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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)

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ABSTRACT

The non-target effects of the plant growth regulator gibberellic acid (GA_3) on host and parasitoid pre-adult development along with adult parasitoid longevity and size were examined using the endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae) reared on its host *Galleria mellonella* (Lepidoptera: Pyralidae) treated with different doses of the GA_3 in diet. Total protein, carbohydrate, and lipid content of hemolymph from parasitoid and host larvae were also evaluated with respect to GA_3 doses. Host development from egg to adult took 86-112 d. However, adult completed pre-adult development at an average of 34 d earlier than controls at the highest dose of 5,000 ppm. GA_3 treatment had the most significant effect on the egg-larval developmental time of host with more than 35% decrease at doses >1,000 ppm. GA_3 treatment did not affect the adult emergence time of parasitoids reared on hosts exposed to different doses. Adult longevity of wasps increased at 50, 100, and 200 ppm, whereas a decrease in longevity was apparent at 2,000 ppm with respect to controls. GA_3 treatment did not affect the adult length of wasps at doses ≤200 ppm however, a decrease in length was observed beyond 200 ppm but not at the highest dose 5,000 ppm. Hemolymph lipid at all and carbohydrate at most of the doses decreased in host larvae upon exposure to GA_3. The increase in hemolymph protein content of host larvae is likely to indicate a physiological adaptability to compensate for GA_3-induced stress. Total protein of wasp larvae fluctuated among treatment doses, was significantly lower at all doses except for the insignificant decrease at 2,000 ppm and increases at 50 and 200 ppm. Total lipid content of GA_3 treated groups increased significantly at only 100 ppm. Except for 100 ppm, there appeared insignificant decreases at doses ≤500 ppm and increases at doses >500 ppm. Total carbohydrate of wasp larvae increased at 100 and 1,000 ppm but decreased at 200 and 5,000 ppm. The potential significance of GA_3 on pest species and its natural enemy which may be used in Integrated Pest Management programs is discussed.

Key words: Pimpla turionellae, *Galleria mellonella*, gibberellic acid, toxicity, parasitoid, sublethal effects.

INTRODUCTION

The progress in the use of synthetic plant growth regulators (PGRs) in agriculture has brought the subject of whether these compounds have any effect on different

biological traits of insects that feed on them into question. Currently, there is evidence that PGRs cause death of insects by their toxic impacts (Honeyborne, 1969; Önder *et al*., 1987), deformations, weight reduction (Carlisle *et al*., 1969; Awad and Taha, 1976), antifeedant effect (Tahori *et al*., 1965; Mansour and Dimetry, 1976), retardation in morphological and sexual development (Carlisle *et al*., 1969; Van Emden, 1969), sterility and reduction in fertility (Bhalla and Robinson, 1968; Honeyborne, 1969; Carlisle *et al*., 1969; El-Ibrashy, 1972; Scheurer and Aschermann, 1976; Prasad *et al*., 1977; Dreyer *et al*., 1984), and obstruction of going through diapose (Bariola *et al*., 1976). Moreover, Posnova (1974) stated that PGRs might also be used in integrated pest management (IPM) programs aimed at economically important insect pests. Recent studies seemed to support this presumption that when combined with insecticides, PGRs caused additive or synergistic effects on insects (McDonald *et al*., 1988; Ahmad *et al*., 2003; Silva *et al*., 2003; Paulson *et al*., 2005; Gupta *et al*., 2009; Uçkan *et al*., 2011). Nevertheless, our latest studies also revealed that these compounds that act as plant growth regulators and against pests might also have significant effects on the natural enemies of insect pests. The effects on parasitoids and predators that result from contamination in their diet by trophic exposure and by exogenous application, in turn, may disrupt the ecology of the interaction among natural enemies-herbivores and plants (Barbosa *et al*., 1991).

Recently, we examined the impact of gibberellic acid (GA_3) (Uçkan *et al*., 2008) and indol-3-acetic acid (IAA) (Uçkan *et al*., 2011), on the development and reproduction of the solitary koinobiont larval endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) reared on the lesser wax moth, *Achoria grisella* Fabr. (Lepidoptera: Pyralidae) larvae that ingested the growth regulator in their diet and found dose dependent changes in adult parasitoid longevity and length of development from egg-to-adult emergence, and F_2 progeny decreased by >40% when compared with the controls in both treatments at high doses. GA_3 belongs to one of the broadest groups of PGRs, gibberellins which are tetracyclic diterpenoid structured PGRs that play role in many mechanisms of growing and development of the plants (Sun and Gubler, 2004). GA_3 is commonly used in many commercial fields such as speeding up stem extension, germination event, blooming, and growth of the fruit (Salisbury and Ross, 1992). The influence of GA_3 treatment on development, survival, longevity, and reproductive potential of pest insects has recently been studied in a number of insect pests (Kaur and Rup, 2002; 2003a; Harikesh and Bhattacharya, 2003). Kaur and Rup (2002) treated the larvae of the melon fruitfly, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) with GA_3, IAA, kinetin, and coumarin and found they can influence the insects reproductive potential. Harikesh and Bhattacharya (2003) reported inhibitory effects on growth, development, and survival of larval *Spodoptera litura* F. (Lepidoptera: Noctuidae). However, except our studies there is no information on their physiological and biochemical effects towards parasitoids.

