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# The Effect of Indole-3-Acetic Acid on Some Biological Features of *Bracon hebetor* (Say) (Hymenoptera: Braconidae)

## İndol-3-Asetik Asitin *Bracon hebetor* (Say) (Hymenoptera: Braconidae)'un Bazı Biyolojik Özelliklerine Etkisi

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## THE EFFECT OF INDOLE-3-ACETIC ACID ON SOME BIOLOGICAL FEATURES OF *BRACON HEBETOR* (SAY) (HYMENOPTERA: BRACONIDAE)


### ABSTRACT

The impacts of indole-3-acetic acid (IAA) on some biological characteristics of the parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) were examined. Different concentrations of IAA (5, 50, 100, 500 and 1000 mg $L^{-1}$) were added to the synthetic diet of host larvae. The developmental period, adult longevity, fecundity, and sex ratio of parasitoids reared on these hosts were evaluated with respect to IAA concentrations. IAA treatment shortened the developmental period of female wasps at almost all concentrations except for 50 mg $L^{-1}$. The developmental period of males showed fluctuations among IAA-treated groups with a significant decline at 5 and 500 mg $L^{-1}$ and a considerable increase at 50 mg $L^{-1}$ with respect to the control group. IAA showed a significant negative effect on the fecundity of *B. hebetor*. However, the sex ratio of the parasitoid did not differ among the IAA-treated and untreated groups. *B. hebetor* females reared on IAA-treated hosts had lower longevity at 50, 100, 500 and 1000 mg $L^{-1}$ than the females reared on untreated hosts. For males, a significant decrease in longevity was also determined at all concentrations compared to the controls.


***Keywords:*** *Bracon hebetor*, Development, Fecundity, Indole-3-Acetic Acid, Longevity.

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## İNDOL-3-ASETİK ASİTİN *BRACON HEBETOR* (SAY) (HYMENOPTERA: BRACONİDAE)'UN BAZI BİYOLOJİK ÖZELLİKLERİNE ETKİSİ


### ÖZ:

İndole-3-asetik asitin (IAA) parazitoit *Bracon hebetor* Say (Hymenoptera: Braconidae)'un bazı biyolojik özelliklerine etkileri araştırılmıştır. Farklı konsantrasyonlarda IAA (5, 50, 100, 500 ve 1000 mg $L^{-1}$) konukçu larvaların yapay besinine ilave edilmiştir. Bu konukçularda yetiştirilen parazitoitlerin gelişim süresi, ergin ömür uzunluğu, verim ve eşey oranı IAA konsantrasyonlarına göre değerlendirilmiştir. IAA uygulaması, 50 mg $L^{-1}$ dışındaki tüm konsantrasyonlarda dişi parazitoitlerin gelişim süresinde kısalmaya neden olmuştur. Erkeklerin gelişim süresi, kontrol grubu ile karşılaştırıldığında, 5 and 500 mg $L^{-1}$ konsantrasyonda önemli bir azalma ve 50 mg $L^{-1}$ konsantrasyonda dikkate değer bir artış ile IAA uygulanan gruplar arasında dalgalanmalar göstermiştir. IAA *B. hebetor*'un veriminde olumsuz bir etki yapmıştır. Buna karşın, parazitoidin eşey oranı IAA uygulanan ve uygulanmayan gruplar arasında farklılık göstermemiştir. IAA uygulanmış konukçular üze-


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rinde yetiştirilen *B. hebetor* dişilerinin ömür uzunluğu 50, 100, 500 ve 1000 mg $L^{-1}$ konsantrasyonlarında IAA uygulanmamış konukçular üzerindekilere oranla daha kısadır. Erkeklerin ömür uzunluğunda tüm konsantrasyonlarda kontrol grubu ile karşılaştırıldığında önemli bir azalma belirlenmiştir.

***Anahtar Kelimeler:*** *Bracon hebetor*, Gelişim, Verim, İndol-3-Asetik Asit, Ömür Uzunluğu.

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## 1. INTRODUCTION

*Bracon hebetor* Say (Hymenoptera: Braconidae) is a gregarious, synovigenic, idiobiont larval parasitoid of many pyralid species. High reproductive performance and short generation time of *B. hebetor* make it an excellent biological control agent for different pest species, including *Galleria mellonella* (Linnaeus) (Lepidoptera:Pyralidae).

