Original article

Plasticity in life features, parasitism and super-parasitism behavior of *Bracon hebetor*, an important natural enemy of *Galleria mellonella* and other lepidopteran host species

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ABSTRACT

The wasp, *Bracon hebetor* Say, is an important potential biocontrol agent of a wide range of lepidopteran insect species. The current study was subjected to compare these ectoparasitoid fitness traits on different host species belonging to the order Lepidoptera. Moreover, we determine the population dynamic with different host densities, sex ratio patterns, super-parasitism, longevity, paralysis success, and reproductive potential under laboratory conditions. Our results revealed that oviposition increased with an increase in host density, while the adult emergence and egg hatching were decreased due to the super-parasitism on host larvae. A higher male and female-biased population were observed when virgin and mated females offered fresh hosts. Adults' longevity was recorded more in females than males when kept only with bee honey + royal jelly + host larvae. The mean duration of egg-adult development was recorded higher on *Galleria mellonella* and lowest on *Busseola fusca*. The parasitization rate, super-parasitism, and cumulative fecundity of mated and virgin female wasps with different host species were observed higher on specific host *Galleria mellonella* while it was lower on *Phthorimaea operculella*. Furthermore, the parasitoids having mating experience preferred fresh, while the parasitoids' lack of mating experiences preferred paralyzed host under olfactometer test. Besides, this research has produced novel facts on the biology of parasitic wasp, *B. hebetor* that may guide the advancement of sustainable biological control programs to control lepidopteran pests.

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1. Introduction

Insect pollinators regulate agricultural yields and biodiversity (Myers, 1996; Klein et al., 2007), and there are many thriving concerns over the consequences and cause of declining insect pollinators (Allen-Wardell et al., 1998; Gallai et al., 2009). The honeybee species are the main constituent of global biodiversity, providing required ecosystem services that are critical to human survival and crops for producing 15–30% of the food supply (Kremen et al., 2002; Greenleaf and Kremen, 2006; Gallai et al., 2009; Potts et al., 2010).

Feral and wild honeybees, which are considered the most important single species for crop pollination, accumulating

empirical data indicate a decline in regional populations over the past few decades (Kwadha et al., 2017; Geldmann and González-Varo, 2018). This population reduction has been associated with a myriad of interacting components among which multiple applications of pesticides (Meixner, 2010; Goulson et al., 2015; Johnson, 2015), fragmentation and habitat loss (Goulson et al., 2015), genetic mismatches (Meixner, 2010), trade-in honey bee and hive products (Pirk et al., 2016), as most important honeybee pests and pathogens, are examined key (Shimanuki et al., 1980; Meixner, 2010; Goulson et al., 2015; Pirk et al., 2016).

The greater wax moth, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) is considered one of the most destructive insect pests of the honeybee's hives owing to the ruinous feeding tendency of its larvae (Station and Beard, 1952; Williams, 1997, Lalita and Yadav, 2018). *G. mellonella* is a typical holometabolous pest that develops over four apparent life stages i.e., egg, larva, pupa, and adult (Gulati and Kaushik, 2004), first reported in the hive of *Apis cerana* (Fabricius, 1793) (Paddock, 1918), later this pest was spreading expeditiously around the globe (Chantawannakul et al., 2016; Makori et al., 2017). Wax moth larvae feed on the wax comb, honeybee pupal skin, pollens collected by workers, honey and broods (Nielsen and Brister, 1979; Türker et al., 1993), frequently exists in all apiaries places (Charrière et al. 2004). Large-scale devastation of bee colonies by wax moth larvae often edge to absconding, colony loss and decline in the proportion of seasonal bee swarms (Paddock, 1918; Nielsen and Brister, 1979; Türker et al., 1993; Gulati and Kaushik, 2004; Chantawannakul et al., 2016; Charrière et al., 2004; Makori et al., 2017; Nagaraja and Rajagopal, 2019). The most effective control measure has been based on the use of chemical fumigants (Ritter and Akratanakul, 2006), sanitation practices (Gulati and Kaushik, 2004), thermal control (Williams, 1997), biological control i.e., *Bacillus thuringiensis* (Harding et al., 2013; Dubovskiy et al., 2016), *Trichogramma* species (Hood et al., 2003), red imported fire ants, *Solenopsis germinita* and *S. invicta* (Van Lenteren et al., 2003), sterile insect techniques (Jafari et al., 2010), semiochemicals (Roller et al., 1968; Svensson et al., 2014), and *Bracon hebetor* and *Apanteles galleriae* (Ghimire and Phillips, 2010). Biological control tactics have been reported as an ecological approach for effective pest management by utilizing solid knowledge about insect pests, however, a lucrative and sustainable biological control agent of wax moth, *G. mellonella* is still ongoing (Kwadha et al., 2017).

Braconidae is a family of parasitoid wasps belongs to Hymenoptera encompasses more than 21,000 certain species (Quicke, 2015; Chen and Achterberg, 2019) out of which have potential roles in the bio-management of lepidopterans species (Ghimire and Phillips, 2010).

The wasp *Bracon hebetor* (Say, 1836), belong to the family Braconidae (Nees, 1811), has been recognized as one of the potential biological control agents due to the short generation time with a tremendous reproductive ability (Dweck et al., 2008) that assault the fourth and fifth instar of wax moth (Awadallah et al., 1985). Female wasps paralyzed host by stinging before oviposition, parasitoids larvae feed on the internal body parts of paralyzed host larvae and may carry on living up to one month (Manishkumar et al., 2013). The biology of these ectoparasitoid differs from the nutritional value, age, and newness of host *G. mellonella* (Alam et al., 2016). Disparate agroecological functions execute significant aspects for the successfulness of bio-control agents in case of augmentative releases like resistance to pesticides and residual consequence (Hasan and Ansari, 2017), ability to distribute in augmentative sites (Zappalà et al., 2012), fitness behavior of biological control agent (Borzoui et al., 2016), their interactions with others bio-control agents (Vanaclocha et al., 2013), and their resilience to

biotic and abiotic aspects (Hasan and Shafiq Ansari, 2016). The functional response of an ectoparasitoid in various ecological zones can decide the potential of a parasitoid to act as a bio-control agent (Fernández-Arhex and Corley, 2005). Practically a successful and sustainable biological control is only attainable when there is a strong association between a host and parasitoid (Wiedenmann and Smith, 1997). Dweck et al. (2008) concluded that female ectoparasitoid, *B. hebetor* use male secreted sex pheromones to find out the location of a host, wax moth. Ghimire and Phillips (2010) reported that ectoparasitoid, *B. hebetor* have a high oviposition rate on lepidopteran hosts and significantly more ectoparasitoid survival on the wax moth, *G. mellonella* as correlating with other hosts species.

