

## Effects of Indol-3-Acetic Acid on the biology of *Galleria mellonella* and its endoparasitoid *Pimpla turionellae*

Fevzi Uçkan<sup>1,\*</sup>, Rabia Özbek<sup>1</sup>, Ekrem Ergin<sup>2</sup>

<sup>1</sup> Department of Biology, Faculty of Science and Literature, Kocaeli University, Kocaeli, 41380, TURKEY

<sup>2</sup> Nursing School, Gülhane Military Medical Academy, Ankara, 06018, TURKEY (e-mails: ozbekrabia@gmail.com, ekremxergin@hotmail.com)

\* Corresponding author: fevzi.uçkan@kocaeli.edu.tr

**ABSTRACT.** The effects of indol-3-acetic acid (IAA) were investigated on biological parameters of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) and its endoparasitoid *Pimpla turionellae* (Linnaeus 1758) (Hymenoptera: Ichneumonidae) reared on hosts treated with 50 to 10,000 ppm of IAA in their diet. Percent fecundity of first generation of *G. mellonella* females increased by 12% at 50 ppm whereas the most effective decrease, by >33%, was observed at 5,000 ppm. Percent fertility decreased by >20% at 1,000 ppm. All treatments displayed  $\pm 5\%$  variations when compared to controls except for a >20% increase in corrected percent sterility at 1,000 ppm. The results did not reveal any considerable effect of IAA on egg hatching, larval and pupal developmental, or adult emergence times. The most striking effect observed was a decline in second generation pupal developmental time by >47% at 1,000 and 10,000 ppm. No dose-wise alterations were observed in adult longevity, weight, size and female sex ratio of *G. mellonella*. Treatment with IAA caused a slight prolongation in adult emergence and decrease in longevity of *P. turionellae* reared on hosts; longevity of wasps declined by >27% at higher doses. Neither wasp size nor weight displayed significant changes upon IAA treatment.

**KEY WORDS:** Biology, *Galleria mellonella*, Indole-3-acetic acid, *Pimpla turionellae*.

### INTRODUCTION

Synthetic chemicals acting as plant growth regulators (PGRs) have been widely used to obtain high levels of agricultural productivity. However, these chemicals not only affect plant growth and development, but they also negatively affect the development, survival, longevity, reproductive potential, hemocytes, and hemolymph metabolites of insects and other animals (AHMED et al, 2003; PAULSON et al., 2005; GUPTA et al., 2009; UÇKAN et al., 2008, UÇKAN et al., 2011a, b; ALTUNTAŞ et al., 2012). The toxic effects of PGRs, including indole-3-acetic acid (IAA), on the development and reproductive potential of the pest species, *Bactrocera cucurbitae* (COQUILLET, 1849) were investigated and the authors suggested that these eco-friendly compounds are promising candidates to replace pesticides in Integrated Pest Management (IPM) programs of economically

important pest insect (KAUR & RUP, 2002). GUPTA et al. (2009) also reported that gibberellic acid (GA<sub>3</sub>) and siapton (an amino acid based plant growth stimulant) caused an increase in the larval period of the hairy caterpillar, *Spilarctia obliqua* (WALKER, 1855) at high doses. However, the overall effects of PGRs on insects still need to be clarified to improve the strategies for pest control and to know what kind of effects these compounds have especially on natural enemies of pest species. For example, UÇKAN et al. (2008; 2011a) demonstrated that egg to adult developmental time of the wasp species, *Apanteles galleriae* (WILKINSON, 1932) reared on *Achoria grisella* (FABRICIUS, 1794) larvae exposed to high doses of GA<sub>3</sub> and IAA increased by 40% and 30%, respectively when wasps were reared on larvae exposed to higher doses of the PGRs. Furthermore, the adult longevity of *A. galleriae* decreased by >50% when wasps were reared on IAA- or GA<sub>3</sub>-treated hosts (UÇKAN et

al., 2008, 2011a). Recently, UÇKAN et al. (2011b) also stated that higher doses of GA<sub>3</sub> negatively affected the adult longevity of the wasp species, *Pimpla turionellae* (LINNAEUS, 1758) (Hymenoptera: Ichneumonidae). A decrease in length was also apparent at most of the treatment levels. Their results also revealed that hemolymph carbohydrate at most of the dose levels, and lipid at all decreased in host larvae upon exposure to GA<sub>3</sub>. KAUR & KAUR (2013) reported that the braconid parasitoid, *Bracon hebetor* (SAY, 1836) reared on coumarin-treated host, *Spodoptera litura* (FABRICIUS, 1775) displayed declines in reproductive potential, female life span, and egg hatching with an extended developmental period at higher concentrations. PRADO & FRANK (2013) also showed that PGRs had adverse effects on parasitoid fitness and caused a decrease in parasitism incidence. Evidence from our and other earlier studies strongly supports the idea that exposure to PGRs via the host diet influences physiological and biochemical mechanisms and plays roles in maintaining survival activities of both pests and their natural enemies.