Plant Growth Regulators (PGRs) present in the plant tissue possibly regulate phytophagous insects's physiology, behaviour and biochemistry. This presumption receives support from reports in the literature by demonstrating their influence on these characteristics when added to the diet or applied exogenously on some insect species (Honeyborne, 1969; Abdellaoui et al., 2009; Tsagkarakis et al., 2012; Abdellaoui et al., 2015; Gündüz and Özcan, 2018). For instance, Kaur and Rup (2003) reported that PGRs like gibberellic acid ($GA_3$), indole-3-acetic acid (IAA), coumarin and kinetin adversely affect the growth and development of Melon Fruit Fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). In the same way, Bhatnagar et al. (2012) showed that miraculan negatively affected the development and survival of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Abdellaoui et al. (2009) noted that $GA_3$ significantly reduced both fecundity and fertility of *Locusta migratoria migratoria* (Linnaeus) (Orthoptera: Acrididae). Tsagkarakis et al. (2012) demonstrated that the fecundity and survivorship of Asian Citrus Psyllid reared on *Citrus volkameriana* (Tan. and Pasq.) (Sapindales: Rutaceae) trees treated with prohexadione calcium and mefluidide considerably decreased.

Several studies have reported that PGRs also have considerable effects on natural enemies of pest species by changing their abundance, fitness, and efficacy. Honeyborne (1969) reported that the use of ethylene bisnitrourethane and chloromequat chloride on broad beans had a negative impact on aphid size. Host size is one of the main parameters influencing offspring size in many parasitoid species, and the parasitoids feeding on these smaller hosts may also be reduced in size (Sequeira and Mackauer, 1992; Godfray, 1994). Ellers et al. (1998) showed that large parasitoids have higher fecundity and higher fat reserves than small parasitoids.

Additionally, Jarosik et al. (2003) found that host size was also positively correlated with sex ratio and survival of parasitoids. In another study, Prado and Frank (2013) showed that incorporation of plant growth regulators into the diet of *Mysuz persicae* (Sulzer) (Hemiptera: Aphididae) resulted in a decrease in the fitness and parasitism of parasitoid *Aphidius colemanii* (Viereck) (Hymenoptera: Aphididae). Similarly, Zhao et al. (2017) found that emergence rate, parasitism potential and female ratio of parasitoid *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) were decreased when reared on *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) feeding on PGRs-supplemented wheat plants. Kaur and Kaur (2013) also observed that the emergence and parasitism capacity of *B. hebetor* decreased when reared on *S. litura* larvae feeding on coumarin-treated diets. Based on these previous studies, our hypothesis is that PGR treated hosts would have indirect effects on some biological features of parasitoid. The purpose of this study is to determine the influence of IAA treatment on some selected biological parameters of *B. hebetor*.

## 2. MATERIALS AND METHODS

Parasitoid *B. hebetor* were reared on the last stage larvae of Greater Wax Moth, *G. mellonella*, as described by Gündüz and Gülel (2004). Host larvae were reared on the diet described by Bronskill (1961) and modified by Sak et al. (2006). All experiments were carried out in a laboratory with a temperature of 25±2°C, a relative humidity of 60± 5%, and a continuous light source.

To determine the effects of IAA on development, fecundity, sex ratio, and longevity of *B. hebetor* five concentrations (5, 50, 100, 500 and 1000 mg $L^{-1}$) of IAA were added to the artificial diet of hosts. The control group was reared on IAA-free diet. Newly emerged females were placed in a vial along with a male and a honey soaked cotton ball. Two days later, parasitoids were transferred into a host containing vial. This procedure was continued until the females death. The vials were observed daily to determine the developmental period of the parasitoid. Total number of progeny and sex ratio of parasitoids were recorded. Three replications for all IAA concentrations were conducted on eight pairs of adult parasitoids during the study.

For the longevity experiments, newly emerged adult parasitoids, selected from each group, were placed into test tubes with honey solution saturated cotton balls. Parasitoids were observed every day until the death of all parasitoids. Each experiment was replicated three times with 20 females and 20 males selected randomly from different populations at different times.

All data were statistically analyzed in SPSS (version 20). The significance between control and IAA treated insects was compared using the Student-Newman-Keuls (SNK) test at a 5% level.

## 3. RESULTS AND DISCUSSION

The impact of IAA on total developmental time (from egg to adult) of *B. hebetor* females and males are presented in Figure 1a and b, respectively.

