

## EFFECT OF INDOL-3 ACETIC ACID ON THE BIOCHEMICAL PARAMETERS OF *Achoria grisella* HEMOLYMPH AND *Apanteles galleriae* LARVA

Fevzi Uçkan, Havva Kübra Soydaş\* Rabia Özbek

Kocaeli University, Faculty of Arts and Science, Department of Biology, 41380,  
Kocaeli, Turkey

E-mail\*: havvasoydabas@gmail.com; kubra.soydabas@kocaeli.edu.tr

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### ABSTRACT

Biochemical structures such as lipid, protein, sugar and glycogen are known to play a pivotal role on the relationship between host and its parasitoid. Any changes in these parameters may have potential to alter the balance of the host-parasitoid relation. Taking this into account, the effects of plant growth regulator, *indol-3 acetic acid* (IAA) on the biochemical parameters of the host and parasitoid were investigated. *Achoria grisella* Fabricius (Lepidoptera: Pyralidae) is a serious pest and causes harmful impacts on honeycomb. Endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) feeds on the hemolymph of the *A. grisella* larva and finally causes mortality of the host. Different concentrations (2, 5, 10, 50, 100, 200, 500, and 1,000ppm) of IAA were added to the synthetic diet of host larvae. Protein, lipid, sugar, and glycogen contents in hemolymph of host and in total parasitoid larvae were determined by Bradford, vanillin-phosphoric acid, and hot anthrone tests using UV-visible spectrophotometer, respectively. Protein level in host hemolymph increased upon supplement of each doses of IAA except for 10ppm. IAA application enhanced the level of sugar at 100 and 200ppm whereas a decrease was observed in lipid at 5, 10, 200, and 1,000ppm doses in host. All doses were effective on the parasitoid larvae. Nevertheless, the most effective dose was 50ppm, which increased glycogen but decreased lipid amount. Similarly, treatment of 1000ppm IAA increased protein level and 100ppm reduced level of sugar. Our study indicated that application of IAA resulted in different effects on the amount of biochemical structures associated with the hemolymph of pest species and its natural enemy. Therefore, results showed that not only the target but also the non-target organisms exposed to widely-used plant growth regulators may be affected and this, in turn may also change the host-parasitoid interaction.

Key words: *Achoria grisella*, *Apanteles galleriae*, Indol-3-acetic acid, biological control.

### INTRODUCTION

Plants are major sources for the production of various natural compounds which can be used for many purposes. Among them, the plant growth regulators (PGRs) which directly or indirectly affects the development of plants via various biochemical pathways. PGRs have influence on the pat-

terns of growth and reproduction in herbivorous insects (Isman *et al.*, 1983; Kaur and Rup, 2002). Nowadays focus increases on natural products due to the disadvantages of synthetic pesticides, because they cause environmental pollutions and pesticide-induced resistance as well as they reflects adverse effects

on nontarget organisms. Eco-friendly compounds may also support the yield and therefore can be used in integrated pest management (IPM) programs to control the pests (Laher, *et al* 2000; Akol, *et al.*, 2002; Kaur and Rup, 2002; Uçkan *et al.*, 2011a). It is reported that PGRs have the capability to inhibit antioxidative defense system and block or activate immune potential enzymes in rat's spleen and lung tissue (Celikand Tuluçe, 2007). It is evident that fecundity, longevity and egg viability have also been changed in insects with the supplement of PGRs (Visscher, 1980; 1983). Auxins are the main class of PGRs, responsible for regulating many developmental processes, such as apical dominancy, phototropism, and cell division in plants. Among them indol-3-acetic acid (IAA) is the most important natural auxin produced in plants, microorganisms, insects, and animals (Sugawara, *et al.*, 2009; Pugine, *etal.*, 2007). It is investigated that IAA has various effects on the development and reproduction of the solitary koinobiont larva endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) reared on the lesser wax moth, *Achoria grisella* Fabr. (Lepidoptera: Pyralidae) (Uçkan *et al.*, 2008; Uçkan *et al.*, 2011b). Investigations has not yet completed without estimating the changes in the amount of four important biochemical components (i.e. lipids, free sugars, glycogen and proteins) in the same individual (Foray *et al.*, 2012). Proteins be represent an important energy producing source in