Many advantages of *Galleria mellonella* (LINNAEUS, 1758) (Lepidoptera: Pyralidae) caterpillars have convinced an increasing number of researchers to favor this species as a powerful, reliable, and proven model system to evaluate the effects of toxic substances on both target species and non-target beneficial ones (SAK et al., 2006; BÜYÜKGÜZEL et al., 2007; ERGIN et al., 2007; UÇKAN et al., 2008; 2011a, b; ALTUNTAŞ et al., 2012). Larvae of the host species are serious pests in beehives. They damage the combs by boring into the hive and leaving silk-lined tunnels or galleries (SANFORD 1987). The solitary idiobiont pupal endoparasitoid *P. turionellae* is utilized for biological control of a number of lepidopteran pest species involving *G. mellonella* (KANSU & UĞUR 1984, FISHER, 1987). Upon paralyzation by the wasp species at the time of oviposition (HAESSELBARTH 1979), the paralyzed host provides food and a living space for larval parasitoids and sometimes food for the adult parasitoid (SLANSKY, 1986). Some host species of *P. turionellae* also feed on plants

and the adult wasps feed on plant nectar and host pupae in nature. Therefore, the accumulation of environmental pollutants and transmission of these compounds to wasps by both feeding in/on a host directly and by feeding on plant nectar, indirectly, are likely to occur (SAK et al. 2006, ERGIN et al. 2007). Thus, the present work was carried out to determine IAA-induced changes in reproductive potential of first generation (F<sub>1</sub>) of *G. mellonella* and egg hatching time, larval and pupal developmental time, adult emergence time, adult longevity, female sex ratio, adult weight and size of second generation (F<sub>2</sub>) of *G. mellonella*, as well as immature developmental time, adult longevity, adult weight and size of *P. turionellae*.

## MATERIAL AND METHODS

### Parasitoid and Host Rearing

Laboratory colonies of the host, *G. mellonella* and the parasitoid, *P. turionellae* were established from adults reared at 25 ± 5°C, 60 ± 5% RH, and with a photoperiod of 12: 12 (L:D) h in our laboratory in Kocaeli University, Turkey. Larvae of *G. mellonella* were maintained by feeding the insects with a diet described by BRONSKILL (1961) and modified by SAK et al. (2006). A piece of honey comb was also added for egg deposition and feeding of the newly hatched larvae. *P. turionellae* were mass reared on the pupae of the host, *G. mellonella* in cages (25 x 25 x 25 cm). Adults of parasitoids were fed a 30% (wt: vol) honey solution and provided with host pupae (four pupae for every 10 female wasps once every 3 d) (SAK et al., 2006).

### Bioassays

An individual mating pair of the host, 1- or 2-day-old *G. mellonella* was placed in 1-L jars containing 2 g honeycomb to provide a mating and oviposition substrate. Adults were removed from the jars on the seventh day. Newly hatched first generation (F<sub>1</sub>) larvae of *G. mellonella* in

jars were exposed to 10 g of the host diet (SAK et al., 2006) treated with 50, 500, 1,000, 5,000 and 10,000 ppm IAA (Merck 10 g, Darmstadt, Germany) homogenized with doses in separate jars. Larvae reared on IAA-free diet were controls. Both experimental and control group diets were replenished daily.

Last instars of  $F_1$  of *G. mellonella* ( $0.18 \pm 0.02$  g) were randomly selected in groups of five, transferred to sterile Petri dishes, and controlled every day until adult emergence. To determine the total number of eggs laid per female, percent fecundity, percent fertility, and corrected percent sterility of  $F_1$  and egg hatching, larval, and pupal developmental time, adult emergence time, adult longevity, sex ratio, adult weight and size of second generation ( $F_2$ ) of *G. mellonella*, an individual mating pair of  $F_1$  adults of *G. mellonella* ( $n=30$ ) was placed into 210 ml cups. A piece of paper was placed into each cup as a deposition substrate then cups were covered with gauze to allow air exchange. *G. mellonella* females were allowed to deposit eggs on papers. The papers on which eggs had been oviposited were changed daily and the number of eggs on them was counted every two days for 16 d. The data were tabulated for the total number of eggs deposited by each female, percent fecundity, and percent fertility in experimental and control groups. Corrected percent fertility was calculated according to KAUR & RUP (2002) by applying the following formula:

$$\text{Corrected percent sterility} = \frac{\% \text{ Fertility in control} - \% \text{ Fertility in treatment}}{\% \text{ Fertility in control}} \times 100$$

From each treatment and control group five  $F_2$  eggs were randomly selected and individually transferred to sterile Petri dishes including 2 g of host diet treated with different doses of IAA and observed daily until egg hatching, and the time between egg laying and hatching recorded as egg hatching time. Diet was replenished daily until larvae pupated and the time between egg hatching and pupation recorded as larval developmental time. The pupated individuals were observed until adult emergence, and pupal

developmental times were recorded. Then, all Petri dishes were observed at 24-h intervals until all individuals died, and the time between adult emergence and death were recorded as adult longevity. Female sex ratio and adult weight of the newly emerged adults were also assessed. Adult body size of *G. mellonella* was determined by measuring the length from head to the tip of the abdomen using an Olympus SZ51 (Olympus, center Valley, PA) stereo dissecting microscope equipped with a calibrated eyepiece micrometer.