P. turionellae uses prepupae and pupae of hosts from an extremely wide range of lepidopteran species (Kansu and Uğur, 1984; Uçkan and Gülel, 1990). Larvae of the host species are serious pests in beehives because they feed on combs, wax,

and honey. Hosts also feed on plants during larval stages and the accumulation of environmental pollutants and transmission of these compounds to their parasitoids is likely to occur (Sak *et al*., 2006; Ergin *et al*., 2007). Therefore, it is conceivable that *P. turionellae* adults feeding on honey, fruit nectar, and host larvae may also be exposed to GA_3 broadly used in orchards. Here, we aimed to examine the effects of GA_3 on adult emergence time, adult longevity, and body length of the solitary idiobiont pupal endoparasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) reared on its host, greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) treated with different doses of the PGR and observed differences for egg-larval, pupal developmental, and adult emergence time. Total protein, carbohydrate, and lipid content of hemolymph from parasitoid and host species were also evaluated with respect to GA_3 doses.

MATERIAL AND METHODS

Insect Rearing

G. mellonella original stock came from colonies kept in our laboratory at the Kocaeli University, Kocaeli, Turkey. A laboratory colony of *P. turionellae* was established from parasitized pupae of *G. mellonella* maintained from stock cultures at Kocaeli University. *P. turionellae* were mass reared on the pupae of the host, *G. mellonella* in cages (25 x 25 x 25 cm) located in an environmental chamber at 25 ± 2°C, 60 ± 5% RH, and a photoperiod of 12:12 (L: D) h. Adults of *P. turionellae* 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). Parasitoid wasps were kept under the same rearing conditions as the host. Host colony was 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 added for egg deposition and feeding of the newly hatched larvae.

Bioassays

An individual mating pair of the host, 1-2-d-old *G. mellonella* was placed in 1-L jars containing 1 g honeycomb to provide a mating and oviposition substrate. The adults were exposed to 10 g of host diet (Sak *et al*., 2006) treated with 50, 100, 200, 500, 1,000, and 5,000 ppm GA_3 (Merck 5 g, Darmstadt, Germany) homogenized with doses in separate jars and removed from the jars on the fifth day. Hosts reared on GA_3-free diet were controls. To determine the impact of GA_3 on the duration of egg-larval, pupal developmental and adult emergence, last instars of *G. mellonella* (0.18 ±0.02 g) were randomly selected and individually transferred to sterile petri dishes (90 x 15 mm). Larvae were exposed to 2 g of host diet (Sak *et al*., 2006) treated with different doses of GA_3 and the diet was replenished at every day until larvae pupated. Petri dishes were covered with a mesh cloth (100 mesies/cm^2) and held under the environmental conditions mentioned above. Larvae were observed at 24-h intervals until adult emergence. The day the host females deposited eggs was considered as the first day of the hosts' pre-adult developmental time. The time required for completion of egg-larval and pupal stages were recorded as were the time of adult emergence per

larvae. Assays for each experimental and control group were replicated six times with twenty larvae completely randomized from different populations at different times. Larvae reared on GA_3-free diet were controls.

In a parallel set of experiments, twenty last instars of *G. mellonella* (0.18 ±0.02 g) were randomly selected from each treatment and control group, and individually transferred to 1-L jars including 2 g of host diet treated with different doses of GA_3. Larvae reared on GA_3-free diet were controls. The jars were covered with a mesh cloth (100 mesies/cm^2) and held under the environmental conditions mentioned above. The diet was replenished at 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 10-20-d-old wasp female. Parasitized pupae were observed until the emergence of adults. The time required for completion of parasitoid development from egg deposition to adult eclosion of parasitoids was recorded. Longevity of newly emerged adult female and male wasps from each treatment was assessed by placing an individual mating pair (n = 20) into a 210-ml cup containing a cotton ball saturated in a 50% (wt/vol) honey solution. Cups were covered with a mesh cloth (100 mesies/cm^2) and held under the environmental conditions mentioned above for the stock cultures. Food was replenished at every day. Parasitoids were observed at 24-h intervals until all parasitoids had died. Adult body lengths of GA_3-treated wasps and controls were determined by measuring length from head to the tip of the abdomen using an Olympus SZ51 (Olympus, Center Valley, PA) stereodissecting microscope equipped with a calibrated eyepiece micrometer.

Hemolymph Collection

To investigate the effects of GA_3 on the hemolymph total protein, carbohydrate, and lipid content, hemolymph samples obtained from *G. mellonella* last instars and *P. turionellae* newly-ecdysed final instars dissected from parasitized host pupae exposed to different doses GA_3 were used. Host and parasitoid larvae reared on GA_3-free diet were controls. Host and parasitoid larvae were sterilized with ethanol and anaesthetized on ice for 10 min. Host larvae were bled by piercing the third proleg and parasitoid larvae on the front part of body with micro-scissors. Care was taken to avoid rupturing the larval gut or dislodging fat body. Ten microliters of hemolymph from each individual host and parasitoid larvae were collected with a 10-µl glass microcapillary tube (Sigma, St. Louis, MO) and transferred to a micro centrifuge tube placed on ice. To avoid hemocyte aggregation, hemolymph samples were immediately mixed to 0.001 mg 1-phenyl-2-thiourea and kept at 4°C. Prior to analysis, all hemolymph samples were centrifuged at 10,000 g for 5 min at 4°C. Assays for each treatment and control group were replicated nine times with 10 host and parasitoid larvae completely randomized from different populations at different times.