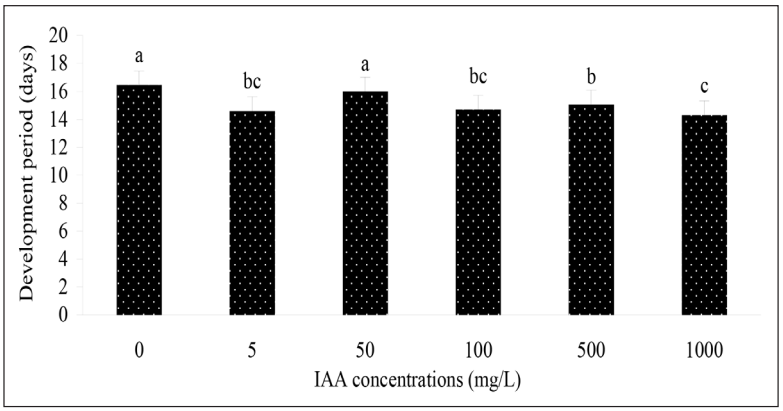


**Figure 1a.** Impact of indole-3-acetic acid on development time (mean ± SE) of *B. hebetor* females. Different letters denote significant differences (SNK test, $P \leq 0.05$).

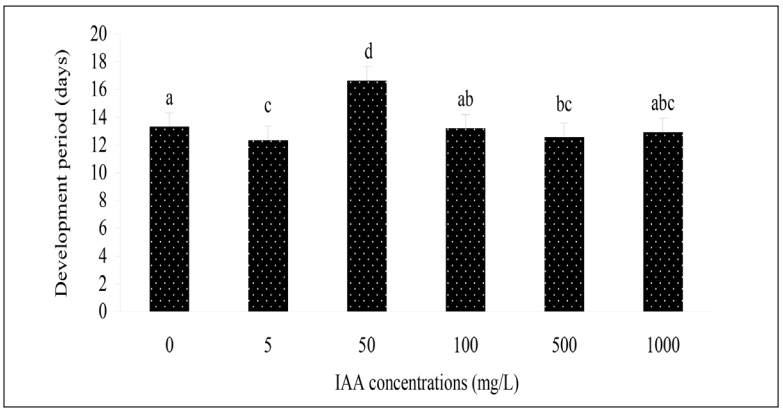


**Figure 1b.** Impact of indole-3-acetic acid on development time (mean ± SE) of *B. hebetor* males. Different letters denote significant differences (SNK test, $P \leq 0.05$).

IAA-treatment led to changes in the developmental period of parasitoids. For females, the mean values at 5, 100, 500 and 1000 mg $L^{-1}$ IAA concentrations were significantly shortened ($P \leq 0.05$). (Figure 1a). However, we did not determine the same trends in males since their developmental times showed some fluctuations among IAA-treated groups, with a significant increase at 50 mg $L^{-1}$ and a decrease

at 5 and 500 mg $L^{-1}$ when compared to controls ($P \leq 0.05$) (Figure 1b). Uçkan et al. (2011a) found that $GA_3$ treatment declined the immature developmental period of *G. mellonella*. Sepperumal and Sukumar (2014) reported that V instar larval duration was declined by one day in *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae) after exposure to kinetin and $GA_3$. In a previous study, Osborne et al. (1968) suggested that plant growth regulators may affect insect's neuroendocrine system. In addition, De Man et al. (1981) reported that dietary supplementation with plant growth regulators may alter the rate of DNA synthesis which helps in protein synthesis and/or the rate of synthesis of insect moulting hormone. These previous studies could help to explain the reason for the faster development in IAA-treated groups. However, Kaur and Kaur (2013) reported a significant prolongation in the developmental period of *B. hebetor* when their host was reared on diet contaminated with a high concentration of coumarin. Similarly, Özcan and Gündüz (2018) proved that incorporation of $GA_3$ into the host diet caused a significant lengthening of the developmental time of *B. hebetor*. In another study, Gupta et al. (2009) observed parallel results in *Spilarctia oblique* (Walker) (Lepidoptera: Arctiidae) by using high concentrations of Siapton® and $GA_3$. On the other hand, Çelik and Sak (2020) showed that kinetin treatment had no effect on the developmental period of *Achroia grisella* (Fabricius) (Lepidoptera: Pyralidae). It may be concluded that there could be different toxicity sensitivities between different species and PGRs.

Analysis of data for the fecundity and sex ratio of *B. hebetor* reared on IAA-treated and untreated hosts are illustrated in Figure 2a and b, respectively.

