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Influence of gibberellic acid on some biological parameters of ectoparasitoid, *Bracon hebetor* (Say, 1836) (Hymenoptera: Braconidae)

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Abstract: Different concentrations (2, 5, 10, 50, 100, 200, 500 and 1000 mg/L) of gibberellic acid (GA_3) were used to determine the effects of plant growth regulators on development, fecundity, sex ratio and longevity of parasitoid *Bracon hebetor* (Hymenoptera: Braconidae) reared on greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae). Treatment with GA_3 prolonged the developmental period of female wasps at 2, 50, 500 and 1000 mg/L and at 10, 500 and 1000 mg/L in male wasps. However, GA_3 did not negatively affect the fecundity and sex ratio of parasitoid. *Bracon hebetor* females reared on GA_3-treated hosts had lower longevity at 10, 500 and 1000 mg/L than the females reared on untreated hosts. For males a significant decline in longevity was also recorded at 5 and 10 mg/L with respect to controls.

Keywords: Gibberellic acid, Bracon hebetor, Developmental time, Fecundity, Longevity.

Introduction

Plant growth regulators (PGRs) directly or indirectly influence the growth and reproduction of associated phytophagous insects (Honeyborne, 1969; Abdellaoui, 2009a; Tsagkarakis and Rogers, 2012). Harikesh and Blattacharya (2001) reported negative effects of PGRs such as miraculan and milstim on survival and developmental parameters of *Spodoptera litura* (Lepidoptera: Noctuidae). Similarly, Kaur and Rup (2002, 2003) found that reproductive potential and development of *Bactrocera cucurbitae* (Diptera: Tephritidae) significantly reduced with the applications of four different PGRs such as coumarin, kinetin, gibberellic acid (GA_3) and indole-3-acetic acid (IAA). Negative effects of PGRs are also determined with other insect species such as *Aphis fabae* (Hemiptera: Aphididae) (Honeyborne, 1969), *Locusta migratoria migratoria* (Orthoptera: Acrididae) (Abdellaoui et al., 2009a) and *Diaphorina citri* (Hemiptera: Psyllidae) (Tsagkarakis and Rogers, 2012). Some researchers have recommended the use of plant growth regulators as successful chemosterilants against some insect pests and can be proposed in an integrated pest management (IPM) program (Kaur and Rup, 2002; Ahmad et al., 2003; Tsagkarakis and Rogers, 2012). Recent studies suggest that PGRs may also have significant impacts on species of the third trophic level by changing abundance, fitness, and efficacy of natural enemies of pest species. For instance, the fitness and parasitism of the braconid *Aphidius colemanii* were significantly reduced when reared on *Mysuz persicae* (Hemiptera: Aphididae) feeding on diets containing four different plant growth regulators: ancymidol, paclobutrazol, uniconazole, and flurprimidol (Prado and Frank, 2013). Likewise, Uçkan et al. (2011a) demonstrated that IAA increased the development period and decreased the longevity of the endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae). Kaur and Kaur (2013) previously showed that coumarin treatment caused a decline in reproductive potential of the parasitoid *Bracon hebetor* (Hymenoptera: Braconidae) reared on *S. litura*. However, the influence of GA_3 on development, reproduction and longevity of *B. hebetor* reared on *Galleria mellonella* (Lepidoptera: Pyralidae) has not been studied. Hence, the present study was designed to investigate the influence of GA_3 on some biological parameters of gregarious, larval ectoparasitoid *B. hebetor*.

Materials and Methods

Insects: *Bracon hebetor* was used as the parasitoid and the last stage larvae of *G. mellonella* was used as host. Insects

Table 1. Effect of GA3 on development time (mean±SE) of *Bracon hebetor* females and males. Different letters denote significant differences (Tukey's HSD test, $P \leq 0.05$).

GA_3 (mg/L)	Developmental time (days)			
	Female		Male	
	Range	Mean±SE	Range	Mean±SE
0	11-19	14.44±0.05 ab	11-18	14.29±0.06 ab
2	13-19	15.67±0.07 c	13-18	14.48±0.05 b
5	11-18	14.16±0.05 d	12-18	14.21±0.04 ac
10	12-17	14.52±0.06 bc	12-20	15.43±0.07 d
50	13-18	14.77±0.05 c	13-17	14.40±0.04 ab
100	12-17	14.52±0.04 bc	12-17	14.01±0.04 c
200	11-18	14.23±0.04 ac	12-19	14.20±0.03 ac
500	14-22	17.36±0.08 f	14-19	16.16±0.04 e
1000	12-23	15.85±0.09 c	13-19	15.08±0.06 f

were obtained from a successive stock culture, which had been reared in Ondokuz Mayıs University Animal Physiology Research Laboratory for several years.