The consequences of host quality and quantity concerning to biology and ecology of *B. hebetor* have been described from different research (Doutt, 1959; Yu et al., 2003). For instance, Eliopoulos and Stathas (2008) revealed the life table parameters of *B. hebetor* reared on *Plodia interpunctella* (Hubner, 1813) (Lepidoptera: Pyralidae), while Khalil et al. (2016) reported the effect of larval densities on the functional response of *B. hebetor* and Jamil et al. (2015) described the effect of different host species on the life history of *B. hebetor*. Moreover, Manzoor et al. (2016) checked the life pattern like sex ratio, longevity and reproduction of *B. hebetor* parasitizing *G. mellonella*. Most importantly, their findings were based on the differentiation of life duration parameters of the parasitoid, however, the effect of host condition on the life history of *B. hebetor* and parasitizing capacity of mated or virgin female parasitoid remained unattended. The diet concentration mostly affects the reproductive performance and longevity of bio-control agents, however, no sufficient work has been done to check the diet preference effects on this parasitoid's fecundity and reproductive potential of this parasitoid, *B. hebtor* (Gündüz and Gülel, 2005; Magro et al., 2006; Çinar et al., 2015; Abd El-Wahab et al., 2016).

This present research was focused on answering very important questions that how does a host condition affect the parasitizing potential of mated or virgin female parasitoids? We hypothesized that the host condition could affect the olfactory response of virgin parasitoids. Moreover, we evaluated the multiple effects of host densities on egg dispersion, sex ratio patterns on different states of the host, dietary composition effects on male and female parasitoids and development of *B. hebetor* on different host species. This project was designed to generate some novel information on *B. hebetor* which could help the effective management of lepidopteran pest species.

2. Materials and methods

2.1. Rearing of Galleria mellonella

Adults and their immature stages, i.e., larvae, pupae, eggs, of wax moth (*G. mellonella*) were sampled from infested honeybee hives of *Apis mellifera* (Linnaeus) from various apiary places located at Faisalabad, Pakistan. Couples of adult wax moths (Achterberg, 1993) were introduced into individually ventilated cages (14 cm × 11 cm) having fresh bee wax, propolis and honey under a controlled environment (28.0 ± 2 °C, 70 ± 5%). Females start oviposition after 24 h of mating. Eggs were transferred into the incubator (28 ± 2 °C, 70 ± 5% RH and constant dark period) for hatching. Upon hatching, the neonates were transferred to a glass jar (3.7 L) containing natural food i.e., honeybee wax. Normally larvae required about two weeks to complete development at 28 °C. Fully developed 5th instar larvae of *G. mellonella* were used in our experiments.

2.2. Rearing of parasitoid

The wasp, *B. hebetor* was reared on the fifth larval instars of the wax moth as previously described by (Jamil et al., 2015) with little modifications. Adult parasitoid wasps were collected directly from leguminous crops, *Trifolium* spp. and honeybee hives located at Agronomic Farm, Agriculture University, Faisalabad, Pakistan. Wasps were spotted based on visual parameters and available taxonomic keys (Achterberg, 1993). Parasitoids were reared in glass jars (500-ml) with 28 ± 2 °C temperature, 70 ± 5% relative humidity (RH) and 12 h L/D period. Only fifth instar larvae of wax moth were used to raise the wasp population described previously (Manzoor et al., 2016). Pairs of parasitoids were concealed into vials (2 cm × 10 cm) and supplied with fifth instar larvae of *G. mellonella* paralyzing of its host and larvae becomes sluggish then eggs were deposited. The larvae parasitized by a wasp were transferred to new glass jars for egg hatching. The newly hatched parasitoid larvae were supplied with water and honey solution by plugging dipped cotton bolls in the vials, while the cotton bolls were replaced by a new one every day.

2.3. Collection of alternative host species

Specimens of the host species used in our experiment were collected from different fields of the University of Agriculture, Faisalabad (UAF) and research centers located relatively at 71.65°E, 31.07°N, and 190 m altitude from (UAF). *Peridroma saucia* (Hübner, 1808) and *Tuta absoluta* (Meyrick, 1917) were collected from the vegetable fields of UAF. *Phthorimaea operculella* (Zeller, 1873) and *Busseola fusca* (Fuller, 1901) were sampled from potato and maize fields of UAF. In contrast, *Spodoptera litura* (Fabricius, 1775) was obtained from Central Cotton Research Institute (CCRI) Multan. All the host species used in our research experiments were collected from March to April and maintained under laboratory conditions (28 ± 2 °C, 70 ± 5% RH, and a 16/8h L:D period) on artificial diets as described previously by (McMorran, 1965; Grisdale, 1973). Each host species was reared in a separate jar (3.7 L). Every two days, the jars were cleaned and replaced with a fresh diet. The young, healthy, and homogenized population of each host species was used for experiments

2.4. Host density experiments and parasitization

The newly emerged wasps were paired (male and female) and allowed to mate for 24 h in glass jars (10 cm × 20 cm). On the second day, the homogenized, equal weights, fresh, and healthy 5th instar reared population larvae of *G. mellonella* were introduced in five treatment glass jars (5 cm × 10 cm) numbered (D1-D5) (as D1 = 2 larvae/jar, D2 = 6 larvae/jar, D3 = 8 larvae/jar, D4 = 12 larvae/jar, and D5 = 16 larvae/jar). Fifteen-gram fresh wax was provided in each jar and larvae were allowed to settle for 3 h. After that, a mated female wasp was provided into each jar and left freely to locate and attack the host for 24 h oviposition period. The total oviposition of female wasp was scored and all the parasitized host larvae were incubated separately at 28 ± 2 °C, 70 ± 5% RH, and a 12 h L:D period. Eggs hatching and adult emergence per host were scored daily.

In another experiment, the sex ratio pattern of *B. hebetor* offered with a fresh or paralyzed host of wax moth was determined. A pair of fresh or paralyzed (stung by wasps) *G. mellonella* larvae were transferred to glass jars (5 cm × 10 cm) individually while five-gram fresh wax was provided in each jar and larvae were allowed to settle for 3 h. A mated or virgin (unmated) female wasp was introduced to each glass jars individually and allowed to parasitize the hosts for 24 h. All the parasitized host larvae (fresh or paralyzed) by mated or virgin wasps were incubated separately at 28 ± 2 °C, 70 ± 5% RH, and a 12 h L:D period. The male and female adult population of wasp emerged per host were scored daily. All the treatments were replicated fifteen times.