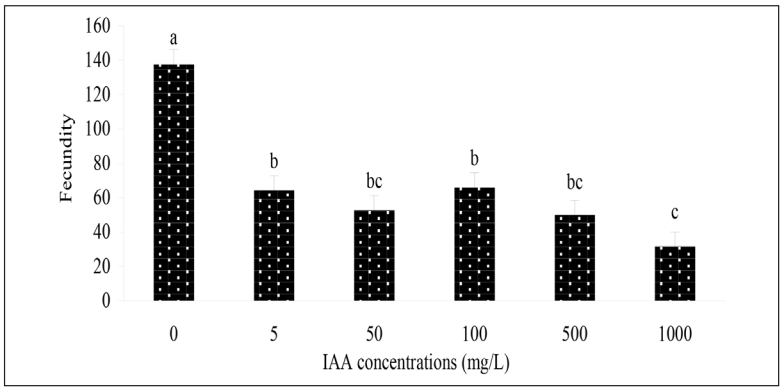


**Figure 2a.** Impact of indole-3-acetic acid on fecundity (mean ± SE) of *B. hebetor.* Different letters denote significant differences (SNK test, $P \leq 0.05$).

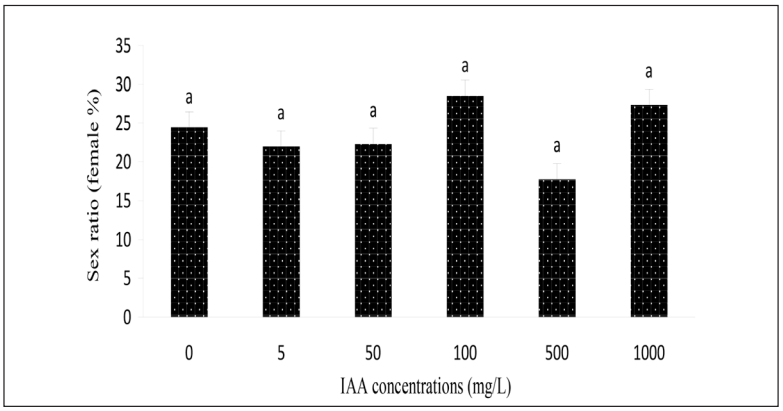


**Figure 2b.** Impact of indole-3-acetic acid on sex ratio (mean ± SE) of *B. hebetor.* Different letters denote significant differences (SNK test, $P \leq 0.05$).

Each female parasitoid reared on untreated hosts produced 137.9 offspring throughout its adult life. We observed that treatment with IAA significantly reduced the offspring production of female parasitoids ($P \leq 0.05$) (Figure 2a). A similar adverse effect of IAA on the total number of both F1 and F2 progeny of *Apanteles galleriae* (Wilkinson) (Hymenoptera: Braconidae) was also reported by Uçkan et al. (2011b). Kaur and Kaur (2013) also observed that fecundity of *B. hebetor* females declined when they were reared on hosts that fed on coumarin-treated diet. Thakur and Mann (1982) showed that topical incorporation of IAA on newly-emerged oriental fruit flies reduced the size of gonads. In another study, Thakur and Kumar (1984) suggested that adverse effect of IAA may be associated with its interference in endocrinal metabolic processes involved in reproduction. Moreover, Uçkan et al. (2014) reported IAA-related changes in the biochemical composition of *A. galleriae*. All of these findings would help us to explain the cause of the reduced fecundity in the present investigation. However, different PGRs have different effects on the reproduction capacity of parasitoids. For instance, Uçkan et al. (2008) did not record any significant effect on the offspring production of *A. galleriae* when its host *A. grisella* was fed on artificial diets treated with $GA_3$. Likewise, Özcan and Gündüz (2018) demonstrated that exposure to different $GA_3$ concentrations in host diet did not affect the progeny production of *B. hebetor*.

Sex ratio was determined as male biased in all groups and showed some weak but insignificant fluctuations among groups ($P > 0.05$) (Figure 2b). This result appears to be consistent with the observations of Kaur and Kaur (2013)'s and Özcan and Gündüz (2018)'s. Contrary to our findings, Zhao et al. (2017) showed that the percentages of females of *Aphidius gifuensis* reduced following the application of paclobutrazol (PBZ), naphthalene acetic acid (NAA) and IAA. Similarly, Prado

and Frank (2013) demonstrated a significant reduction in the female ratio of *A. colemani* due to exposure to paclobutrazol and uniconazole.

Our results demonstrated that IAA had negative effects on the longevity of female and male parasitoids (Figure 3a and b). All the IAA concentrations significantly reduced ($P \leq 0.05$) the longevity of female parasitoids except for the lowest concentration ($P > 0.05$) (Figure 3a). Similarly, the longevity of male parasitoids decreased in all IAA-treated-groups compared with controls ($P \leq 0.05$) (Figure 3b). These results corresponded to those reported for *A. galleriae* (Uçkan et al., 2011a), *S. litura* (Bhatnagar et al., 2012) and *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae)(Tsagkarakis et al., 2012).

