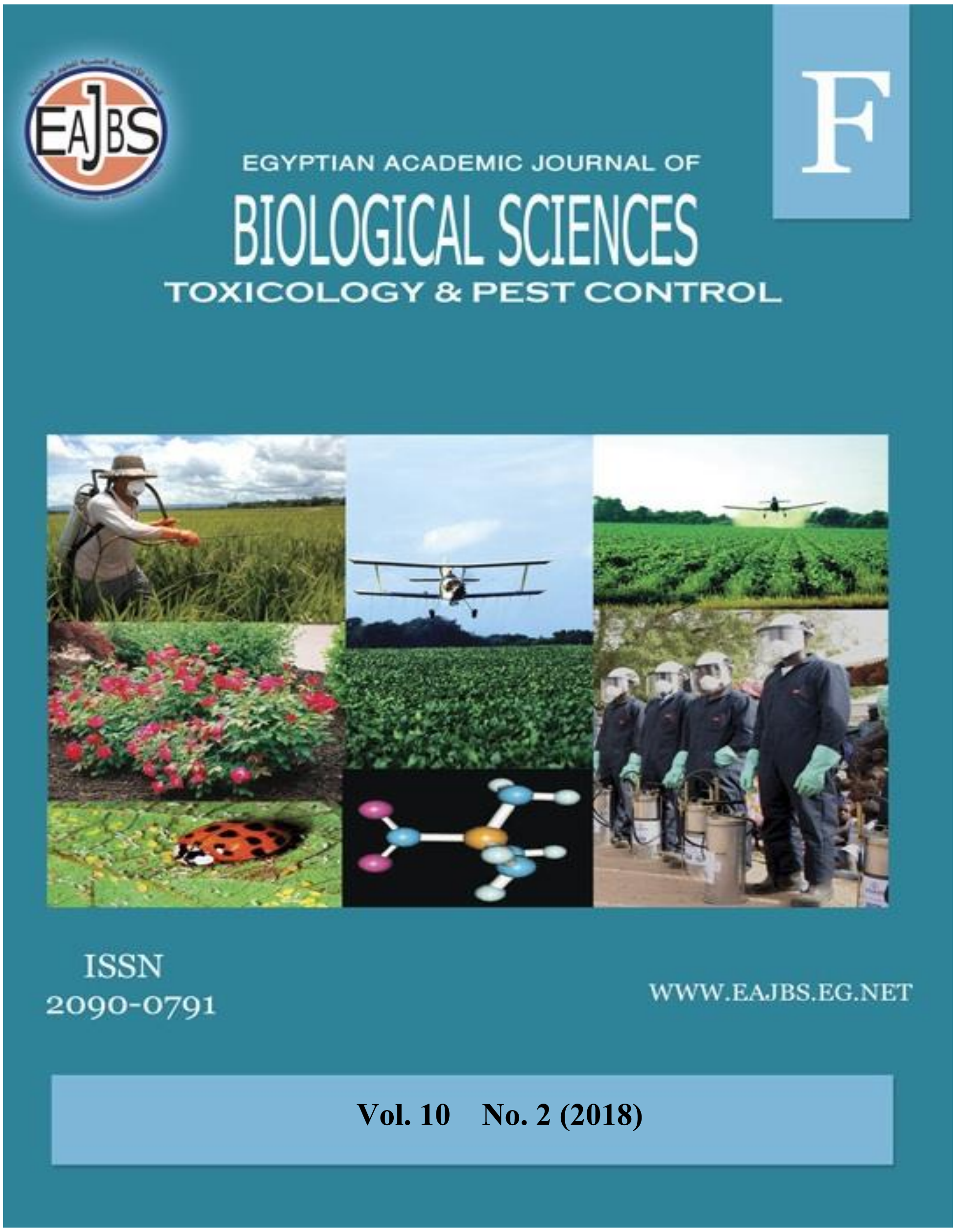

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Evaluation of the Biological Parameters of the Interaction the Parasitoid, *Bracon brevicornis* Wesmael (Braconidae: Hymenoptera) vs. Four Different Hosts under the No-Choice Situation

Mervat A. kandil; Ayman, M. Adly, Ashraf F. Ahmed and Mostafa A. M. Shalaby
Plant Protection Research Institute, Dokki, Giza,Egypt
E-mail: mostafashalaby9@yahoo.com

