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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)

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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 responses 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 (GA_3), 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, GA_3, and ETF, at sublethal doses caused adverse impacts on various antioxidant enzymes and led to oxidative stress in model insect *G. mellonella* (Altuntaş, 2015b; Altuntas *et al.,* 2016). For these reasons, the adverse biological effects of IAA on insects

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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 idiobiont, 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 on 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 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 6d 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 per 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 H_2O_2 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 per 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 $p \leq 0.05$.

Table 1 Effects of various doses of IAA on CAT, SOD, GST activities and MDA level in larval hemolymph of *G. mellonella*

	Mean ± SE*			
IAA doses (ppm)	CAT	SOD	GST	MDA
0	0.62 ± 0.04a	8.51 ± 0.23a	1.15 ± 0.10a	69.22 ± 17.81a
50	0.56 ± 0.03a	8,51 ± 0,00a	0.82 ± 0.02ab	156.28 ± 24.13b
500	0.34 ± 0.02b	5.06 ± 0.23b	0.65 ± 0.12b	197.03 ± 20.05b
1,000	0.38 ± 0.02b	6.44 ± 0.23c	0.43 ± 0.07b	194.02 ± 27.52b
5,000	0.42 ± 0.02b	5.98 ± 0.23bc	0.48 ± 0.12b	157.65 ± 27.02b
10,000	0.43 ± 0.02b	5.75 ± 0.23bc	0.56 ± 0.09b	175.81 ± 37.01b

*Means ± standard errors within each column followed by the different letter (a-c) indicate significant differences ($p \leq 0.05$, LSD test). CAT: Catalase (mmol/min/mg protein), SOD: Superoxide dismutase (U/mg protein), GST: Glutathione S transferase (µmol/min/mg protein), MDA: Malondialdehyde (µM/mg protein)

Results and Discussion

Our data showed that treatment of *G. mellonella* larvae with diet containing IAA caused a decrease in the activities of antioxidant enzymes; CAT ($F = 16.351$; df = 5, 18; $p < 0.05$), SOD ($F = 56.692$; df = 5, 18; $p < 0.05$) and GST ($F = 8.448$; df = 5, 18; $p < 0.05$) at high doses 500, 1000, 5000 and 10000 ppm as compared to control. In particular, significant reductions in CAT and SOD enzyme activities in the hemolymph of *G. mellonella* larvae were observed at 500 ppm (> 40 %), also, GST enzyme activity increased by more than 60 % at 1000 ppm. Similar to present findings, Altuntaş (2015) revealed that activities of antioxidant enzymes in larval hemolymph of *G. mellonella* did not change at higher doses of dietary GA_3 but increased at lower doses of this PGR. On the other hand, Shayegan et al. (2019) also showed dose-dependent inducing effects of GA_3 on SOD and CAT activity of *Helicoverpa armigera* larvae. Further, an important finding presented in this study was the sharp decrease observed in CAT and SOD activities in host hemolymph at 500 ppm dose of IAA similar with GA_3 doses reported previously by Altuntaş (2012). IAA treatment also caused an increase in *G. mellonella* MDA levels at all doses. As compared to control, the most effective IAA dose 500 ppm increased MDA levels in hemolymph of larvae by 185% ($F = 2.918$; df = 5, 18; $p < 0.05$) (Table 1.). Similar to previous studies conducted with mammals showed that exposure to different IAA concentrations increased the lipid peroxidation, inhibited antioxidant response in various rat tissues (Tuluce and Celik, 2006), and also decreased CAT activity in the kidney of the F_2 generation of mice (Yılmaz *et al.,* 2004). Altuntaş (2015) also reported that activities of antioxidant enzymes in larval hemolymph of *G. mellonella* did not change at higher doses of dietary GA_3 but increased at lower doses of this PGR. On the other hand, Shayegan *et al*. (2019) showed dose-dependent inducing effects of GA_3 on SOD and CAT activity of *Helicoverpa armigera* larvae. Therefore, our findings demonstrated that exposing *G. mellonella* to IAA via larval diet leads to oxidative stress by elevating MDA levels; It is known that an increase in MDA levels is an important marker for oxidative stress and occurs naturally during lipid peroxidation. Keeping in mind the similarity between the response of model insect *G. mellonella* and mammals to IAA may assist in the improvement of novel insect-based screening systems to measure the toxicity of PGRs or other environmental chemicals instead of using of mammals in biomonitoring tests. In addition, induced oxidative stress by xenobiotics causes cell death either by necrosis or apoptosis mechanisms (Kannan and Jain, 2000). Increases in apoptotic activities in different tissue cells of mice treated with IAA were observed in early studies by Furukawa *et al.* (2004). In another study, it was also reported that a plant growth regulator, GA_3 induced apoptotic and necrotic cell death and reduced cell viability in GA_3 treated *G. mellonella* larvae when compared to untreated larvae (Altuntaş *et al.,* 2012). Furthermore, Çelik *et al.* (2017) demonstrated that lower doses of dietary IAA caused an increase in apoptotic indices in *Achoria grisella* (Lepidoptera: Pyralidae) larvae. For these reasons, these findings imply that dietary IAA treatment may cause excessive apoptosis by suppressing the antioxidant defense system in the host *G. mellonella* larvae.