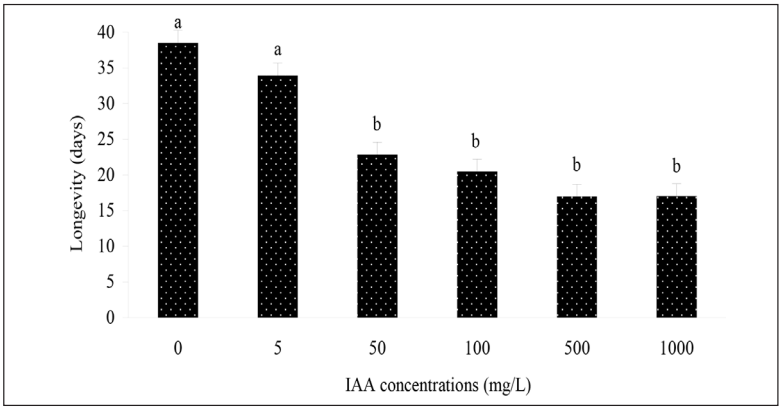


**Figure 3a.** Impact of indole-3-acetic acid on longevity of *B. hebetor* females (mean ± SE). Different letters denote significant differences (SNK test, $P \leq 0.05$).

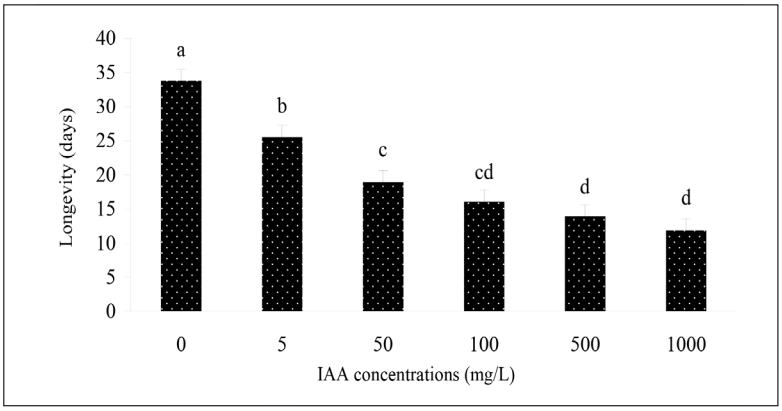


**Figure 3b.** Impact of indole-3-acetic acid on longevity of *B. hebetor* males (mean ± SE). Different letters denote significant differences (SNK test, $P \leq 0.05$).

## 4. CONCLUSION

In the current study, different concentrations of IAA were added to the host diet and development period, adult longevity, fecundity and sex ratio of parasitoids reared on these hosts were examined. *B. hebetor* females emerge with a very limited number of mature eggs and egg production and maturation continue during their lifetime. Since host feeding enables the females to obtain proteins associated with egg production and maturation, host quality strongly influences their reproductive success. Our findings showed that females were able to paralyze and subsequently oviposit on all of IAA-treated larvae that were offered to them during experiments. For insect parasitoids host is the unique nutrient source for the developing immature. Therefore, physiological suitability of the host is crucial for the successful development of parasitoid progeny. Host quality also influences the fitness of the emerging parasitoid by affecting longevity, fecundity, and offspring sex ratio. We determined that IAA application caused a noticeable decline in the development period of female wasps. Fast development may provide the fitness benefit of avoiding high predation risk during immature stages; however, it may also result in a loss of fitness in smaller individuals and lower fecundity when compared to larger ones. We do not know whether the IAA application had a negative affect on adult size in this study, but fecundity and longevity of emerging wasps declined considerably.

Overall, the above findings showed that IAA application via host diet has a considerable effect on selected properties of *B. hebetor*. However, due to the complexity of PGRs in tritrophic interactions, detailed experiments are required in order to understand the cause of these changes, determine the duration of efficacy, and their long-term effects in biological control.

### Conflict of Interest

The authors declare that there is no conflict of interest.

### Ethics

This study does not require ethics committee approval.

### Author Contribution Rates

Design of Study: NEAG (%50), DS (%50)

Data Acquisition: NEAG (%50), DS (%50)

Data Analysis: NEAG (%60), DS (%40)

Writing Up: NEAG (%80), DS (%20)

Submission and Revision: NEAG (%85), DS (%15)

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