In a parallel set of experiments, upon molting to the last stage, larvae ( $0.18 \pm 0.02$  g) of *G. mellonella*  $F_1$  were randomly selected from the jars and transferred in groups of five to sterile Petri dishes and controlled every day until larvae pupated. Then, parasitization was performed on day 1 or 2 of the host pupae by exposing an individual host pupa to an individual 10- to 20-d-old wasp female. Parasitized pupae were observed until adult emergence. The time required for completion of development from egg deposition to adult eclosion of parasitoids was recorded as immature developmental time. Newly emerged female and male parasitoid weight from each treatment and control group was recorded as adult weight. Then, individual mating pairs of five for each experimental and control group were placed in a 210 ml cup containing a cotton ball saturated in a 30% (wt: vol) honey solution. Cups were covered with a mesh cloth and food was replenished every day. Parasitoids were observed at 24-h intervals until all parasitoids died and adult longevity was recorded. Adult body size of *P. turionellae* was also determined by the method described above for host species.

### Statistical Analysis

The experiments were repeated three times with specimens taken from different populations at different times. Means were compared using one-way analysis of variance (ANOVA) of SPSS V.18 for Windows. Means were subjected to Tukey's Honestly Significant Difference (HSD)

Table 1

IAA-related changes in total number of eggs/female, percent fecundity, percent fertility and corrected percent sterility of *G. mellonella*.

IAA (ppm)	Total no. of eggs/female Mean <sup>a</sup> ± SE <sup>b</sup>	Percent fecundity Mean <sup>a</sup> ± SE <sup>b</sup>	Percent fertility Mean <sup>a</sup> ± SE <sup>b</sup>	Corrected percent sterility Mean <sup>a</sup> ± SE <sup>b</sup>
0	699.53±77.98a	100.00±0.00ab	88.04±2.00a	—
50	784.67±82.85a	112.17±11.84b	92.75±0.89a	-5.35±1.01a
500	581.67±80.45a	83.15±11.50ab	89.20±1.42a	-1.32±1.61a
1,000	571.53±84.62a	89.47±12.18ab	70.07±8.18b	20.41±9.29b
5,000	465.33±74.05a	66.52±10.59a	83.25±4.01ab	5.45±4.56ab
10,000	546.87±66.14a	78.18±9.46ab	87.57±2.74a	0.53±3.12a

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

<sup>b</sup> Average of 15 individuals per treatment.

test when variances were homogenous, but Tamhane T2 tests otherwise were used to assess the significance of the effects of IAA doses ( $P<0.05$ ).

## RESULTS

The total number of eggs laid by a single host female fed on IAA-free diet was  $699.53 \pm 77.98$ . Treatment of IAA did not considerably affect the number of eggs laid by *G. mellonella* regardless of the dose tested ( $F = 2.164$ ;  $df = 5, 84$ ;  $P = 0.066$ ). The number of eggs laid per female did decrease at all dose levels except 50 ppm, however the differences were not significant with respect to controls (Table 1). The percent fecundity of the control group was assumed as 100% in order to determine the relative percent fecundity of assays. The lack of a decrease in total number of eggs at 50 ppm of IAA treatment also led to an increase in percent fecundity and fertility where fecundity was increased to  $112.17 \pm 11.84\%$  with  $92.75 \pm 0.89\%$  fertility at the lowest concentration. This trend was not the

same for other dose treatments with a fluctuation among doses, reduced significantly at only 5,000 ppm for percent fecundity ( $F = 2.528$ ;  $df = 4, 70$ ;  $P = 0.035$ ) (Table 1). Percent fertility decreased significantly only at 1,000 ppm ( $F = 3.940$ ;  $df = 5, 84$ ;  $P = 0.003$ ) with a considerable increase in corrected percent sterility ( $F = 4.146$ ;  $df = 4, 70$ ;  $P = 0.005$ ) reaching  $20.41 \pm 9.29\%$  when compared to other doses (Table 1).