The endoparasitoid, idiobiont and solitary wasp *P. turionellae* 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. truionellae* 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

Table 2 Effects of various doses of IAA on CAT, SOD, GST activities and MDA level in larval hemolymph of *P. turionellae*

	Mean ± SE*			
IAA doses (ppm)	CAT	SOD	GST	MDA
0	0.011 ± 0.003a	8.48 ± 0.36a	0.03 ± 0.01a	31.75 ± 7.65a
50	0.026 ± 0.008b	8.10 ± 0.39a	0.04 ± 0.01a	77.54 ± 11.03a
500	0.010 ± 0.003a	6.49 ± 1.06b	0.09 ± 0.02b	116.60 ± 39.49b
1,000	0.009 ± 0.005a	6.79 ± 1.19b	0.07 ± 0.02b	257.26 ± 53.10bc
5,000	0.006 ± 0.001a	4.14 ± 0.49b	0.05 ± 0.01a	241.28 ± 24.74bc
10,000	0.008 ± 0.0016a	5.75 ± 0.94b	0.04 ± 0.01a	305.15 ± 30.86c

*Means ± standard errors within each column followed by the different letter (a-c) are significantly different ($p \leq 0.05$, LSD test). CAT: Catalase (mmol/min /mg protein), SOD: Superoxide dismutase (U/mg protein), GST: Glutathione S transferase (µmol/min/mg protein), MDA: Malondialdehyde (µM/mg protein)

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 GA_3, has negative effects on the parasitoids by reducing parasitism rates/abilities, emergence rate, and proportion of females. These negative influences of IAA_3 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 increased depending on the IAA_3 doses the larvae of *P. turionellae* were exposed to through the host pupae. As a consequence, the IAA_3-mediated toxic effects 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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References

Altuntaş H, Kiliç A, Uckan F, Ergin E. Effects of gibberellic acid on hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Environ. Entomol. 41: 688-696, 2012.

Altuntaş H. Effects of ethephon on the hemolymph metabolites of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae). Acta. Physiol. Pol. A. 128**,** 182-183, 2015a.

Altuntaş H. Determination of gibberellic acid (GA 3)-induced oxidative stress in a model organism *Galleria mellonella* L. (Lepidoptera: Pyralidae). Environ. Entomol. 44: 100-105, 2015b.

Altuntaş H, Demirci SNŞ, Duman E, Ergin E. Toxicological and physiological effects of ethephon on the model organism, *Galleria mellonella* L. 1758 (Lepidoptera: Pyralidae). Turk. Entomol. Derg. 40: 413-423, 2016.

Çelik D, Özbek R, Uçkan F. Effects of Indole-3-Acetic Acid on Hemocytes of *Achoria grisella* Fabr. (Lepidoptera: Pyralidae). J. Entomol. Res. Soc. 19: 83-93, 2017.