insects and they take part in fuel, egg production and diapause (Hahn *et al.*, 2008; Suarez *et al.*, 2005; Bernstein and Jervis, 2008; Hahn and Denlinger, 2007). To maintain the survival of insects, energy is required in different processes such as moulting, adult, gonadal and reproductive growth. Muscular activity and fasting also increase the metabolism. The biological effects of IAA on the biochemical parameters in different developmental stages, number of off spring produced and sex ratio of the koinobiont, solitary, larval endo-parasitoid *A. galleriae* reached on it shost *A. grisella* which is a serious pest because it feeds on combs, wax, and honey as reported by Uçkan *et al.*, (2011a). Additionally, a correlative study of host-parasitoid biochemical parameters like total protein, lipid, sugar and glycogen need attention to investigate.

## MATERIALS AND METHODS

**Insects:** experimental colonies of the host *A. grisella* and the parasitoid *A. galleriae* were established from successive colonies in Kocaeli University animal physiology research laboratory. The host colony was maintained by feeding the insects with a synthetic diet modified by Sak *et al.*, (2006) and bit of honeycomb was added for egg deposition and feeding of the newly hatched larvae. Both host and parasitoid species were reared in the same environmental chamber at  $25 \pm 2$  °C,  $60 \pm 5$  % relative humidity, and photoperiod of 12 : 12 (L : D) hours. Early

instars of *A. grisella* were provided for *A. galleriae* adults to lay their eggs in host larvae. For feeding of *A. galleriae* adults a piece of cotton soaked in a honey solution (50 %) was placed inside the jars (Uçkan and Ergin (2003). The method, which was described by Uçkan and Gülel (2000) used to establish and maintain successive colonies of both host and parasitoid species.

**Exposing to Indol-3-acetic acid:** One to two days old *A. grisella* adults were left in 210 ml jars containing 01 g of honey comb for mating and oviposition. On the 5 th day of incubation host adults were removed from the jars. These series were used to take hymolymph from host larvae. For collecting parasitoid larvae, the same incubating steps were applied as previously described for host larvae. Additionally in 7 th day, 1-2 days old parasitoids were placed into the jars. Honey:distilled water (1:1) feed was supplemented to parasitoid adults. The parasitoids were removed from the jars on the 9 th day. Early instars of the host were exposed to 5 g of host diet (Sak et al., 2006) supplemented with 2, 5, 10, 50, 100, 200, 500 and 1000ppm IAA (Merck) at the dark period of time and same feeding procedure repeated once at week until collection of both hemolymph from non-parasitized host and parasitoids from parasitized host larvae. IAA was indirectly taken by the parasitoid larvae from host hemolymph. The controls of both non-parasitized and parasitized host larvae were fed on a IAA-free diet. Both host and para-

sitoids were reared in the same environmental conditions at  $25\pm 2$  °C,  $60\pm 5$  RH and 12:12 (L:D) hours photoperiod.

**Sample Collection:** To investigate the effects of different doses of IAA on the hemolymph of host larvae, 0.02-0.04 g individuals were randomly selected from different colonies at different times. Host larvae were washed with 70% ethanol and punctured on the first hind leg with a steril insect pin (Altuntaş, et al., 2012). From each host larvae 3µl hemolymph was collected with a glass micro capillary tube (Sigma Aldrich). Sterilized microcentrifuge tubes having 0.005 g N-phenylthiourea (Sigma Aldrich) were used to store hemolymph at -20 °C (Vogelweith, et al., 2014). Parasitized host larvae were randomly selected from different colonies at different times to obtain parasitoid larvae. Under stereo microscope parasitized host larvae dissected between head capsule and first thoracic segment, so parasitoid larvae were moved outside from the host body. Sterilized microcentrifuge tubes containing 500 µl 10 % trichloroacetic acid was used to store the larvae, which were used for protein assay. Parasitoid larvae stored at -20 °C with out any treatment were used to assay the glycogen, total sugar and lipid.