Exposure to IAA in host diet resulted in an increase in egg hatching time of  $F_2$  females of *G. mellonella* only at 1,000 and 10,000 ppm doses ( $F = 19.731$ ;  $df = 5, 264$ ;  $P = 0.00$ ) (Table 2). Similarly, larval developmental time increased ( $F = 29.916$ ;  $df = 5, 264$ ;  $P = 0.00$ ) at 500 and 5,000 ppm with respect to control (Table 2). However, pupal developmental time of *G. mellonella* decreased ( $F = 86.128$ ;  $df = 5, 264$ ;  $P = 0.00$ ) by  $>47\%$  at 1,000 and 10,000 ppm (Table 2). On other hand, adult emergence time fluctuated among treatments ( $F = 48.676$ ;  $df = 5, 264$ ;  $P = 0.00$ ) and significantly decreased at 1,000 and 10,000 ppm and increased at 50 and 500 ppm with respect to controls (Table 2).

TABLE 2

IAA-related changes in egg hatching, larval, pupal developmental and adult emergence times (day) of *G. mellonella*.

IAA (ppm)	Egg hatching time	Larval developmental time	Pupal developmental time	Adult emergence time
	Mean <sup>a</sup> ± SE <sup>b</sup>			
0	10.16±0.12a	44.13±0.43a	13.18±0.27a	67.47±0.40b
50	9.96±0.07a	45.18±0.20ab	14.78±0.79a	69.91±0.74c
500	10.16±0.05a	46.93±0.20c	13.31±0.26a	70.40±0.30c
1,000	12.84±0.68b	43.00±0.30a	6.80±0.29b	62.78±0.87a
5,000	10.20±0.08a	45.62±0.23b	13.22±0.29a	69.04±0.32bc
10,000	12.27±0.10b	43.27±0.22a	6.36±0.16b	61.89±0.15a

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

<sup>b</sup> Average of 45 individuals per treatment.

IAA-treated *G. mellonella* F<sub>2</sub> adults lived for shorter times than controls at all doses tested, however this decline in longevity of adults was not significant ( $F = 2.196$ ;  $df = 5, 264$ ;  $P = 0.055$ ). This trend was also similar in the female sex ratio of adults, showing variations among doses but the ratio did not differ significantly ( $F = 2.137$ ;  $df = 5, 12$ ;  $P = 0.130$ ) upon exposure to different doses of IAA (Table 3). On the other hand, adult weight of *G. mellonella* significantly decreased ( $F = 4.538$ ;  $df = 5, 264$ ;  $P = 0.001$ ) at 500 ppm with respect to controls (Table 3). Adult size of *G. mellonella* did not differ significantly when compared to controls, but it did increase at 10,000 ppm with respect to 500 and 5,000 ppm ( $F = 3.610$ ;  $df = 5, 264$ ;  $P = 0.004$ ) (Table 3).

Immature developmental time of *P. turionellae* females reared on *G. mellonella* pupae exposed to different doses of IAA was not significantly different to that of female parasitoids reared on untreated hosts ( $F = 1.418$ ;  $df = 5, 174$ ;  $P = 0.198$ ) (Table 4). However, males at 1,000 ppm ( $F = 4.472$ ;  $df = 5, 174$ ;  $P = 0.001$ ) completed their immature development later than did those in control and other experimental groups. Wasp

development from egg to adult at 25°C normally required 13-23 d in the control group. Treatment with IAA increased immature developmental time of parasitoids, especially at doses >500 ppm ( $F = 4.259$ ;  $df = 5, 354$ ;  $P = 0.001$ ) and parasitoids reared on hosts exposed to 1,000 ppm IAA emerged 2-4 d later than did controls (Table 4).

The mean longevity of IAA-treated adults decreased significantly at doses of 1,000 and 10,000 ppm ( $F = 9.292$ ;  $df = 5, 354$ ;  $P = 0.000$ ) compared with lower doses tested and wasps reared on untreated hosts. Adults lived 30% and 27% shorter lives than did controls at doses of 1,000 and 10,000 ppm, respectively (Table 4). All IAA-treated females except for those at 500 ppm lived shorter lives than did controls, however there was only a considerable decline in the longevity of females ( $F = 8.901$ ;  $df = 5, 174$ ;  $P = 0.001$ ) at 1,000 and 10,000 ppm. The longevity of females declined by 28 and 33%, respectively. Male longevity fluctuated among doses ( $F = 4.160$ ;  $df = 5, 174$ ;  $P = 0.001$ ) with a significant decrease only at 1,000 ppm such that males lived 33% shorter lives than did controls (Table 4).

Table 3

IAA-related changes in adult longevity, female sex ratio, adult weight and size of *G. mellonella*.

IAA (ppm)	Adult longevity (day)	Female sex ratio (%)	Adult weight (g)	Adult size (mm)
	Mean <sup>a</sup> ± SE <sup>b</sup>			
0	22.49±1.29a	86.66±6.67a	0.09±0.00a	12.32±0.19ab
50	18.73±0.96a	93.33±6.67a	0.09±0.00a	12.39±0.17ab
500	19.42±0.89a	88.89±5.88a	0.07±0.00b	11.93±0.18a
1,000	20.38±0.93a	68.89±8.01a	0.08±0.00a	12.61±0.20ab
5,000	22.07±1.13a	84.44±4.44a	0.08±0.00ab	12.10±0.17a
10,000	18.73±1.39a	73.33±6.67a	0.09±0.00a	12.88±0.16b

<sup>a</sup> Means within each column followed by the same letter are not significantly different ( $P>0.05$ ).<sup>b</sup> Average of 45 individuals per treatment.