Determination of total protein

Total protein of each hemolymph sample from host and parasitoid larvae was estimated by the method of Lowry *et al*., (1951) using bovine serum albumin (BSA)

(Merck) as standard at a concentration range of 10-200 µg/ml. Following centrifugation, 1 µl of hemolymph from each sample was placed into a 1.5-ml spectrophotometer cuvette and Lowry reagent added. The optical density of the mixture was measured photometrically using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 695 nm.

Determination of total lipid

Total lipid content of each hemolymph sample from host and parasitoid larvae was determined by the vanillin-phosphoric acid method of Van Handel (1985b). To 1 µl of the hemolymph from each sample placed in 1.5-ml micro centrifuge tubes, 50 µl of 2% sodium sulphate and 450 µl of chloroform-methanol 2:1 (vol/vol) were added. The mixture was centrifuged at 14,000 rpm for 2 min. After centrifugation, 200 µl of the supernatant in each tube was transferred to different 1.5-ml micro centrifuge tubes for the lipid assay. All samples were heated at 90°C until the solution evaporated from the tubes. The lipid precipitate in tubes was mixed with concentrated sulphuric acid (40 µl). The mixture was stirred and heated in boiling water bath for 10 min. Then 40 µl of concentrated H_2SO_4 was added and the tubes were heated in boiling water bath for 2 min. The tubes were cooled on ice and 960 µl of vanillin-phosphoric acid reagent was added in each. The solution in each tube was held at room temperature for 30 min and then transferred to 1.5-ml cuvettes. The optical density was measured using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at a wavelength of 525 nm. The corn oil solution (Merck, Darmstadt, Germany) was used for the standard curve at a concentration range of 5-50 µg/ml.

Determination of total carbohydrate

An anthrone test developed by Van Handel (1985a) was used for the detection of carbohydrate in the hemolymph obtained from host and parasitoid larvae using glucose (Merck, Darmstadt, Germany) as standard at a range of 1-25 µg/ml. To 1 µl of the hemolymph from each sample placed in 1.5-ml micro centrifuge tubes, 50 µl of 2% sodium sulphate and 450 µl of chloroform-methanol 2:1 (vol/vol) were added. The mixture was centrifuged at 14,000 rpm for 2 min. After centrifugation, 200 µl of the supernatant in each tube was transferred to different 1.5-ml micro centrifuge tubes for the carbohydrate assay. All samples were heated at 90°C until approximately 50 µl of the solution remained in the tubes. Then 950 µl of anthrone reagent was added and the tubes were heated at 90 C° for 15 min. The solution in each tube was cooled on ice and then transferred to 1.5-ml cuvettes. The optical density was measured using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at a wavelength of 625 nm.

Statistics

GA_3-induced variations in egg-larval, pupal developmental and adult emergence time of the host were inferred using one-way analysis of variance (ANOVA) (SPSS 1999) as were the total protein, lipid, and carbohydrate content of hemolymph samples from host and parasitoid species. Data pooled for both parasitoid sexes for egg-to-adult developmental time, adult longevity, and length were subjected to two-way ANOVA to

determine the main effects of GA_3 doses, sex, and their interaction on adult emergence, longevity, and length. In case there was an influence of sex on the interaction between GA_3 doses and parasitoid adult emergence, longevity, and length, t-tests were also performed between sexes. Subsequently, means were subjected to Tukey's Honestly Significant Difference (HSD) tests when the variances are homogenous, but Tamhane tests otherwise to assess the significance of the effects of GA_3 doses. Dunnett's t-tests were also inferred to determine the significance of the effects of GA_3 doses on protein, lipid, and carbohydrate content of host and parasitoid larvae with respect to control group (SPSS, 1999).

RESULTS

Exposure of *G. mellonella* to a diet containing GA_3 resulted in a remarkable shortage of the egg-larval (F = 194.38; df = 7, 952; P < 0.001), pupal (F = 94.48; df = 7, 952; P < 0.001) development, and adult emergence (F = 128.51; df = 7, 952; P < 0.001) time. Host development from egg to adult at 25°C normally required 86 to 112 d. However, adult emergence of hosts reared on GA_3-treated diet occurred earlier than that of controls at all doses. Adults completed pre-adult development at an average of 34 d earlier than controls at the highest dose of 5,000 ppm. GA_3 treatment did the most significant effect on the egg-larval developmental time of host with more than 35% decrease at doses >1,000 ppm with respect to those reared on untreated diet (Table 1).

Table 1. Changes in egg-larval, pupal developmental and adult emergence time of *G. mellonella* fed on different doses of GA_3 incorporated diet.