#### **Biochemical assays:**

**Protein:** Plummer (1971) method was applied for the extraction of protein. Parasitoids larvae were homogenized (QIAGEN Tissue Lyser LT) in 500 µl of 10 % trichloroacetic acid solution at 50 Hz for 30 min and centrifuged (Beck-

man Coulter Microfuge 22R) at 3500 rpm at room temperature for 15 min. The supernatant was discarded and 500 µl of 96 % ethanol was added to the tubes. These were then vortexed for 5 min and centrifuged at 3500 rpm for 15 min. After centrifugation the supernatant was discarded and the pellet was dissolved in distilled water. The quantity of protein was determined by using Bradford reagent, as described by Bradford (1976). Bovine serum albumin was used as the standard protein. Absorbance was taken at 595 nm with UV-visible spectrophotometer (Shimadzu UV-mini 1240).

**Lipid, Glycogen and Sugar:** Parasitoid larvae were transferred in 2ml microcentrifuge tubes with 50 µl sodium sulphate, and homogenized at 50 Hz for 5 min then 450µl of chloroform : methanol (1:2) was added and again homogenized at 50 Hz for 25 min. The tubes were vortexed and centrifuged at 14,000 rpm for 2 min. The supernatant was used to determine total lipid and sugar. The precipitates were used to investigate the glycogen. Supernatant from each sample 200 µl, was transferred to a 2 ml microcentrifuge tube and then heated at 90 °C until the solution completely evaporated. Samples were then dissolved in 40 µl of sulfuric acid and heated at 90 °C for 2 min. The tubes were cooled on ice and 960 µl of vanillin phosphoric acid reagent was added (van Handel, 1985a). Corn oil was used as the standard lipid. Each tube was left at room temperature for 30 min, and then

absorbance was taken at 525 nm through UV-vis spectrophotometer. For the determination of glucose amount 200 µl of the supernatant was transferred to a 2 ml microcentrifuge tube and heated at 90 °C until 50 µl remained. 950 µl of anthrone reagent (vanHandel, 1985b) was added to supernatant, heated at 90 °C for 15 min., cooled on ice and absorbance was noted at 625 nm. Glucose was used as the standard sugar. The precipitates were heated at 90 °C until dry and 1 ml anthron reagent (van Handel, 1985b) was added. These tubes were heated at 90 °C for 15 min., cooled on ice and absorbance was noted at 625 nm. Glycogen from oyster was used as the standard glycogen

**Statistical Analysis:** Experiments were repeated three times with specimen taken from different populations at different times. Means were compared using one-way analysis of variance (ANOVA) of SPSSV.18 for Windows. Means were subjected to Tamhane T2 tests to assess the significance of the effects of IAA doses ( $P < 0.05$ ).

## RESULTS

Exposure to IAA in host diet resulted in an increase in hemolymph total protein levels of *A. grisella* at 2, 5, 50, 100, 200, 500 and 1,000 ppm ( $F=196, 254$ ;  $df=8, 531$ ;  $P=0,000$ ) (Table1). However, hemolymph total lipid levels of *A. grisella* decreased ( $F=9, 021$ ;  $df=8, 53$ ;  $P=0,000$ ) at 5, 10, 200 and 1000 ppm when compared to control (Table 1). On other hand, hemolymph total sugar

levels fluctuated among treatments with a significant increase ( $F=58,139$ ;  $df=8, 531$ ;  $P=0,000$ ) at 100 and 200ppm and decrease at 10, 50 and 500ppm with respect to control (Table 1).