Treatment with IAA did not affect the adult weight of males ( $F = 1.073$ ;  $df = 5, 174$ ;  $P = 0.377$ ) and both sexes combined ( $F = 1.177$ ;  $df = 5, 354$ ;  $P = 0.177$ ) when compared to controls. However, there was only a significant decrease in female weight at 5,000 ppm ( $F = 2.676$ ;  $df =$

5, 174;  $P = 0.023$ ) when compared to 500 and 10,000 ppm (Table 5).

Adult wasp females reared on IAA-treated hosts did not differ in length ( $F = 1.160$ ;  $df = 5, 174$ ;  $P = 0.331$ ), while there were only significant

Table 4

IAA-related changes in immature developmental time and adult longevity of *P. turionellae*.

IAA(ppm)	Immature Developmental Time (day)			Adult Longevity (day)		
	Female	Male	Both sexes	Female	Male	Both sexes
	Mean <sup>a</sup> ± SE <sup>b</sup>	Mean <sup>a</sup> ± SE <sup>b</sup>	Mean <sup>c</sup> ± SE <sup>b</sup>	Mean <sup>a</sup> ± SE <sup>b</sup>	Mean <sup>a</sup> ± SE <sup>b</sup>	Mean <sup>c</sup> ± SE <sup>b</sup>
0	19.50± 0.31a	17.17 ±0.43a	18.33±0.30a	56.20±3.10ab	42.10±2.24b	49.15±2.11a
50	19.50±0.27a	17.97±0.30ab	18.73±0.22ab	55.60±2.88ab	38.53±2.78ab	47.07±2.27a
500	18.90±0.27a	17.60±0.18a	18.25± 0.18a	63.33±4.72ab	41.20±3.53b	52.27±3.26a
1,000	19.93±0.30a	19.13±0.38b	19.53± 0.25b	40.70±2.59cd	28.03±2.09a	34.37±1.85b
5,000	19.80±0.35a	18.53±0.36ab	19.17± 0.26ab	54.17±3.99bc	33.67±2.88ab	43.92±2.78ab
10,000	19.50± 0.31a	17.17 ±0.43ab	19.05± 0.22ab	37.73±2.87d	33.47±2.06ab	35.60±1.77b

<sup>a</sup> Means within each column followed by the same letter are not significantly different ( $P>0.05$ ).<sup>b</sup> Average of 30 individuals per treatment.<sup>c</sup> Average of 60 individuals per treatment.

Table 5

IAA-related changes in adult weight and adult size of *P. turionellae*.

IAA (ppm)	Adult weight (g)			Adult size (mm)		
	Female Mean <sup>a</sup> ± SE <sup>b</sup>	Male Mean <sup>a</sup> ±SE <sup>b</sup>	Both sexes Mean <sup>c</sup> ±SE <sup>b</sup>	Female Mean <sup>a</sup> ± SE <sup>b</sup>	Male Mean <sup>a</sup> ± SE <sup>b</sup>	Both sexes Mean <sup>c</sup> ± SE <sup>b</sup>
0	0.03±0.00ab	0.02±0.00a	0.02±0.00a	10.82±0.18a	9.87±0.15ab	10.36±0.13ab
50	0.03±0.00ab	0.02±0.00a	0.02±0.00a	10.52±0.15a	9.85±0.22ab	10.19±0.14ab
500	0.03±0.00b	0.02±0.00a	0.03±0.00a	11.00±0.15a	10.35±0.19b	10.68±0.13b
1,000	0.03±0.00ab	0.02±0.00a	0.02±0.00a	10.60±0.18a	9.42±0.19a	10.01±0.15a
5,000	0.02±0.00a	0.02±0.00a	0.02±0.00a	10.60±0.19a	10.15±0.20ab	10.38±0.14ab
10,000	0.03±0.00b	0.02±0.00a	0.02±0.00a	10.75±0.15a	9.58±0.20ab	10.17±0.14a

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

<sup>b</sup> Average of 30 individuals per treatment.

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

variations among doses in males ( $F = 3.183$ ;  $df = 5, 174$ ;  $P = 0.000$ ) and both sexes combined ( $F = 2.724$ ;  $df = 5, 354$ ;  $P = 0.020$ ). However, IAA treatment also did not affect the size of males and both sexes combined when compared to that of controls (Table 5).