2.5. Bracon hebetor longevity test

To check the longevity of *B. hebetor*, newly emerged males and females were coupled and transferred to a glass jar (10 cm × 20 cm). All the treatments were numbered (A1-A9) to check each of the specified food types: 1) without food (starvation); 2) water only; 3) bee honey only; 4) host larvae only; 5) bee honey + host larvae; 6) royal jelly; 7) royal jelly + bee honey; 8) bee honey + royal jelly + host larvae; 9) sugar solution only. Two pairs of *G. mellonella* larvae were used as host larvae and wasps were monitored daily to score each longevity at 28 ± 2 °C, 70 ± 5% RH, and a 12 h L:D period. All the treatments were replicated fifteen times.

2.6. Hosts suitability experiments

Different hosts species (*P. saucia, P. operculella, S. litura, B. fusca* and *T. absoluta*), including specific host *G. mellonella,* were used to determine the biological parameters of *B. hebetor* under controlled environmental conditions (28 ± 2 °C, 70 ± 5% RH, and a 12 h L:D period). The fresh, healthy and equal weight larvae were selected for the experiments. Two pairs of 24 h old-mated *B. hebetor* was transferred to a glass jar (10 cm × 20 cm). A cotton swab containing 70% honey solution was placed inside the glass jars for adult feeding. Fifteen larvae of each host species were transferred to each jar and the jar's opening was covered with a piece of muslin cloth and tightened with a rubber band to prevent the larvae escape. Parasitoids were allowed to oviposit for 24 h. In comparison, the same type of experiment was performed for virgin female wasps. Every day, each host's larvae were replaced by 15 new ones planned for five running days, while the jars opening were tightened quickly to prevent the wasp's escape. The larvae parasitoid by wasp were placed individually in each Petri dish (5 cm × 3.5 cm) and were incubated under the control chamber (28 ± 2 °C, 70 ± 5% RH, and a 12 h L:D period) for further observations. The total larvae paralyzed or oviposited for each day and over five consecutive days as well as the total adult population emerged were observed. The fresh and parasitized host larvae were fed with prepared artificial diets as previously described by (McMorran, 1965; Grisdale, 1973). Moreover, the progeny development time from the egg-adult from all the respective host species were also recorded. Fifteen replicate sets were made for each host species.

2.7. Bracon hebetor behavioral study under glass olfactometer test

A glass olfactometer was used to check the attraction of mated and virgin female's parasitoids toward fresh or paralyzed host species (Fig. 1). A day before the experiment, 20 larvae of each host species (*G. mellonella, P. saucia, P. operculella, S. litura, B. fusca* and *T. absoluta*) were transferred into a separate glass jar holding five pairs of mated females and allowed to oviposit for 24 h to obtain paralyzed host larvae. For parasitoids, newly emerged young couples of wasps were introduced into a glass jar (10 cm × 20 cm) to mate for 24 h. The paralyzed larvae of each host species individually were introduced into an olfactometer glass jar (10 cm × 20 cm), while mated and virgin female parasitoids were allowed through the glass tube to check the preference toward a fresh or paralyzed host. Each parasitoid's initial choice that walked into a choice arm and remained there at least for one minute was recorded. If a female parasitoid did not choose within 10 min after being released into glass tubes, it was considered as "no response". Each larva was utilized to make five choices; after that, a new one
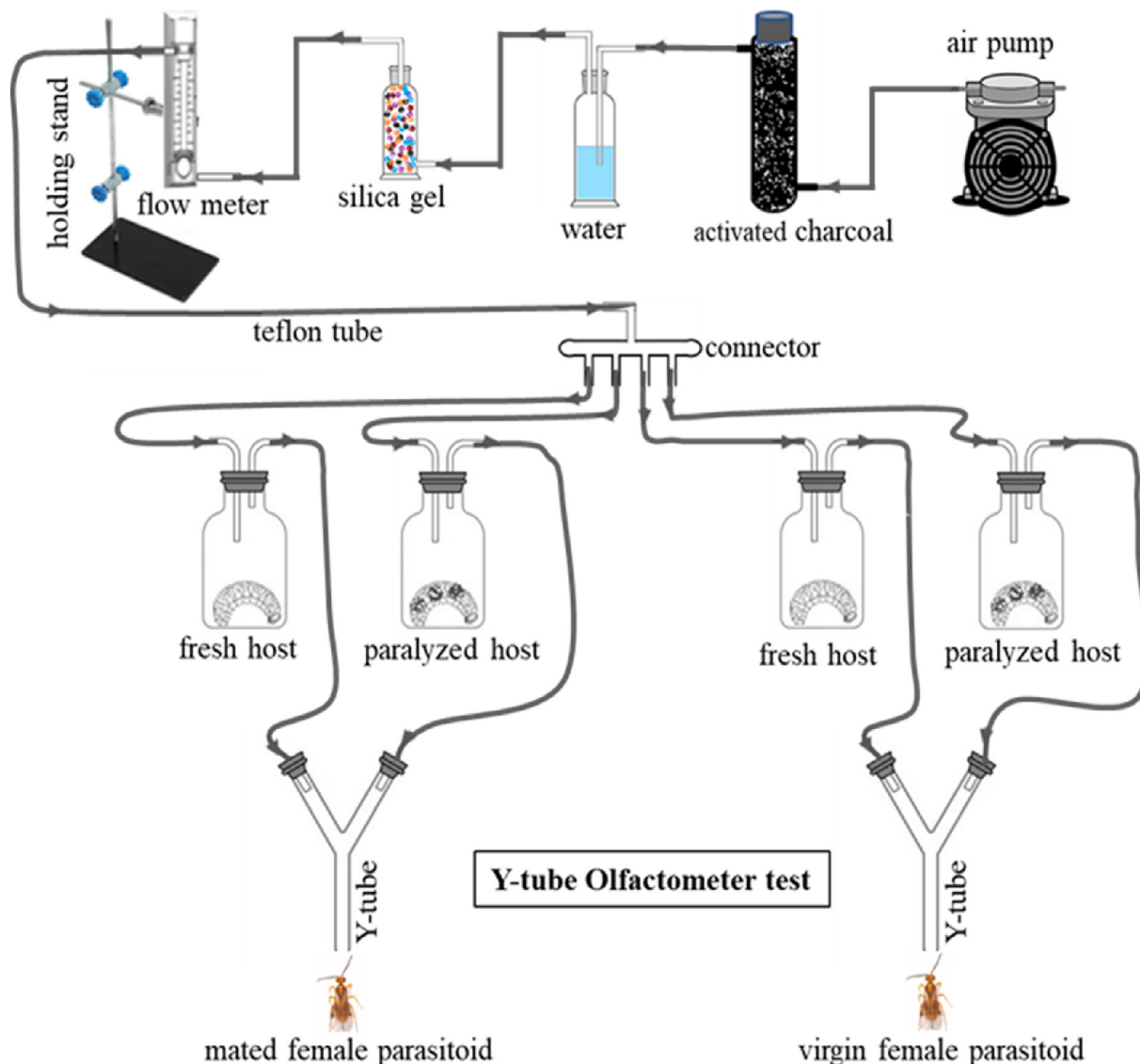
Fig. 1. Schematic design of Y-tube olfactometer used to determine the behavioral response of mated and virgin female parasitoids. The glass olfactometer contains two central tubes (10 cm long), connected separately to each glass jar. A thick black cloth was used to cover Y-tubes to create a dark environment. A small air pump was used to pump air, while pumped air was cleaned in a series of steps with activated charcoal; distilled water, while silica gel was used to absorb moisture contents from the air coming through the water. An airflow pressure of 0.3 L min-1 was adjusted using a flowmeter, reached through a Teflon tube. Tested host larvae were placed inside the glass jars, while parasitoids were released individually at the Y-tube arm entrance and were considered to have made a choice when female wasp walked into a choice arm.