GA_3 (ppm)	Egg-larval developmental time (day)		Pupal developmental time (day)		Adult emergence time (day)	
	Range	(mean[a] ± SE)[b]	Range	(mean[a] ± SE)[b]	Range	(mean[a] ± SE)[b]
0	72–92	82.30 ± 0.53a	8–23	14.98 ± 0.23a	86–112	97.28 ± 0.69a
50	40–72	60.14 ± 0.69b	9–26	18.19 ± 0.27b	58–92	78.33 ± 0.75b
100	51–67	59.77 ± 0.39b	17–33	23.51 ± 0.37c	70–100	83.28 ± 0.69c
200	44–76	59.98 ± 0.84b	10–35	23.99 ± 0.59c	60–111	83.97 ± 1.17c
500	44–82	62.12 ± 0.89b	10–31	19.78 ± 0.39d	54–100	81.89 ± 1.10bc
1000	45–67	56.54 ± 0.55c	8–24	15.02 ± 0.34a	58–91	71.56 ± 0.71d
2000	32–75	51.59 ± 0.91d	10–26	17.68 ± 0.39b	54–90	69.28 ± 1.17d
5000	32–69	48.38 ± 0.83d	10–24	15.00 ± 0.34a	48–88	63.38 ± 0.97e

[a] Average for six assays, each with 20 larvae per treatment.

[b] Means within a column followed by the same letter are not significantly different (P > 0.05; Tamhane test).

The effect of GA_3 on egg-to-adult developmental time of parasitoid wasps was not dose dependent (F = 1.72; df = 7; P > 0.05) but sex dependent (F = 16.43; df = 1; P < 0.001), and the relationship between GA_3 dose and egg-to-adult developmental time

was not influenced by gender (F = 0.78; df = 7; P > 0.05). Wasp development from egg to adult at 25°C normally required 17-28 d. GA_3 treatment did not affect the adult emergence time of parasitoids reared on hosts exposed to different doses. Only at 1000 ppm female wasps emerged later than males (Table 2).

Table 2. Changes in egg-to-adult developmental time of *P. turionellae* reared on *G. mellonella* fed on different doses of GA_3 incorporated diet.

GA_3 (ppm)	Egg-to-adult developmental time (day)					
	Male		Female		Both Sexes	
	Range	(mean ± SE)[b]	Range	(mean ± SE)[b]	Range	(mean[a] ± SE)[b]
0	17–22	20.29 ± 0.61x	18–28	22.77 ± 1.13x	17–28	21.90 ± 0.80a
50	17–24	19.27 ± 0.56x	18–24	20.00 ± 0.65x	17–24	19.60 ± 0.42a
100	18–25	19.64 ± 0.59x	18–29	22.11 ± 1.48x	18–29	20.75 ± 0.77a
200	16–23	18.88 ± 0.91x	16–22	20.33 ± 0.50x	16–23	19.75 ± 0.49a
500	16–26	20.00 ± 0.97x	19–26	22.57 ± 1.13x	16–26	20.90 ± 0.78a
1000	15–22	18.36 ± 0.79x	18–25	21.11 ± 0.72y	15–25	19.60 ± 0.61a
2000	18–23	20.33 ± 0.43x	18–25	22.00 ± 1.10x	18–25	21.00 ± 0.53a
5000	18–24	20.36 ± 0.65x	18–23	20.11 ± 0.45x	18–24	20.25 ± 0.40a

[a] Average for 20 individuals per treatment.

[b] Means within a column followed by the same letter are not significantly different (P > 0.05; Tukey's HSD test).

The effect of GA_3 on adult longevity of parasitoid wasps was dose dependent (F = 45.99; df = 7; P < 0.001) and sex dependent (F = 696.92; df = 1; P < 0.001), and the relationship between GA_3 dose and adult longevity was influenced by gender (F = 29.89; df = 7; P < 0.001). Male longevity considerably decreased at doses >1,000 ppm (F = 7.91; df = 7, 76; P < 0.001; Tukey's HSD test) whereas females lived longer at doses 50 to 200 ppm (F = 49.36; df = 7, 68; P < 0.001; Tamhane test) with respect to control and other treatment doses. Females also considerably lived longer than males at all doses tested (t-tests, P < 0.05) however the same trend was also observed for untreated wasps. Adult longevity of wasps increased at 50, 100, and 200 ppm, whereas a decrease in longevity was apparent at 2,000 ppm with respect to controls (Table 3).

The effect of GA_3 on adult length of parasitoid wasps was dose dependent (F = 4.32; df = 7; P < 0.001) and sex dependent (F = 64.54; df = 1; P < 0.001), and the relationship between GA_3 dose and adult length was influenced by gender (F = 3.57; df = 7; P < 0.01). Male length was significantly lower at 200 ppm (F = 2.69; df = 7, 76; P < 0.05), however females with an average highest length of 12 mm did not differ from that of control but other treatment groups (F = 4.66; df = 7, 68; P < 0.001). Adult length of GA_3-treated females differed from that of males at all treatment doses (t-tests, P < 0.05) except for 1,000 and 2,000 ppm. GA_3 treatment did not affect the adult length of wasps at doses ≤200 ppm with respect to those parasitoids reared on

untreated hosts. However, a considerable decrease in length was observed beyond 200 ppm but not at the highest dose 5,000 ppm (Table 4).

Table 3. Changes in adult longevity of of *P. turionellae* reared on *G. mellonella* fed on different doses of GA_3 incorporated diet.