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ABSTRACT

Bracon brevicornis Wesmael (Braconidae: Hymenoptera) is a generalist idiobiont ecto-parasitoid that parasitizes a wide range of Lepidoptera. In the present context, our study aimed to examine under laboratory conditions the effect of host size on the biological parameters of parasitoids *B. brevicornis* for efficient laboratory rearing of that parasitoid. Under the no-choice situation, we compared biological parameters of this parasitoid wasp on four host species belonging to the Lepidoptera, viz. *Pectinophora gossypiella* (Gelechiidae) and Earias insulana (Noctuidae) as small size hosts, *Helicoverpa armigera* (Noctuidae) and *Spodoptera littoralis* (Noctuidae) as large size hosts. The biological parameters of the parasitoid on *P. gossypiella or E. insulana* (Small size) in most case proved to have a significant effect on their egg-adult developmental stages compare to its interaction vs. H. armegra or *S. littoralis* host (large size). The hosting on *P. gossypiella* or *E. insulana* the parasitoid produced excellent paralyzing and parasitism capacities but this capacity was less efficient on large size hosts and they received a significantly lower number of eggs as compared to *H. armegra or S. littoralis* which they received a good number of eggs. No variation was observed considering the egg-to-adult development time of *B. brevincornis* parasitizing *P.gossypiella* (small size) and *S. littoralis* (large size), but the lowest egg-to-adult development time was observed on H. armegra (large size). The biochemical analysis showed that the larger size hosts contained more resources for the growing the parasitoid, this was reflected only on fecundity. The other biological parameters: hatchability, longevity, lifetime and the percentage of hosts producing parasitoid offspring and sex ratio were not dependent on host size regardless of parasitoid generation. The results indicate that the parasite's behaviour of *B.brevicornis* can develop under no-choice situation equally successfully on both small and large size host.

INTRODUCTION

In Egypt and in recent years, biological control began to take its place in the cotton fields, especially for control of the bollworms, Pink bollworm (*Pectinophora gossypiella* (Saund.) Lepidoptera: Gelechiidae) and Spiny bollworm (*Earias insulana*

(Boisd) Lepidoptera: Noctuidae). The Ministry Egyptian of Agriculture has established several mass rearing laboratories for production *Trichogramma evanescence* in most provinces of Lower and Upper Egypt. Among the various groups of biocontrol agents, braconid parasitoids are well known for the management of different lepidopteran larvae (Yu *et al.,* 2002; Gül and Gülel, 1995; Heimpel *et al.,* 1997; Darwish *et al.,* 2003; Altuntas et al., 2010 and Thanavendan and Jeyarani, 2010). *Bracon brevicornis* Wesmael (Hymenoptera, Braconidae) *has* attracted interest as a potential biological control agent, is an idiobiontic and develops as a gregarious ectoparasite on its host. There are many biological studies on *B. brevicornis* and the relative impact of parasitism on pink bollworm and different lepidopteran larvae were reviewed by (Abbas, 1980; Temerak, 1981a and b; Temerak, 1983; Jackson and Butler, 1984; and Thanavendan and Jeyarani, 2010). With the wide application of biological control technologies, natural enemy insects mass-reared on substitute hosts are an important part of biological control success (Yang *et al.,* 2014). Some biological control studies have recognized the important role of the behavioural and physiological interactions between parasitoids and different hosts to ensure the success of biological control programs (Uckan and Gulel, 2002 and Quicke 2015). Host-size-dependent host-selection is common among parasitoids because the size of hosts is a determinant of host quality (Charnov & Skinner, 1984; Godfray, 1994; Goubault *et al.,* 2004; Rehman & Powell, 2010; Liu *et al.,* 2011). In contrast, recent work has shown that host size at the time of oviposition may have little influence on the fitness functions in some of the koinobiont species (Harvey 2000, Harvey *et al.,* 2004). Also, understanding such host-size effects is important for biological control using parasitoids (Jervis & Kidd, 1996; Heimpel & Lundgren, 2000; Ode & Heinz, 2002; Hajek, 2004). The aim of our study was to examine the effect of host size on the biological parameters of parasitoids *B. brevicornis* on four different host species belonging to the Lepidoptera, viz. *Pectinophora gossypiella (*Gelechiidae) and *Earias insulana* (Noctuidae) as small size hosts, *Helicoverpa armigera* (Noctuidae) and *Spodoptera* ***littoralis*** (Noctuidae) as large size hosts.

MATERIALS AND METHODS

1. Parasitoid Origin and Rearing:

B. brevicornis used in this study originated from field-collected dried boll samples from the cotton crop in Qaliubiya province, Lower Egypt. To form stock populations the larval parasitoid *B. brevicornis* was cultured on the pink bollworm full-grown larvae and grew up several generations in a glass jar (5 cm diameter x 12 cm height) under laboratory condition (25 ± 2ºC). As food for the adult parasitoids, three drops of honey was provided on the inside wall of the jar.

2. Hosts:

The host species studied in this experiment was pink bollworm *P. gossypiella*, spiny bollworm, *E. insulana*, American bollworm, *H. armegira*, and cotton leafworm, *S littoralis*. They obtained from rearing laboratories of plant protection research institute (PPRI), Agricultural research centre, Egypt.