**Table-1:** IAA-induced changes in hemolymph total protein, lipid and sugar ( $\mu\text{g/ml}$ ) of *Achoria grisella*

IAA ppm	<i>Achoria grisella</i>		
	Hemolymph Total Protein <sup>a</sup> (Mean $\pm$ SE) <sup>b</sup>	Hemolymph total Lipid <sup>a</sup> (Mean $\pm$ SE) <sup>b</sup>	Hemolymph Total Sugar <sup>a</sup> (Mean $\pm$ SE) <sup>b</sup>
0	101.75 $\pm$ 2.58a	261.34 $\pm$ 15.73a	66.13 $\pm$ 2.61ab
2	161.69 $\pm$ 2.07b	209.26 $\pm$ 11.44ab	73.94 $\pm$ 2.52a
5	139.88 $\pm$ 1.21c	184.95 $\pm$ 11.38bcd	72.74 $\pm$ 0.42a
10	107.78 $\pm$ 1.03a	146.41 $\pm$ 13.78c	62.32 $\pm$ 1.81bc
50	168.60 $\pm$ 1.31b	246.30 $\pm$ 12.02ae	57.51 $\pm$ 1.74b
100	178.35 $\pm$ 0.55d	201.16 $\pm$ 13.68abc	102.38 $\pm$ 3.14d
200	153.53 $\pm$ 1.84bd	171.06 $\pm$ 7.54bc	98.78 $\pm$ 2.58d
500	136.86 $\pm$ 2.72c	224.31 $\pm$ 7.75ad	55.31 $\pm$ 2.15b
1000	156.54 $\pm$ 2.30b	191.90 $\pm$ 12.44bcde	70.33 $\pm$ 1.66ac

<sup>a</sup>Means within each column followed by the same letter are not significantly different ( $P>0.05$ ).

<sup>b</sup>Average of 60 individuals per treatment.

Total lipid levels of *A. galleria* reared on *A. grisella* larvae exposed to different doses of IAA was significantly higher ( $F=38, 284$ ;  $df=8, 531$ ;  $P=0,000$ ) at 5, 50, 100, 200 and 1000 ppm than parasitoids reared on untreated host (Table 2). Total protein levels of *A. galleria* showed variations among doses with a significant ( $F=106, 904$ ;  $df=8, 531$ ;  $P=0,000$ ) increase at 100 ppm and decrease at 10, 50, 500 and 1,000 ppm. This trend was also similar in total

sugar and glycogen levels (Table 2). The mean total sugar levels of IAA exposed *A. galleria* parasitoids reduced at 2, 500 and 1000 ppm ( $F=101, 923$ ;  $df=8, 531$ ;  $P=0,000$ ) and increased at 5, 100 and 200 ppm compared with wasps reared on untreated hosts (Table 2). Treatment of IAA induced decrease in total glycogen levels of parasitoids ( $F=106, 361$ ;  $df=8, 531$ ;  $P=0,000$ ) at 2, 5, 50 and 500 ppm and increase at only 200ppm (Table 2).

**Table -2:** IAA-induced changes in total protein, lipid, sugar and glycogen ( $\mu\text{g/ml}$ ) of *Apanteles galleria*