The host, *G. mellonella* larvae were maintained on the diet described by Bronskill (1961) and modified by Sak et al. (2006). Newly emerged adult parasitoids were confined in pairs to the test tubes. A piece of cotton ball soaked with 50% honey solution and one last instar host larvae were placed inside the tubes. All cultures were maintained at 25±2°C, and 60±5% relative humidity and continuously illuminated laboratory.

Treatments: The effects of GA_3 on development, fecundity, sex ratio, and longevity of *B. hebetor* were investigated by exposing the adult parasitoids to the last instars *G. mellonella* larvae fed on artificial diet incorporated with eight concentrations of GA_3 which are 2, 5, 10, 50, 100, 200, 500, 1000 mg/L. In addition to these eight treatments, a control diet without GA_3 was also tested. 10-15 *G. mellonella* adults were transferred into 500 mL glass jars containing diet for oviposition. Last instar larvae were used for experiments. To determine the effects of GA_3 on immature development time, fecundity and sex ratio of parasitoid, randomly selected newly emerged adults from the GA_3-treated and untreated hosts were used. Insects were paired and transferred in test tubes (15x100 mm) containing one last instar host larvae reared on diet supplemented with different concentrations of GA_3. They were also provided with a honey saturated cotton pad. Every other day, parasitoids were removed from the tubes and fresh hosts were given to the wasps until the female died. Larvae reared on untreated diet served as control for each treatment. Parasitized hosts were placed under the same conditions described above for mass rearing and controlled daily to determine the time required for immature development. To the emergence, the number of emerged F1 adult wasps each sex was counted and noted in the control and the treated groups. The data were used for fecundity rate (total number of wasps per female) of parasitoid. The experiment was replicated three times with eight pairs per replicate for both treated and untreated groups.

In order to investigate the impact of GA_3 on the longevity of adult parasitoids, randomly selected newly emerged female and male wasps from each treatment were placed in test tubes, with cotton ball saturated with a honey solution. The tubes were kept under the same conditions mentioned above. Each tube checked every day until all parasitoids had died and adult longevity was recorded.

Statistical Analysis: All the bioassays were repeated three times and the results are expressed as means±standard errors (SE). Data were submitted to the one-way analysis of variance (ANOVA) using SPSS (Version 20.0). The significance between control and treated series was made by Tukey's HSD test at 5% level.

Results

The influence of GA_3 on development time of parasitoid is presented in Table 1. The results revealed that treatment with GA_3 influenced the development of both female and male parasitoids. For females, GA_3 caused a significant lengthening of the development period at the concentration of 2, 50, 500 and 1000 mg/L, compared to controls ($P \leq 0.05$). Similarly, development period of males fluctuated among treatments with a significant prolongation at 10, 500 and 1000 mg/L and shortening at 100 mg/L when compared to controls ($P \leq 0.05$).

The effect of GA_3 on the fecundity and sex ratio of *B. hebetor* is shown in Table 2. Each female parasitoid reared on untreated hosts produced 136.53±9.49 offspring

Table 2. Effect of GA3 on fecundity and sex ratio (mean±SE) of *Bracon hebetor*. Different letters denote significant differences (Tukey's HSD test, $P \leq 0.05$).

GA_3 (mg/L)	Fecundity and sex ratio					
	Female		Male		Total fecundity	Female sex ratio (%)
	Range	Mean±SE	Range	Mean±SE	Mean±SE	Mean±SE
0	15-62	33.05±2.93 a	20-168	103.47±9.74 a	136.53 ±9.49 a	26.96±3.13 a
2	6-46	23.33±2.53 a	32-211	117.62±12.33 a	142.95±13.55 a	19.33±1.99 a
5	2-41	21.2±2.59 a	20-191	99.60±11.49 a	120.80±12.87 a	18.96±1.82 a
10	2-41	21.64±2.40 a	19-204	89.68±10.70 a	111.32±11.34 a	22.52±2.85 a
50	5-55	26.68±3.20 a	40-157	90.91±6.48 a	117.59±7.35 a	22.72±2.29 a
100	9-96	28.89±5.10 a	18-241	118.11±13.86 a	147.00±16.45 a	21.38±3.10 a
200	12-66	34.53±3.34 a	26-231	120.63±15.02 a	155.16±16.60 a	25.80±2.68 a
500	5-48	24.94±2.74 a	13-146	73.82±8.08 a	98.76±9.42 a	26.82±2.47 a
1000	8-51	24.67±2.62 a	20-218	88.76±10.35 a	113.43±10.09 a	25.18±3.41 a

Table 3. Effect of GA3 on adult longevity (mean±SE) of *Bracon hebetor*. Different letters denote significant differences (Tukey's HSD test, $P \leq 0.05$).