GA_3 (ppm)	Adult longevity (day)					
	Male		Female		Both Sexes	
	Range	(mean[a] ± SE)[b]	Range	(mean[a] ± SE)[b]	Range	(mean[c] ± SE)[b]
0	39–58	47.43 ± 2.76a x	56– 67	62.15 ± 1.06a y	39-67	57.00 ± 1.97ab
50	31–54	39.91 ± 2.17abc x	106–136	116.00 ± 4.05b y	31–136	74.15 ± 8.94c
100	34–54	47.36 ± 2.13a x	100–115	106.89 ± 2.10b y	34–115	74.15 ± 6.95c
200	42–59	49.75 ± 1.71a x	79–109	92.42 ± 2.91c y	42–109	75.35 ± 5.14c
500	39–59	47.85 ± 1.79a x	76–104	87.86 ± 3.69cd y	39–104	61.85 ± 4.69b
1000	29–59	43.55 ± 2.53ab x	40–88	63.11 ± 5.77ad y	29–88	52.35 ± 3.63a
2000	23–45	32.67 ± 2.13c x	35–64	50.00 ± 4.17a y	23–64	39.60 ± 2.82d
5000	23–59	36.18 ± 2.49bc x	56–78	65.56 ± 2.35a y	29–78	49.40 ± 3.75a

a Average for 20 individuals per treatment.

b Means within a row (x-y) (P > 0.05; t-tests) and column (a-d) followed by the same letter are not significantly different (P > 0.05; Tamhane test for females, Tukey's HSD test for males and both sexes).

Table 4. Changes in adult lenght of of *P. turionellae* reared on *G. mellonella* fed on different doses of GA_3 incorporated diet.

GA_3 (ppm)	Adult length (mm)					
	Male		Female		Both Sexes	
	Range	(mean[a] ± SE)[b]	Range	(mean[a] ± SE)[b]	Range	(mean[c] ± SE)[b]
0	9–12	10.43 ± 0.37a x	9–13	11.15 ± 0.30ab x	9–13	10.90 ± 0.24ab
50	8–11	9.55 ± 0.28ab x	9–13	11.22 ± 0.40ab y	8–13	10.30 ± 0.30abc
100	9–12	10.55 ± 0.25a x	11–13	12.00 ± 0.29b y	9–13	11.20 ± 0.25a
200	7–10	8.88 ± 0.40b x	10–13	11.92 ± 0.31b y	7–13	10.70 ± 0.42abc
500	8–12	9.69 ± 0.36ab x	10–12	10.86 ± 0.26ab y	7–12	10.10 ± 0.28bc
1000	8–10	9.55 ± 0.28ab x	9–12	10.44 ± 0.38a x	8–12	9.95 ± 0.25bc
2000	9–10	9.67 ± 0.14ab x	9–11	9.88 ± 0.30a x	9–11	9.75 ± 0.14c
5000	9–12	9.91 ± 0.28ab x	10–12	10.89 ± 0.26ab y	9–12	10.35 ± 0.22abc

a Average for 20 individuals per treatment.

b Means within a row (x-y) (P > 0.05; t-tests) and column followed by the same letter are not significantly different (P > 0.05; Tukey's HSD test).

Total protein from host larvae increased on exposure to different doses of GA_3 in diet (F = 75.27; df = 7, 64; P < 0.001) except for the significant decline at 5,000 ppm when compared to that of control. However, this trend in increase was not dose-wise and was not significant at 200 and 1,000 ppm (Fig. 1). Total lipid content of GA_3 treated groups was significantly lower than that of the control group for host larvae (F = 45.17; df = 7, 64; P < 0.001) (Fig. 1). There was also a significant decrease in total carbohydrate content of larvae at 50, 200, 2,000, and 5,000 ppm (F= 18.53; df= 7, 64; P < 0.001) with respect to the control group. However, GA_3 treated larvae had higher carbohydrate content than untreated ones at 100, 500, and 1,000 ppm with a significant increase only at 500 ppm (Fig.1).

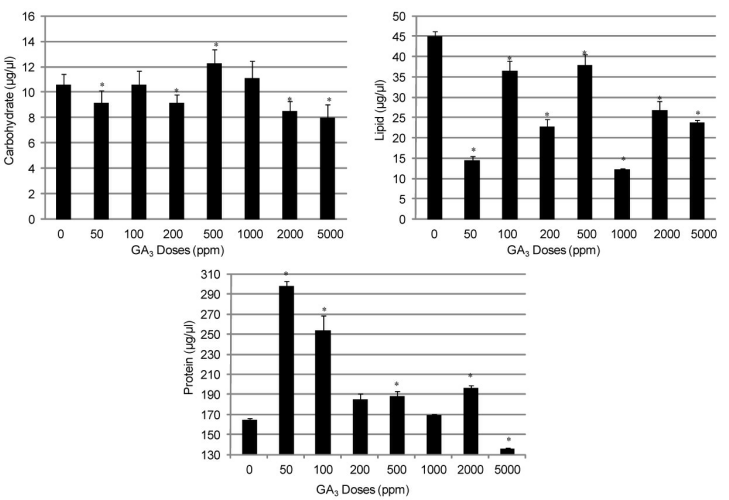

Fig. 1. GA_3-related changes in total protein, lipid, and carbohydrate amount (µg/µl) of *G. mellonella* larvae. Each bar represents mean ± SE of three replicates.

