlargement, root initiation, vascular tissue differentiation and flowering (Davies, 2010). Indole-3-acetic acid (IAA) is also one of the important natural auxins in most plants (Davies, 2010). Synthetic IAA products are used widely in agricultural processes like plant growth and development in order to increase productivity (Kumar et al., 2001). Because of the wide usage of these indolic compounds as phytohormones or PGRs in the environment, non-target organisms such as biological control agents could be affected negatively. Several previous studies reported that IAA caused adverse effects on survival, longevity, developmental time, hemocytes studies and hemolymph metabolites of various Lepidopteran pest species (Rup et al., 2002; Kaur and Rup, 2003; Uçkan et al., 2011a; Uçkan et al., 2014; Uçkan et al., 2015; Çelik et al., 2017). Various authors also suggested that PGRs such as gibberellic acid (GAs), ethephon (ETF) and IAA could be used instead of insecticides to control lepidopteran pests in Integrated Pest Management programs (Uçkan et al. 2011b; 2014; Altuntaş et al., 2012; Altuntaş, 2015a). On the other hand, Uçkan et al. (2011a) reported that IAA adversely affects the life history traits of the endoparasitoid Apanteles galleriae (Hymenoptera: Braconidae), an important natural enemy of G. mellonella. It is possible that these observed biological effects of IAA on various pests and parasitoid species could be associated with their immune responses. Antioxidant systems and immune mechanisms in insects play an important role in the detoxification of several organic and inorganic environmental pollutants (Felton, 1995). Previously, researchers also showed that dietary PGRs, GAs and ETF, at sublethal doses caused adverse impacts on various antioxidant enzymes and led to oxidative stress in model insect G. mellonella (Altuntas, 2015b; Altuntas et al., 2016). For these reasons, the adverse biological effects of IAA on insects
might be related to their physiological antioxidant capacities. In order to provide information about toxic modes of action of IAA on target and non-target insects, we used the model host (greater wax moth) G. mellonella L. (Lepidoptera: Pyralidae) and idobiont, solitary, pupal endoparasitoid P. turionellae L. (Hymenoptera: Ichneumonidae).

It is well known that G. mellonella larvae are used as a model insect in ecotoxicological and ecophysiological investigations or model host for several parasitoid species in biological control programs because their culture rearing conditions are economic, easy and faster in the laboratory (Altuntaş et al., 2016; Kwadha et al., 2017). Therefore, the effects of various doses (50-10,000 ppm) of dietary IAA on activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferases (GSTs) and also lipid peroxidation level in host Galleria mellonella and its pupal endoparasitoid Pimpla turionellae larvae were investigated for the first time in this study.

Materials and Methods

Insect rearing

The laboratory colonies of the model host Galleria mellonella and pupal endoparasitoid Pimpla turionellae were reared at 25 ± 5 °C, 60 ± 5 % RH, and with a photoperiod of 12: 12 (L: D) in Kocaeli University, Turkey as described before (Uçkan et al., 2015). First instar larvae of the host were maintained by feeding artificial diet as described by Altuntaş et al. (2016). The last instar larvae of G. mellonella were collected and put in jars including folder paper to facilitate pupation. Then, host pupae were used for parasitization by female adult P. turionellae. Adult parasitoids were also reared on 50% (wt: vol) honey solution in cages (25 x 25 x 25 cm).

Bioassays

Selected doses (0, 50, 500, 1,000, 5,000, and 10,000 ppm) of IAA (Merck 10 g, Darmstadt, Germany) were used in all experimental analyses. All doses of IAA were prepared in distilled water and homogenized with an artificial diet (Altuntaş et al., 2016). To determine the effects of IAA on the antioxidant enzyme activity in G. mellonella and its parasitoid P. turionellae, newly hatched G. mellonella larvae were exposed to 5 g of host artificial diet including the selected doses of IAA. In parallel experiments, larvae were fed with an artificial diet containing distilled water; these were treated as the control group. Thus, for each experimental and control assay, eight last instars G. mellonella larvae (0.25 – 0.30 mg) were used in three replicates (n = 24). In addition, selected last stage G. mellonella larvae treated or untreated with IAA doses were used pupated and provided as host to P. turionellae for parasitoid experiments. Therefore, in each experimental analysis, eight 6th old P. turionellae larvae (almost 8 d after parasitization) were used in three replicates (n = 24 larvae).

Hemolymph collection and storage

Hemolymph samples were collected from the last stage G. mellonella and P. turionellae larvae for experimental analyses. Each larva was pierced on the second foreleg with a sterile microneedle and the was hemolymph collected via a 10 μL glass microcapillary tube (Sigma, St. Louis, MO). Ten microliters of hemolymph were collected from each of the eight larvae and immediately transferred into the same two mL eppendorf tube containing 0.001 mg 1-phenyl-2-thiourea in order to avoid hemocyte aggregation and melanization. During hemolymph collection, collection tubes were kept on ice and then stored at -80 °C until enzyme activity assays. On the same day of assays, samples were centrifuged at 7000 rpm for 10 min at 4 °C and the supernatant transferred to a new collection tube and keep on ice.