replaced it. After every five replicates, the glass tubes were replaced by each other to randomize any positional error. Following 10 replicates, the olfactometer was cleaned and a new one replaced by glass tubes. Each treatment was repeated twenty times.

2.8. Data analysis

All the data were arcsine transformed to normalize the count values before the analysis. The analysis was undertaken for transformed data, while the back-transformed data was only presented. The population dynamics, longevity on different food types, the performance and development of *B. hebetor* on different host species were subjected to GLM (R development core team, 2020), When ANOVAs were significant, the relevant comparisons of means were made using the Tukey test at 5% significance level. Independent T-test was used to evaluate the significance between

sex ratio pattern and behavioral response of *B. hebetor* under Y-tube olfactometer tests.

3. Results

3.1. The population dynamics of Bracon hebetor at different host densities

The analyzed data concerning the total number of eggs laid, egg hatching and adult emergence of *B. hebetor* on distinct host *G. mellonella* densities were presented in Fig. 2. The results showed significant differences among egg-laying, egg hatching and adult emergence from different host densities. The oviposition rate was increased as host density increased whereas the maximum mean number of eggs laid by an individual female wasp was (128 ± 2.07) on the highest host density in comparison to the lowest host density which recorded a minimum (20.08 ± 0.66) as shown in Fig. 2A. Egg hatching and adult emergence were recorded
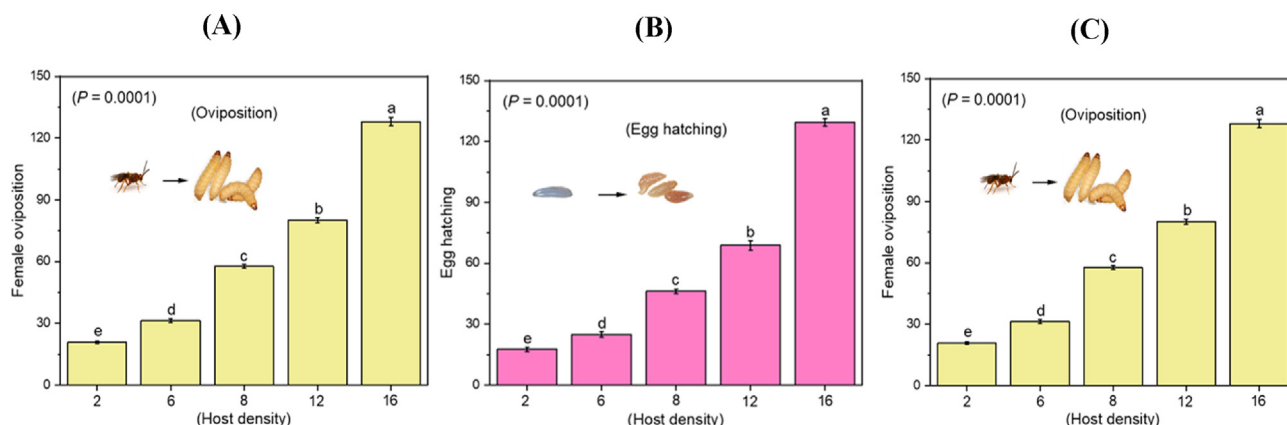
Fig. 2. The increase in host densities higher the parasitizing capacity and development performance by *B. hebetor*. (A) Oviposition, (B) Egg hatching and (C) Adult emergence from the pupa of *B. hebetor* with different host densities. Values are indicated as mean ± SE. Error bars indicating different letters are significantly different using ANOVAs, while the means were separated by the Tukey test ($P \leq 0.05$).

Table 1
Host species used for parasitism study.

Common name	Scientific name	Family	Larval instars
Variegated cutworm	*Peridroma saucia* (Hübner, 1808)	Noctuidae	5th
Potato tuber moth	*Phthorimaea operculella* (Zeller, 1808)	Gelechiidae	4th
Tobacco budworm	*Spodoptera litura* (Fabricius, 1775)	Noctuidae	5th
Maize stalk borer	*Busseola fusca* (Fuller, 1901)	Noctuidae	5th
Tomato pinworm	*Tuta absoluta* (Meyrick, 1917)	Gelechiidae	4th

maximum (129.40 ± 1.72 and 119.20 ± 0.73 respectively) at the highest host densities (Fig. 2B, C).

3.2. Sex ratio pattern of Bracon hebetor

The highest male-biased population of *B. hebetor* was recorded when a virgin (19.20 ± 2.42) and mated (18.86 ± 0.81) female

wasp were allowed to oviposit fresh host *G. mellonella* larvae as shown in Fig. 3A. The female-biased population of *B. hebetor* was higher (15.64 ± 1.58) and lower (9.20 ± 1.28) when mated and virgin females oviposit on fresh host larvae, respectively (Fig. 3B). It was recorded that the mated and virgin female wasp deposited the highest number of eggs on fresh host larvae as compared to the paralyzed host which in turn recorded the highest male-biased population.