Davies PJ. Plant Hormones, Physiology, Biochemistry, and Molecular Biology, 2nd edn. Kluwer Academic Publishers, Dordrecht, Netherlands. Davies PJ. (2010). The Plant Hormones: Their Nature, Occurrence and Functions, (eds. P.J. Davies), 3rd edn. Kluwer Academic, New York, USA, 1995.

Felton GW, Summers CB. Antioxidant systems in insects. Arch. Insect Biochem. Physiol. 29: 187-197, 1995.

Furukawa S, Abe M, Usuda K, Ogawa I. Indole-3-acetic acid induces microencephaly in rat fetuses. Toxicol Pathol 32: 659-667, 2004.

Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology. 7: 153-163, 2000.

Kaur R, Rup PJ. Evaluation of regulatory influence of four plant growth regulators on the reproductive potential and longevity of melon fruit fly *Bactrocera cucurbitae*. Phytoparasitica 30: 224-230, 2002.

Kaur R, Rup PJ. Influence of some plant growth regulators (PGR) on biochemical profile in the larvae of melon fruit fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Trypetidae). Entomon. Trivandrum. 28: 89-95, 2003.

Kumar B, Pandey D, Goswami C, Jain S. Effect of growth regulators on photosynthesis, transpiration and related parameters in water stressed cotton. Biol. Plant. 44: 475-478, 2001.

Kwadha CA, Ong'amo GO, Ndegwa PN, Raina SK, Fombong AT. The Biology and Control of the Greater Wax Moth, *Galleria mellonella*. Insects. 8: 1-17, 2017.

Rup PJ, Sohal SK, Kaur G, Dhillon M. The influence of allelochemicals and plant growth regulators on emergence and development of mustard aphid, *Lipaphis erysimi* (Kalt.). Allelopathy J. 10: 53-58, 2002.

Shayegan D, Sendi JJ, Sahragard A, Zibaee A. Immunological and antioxidant responses of larval *Helicoverpa armigera* (Lepidoptera: Noctuidae) to gibberellic acid in the diet. Invertebrate Surviv. J. 16: 48-59, 2019.

Tülüce Y, Çelik I. Influence of subacute and subchronic treatment of abscisic acid and gibberellic acid on serum marker enzymes and erythrocyte and tissue antioxidant defense system and lipid peroxidation in rats. Pest Biochem. Physiol. 86: 85-92, 2006.

Uçkan F, Haftacı İ, Ergin E. Effects of indole-3-acetic acid on biological parameters of the larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae). Ann. Entomol. Soc. Am. 104: 77-82, 2011a.

Uçkan F, Öztürk Z, Altuntaş H, Ergin E. Effects of gibberellic acid (GA_3) on biological parameters and hemolymph metabolites of the pupal endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae) and its host *Galleria mellonella* (Lepidoptera: Pyralidae). JERS. 13: 1-14, 2011b.

Uçkan F, Soydabaş HK, Özbek R. Effect of Indol-3-acetic acid on the biochemical parameters of *Achoria grisella* hemolymph and *Apanteles galleriae* larva. *Pak. J. Biol. Sci.* 11: 163-171, 2014.

Uçkan F, Özbek R, Ergin E. Effects of indole-3-acetic acid on the biology of *Galleria mellonella* (Lepidoptera: Pyralidae) and its endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae). Belgian J. Zool. 145: 49-58, 2015.

Yilmaz H, Yüksel R, Türköz EY. F_2 nesil farelerde indol-3-asetik asitin böbrek katalaz, süperoksit dismutaz ve glutatyon peroksidaz aktiviteleri üzerine olan etkisi. Van Tıp Dergisi. 11: 64-68, 2004.

Zhao H, Cao HH, Pan MZ, Sun YX, Liu TX. The Role of Plant Growth Regulators in a Plant–Aphid–Parasitoid Tritrophic System. J. Plant Growth Regul. 36: 868-876, 2017.