Antioxidant enzyme activities and Malondialdehyde levels

Protein concentrations of hemolymph samples were determined by using Bradford reagent (Sigma) according to 96 well plate method, and bovine serum albumin was used to create a standard curve. SOD and GST activities were evaluated by using commercial kits from CAYMAN (Cayman Chemical, Ann Arbor, MI). The SOD activity was measured at 450 nm in a Microtiter plate (BMG Labtech) using xanthine and xanthine oxidase systems and defined as U/mg protein. GST activity was read continuously at 340 nm for 5 min in a Microtiter plate (BMG Labtech) using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates and the activity was defined as μmol/ min/mg protein. CAT activity analysis was performed according to Chance and Maehly (1995). The decrease in absorbance over a 10 min period at 240 nm due to H2O2 decomposition was measured in this assay. The absorbance of CAT activity was read in UV/VIS spectrophotometer and thus, the activity was defined as mmol/min/mg protein. Malondialdehyde (MDA, a product of lipid peroxidation) levels in hemolymph samples were also determined using a commercially available kit protocol (Cayman Chemical, Ann Arbor, MI). According to the protocol, MDA in hemolymph samples was incubated with thiobarbituric acid (TBA) at 95 °C and thus absorbance was read at 530 nm in a microtiter plate (BMG Labtech). The content of MDA was determined as the μM/mg per protein.

Statistical analysis

All data were represented as mean ± standard error (SE). The SPSS software program (version 18.0 for Windows, Chicago, IL) was used for statistical analysis. Dose-dependent changes in the antioxidant enzymes and MDA level were verified to be normally distributed. To compare means, ANOVA (one-way analysis of variance) and to determine the significant differences LSD-post hoc tests (Least Significant Difference) were conducted. The results obtained in the experiments were evaluated as being statistically significant at a 95 % confidence interval with p ≤ 0.05.
The endoparasitoid, idiobiont and solitary wasp *P. truniorum* is the most effective biological control agent against several lepidopteran pest species including model insect and storage pest *G. mellonella*. Therefore, it is conceivable that *P. truniorum* could be exposed to IAA broadly used in agriculture during the adult stage that feeds on honey, fruit, and nectar or during the larval stage.
that feeds on host’s pupae. Researchers have already shown the effects of IAA on different physiological properties such as developmental times, biochemical parameters, total, and differential hemocyte counts and apoptosis of different insects in the host-parasitoid relationship (Uçkan et al., 2011a; 2014; 2015; Çelik et al., 2017). Zhao et al. (2017) also reported that treatment of aphids with dietary PGRs including IAA, naphthalene acetic acid (NAA) and GA3, has negative effects on the parasitoids by reducing parasitism rates/abilities, emergence rate, and proportion of females. These negative influences of IAA on life-history parameters of parasitoids may be related to the suppression of antioxidant defense as well as immunological response. Data obtained from this study also support the explanation stated above. Therefore, in accordance with these previous studies (Uçkan et al., 2011a; 2014; 2015; Çelik et al., 2017; Zhao et al., 2017), exposure of the endoparasitoid to IAA by host pupae increased MDA levels increased significantly in a dose-dependent manner (F = 12.045; df = 5, 18; p < 0.05), and altered SOD, CAT and GST activities in the larval hemolymph of P. turionellae with respect to control (Table 2, p < 0.05). We found that endoparasitoid’s SOD activity decreased in all IAA doses except at 50 ppm in comparison to untreated larvae (F = 3.829; df = 5, 18; p < 0.05). In contrast to the SOD activity results, CAT activity in the larval hemolymph of P. turionellae increased only at 50 ppm IAA dose compared to the control group, but no changes were observed in other doses of IAA. (F = 3.478; df = 5, 18; p < 0.05). Table 2). However, GST activity in larval hemolymph of endoparasitoid increased at 500 and 1000 ppm doses of IAA as compared with control (F = 3.482; df = 5, 18; p < 0.05). MDA level in larval hemolymph of P. turionellae also. This increase in MDA level reached nearly more than 10 times that of the control group at the highest dose of IAA (Table 2). Interestingly, these results indicated that increase in lipid peroxidation was not inhibited despite the increase in GST activity, an important detoxification enzyme in the hemolymph of the larval endoparasitoid, at 500 and 1000 ppm IAA doses treatment. Thus, this study, for the first time, showed that oxidative stress occurred in not only host antioxidant defense system, but also in the endoparasitoid P. turionellae. In addition, our study results are also important for the observation of toxic effects of IAA on trophic interactions between host and parasitoid species.

In conclusion, IAA induced oxidative stress in the host and parasitoid insects could be a potential threat causing the negative influences on the survival of parasitoid species for biological control programs. Thus, adult emergence time may happen in unfavorable environmental conditions. Therefore, our study provides important information for the conscious use of IAA in agriculture so as to conserve the host-parasitoid interactions in the ecosystem.

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