3.3. Longevity of Bracon hebetor on different food types

A significant difference in longevity was observed when *B. hebetor* was offered with varying types of food (Table 2). Female and male wasps considerably recorded higher longevity when fed on bee honey + royal jelly + host larvae diet than those on the honey solution and other diets. When compared between the longevity of males and females according to food types, the results showed a non-significant difference between all the food types except the water only and host larval diets. The lowest longevity was recorded when the female and male wasps were starved.
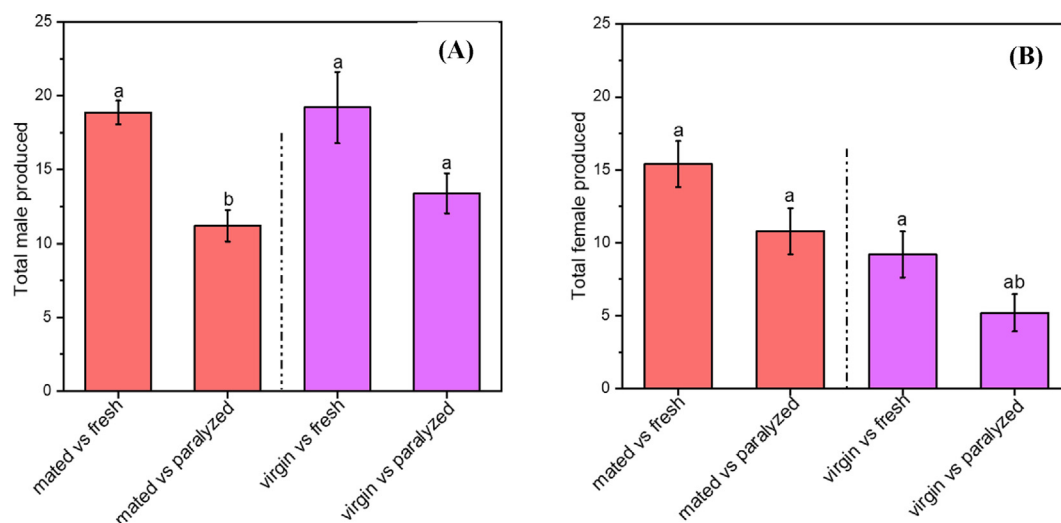

Fig. 3. The change in host condition affects the sex ratio pattern of the parasitoid, *B. hebetor*. (A) The male and (B) female of *B. hebetor* produced when offered with fresh or paralyzed host larvae of *G. mellonella*. Values are indicated as mean ± SE. Error bars indicating different letters are significantly different for paralyzed or fresh host larvae parasitoid by mated or virgin females' wasps using Independent T-test ($P \leq 0.05$).

Table 2
Effect of different food types on the longevity of *Bracon hebetor.*

Food types	Life span (mean ± SE)		F vs M
	Female	Male	$P \leq 0.05$
Without food (starvation)	4.19 ± 0.29 g	3.42 ± 0.21f	0.064
Water only	5.11 ± 0.47 g	3.92 ± 0.80f	0.000
Bee honey only	18.91 ± 1.32c	19.41 ± 0.65c	0.441
Host larvae only	9.96 ± 2.14 e	8.02 ± 0.46 e	0.011
Bee honey + host larvae	15.2 ± 1.07 d	13.79 ± 1.19 d	0.052
Royal jelly	20.21 ± 1.12c	19.82 ± 0.89c	0.649
Royal jelly + bee honey	22.34 ± 0.56b	22.13 ± 1.36b	0.732
Bee honey + royal jelly + host larvae	26.23 ± 1.79 a	24.41 ± 0.17 a	0.049
Sugar solution only	6.96 ± 0.34f	7.43 ± 1.01 e	0.551

Each food type was offered individually to males and females of *B. hebetor*. Values are indicated as mean ± SE. The different letters in columns are significantly different between all the food types for male or female parasitoids using ANOVAs, while the means were separated by the Tukey test ($P \leq 0.05$).

3.4. The host suitability of different lepidopteran species for the performance of Bracon hebetor

The proportion of host oviposited, paralyzed, eggs laid and total adult population emerged each day as well throughout the 5 consecutive days varied significantly with the host species (Fig. 4). The highest paralyzation and oviposition rates/day were observed in mated (14.63 ± 0.69 and 10.35 ± 0.58) and virgin (8.70 ± 0.68 and 6.86 ± 0.35) female parasitoids against the larvae of *G. mellonella* respectively (Fig. 4A, B). While, the other hosts were *P. saucia* (9.59 ± 0.57) and *S. litura* (9.14 ± 0.54) in paralyzation whereas, *S. litura* (7.16 ± 0.46) and *B. fusca* (7.21 ± 0.36) in oviposition respectively showed a non-significant difference under mated choice. The oviposition and paralysis were recorded lower in mated (3.9 ± 0.37 and 5.97 ± 0.96) and virgin (2.86 ± 0.33 and 2.84 ± 0.21) female parasitoids against the *P. operculella* host. However, the virgin females recorded less oviposition and paralyzation as compared to mated females at a single day or for over 5-days (Fig. 4A, B).

A similar observation was recorded regarding the oviposition and paralysis success of female parasitoids for over 5 days (Fig. 4C, D). The paralysis knocks and oviposition results showed significant differences between mated and virgin females' parasitoids.

Same as per/day performance, the host *G. mellonella* recorded the highest oviposition and paralysis for mated (58.20 ± 2.13 and 72 ± 1.09) and virgin (42.80 ± 2.05 and 64.20 ± 3.02) female parasitoids, whereas the host *P. operculella* recorded the lowest oviposition and paralysis for mated (21.80 ± 1.06 and 27.80 ± 1.15) and virgin (14.60 ± 1.93 and 19.40 ± 0.92) female wasps respectively for over five consecutive days (Fig. 4C, D).

The total eggs laid by *B. hebetor* mated female parasitoid over 5 days were higher (154.60 ± 3.5) on the *G. mellonella* host larvae in comparison to *P. operculella* host which recorded lower (58 ± 2.8) (Fig. 4E).

Significantly, the highest adult progeny (77.20 ± 2.08) was recorded for *G. mellonella* followed by *P. saucia* (61.40 ± 2.06) and *S. litura* (46.8 ± 3.07). However, the host larvae of *P. operculella, B. fusca* and *T. absoluta* showed non-significant differences in the mean number of *B. hebetor* adult progenies produced with mated female parasitoids (Fig. 4F). The lowest mean *B. hebetor* adult progeny was recorded on the host *P. operculella* (26.60 ± 1.86) for mated and by the *T. absoluta* (11.60 ± 1.36) for virgin female wasps.

3.5. The development of Bracon hebetor on different host species

The total development times egg-adult for *B. hebetor* was non-significant with all the offered host species (F = 0.424; P = 0.823).

However, the development time was shortest on all host species except for the *G. mellonella* host which recorded higher (Table. 3). The parasitoid grows faster at egg-larvae-pupa stages on *G. mellonella* host except for the adult stage which recorded the longest time. Moreover, there were no significant differences observed in larval development times among all host species (Table.3).