GA_3 (mg/L)	Adult longevity (day)			
	Female		Male	
	Range	Mean±SE	Range	Mean±SE
0	21-107	59.01±2.61 a	18-91	46.98±1.63 ab
2	14-104	62.26±2.73 a	14-95	64.47±2.52 c
5	18-132	63.81±6.33 a	15-91	32.21±1.78 d
10	14-104	41.49±3.72 bc	11-46	27.81±1.42 d
50	15-91	52.83±2.31 ab	13-75	46.15±1.22 ab
100	13-89	52.16±1.91 ab	19-89	49.38±1.47 a
200	9-90	52.93±2.09 ab	9-77	41.80±1.40 ab
500	12-85	40.30±2.97 bc	12-76	41.05±1.78 b
1000	10-58	35.92±1.64 c	10-74	43.17±1.50 ab

throughout its adult life. Analysis of data revealed that treatment with GA_3 did not significantly change the offspring production of female parasitoids ($P>0.05$). Sex ratio was determined as male biased at all groups and showed some weak but insignificant fluctuations among groups.

Analysis of data for the longevity of adult parasitoids reared on GA_3-treated and untreated hosts are illustrated in Table 3. Female parasitoids lived shorter at 10, 500 and 1000 mg/L when compared to controls ($P \leq 0.05$). The longevity of male parasitoids had some variations among treated-groups with a significant increase at 2 mg/L and decrease at 5, 10 mg/L compared with controls ($P \leq 0.05$).

Discussion

In this study we examined the influence of GA_3 on development, fecundity, sex ratio and longevity of larval ectoparasitoid *B. hebetor*. Incorporation of GA_3 into the diet of host species significantly prolonged the immature developmental time of parasitoids. These findings corresponded to the results of Uçkan et al. (2008). They showed that high concentrations of GA_3 prolonged development of *A. galleriae* (Hymenoptera: Braconidae), a parasitoid of *Achoria grisella* (Lepidoptera: Pyralidae). Similarly, *B. hebetor* had prolonged development time when its host, *S. litura*, reared on high concentrations of coumarin added to the diet (Kaur and Kaur, 2013). Gupta et al. (2009) showed that five different PGRs differed in their effect on larval development time of *Spilarctia obliqua* (Lepidoptera: Arctiidae). High concentrations of gibberellic acid and siapton caused significant lengthening of development time, whereas triacontanol, chlormequat chloride and mepiquat chloride had no effect. Hence, it can be said that plant growth regulators affect insects in different ways. Some researchers presumed that the negative effects of GA_3 on life parameters of different insects may be caused by the chemical similarity to the juvenile hormone (JH) (Visscher, 1980; Rossler and Greany, 1990; Hussein, 2005).

We found no significant differences in the progeny production of *B. hebetor* when *G. mellonella* reared on GA_3 added diet. Similarly, Uçkan et al. (2008) showed that offspring production of *A. galleriae* did not vary significantly with GA_3 treatment. However, Honeyborne, 1969 reported that ethylene bisnitrourethane and gibberellic acid treatments caused a significant adverse

effect on the fecundity of *A. fabae*. Similarly, Abdellaoui et al. (2009b) observed that gibberellic acid application markedly declined fecundity and fertility of *L. migratoria migratoria.* Kaur and Kaur (2013) also recorded negative effects on the reproductive potential of *B. hebetor* when its host *S. litura* larvae reared on coumarin amended diet.

The results of present investigation also indicated that GA_3 treatment made significant changes in longevity of both female and male parasitoids compared to controls. Female longevity decreased at 10, 500 and 1000 mg/L and male longevity showed different trends with a significant increase at 2 mg/L and decrease at 5, 10 mg/L compared with controls. Similarly, Uçkan et al. (2008) observed that GA_3 treatment remarkably changed the longevity of endoparasitoid *A. galleriae*. They reported that at GA_3 doses >10 ppm, the lifespan of both female and male parasitoids declined compared to untreated groups. In another study Uçkan et al. (2011b) also indicated that adult longevity of the Ichneumonid parasitoid, *Pimpla turionellae* reared on GA_3 treated host negatively affected at higher concentrations. Abdellaoui et al. (2009b) observed that GA_3 had antifeedant and toxic effects on *Spodoptera littoralis* and *L. migratoria*. They suggested that these negative effects may explain the reason of declining survival in insects.

Consequently, this study supports the findings of different researchers, who reported the adverse influences of PGRs on life history parameters of various insect species. The application of GA_3 through a diet incorporation assay had a negative effect on parasitoid fitness by reducing longevity and increasing immature development period. However, GA_3 treatment did not cause any significant difference in the progeny production and sex ratio of *B. hebetor*. These results suggest that there could be different toxicity sensitivities between different insects and different PGRs. Because of this, detailed studies are necessary to obtain new knowledge about long-term effects of PGRs in biological systems.

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