IAA Ppm	<i>Apanteles galleria</i>			
	Total Protein <sup>a</sup> (Mean $\pm$ SE) <sup>b</sup>	Total Lipid <sup>a</sup> (Mean $\pm$ SE) <sup>b</sup>	Total Sugar <sup>a</sup> (Mean $\pm$ SE) <sup>b</sup>	Total Glycogen <sup>a</sup> (Mean $\pm$ SE) <sup>b</sup>
0	7.54 $\pm$ 0.18ab	40.66 $\pm$ 0.61a	5.05 $\pm$ 0.14a	22.45 $\pm$ 0.61a
2	6.74 $\pm$ 0.20ac	39.74 $\pm$ 0.61a	4.46 $\pm$ 0.08b	17.97 $\pm$ 0.21b
5	7.73 $\pm$ 0.09b	48.67 $\pm$ 1.50c	6.91 $\pm$ 0.09c	15.73 $\pm$ 0.36c
10	5.61 $\pm$ 0.17d	38.20 $\pm$ 1.54a	4.80 $\pm$ 0.15abc	22.22 $\pm$ 0.45ad
50	5.85 $\pm$ 0.15de	65.10 $\pm$ 3.63b	5.45 $\pm$ 0.18a	13.55 $\pm$ 0.60c
100	8.94 $\pm$ 0.01f	55.06 $\pm$ 0.49b	8.11 $\pm$ 0.23d	24.43 $\pm$ 0.42a
200	7.07 $\pm$ 0.14a	57.91 $\pm$ 1.34b	6.67 $\pm$ 0.14c	27.67 $\pm$ 0.20e
500	6.44 $\pm$ 0.10ce	37.90 $\pm$ 1.81a	4.17 $\pm$ 0.03e	18.05 $\pm$ 0.18b
1000	4.15 $\pm$ 0.01g	57.51 $\pm$ 0.96b	4.46 $\pm$ 0.04b	21.94 $\pm$ 0.57ad

<sup>a</sup>Means within each column followed by the same letter are not significantly different ( $P>0.05$ ). <sup>b</sup>Average of 60 individuals per treatment.

## DISCUSSION

Our results indicated that IAA were detected in the hemolymph of treatment affected both host and paralarvae of *A. grisella*. Rup et al., (1998) also showed increase in protein and a decline in both lipid and glycogen level following  $\text{GA}_3$  treatment in *Zapri- onus paravittiger* (Godole and Vaidya). In another study, it was reported that  $\text{GA}_3$ , IAA, kinetin and coumarin treatment induced changes in protein and carbohydrate content of *Bactrocer acurbitae* (Coquillett) and caused alteration in molting and metamorphosis (Kaur and Rup, 2003a,b). Rup et al., (2000, 2002) suggested that the decrease in metabolites as a result of stress induced by PGRs. So, the decrease in energy reserves due to IAA

induced stress may lead a alternation in biological parameters both host and parasitoid. We previously displayed that IAA had growth and developmental inhibitory effects on *G. mellonella* larvae, which is the most harmful stage of this pest, with prolongation in egg hatching time and larval development time, but shortening in pupal development time (Uçkan et al., unpublished). On the contrary of IAA,  $\text{GA}_3$  treatment caused a decline in preadult development time of *G. mellonella* (Uçkan et al., 2011b). So, effects of PGRs are not the same on insects. We observed that bio-chemical contents of parasitoid wasps, which reared on larvae exposed to IAA in diet, altered with respect to control. We observed fluctuation in

lipid, protein, sugar and glycogen levels. Uçkan et al., (2011a) previously reported that IAA treatment caused a prolongation in adult emergency time and a decline in longevity of wasp. GA<sub>3</sub> treatment also changed protein, lipid and carbohydrate levels and adult longevity of the para-sitoid was *Pimpla turionellae* (Uçkan et al., 2011b) and caused decrease in life span and increase in egg to adult development time of *A.galleriae* (Uçkan et al., 2008). Uçkan and Ergin (2003) suggested that carbohydrates are necessary for an extended adult longevity of *A.galleria*. Thus, IAA related changes in biochemical profile of wasp may cause detrimental effects on the development and longevity of adult *A. galleriae*.

## CONCLUSION

Here it was concluded that IAA induced changes in biochemical parameters of the pest host *A. grisella* and its natural enemy the wasp species *A. galleriae*. Other evidences and our investigations brings to mind that IAA may also cause changes in the hormonal balance, which is effective on the development and metabolism of the insects. The biochemical defects may lead to changes in egg to adult development all time and longevity especially for the wasp species. This may in turn disturb the effectiveness of parasitoid species in the biological relationships between host and parasitoid.

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