3.6. Behavioral response of Bracon hebetor under Y-tube olfactometer

The results of the olfactometer experiments that tested the behavior of *B. hebetor* to the fresh or paralyzed hosts of different lepidopteran species are shown in (Fig. 5). For mated female parasitoids, significant differences were recorded in the parasitoids choice for fresh hosts over paralyzed ones (Fig. 5A). The *B. hebetor* showed the highest attraction for the *G. mellonella* host (31.8 ± 2.35), whereas the response was found lower for *P. operculella* (17.2 ± 2.1). In another choice test of virgin female parasitoids, the parasitoids were significantly more attracted to paralyzed host species than the fresh ones (Fig. 5B). However, same as the previous test, the parasitoids response was higher for the *G. mellonella* host but not for *P. operculella*. The parasitoid attraction for the host *P. saucia, P. operculella* and *S. litura* were found non-significant, whereas the *T. absoluta* recorded the lowest (6.2 ± 1.06) attraction for virgin parasitoids (Fig. 5B).

4. Discussion

The successful implementation of bio-control tactics from past years, controlling insect pests with natural enemies, has become a hot topic among researchers. Currently, there are a lot of researchers are in efforts that how we could promote the reproductive potential and pollination abilities of natural enemies by adding some minerals as a plant nutrient or by modifying plants genetically that could produce multiple flowers with the highest attraction to natural enemies (Sivinski et al., 2011; Han et al., 2016).

The *B. hebetor* has wide host species on several lepidopteran larvae, short generation period and high productivity researchers usually use this parasitoid to study the host-parasitoid interaction (Gunduz and Gülel, 2005). Here, we focused on the factors that affect the biology, reproduction and efficiency of *B. hebetor* parasitism on different lepidopteran pest species (Table 1).

The relation between host density and population of the parasitoid was studied by Solomon (1949). He described this natural two sides response: (i) the Numerical response is a change in the parasitoid density in response to change in the density of the host population and the Functional response which is the response of each parasitoid to change the density of the host. Our obtained results confirmed the previous hypothesis. It may be hypothesized that an increase in egg hatching and change in percentage of adult emergence is due to less crowding of eggs on the exposed host larvae and less larval competition for food at the highest host density (16 host), so many of them thrived, while the other parasitoid larvae were died because of fewer hosts. Obtained results confirmed that oviposition, egg hatching and adult emergence increases with increasing host densities. Furthermore, the oviposition rate was increased as host density increased and it was the observation that to make minimum crowding of eggs on host larvae, parasitoids optimize their clutch size accordingly. However, sex ratio patterns affect growth rates and the evolutionary trajectories of insect population and are considered the most basic demographic parameter by supplying an intimation of the relative survival of both male and female and the future breeding perspective of a populace. Our results indicated that the male progeny sex ratio of wasp is dependent on the number of eggs laid and host condition. Moreover, the experiments under the super-parasitism check showed less male
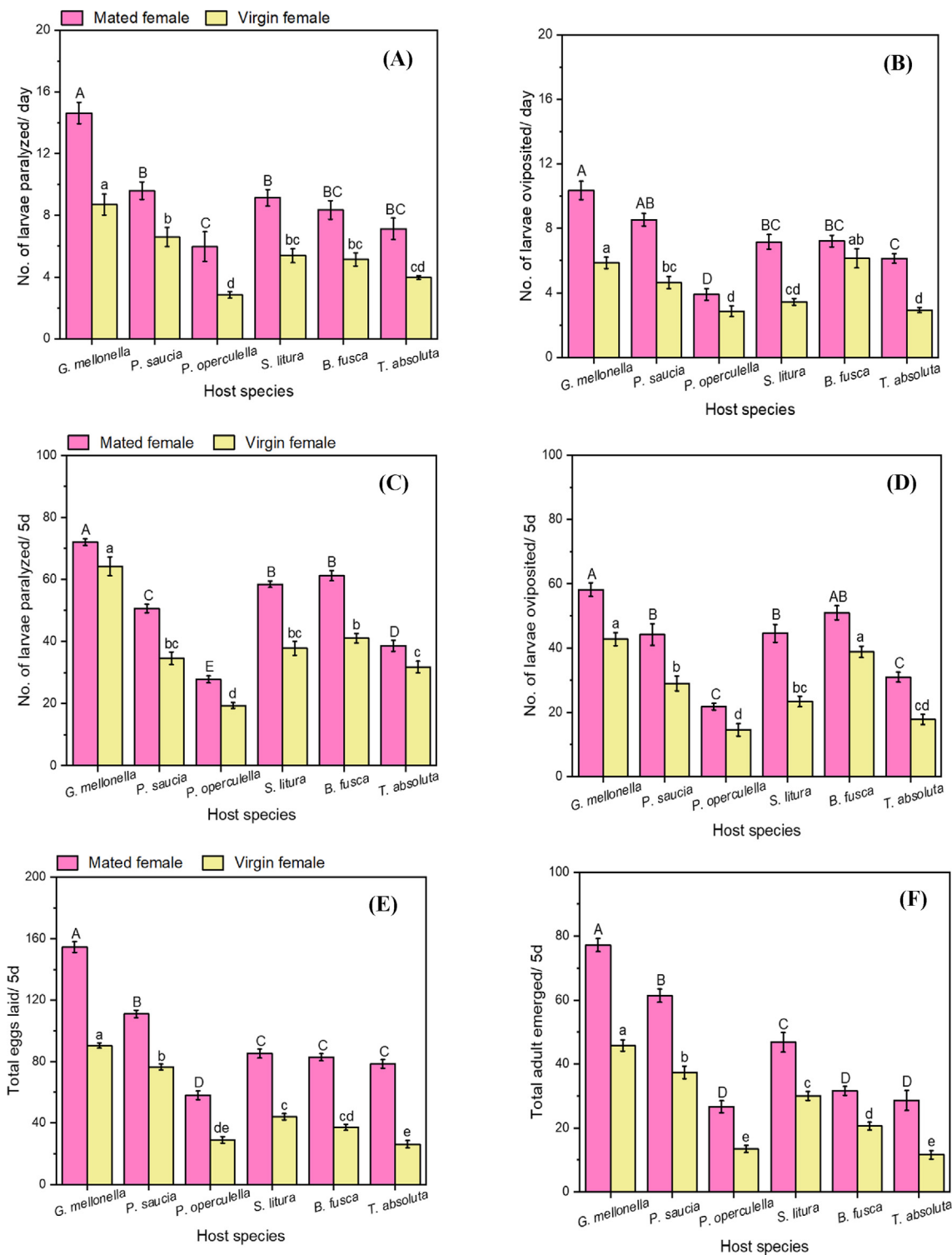
Fig. 4. The growing performance of *B. hebetor* is mostly affected by the different lepidopteran host species. (A, B) The paralyzation and oviposition performance of mated or virgin parasitoid offered with different lepidopteron hosts species. (C, D) Total oviposition and paralyzation performance of parasitoid recorded during 5-days of period time. (E, F) The total eggs laid, and adult progeny produced by per mated or virgin females over 5 days. Values are indicated as mean ± SE. Upper-case letters showing a significant difference between different host mated female's wasp, whereas the lower-case letters indicating a significant difference among different host virgin females. (P ≤ 0.05; ANOVAs).