## DISCUSSION

Previous studies have demonstrated that different PGRs influence the well-being of various insects differently, including pests (KAUR & RUP, 2002; 2003a; 2003b; HARIKESH & BHATTACHARYA, 2003; GUPTA et al. 2009) and those species that are beneficial (UÇKAN et al., 2008; UÇKAN et al., 2011a; b), and are considered as ecological life vests (UÇKAN & GÜLEL, 2002). Here, we first attempted to explore the effects of IAA on the reproductive potential of the pest species, *G. mellonella*. Analysis of the data for the total number of eggs laid per female revealed that treatment with different IAA doses did not affect the reproductive potential of the pest species. Percent fecundity increased by 12%

at 50 ppm whereas the most effective decrease by >33% was observed at 5,000 ppm. However, both results were not significantly different from that observed in controls. Percent fertility significantly decreased by >20% at 1,000 ppm. Compatible with percent fertility, all treatments displayed  $\pm 5\%$  variations when compared to controls except for a significant increase in corrected percent sterility by >20% at 1,000 ppm.

It is likely that IAA had a low level of adverse activity on the reproductive potential of *G. mellonella*. Similar responses have also been observed in *B. cucurbitae* (KAUR & RUP, 2002) *Dacus dorsalis* (Hendel, 1912) (THAKUR & KUMAR, 1984), *Lipaphis erysimi* (Kaltenbach, 1843) (RUP & DHILLON, 1999) and *Zaprionus paravittiger* (Godbole & Vaidya, 1972) (RUP et al., 1997). Researchers suggested that the impact of IAA may be correlated to its interference with the neurosecretory system, which may affect the reproductive system (THAKUR & KUMAR 1984). Our observations may suggest that IAA does not have a lethal effect toward developing pest progeny across trophic levels.

We previously demonstrated GA<sub>3</sub> had consistent negative effects on the pre-adult developmental time of *G. mellonella* with >35% reduction in overall time to adult eclosion largely due to the decrease in egg-larval developmental time at the highest dose of 5,000 ppm tested (UÇKAN et al., 2011b). However, this study with the same pest species did not find a considerable effect of IAA on the egg hatching, larval, pupal developmental and adult emergence times from treatments; rather, only slight increases in egg hatching and larval developmental times, decreases in pupal developmental time, and both in adult emergence time were recorded at some doses tested. The most striking effect observed was a decline in pupal developmental time of *G. mellonella* by >47% at 1,000 and 10,000 ppm. GUPTA et al. (2009) reported that GA<sub>3</sub> and siapton caused an increase in the larval period of the hairy caterpillar, *S. oblique* at high doses. Furthermore, KAUR & RUP (1999) observed parallel responses in *B. cucurbitae* by using GA<sub>3</sub>. However, GUPTA et al. (2009) reported that triacontanal (a saturated long chain alcohol that is known to have a growth promoting activity) did not cause any significant difference in larval or pupal period at any dose tested. Thus, the effects of plant growth regulators on insect pests are variable.

We have previously suggested that GA<sub>3</sub> could be used as an insecticide against *G. mellonella* since treatment with GA<sub>3</sub> in the diet resulted in a significant influence on the immature developmental time of the pest larva, which is considered the most damaging stage of the pest species (Uçkan et al., 2011b). Further evidence from that study that GA<sub>3</sub> did not affect the egg-to-adult developmental time of parasitoids also supports the assumption that GA<sub>3</sub> would be a successful chemosterilant against pest species. However, in the present study, IAA had a relatively low level of adverse activity on the reproductive potential and immature developmental times and almost no effect was observed on adult longevity and female sex ratio of the pest species. The differences in adult size and weight were also significant at only some doses tested. On the other hand, we observed that IAA treatment

caused a slight prolongation in adult emergence and decrease in adult longevity of parasitoids reared on hosts exposed to different doses; longevity of wasps declined by >27% at higher doses. Our previous work also demonstrated that the egg to adult developmental time of the wasp *A. galleriae* reared on *A. grisella* larvae exposed to high doses ( $\geq 200$  ppm) of GA<sub>3</sub> increased by 40% (UÇKAN et al., 2008), and increased by 30% when wasps were reared on larvae exposed to  $\geq 500$  ppm of IAA (UÇKAN et al., 2011a). Similar responses in the adult longevity of parasitoid wasps have also been observed as a decrease by >50% when wasps were reared on hosts exposed to high doses of IAA and GA<sub>3</sub> treatments (UÇKAN et al., 2008, 2011a). KAUR & KAUR (2013) also observed decreased female life span and extended developmental periods of the braconid wasp *B. hebetor* reared on *S. litura* exposed to high doses of coumarin. These results are expected, because previous studies displayed that PGRs reduce the total lipid and carbohydrate levels in insects (RAUP et al., 1998; KAUR & RUP 2003b). We have observed that lipid at all doses, and carbohydrate at most of the doses, decreased in the hemolymph of *G. mellonella* larvae upon exposure to GA<sub>3</sub> (UÇKAN et al., 2011b). Recently, we also found that GA<sub>3</sub> resulted in different effects on the quantity of free amino acids associated with energy metabolism of *G. mellonella* and *P. turionellae* (ALTUNTAŞ et al., 2014). It is known that stress responses in arthropods are energetically demanding events (KORSLOOT et al., 2004) and RUP et al. (2000, 2002) suggested that PGRs-induced stress may cause decreases in hemolymph components. It is likely that the decrease in energy reserves of the host resulting from IAA-induced stress may cause delay in the immature growth and development and a decline in adult longevity of parasitoid species (UÇKAN & ERGIN 2002; UÇKAN et al., 2007, 2008). Thus, it may be concluded that PGRs influence the life history parameters of various insects differently, depending on a number of factors. In the present case, our data displayed no severe adverse effects on the reproductive potential, development and longevity of pest species, whereas interferences in development