Significant differences are indicated by * (P<0.05)

Total protein of wasp larvae fluctuated among treatment doses, was significantly lower at all doses (F= 10.13; df= 7, 64; *P < 0.001*) except for the insignificant decrease at 2,000 ppm and increases at 50 and 200 ppm with respect to control (Fig. 2). Total lipid content of GA_3 treated groups increased significantly at only 100 ppm (F= 10.35; df= 7, 64; *P < 0.001*). Except for 100 ppm, there appeared insignificant decreases at doses ≤500 ppm and increases at doses >500 ppm (Fig. 2). Total carbohydrate of wasp larvae also fluctuated among treatment doses with significant increases at 100 and 1,000 ppm and decreases 200 and 5,000 ppm. The difference in the amount of other GA_3 treated larvae was not significantly different from that of control (Fig. 2).

DISCUSSION AND CONCLUSIONS

Plant growth regulators, in the diet of herbivore hosts, appear to have detrimental effects on the survival, development, morphology, and metabolism of insect pests

(Mansour and Dimetry, 1976; Kaur and Rup, 2002; 2003a; 2003b; Harikesh and Bhattacharya, 2003; Uçkan *et al*., 2008; Gupta, *et al*. 2009; Uçkan *et al*., 2011). The question how these effects in turn manipulate parasitoid biology has been the subject of our recent studies (Uçkan *et al*., 2008; Uçkan *et al*., 2011). Here, experiments were conducted to determine whether GA_3 in host diet affected the host and parasitoid development and hemolymph content in a fashion that would influence parasitoid-host interactions.

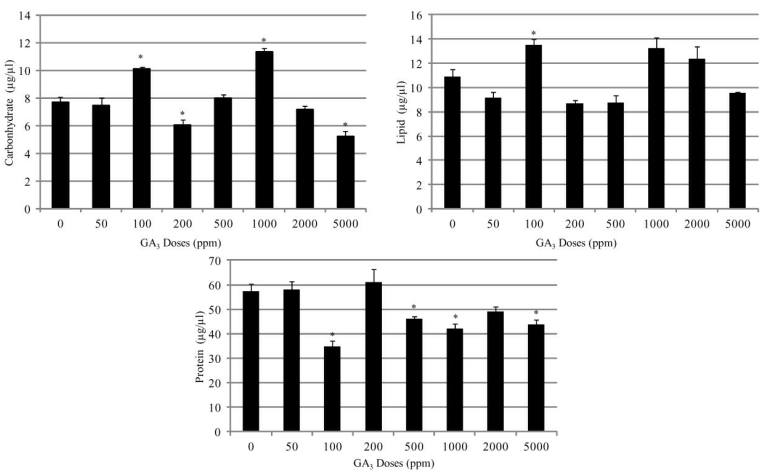

Fig. 2. GA_3-related changes in total protein, lipid, and carbohydrate amount (µg/µl) of *P. turionellae* larvae. Each bar represents mean ± SE of three replicates.

Significant differences are indicated by * ($P<0.05$)

Our data demonstrated that GA_3 had consistent negative effects on the pre-adult developmental time of *G. mellonella* with >35% shortage in overall time to adult eclosion largely due to the decrease in egg-larval developmental time at the highest dose of 5,000 ppm.

There are several lines of evidence that challenge this result and suggest a prolongation in the immature developmental time oriental leafworm moth, *Spodoptera litura* F. (Lepidoptera: Noctuidae) and melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) (Harikesh and Bhattacharya, 2003; Kaur and Rup 2003a) following the application of GA_3. The authors reported growth- and development-inhibitory effects on host species with a considerable decline in larval survival of *S. litura* beyond 200 ppm of GA_3 and 100% mortality in *B. cucurbitae* first instars at high concentrations, respectively. Gupta *et al*., (2009) also reported that GA_3 and siapton (an amino acid-based plant growth stimulant) caused an increase in the larval period of the hairy caterpillar, *Spilarctia oblique* Walker (Lepidoptera: Arctiidae) at high doses but no difference was observed in the pupal period. However, they also observed that triacontanol (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 are variable on insect pests. If PGRs have insecticidal activity, then it is likely to use GA_3 against *G. mellonella* because treatment of pests with GA_3 in their diet resulted in a significant influence on the immature developmental time of larvae which is considered the most damaging stage of this pest species that GA_3 feeds on combs, wax, and honey in beehives. Further evidence from this study that GA_3 did not affect the egg-to-adult developmental time of parasitoids also supports the assumption that GA_3 would be a successful chemosterilant against *G. mellonella* and replace the traditional synthetic organopesticides. However, this result is unexpected, since our previous studies (Uçkan *et al*., 2008; Uçkan *et al*., 2011) found that the overall time to adult eclosion of *A. galleriae* increased by 30-40% when wasps were reared on *A. grisella* larvae fed high doses of GA_3 and IAA, respectively. It is likely that GA_3 does not have a lethal effect towards developing parasitoid larvae across trophic levels, in this case, via their hosts. Because GA_3 is a terpenoid compound like any of the juvenile hormones (JHs) (Visscher, 1980; Kaur and Rup, 2002), it is quite likely that much of the ingested GA_3 may have been degraded or digested by similar esterases and hydrolases that target JHs and other terpenoids in the midgut of *G. mellonella*, and any that was absorbed could have been modified or metabolized by an array of enzymes. The result would be either no effect by the growth regulator or only sub-lethal effects such as increases and decreases in adult longevity of wasps that results from a prolongation in longevity of females at doses 50-200 ppm and a decline in that of males at 2,000 and 5,000 ppm. In addition, decreases in parasitoid size may also be attributed to the non-specific toxicity of diet as amounts of GA_3 increases, resulting in a decline in diet quality and an interference of sufficient food supply from the host by parasitoid (Uçkan and Ergin, 2002; Uçkan *et al*., 2007). It is clear that some GA_3 was absorbed and sequestered by the host as evidenced by the alterations in parasitoid longevity and size.