and female-biased sex ratio when mated and virgin female was allowed to oviposit on paralyzed host larvae. Finally, the *B. hebetor* male-biased progeny sex ratio was recorded more in parasitism and super-parasitism than *B. hebetor* females. Jehan et al. (2020) stated that, especially in parasitoids, the sex ratio is greatly affected by

reproductive decisions. The sex ratio, fecundity rate and generation time of *B. hebetor* were significantly affected when different densities of *Corcyra cephalonica* (Stainton, 1866) (Pyralidae, Lepidoptera) were maintained. However, the longevity of female wasps had no significant differences at different *C. cephalonica* densities (Singh

Table 3
Effect of different host species on the development of Bracon hebetor.

Host species	Time duration (days)				
	Egg	Larvae	Pupa	Adult	Egg - Adult
Wax moth, *G. mellonella*	1.19 ± 0.082 d	3.02 ± 0.069 a	4.07 ± 0.161c	10.61 ± 0.363 a	18.90 ± 0.49
Variegated cutworm, *P. saucia*	1.21 ± 0.035 d	3.09 ± 0.031 a	4.39 ± 0.076c	9.96 ± 0.143 ab	18.67 ± 0.47
Potato tuber moth, *P. operculella*	1.49 ± 0.030 a	3.16 ± 0.041 a	5.21 ± 0.210 a	8.42 ± 0.245c	18.29 ± 0.60
Tobacco budworm, *S. litura*	1.29 ± 0.081 bc	3.12 ± 0.162 a	4.56 ± 0.206 bc	9.22 ± 0.315 abc	18.20 ± 0.70
Maize stalk borer, *B. fusca*	1.26 ± 0.061 bc	3.10 ± 0.131 a	4.51 ± 0.065c	9.16 ± 0.143 bc	18.04 ± 0.86
Tomato pinworm, *T. absoluta*	1.36 ± 0.083b	3.14 ± 0.184 a	5.13 ± 0.121 ab	8.92 ± 0.154 bc	18.57 ± 0.81

The *B. hebetor* was offered with fresh host larvae of different species separately. Values are indicated as mean ± SE. The different letters in columns are significantly different between all the host species using ANOVAs, while the means were separated by the Tukey test ($P \leq 0.05$).

et al., 2016). Furthermore, Rasool et al. (2017) mentioned that used two species host of varying size (*G. mellonella* and *S. litura*) and varying densities of *B. hebetor* affect the sex ratio, number and size of emerging wasp adults. *B. hebetor* females could regulate egg-laying according to the increase of host densities. Also, they could regulate the sex ratio based on the number of deposit eggs/ larvae and the host densities (Aamer et al., 2015). *B. hebetor* usually mate once during their life and after that, they aim to paralyze all offered host larvae before allocating their eggs. At high densities of host larvae, reduction in fecundity occurred from the optimum state maybe this is a result of their efforts and energy expense during paralyzation of all obtainable host larvae (Singh et al., 2016).

Fig. 5. The female parasitoids lack mating experiences their attraction could be mismatched with the paralyzed host. Behavioral response of mated and virgin female parasitoids offered with a fresh or paralyzed host of different lepidopteran species (n = 20). Values are indicated as mean ± SE. Each column bars indicating different letters between fresh or paralyzed host are significantly different using Independent T-test ($P \leq 0.05$).

The results obtained concerning the effect of food types on *B. hebetor* wasps' longevity shows significant differences for bee honey + royal jelly + host larvae. Using this combination of food had the longest longevities (24.41 ± 0.17 and 26.23 ± 1.79 days) for females and males. Many environmental factors such as light, temperature, humidity, population density, and food types of noticeably affected parasitoids' longevity. In general, the development and reproduction of insects depending on quality and type of nutrition. Honeybee products (bee honey, royal jelly and pollens) are rich in nutrients that contain carbohydrates (mainly sugars), water, protein, amino acids, enzymes, vitamins, acetylcholine and others (Hagen et al., 1984; El Sohaimy et al., 2015; Alvarez-Suarez, 2017). Diets containing natural floral nectar and honeybee products are the best diets for parasitoid development (Barbehenn et al., 1999). Feeding females of *B. hebetor* on bee honey and sucrose syrup have affected the level of glycogen and total sugar in female wasps hemolymph (Gündüz et al., 2010). Moreover, El-Wahab et al. (2016) tested honey products as diets for *B. hebetor* and mentioned that these diets promise in the rearing and mass production of this parasitoid. They found that the diets included royal jelly, royal jelly + bee honey and royal jelly + pollen grains positively affected the fecundity, egg hatching and longevity of males and females. Furthermore, bee honey, royal jelly, pollen, propolis and their mixtures have enhanced the longevity, fecundity, sex ratio, and adult emergence rate of three species of *Trichogramma* wasps (Mashal et al., 2019). Our results confirmed that honey combination with royal jelly accommodates the additional protein necessity for female parasitoids while the host larvae employed for continuing eggs production throughout their lifespan as well contribute to escalating the fecundity and longevity of the parasitoids. The combined use of this diet had a significant effect on the reproduction and survival of *B. hebetor* wasp.