and longevity of wasps were abundant at high doses of IAA treatment. Further effects of IAA should be explored for its overall influence on pest status before proposing this plant growth factor as an environmentally-safe compound for use in the management of lepidopteran pest species. The authors are currently attempting to evaluate the effects of IAA on the hemolytic and phenoloxidase activity, hormones controlling insect development and metamorphosis, and antioxidant enzymes detoxifying free radicals of the pest species *G. mellonella*. In addition, investigation of how the same parameters are affected by IAA via host feeding for *P. turionellae* is within the scope of new research.

## REFERENCES

- AHMAD N, RASHDI SMMS & RAJPUT AA (2003). Efficacy of plant growth regulators to manage the insect pests of cotton. *Asian Journal of Plant Sciences*, 2:544-547.
- ALTUNTAŞ H, KILIÇ AY, UÇKAN F & ERGİN E (2012). Effects of gibberellic acid on hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Environmental Entomology*, 41 (3):688-696.
- ALTUNTAŞ H, UÇKAN F, KILIÇ AY & ERGİN E (2014). Effects of gibberellic acid on hemolymph free amino acids of *Galleria mellonella* (Lepidoptera: Pyralidae) and Endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae). *Annals of the Entomological Society of America*, 107 (5):1000-1009.
- BRONSKILL JF (1961). A cage to simplify the rearing of the greater wax moth, *Galleria mellonella* (Pyralidae). *Journal of the Lepidoptera Society*, 15:102-104.
- DOUTT RL (1955). Reviewed Biological Control of Agricultural Pests. *The American Biology Teacher*, 17(4):134-136.
- ERGİN E, ER A, UÇKAN F & RIVERS DB (2007). Effects of cypermethrin exposed hosts on egg-adult development time, number of offspring, sex ratio, longevity, and size of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). *Belgian Journal of Zoology*, 137:27-31.
- FISHER R (1987). Ecological investigations on pupal parasitoids (Hym., Ichneumonidae) of four *Yponomeuta* species (Lepid., Yponomeutidae). *Journal of Applied Entomology*, 103(1-5):515-523.
- GUPTA G, YADAV SR, BHATTACHARYA AK (2009). Influence of synthetic plant growth substances on the survivorship and developmental parameters of *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae). *Journal of Pesticide Science*, 82:41-46.
- HAESSELBARTH E (1979). On the pupal parasites of the Pine Beauty (*Panolis flammea* (Schiff.)), the Pine Looper (*Bupalus piniarius* (L.)) and of *Boarmia bistortata* (Goeze) in Bavarian pine forests. *Zeitschrift für Angewandte Entomologie*, 87(3):186-202.
- HARIKESH S, BHATTACHARYA AK (2003). Negative role of gibberellic acid on the developmental behaviour of *Spodoptera litura*. *Indian Journal of Entomology*, 65: 293-297.
- KANSU IA & UĞUR A (1984). Investigations into the biological relationships between *Pimpla turionellae* (L.) (Hymenoptera - Ichneumonidae) and some lepidopterous pupae hosts. *Doğa Bilim Dergisi*, 8(2):160-173.
- KAUR M & KAUR S (2013). Tritrophic interactions among coumarin, the herbivore *Spodoptera litura* and a gregarious ectoparasitoid *Bracon hebetor*. *BioControl*, 58:755-763.
- KAUR R & RUP PJ (1999). Evaluation of gibberellic acid against immature stages of *Bactrocera cucurbitae* (Coquillett). *Journal of Insect Science*, 12:9-14.
- KAUR R & RUP PJ (2002). 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.
- KAUR R & RUP PJ (2003a). Influence of four plant growth regulators on development of the melon fruit fly, *Bactrocera cucurbitae* (Coquillett). *Insect Science and Its Application*, 23:121-125.
- KAUR R & RUP PJ (2003b). Influence of some plant growth regulators (PGR) on biochemical profile in the larvae of melon fruit fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Trypetidae). *Entomon*, 28:89-95.
- KORSLOOT A, VAN GESTEL CAM & VAN STRAALLEN NM (2004). Environmental stress and cellular response in arthropods. CRC press, Boca Raton, Florida.
- PAULSON GS, HULL LA & BIDDINGER DJ (2005). Effect of a plant growth regulator prohexadione-