Further evidence comes from results that lipid at all and carbohydrate at most of the doses (see Fig.1) decreased in hemolymph of host larvae upon exposure to GA_3. The decrease in energy reserves of the host resulting from GA_3-induced stress can account for the alterations in longevity and size of *P. turionellae* progeny dependent on the host energy stores (Uçkan *et al*., 2008; Uçkan *et al*., 2011). It has also been observed that larval glycogen content is reduced at higher doses of GA_3 treatment in banana fruitfly, *Zaprionus paravittiger* (Godbole and Vaidya) (Diptera: Drosophilidae) (Rup *et al*., 1998) and *B. cucurbitae* (Kaur and Rup, 2003a; 2003b), with a corresponding decrease in total lipid and carbohydrate levels in the latter species. The increase in hemolymph protein content of host larvae may also indicate a physiological adaptability to compensate for GA_3-induced stress (Riberio *et al*., 2001). Because, animals require high energy under stress conditions, the energy demand may have led to the stimulation of protein catabolism. The decrease in protein content might also be due to a mechanism of lipoprotein formation, which will be used to repair damaged cell and tissue organelles (Sancho *et al*., 1998). In the present study, it is not clearly evident that GA_3 has an effect on hemolymph metabolites of parasitoid (see Fig. 2). Because, the magnitude of the differences in protein, carbohydrate, and lipid content

in hemolymph was not dose-wise and fluctuated among treatments. Whatever the differences are, the changes in the level of protein, carbohydrate, and lipid content will adversely affect the growth, development, and reproduction of parasitoid species. This fact, in turn, may disrupt the effectiveness of parasitoid species in biological control programs. Consequently, growth regulatory compounds like GA_3 are bound to play a vital role in the patterns of growth and development of associated phytophagous insects through disruptions of metabolic processes. Together with the data obtained from this point of view, these effects might also release important results on parasitoid biology and physiology. Further studies are necessary to support the idea that GA_3 can be used as a chemosterilant and replace with the traditional synthetic organopesticides. The authors are currently evaluating the effect of GA_3 on the hemolymph chemistry and hemocytes of *G. mellonella* and *P. turionellae*.

REFERENCES

Ahmad, N., Rashdi, S. M. M. S., Rajput, A. A., 2003, Efficiency of plant growth regulators to manage the insect pests of cotton. *Asian Journal of Plant Sciences*, 2: 544-547.

Awad, T. M., Taha, F. A., 1976, The effect of some plant growth inhibitors on the developmental stages of *Spodoptera littoralis* Boisd. (Lep.: Noctuidae). *Zeitschrift für Angewandte Entomologie,* 80: 306-310.

Barbosa, P., Gross, P., Kemper, J., 1991, Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology*, 72: 1567-1575.

Bariola, L. A., Kittock, D. L., Arle, H. F., Vail, P. V, Hennebery T. J., 1976, Controlling pink bollworms: Effects of chemical termination of cotton fruiting on populations of diapausing larvae. *Journal of Economic Entomology*, 69: 633-639.

Bhalla, O. P., Robinson, A. G., 1968, Effect of chemosterilants and growth regulators on the pea aphid and artificial diet. *Journal of Economic Entomology*, 61: 552-555.

Bronskill, J. F., 1961, A cage to simplify the rearing of the greater wax moth, *Galleria mellonella* (Pyralidae). *Journal of Lepidopteran Society*, 15: 102-104.

Carlisle, D. B., Ellis, P. E., Osborne, D. J., 1969, Effects of plant growth regulators on locusts and cotton stainer bugs. *Journal of the Science of Food and Agriculture*, 20: 391-393.

Dreyer, D. L., Campbell, B. C., Jones, K. C., 1984, Effect of bioregulator- treated sorghum on greenbug fecundity and feeding behavior: Implication for host-plant resistance. *Phytochemistry*, 23: 1593-1596.

El-Ibrashy, M. T., 1972, The sterilent activity of certain biologically active compounds against *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae). *Zeitschrift für Angewandte Entomologie*, 71: 326-332.

Ergin, E., Er, A., Uçkan, F., Rivers, D. B., 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.

Gupta, G., Yadav, S. R., Bhattacharya, A. K., 2009, Influence of synthetic plant growth substances on the survivorship and developmental parameters of *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae). *Journal of Pest Science*, 82: 41-46.

Harikesh, S., Bhattacharya, A. K., 2003, Negative role of gibberellic acid on the developmental behaviour of *Spodoptera litura*. *Indian Journal of Entomology*, 65: 293-297.

Honeyborne, C. H. B., 1969, Performance of *Brevicoryne brassicae* on plants treated with growth regulators. *Journal of the Science of Food and Agriculture*, 20: 388-390.