The host species have affected the development and biological activities of parasitoid wasp *B. hebetor*. Here, the minimum time for *B. hebetor* was obtained with the parasite on *G. mellonella* and *P. saucia* larvae. However, *P. operculella* and *T. absoluta* larvae produced the most prolonged period for parasitoid tested wasp development. We determined the behavioral response of parasitic wasp, *B. hebetor* toward all available six-host species in support of our hypothesis. Although mated and virgin females' wasps *B. hebetor* accepted nearly all offered host larvae for oviposition and paralyzation. However, Schmid-Hempel (2009) mentioned that parasitoids deploy their host larvae in various ways and, most importantly, specialized in host choice. Parasitoid wasps are indicated as model organisms for traverse obstructions on arthropods' development and life history. Eliopoulos et al. (2002) reported that the parasitism percentage of *B. hebetor* on lepidopterous pests of stored products; *Plodia interpunctella* (Hübner, 1813), *Ephestia kuehniella* (Zeller, 1879) and *Ephestia elutella* (Hübner, 1796) (Pyralidae, Lepidoptera) was less than 5%. Ghimire and Phillips (2014) found that the oviposition and reproductive performance of *B. hebetor* were significantly different on six hosts of pyralid pests. Authors
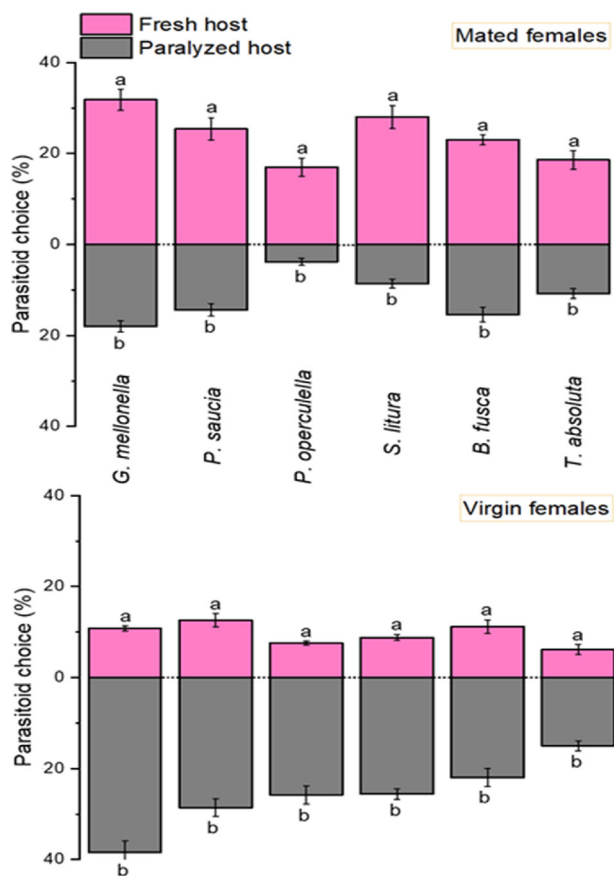
reported that *G. mellonella* has the highest fecundity and lowest parasitoid survival percentage, whereas the *E. elutella* has the lowest fecundity and *Amyelois transitella* (Walker, 1863) (Pyralidae, Lepidoptera) had the highest survival percentage. Also, Milonas (2005) investigated the effect of host species, size and larval competition on parasitoid size, survival and development and reported that *B. hebetor* was a gregarious parasitoid that affects the several lepidopterous pests which attack stored products and field crops as well.

The ethological response of female wasp was determined toward various paralyzed lepidopteran host species. Gündüz and Gülel (2005) mentioned that the *B. hebetor* females could parasite on old and young larvae but they usually prefer the last instar larvae for laying their eggs. However, the overall response rate of mated and virgin female wasp was lower compared to fresh hosts provided as shown in Fig. 4. Whereas, Farag et al. (2015) reported that the number of eggs laid by *B. hebetor* females was affected by the host species. Wasp females deposited the total maximum number of 395.11 eggs on *G. mellonella* while depositing the lowest number of eggs, 56 on *C. cephalonica*. On the other hand, Muslim et al. (2017) reported that female wasps had the highest paralysis and parasitism percentages on larvae of *C. cephalonica*, followed by *G. mellonella*, *E. kuehniella*, and *P. interpunctella*, while these activities were reduced on *S. litura* and *S. littoralis*. Mansour and Saber (2017) found that *Bracon* is highly polyphagous, against various species of order Lepidoptera including *C. cephalonica*, *Sitotroga cerealella* (Olivier, 1789) (Fam.: Gelechiidae), *G. mellonella*, *Helicoverpa armigera* (Fam.: Noctuidae) (Hübner, 1808) and *S. litura*. Adly and Marzouk (2019) evaluated the efficacy of *B. hebetor* on *G. mellonella* under laboratory conditions, honeybee colonies, and stored beeswax combs, the authors mentioned that the pre-ovipositional, ovipositional, and post-ovipositional periods of the parasitoid were 0.27 ± 0.45, 20.87 ± 1.5, and 4.33 ± 0. 48 days, respectively.

In our olfactometry assay, we found that the mated females of *B. hebetor* tended to choose the fresh hosts more frequently than the paralyzed ones. In contrast, the *B. hebetor* virgin females were more attracted to the paralyzed host species than the fresh hosts. This may be due to the lack of mating experience or larval odor that mismatch their paralyzed hosts' attraction with paralyzed hosts. Because the paralyzed hosts have already been oviposited by mated parasitoids, which it can release some cues that could help find a host for virgin parasitoids. Besides, the parasitoid response differs significantly among lepidopteran host species. Our results highlight that mated females of *B. hebetor* could recognize among fresh and paralyzed hosts and the host species. The female wasps of *Trichogramma evanescens* (Westwood, 1833) and *Trichogramma brassicae* (Bezdenko, 1968) were attracted to the virgin adult female odor of *T. absoluta* comparing with clean air jar (Ahmadi and Poorjavad, 2018). In another study, Bodino et al. (2016) found that the learning cues influenced the host selection behaviors of the parasitoid *Necremnus tutae* (Reuter). Moreover, Segura et al. (2007) proposed that visual information is associated with host selection cues by the parasitoid *Diachasmimorpha longicaudata* (Wharton, 1987) and important in host search. In conclusion, they explain that visual cues' learning preferences are imperative for generalist parasitoids in host foraging. Our results predict that the preference of virgin females' parasitoids for the paralyzed host can be due to the lack of visual learning, which they can attain during mating. However, future works are required to confirm this scarcity of research and to provide more information on the host (fresh and paralyzed) chemical cues used during host selection for *B. hebetor* wasp with molecular works. We hope this research will help to develop and implement a successful biological control program using *B. hebetor* as a potential biocontrol agent for controlling several pests in the future.

5. Conclusions

We concluded that the host density, host condition, diet composition and availability of host type largely influence the performance of *B. hebetor*, whereas the virgin parasitoids which are lack mating experiences mismatch their choices with paralyzed hosts in olfactometer tests. Useful knowledge has been generated concerning the exploitation of *B. hebetor* in mass-rearing programs for the successful implementation of biological control approaches in controlling lepidopteran pests.

Declaration of Competing Interest

The authors declare no conflict of interest exists.

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