- calcium on insect pests of apple and pear. *Journal of Economic Entomology*, 98:423-431.
- PRADO SG & FRANK SD (2013). Tritrophic effects of plant growth regulators in an aphid-parasitoid system. *Biological Control*, 66:72-76.
- RUP PJ & DHILLON MK (1999). Morphogenetic and biochemical responses of the mustard aphid, *Lipaphis erysimi* (Kalt.) (Homoptera: Aphididae) to indole-3-acetic acid (IAA). *Journal of Aphidology*, 13:31-38.
- RUP PJ & KALIA S (1993). Effect of gibberellic acid on the development of banana fruit fly, *Zaprionus paravittiger* (Godbole and Vaidya) (Drosophilidae: Diptera). *Pest Management and Economic Zoology*, 1:27-31.
- RUP PJ, KAUR R & KAUR J (1998). Effect of gibberellic acid (GA3) on the protein, lipid and carbohydrate contents of banana fruit fly, *Zaprionus paravittiger* larvae. *Insect Science and Its Application*, 18:145-148.
- RUPPJ, KUMARIS & SOHALSK (1997). Morphogenetic responses of *Zaprionus paravittiger* to indole acetic acid. In: GROVER IS & THUKRAL AS (eds) *Environment and Development*. Scientific Publishers, Jodhpur, India:289-293.
- RUP PJ, SOHAL SK, KAUR G & DHILLON M (2002). The influence of allelochemicals and plant growth regulators on emergence and development of mustard aphid, *Lipaphis erysimi* (Kalt.). *Allelopathy Journal*, 10:53-58.
- RUP PJ, SOHAL SK, SOHI R, KAUR G, SANDHU N, GURM SK, DHINGRA P & WADHWA SK (2000). Influence of PGRs on carbohydrate content in *Lipaphis erysimi* (Kalt.). *Indian Journal of Experimental Biology*, 38:1066-1068.
- SAK O, UÇKAN F & ERĞİN E (2006). Effects of cypermethrin on total body weight, glycogen, protein, and lipid contents of *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). *Belgian Journal of Zoology*, 136:53-58.
- SALAMA HS & EL-SHARABY AM (1972). Gibberellic acid and  $\beta$ -sitosterol as sterilants of the cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae). *Experientia*, 28:413-414.
- SANFORD MT (1987). *Diseases and Pests of the Honey Bee*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.
- SLANSKY JRF (1986). Nutritional ecology of endoparasitic insects and their hosts: an overview. *Journal of Insect Physiology*, 32(4):255-261.
- TANAKA T & MINAKUCHI C (2012). Insecticides and Parasitoids. In: PERVEEN F (ed.) *Insecticides - Advances in Integrated Pest Management*. InTech: 115-140. (ISBN: 978-953-307-780-2)
- THAKUR JN & KUMAR A (1984). Effects of 3-indole acetic acid on the fertility of fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae). *National Academy Science Letters India*, 7:197-199.
- UÇKAN F & ERĞİN E (2002). Effect of host diet on the immature developmental time, fecundity, sex ratio, adult longevity, and size of *Apanteles galleriae* (Hymenoptera: Braconidae). *Environmental Entomology*, 31:168-171.
- UÇKAN F & GÜLEL A (2002). Age-related fecundity and sex ratio variation in *Apanteles galleriae* (Hym., Braconidae) and host effect on fecundity and sex ratio of its hyperparasitoid *Dibrachys boarmiae* (Hym., Pteromalidae). *Journal of Applied Entomology*, 126:534-537.
- UÇKAN F, HEPCORMAN ŞENGÜL Ş, SAK O & KORKMAZ M (2007). Effects of 5-aza-dC on biological parameters of the larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae), and on its host, *Achoria grisella* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America*, 100:265-269.
- UÇKAN F, HAFTACI İ & ERĞİN E (2011a) Effects of indol-3-acetic acid on biological parameters of the larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae). *Annals of the Entomological Society of America*, 104(1):77-82.
- UÇKAN F, ÖZTÜRK Z, ALTUNTAŞ H & ERĞİN E (2011b). Effects of gibberellic acid (GA3) on biological parameters and hemolymph metabolites of the pupal endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae) and its host *Galleria mellonella* (Lepidoptera: Pyralidae). *Journal of the Entomological Research Society*, 13(3):1-14.
- UÇKAN F, TÜVEN A, ER A & ERĞİN E (2008). Effects of gibberellic acid on biological parameters of the larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae). *Annals of the Entomological Society of America*, 101:593-597.

Received: July 14th, 2014

Accepted: February 8th, 2015

Branch editor: Guy Smagghe