Kansu, Y. A., Uğur, A., 1984, *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae) ile Konukçusu Bazı Lepidopter Pupaları Arasındaki Biyolojik İlişkiler üzerinde Araştırmalar. *Doğa Bilim Dergisi,* D2.8: 160-173.

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

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

Lowry, O. H., Rose, Brough, N. J., Farr, A. L., Randall, V. J., 1951, Protein Measurement with the Folin Phenol Reagent. *The Journal of Biological Chemistry*, 193: 265-275.

Mansour, M. H., Dimetry, N. Z., 1976, Effect of three plant growth regulators on the immature stages of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Zeitschrift für Angewandte Entomologie*, 80: 88-93.

McDonald, R. E., Greany, P. D., Shaw, P. E., Schroeder, W. J., Hatton, T. T., Wilson, C. W., 1988, *Use of gibberellic acid for Caribbean fruit fly (Anastrepha suspensa) control in grapefruit. In:* Goren, R., Mendel K., (Eds.). Proceedings of the Sixth International Citrus Congress, Balaban Publications, Rehovot, Israel, 1147-1152.

Önder, F., Karsavuran, Y., Hakerlerler, H., Tezcan, S., 1987, Bitki büyüme regülatörlerinden MLC'nin laboratuvar koşullarında *Dolycoris baccarum* (L) (Het.: Pentatomidae) üzerinde etkileri. *Türkiye 1. Entomoloji Kongresi Bildirileri,* İzmir, 325-334.

Paulson, G. S., Hull, L. A., Biddinger, D. J., 2005, Effect of a plant growth regulator prohexadione-calcium on insect pests of apple and pear. *Journal of Economic Entomology*, 98: 423-431.

Posnova, A. N., 1974, Plant growth regulators and their use in applied entomology. *Trudy Vsesoyuznogo Nauchno-issledovatel'skogo institua*, Zashchity, Rastenii, 40: 135-145.

Prasad, R., Behera, H. N., Das, C. C., 1977, MLC induced meiotic instability in grasshopper spermatocytes. *Current Sciences*, 46: 191-192.

Riberio, S., Sousa, J. P., Nogueira, J. A. A., Soares, A. M. V. M., 2001, Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus*. *Ecotoxicology and Environmental Safety*, 49: 131-138.

Rup, P. J., Kaur, R., Kaur, J., 1998, Effect of gibberellic acid (GA_3) on the protein, lipid and carbohydrate contents of banana fruit fly, *Zaprionus paravittiger* larvae. *Insect Science and Its Applications*, 18: 145-148.

Sak, O., Uçkan, F., Ergin, E., 2006, Effects of cypermethrin on total body weight, glycogen, protein, and lipid contents of *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). *Belgian Journal of Zoo*logy, 136: 53-58.

Salisbury, F. B., Ross, C. W., 1992, *Plant Physiology*. Wadsworth Publishing Company, United States, 1-682.

Sancho, E., Ferrando, M. D., Fernandez, C., Andreu, E., 1998, Liver energy metabolism of *Anguilla anguilla* after exposure to fenitrothion. *Ecotoxicology and Environmental Safety*, 41: 168-175.

Scheurer, S., Aschermann, S., 1976, *The influence of natural and some synthetic plant growth regulators on the reproductive activity of Aphis fabae sucking plant parts. In:* Mahunka, S., (Ed.). Folia Entomologica Hungarica, Luther-Strasse, German Demokratic Republic, 221-226.

Silva, M. T. B., Costa, E. C., Boss, A., 2003, Control of *Anticarsia gemmatalis* Hubner (Lepidoptera: Noctuidae) larvae with insect growth regulators. *Ciencia Rural*, 33: 601-605.

Sun, T., Gubler, F., 2004, Molecular mechanism of gibberellin signaling in plants. *Annual Review of Plant Biology*, 55: 197-223.

SPSS Inc., 1999, SPSS 10.0 Statistics. SPSS, Chicago, IL.

Tahori, A. S., Zeidler, G., Halevy, A. H., 1965, Effect of some plant growth retardants on the feeding oh the cotton leaf worm. *Journal of the Science of Food and Agriculture*, 16: 570-572.

Uçkan, F., Gülel, A., 1990, Endoparazitoid *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae) dişilerinde zehir aparatının yapısı ve zehirinin başlıca kimyasal grubunun tayini. *X. Ulusal Biyoloji Kongresi*, Erzurum. 73-83.

Uçkan, F., Ergin, 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., Hepçorman, 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., Tüven, A., Er, A., Ergin, 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.

Uçkan, F., Haftacı, İ., Ergin, E., 2011, 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: 77-82.

Van Emden, H. F., 1969, Plant resistance to aphids induced by chemicals. *Journal of the Science of Food and Agriculture*, 20: 385-387.

Van Handel, E., 1985a, Rapid Determination of Glycogen and Sugars in Mosquitoes. *Journal of the American Mosquito Control Association*, 1: 299-301.

Van Handel, E., 1985b, Rapid Determination of Total Lipids in Mosquitoes. *Journal of the American Mosquito Control Association*, 1: 302-304.

Visscher, N. S., 1980, Regulation of grasshopper fecundity, longevity and egg viability by plant growth hormones. *Experimential*, 36: 130-131.

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