3. Evaluation of the Biological Parameters of the Parasitoid, *B.Brevicornis* Vs. Four Different Hosts under the No-Choice Situation:

Both *P. gossypiella* and *E. insulana* larvae are distinguished by their small size compared to *H. armegira* and *S littoralis*. The interaction of the parasitoid, *B. brevicornis* with these hosts was evaluated under no-choice situation as follows: Three replicates each of ten pairs (10♀&10♂) of freshly emerged parasitoids were confined with five full-grown pink bollworms, P. *gossypiella* larvae as a host for parasitization in a glass jar closed by gauze cloth. The experiments were conducted under laboratory conditions (25 ± 2ºC). Several droplets of honey bee were streaked on the surface of each glass jars lid as a source of food. The interaction between the parasitoid and the host was assessed on the following approach: Every day, the host exposed to the parasite is replaced with another and the same procedure was followed until parasitoid death. Each replacement larva inspected immediately by stereoscopic and photographs were taken. The following biological parameters were recorded in terms: the number of alive larvae, number of paralyzed larvae, number of larvae that were parasitized and number of the parasitoid eggs they had. As well as immature stages of the parasite were followed daily in terms of development period to pupation, mature stage and sex ratio, also, hatching ratio was calculated. As with the previous manner, the interaction between the parasitoid, B. *brevicornis* Vs. spiny bollworm larvae, *E. insulana* was evaluated. In the same approach, and through three replications for both the *H. armegira* at 6th instar and *S littoralis* at 5th instar, the biological parameters were evaluated for the parasite's interaction on each of them.

4. Biochemical Analyses:

Analyses technique of total soluble protein, lipids, carbohydrate in total homogenate for different kinds of larvae were done at insect Physiology Dept. Plant Protection Researches Institute (PPRI). Samples from Full-grown larvae /*P. gossypiella* and *E. insulana*, 5th instar larvae / *H. armigera* and 6th instar larvae / *S.littoralis* were collected for biochemical analyses. The total soluble protein, lipids, and carbohydrate analyses were determined according to technique methods as described by Knight *et al*. (1972) and Bradford (1976).

5. Statistical Analysis:

The recorded data of the biological characteristics of the interaction of the parasitoid, *B.brevicornis* vs. four different hosts under the no-choice situation were analyzed using analysis of variance (ANOVA) using costat software program, 1990.

RESULTS AND DISCUSSION

1. Effect of Different Host Species on the Biological Parameters of the Parasitoid, *B.Brevicornis* under the No-Choice Situation:

The percentage of both the parasitized and paralyzed host was 91.0, 83.0, 69.0 and 76.0 when the parasitoid, *B.brevicornis* interacted with the larvae of P. *gossypiella, E. insulana, H. armigera and S. littoralis* under no-choice situation (Table 1).

The results in Table 1, as well as the illustrations in Figure 1, shows that the numbers of eggs deposited by the female parasite *B. brevicornis* were varied according to host species, and the number of eggs increased with the presence of the large-sized host. In this context, the daily examination of the replicates showed that the number of eggs during the test period ranged from 8 to 36 / *S. littoralis* and from 12 -26 eggs / *H. armigera* respectively. At the same time, the number of eggs placed on hosts of pink and spiny larvae recorded numbers ranging from 6 – 12 and 8 - 14, respectively. The recorded results showed that the highest percentage of the cocoon formation occurred in the case of interaction whether between the *P. gossypiella* or *E.insulana* larvae and recorded 96 and 93% respectively, whilst it decreased to 76.0 and 63.0 % on the larvae of *S. littoralis* and *H. armigera*, respectively. The results showed that the sex ratio of the offspring of the parasite originating from that different hosts has varied and the percentage of female individuals exceeded the rate of male individuals and recorded 0.65♀: 0.35 ♂/ *P.gossypiella*, 0.73♀: 0.27♂ / *E.*

insulana, 0.69: 0.31♂ / *S. littoralis,* while the sexual ratio was nearly balanced on the, *H. armigera* and recorded 0.56♀: 0.44 ♂.

Table 1: The Biological parameters of the parasitoid, *B.brevicornis* that had hosted on four different species under the no-choice situation.

The parasitoid vs. host	Range of daily numbers of deposited eggs/interaction period	% parasitized & paralyzed / host	% Hatchability	% Pupation	% adult emergence	Sex Ratio	
						♀	♂
Small size host *B.brevicornis vs. P. gossypilla*	6 - 12	91.0	97.0	96.0	98.0	0.65	0.35
B.brevicornis vs. E. insulana	8 - 14	83.0	95.0	93.0	95.0	0.73	0.27
Large size host *B.brevicornis vs. H. armegra*	12 - 26	69.0	89.0	76.5	75.0	0.56	0.44
B.brevicornis vs. S. littoralis	8 - 36	76.0	83.0	63.5	60.0	0.69	0.31

small size host large size host

Fig. 1 Expressive photograph of low fecundity level of the parasitoid *B.brevicornis* on small size host (*P.gossypiella*) compared to good fecundity on large size host (*S. litturalis*)

The recorded results showed that the highest percentage of the cocoon formation occurred in the case of interaction whether between the *P. gossypiella* or *E.insulana* larvae and recorded 96 and 93% respectively, whilst it decreased to 76.0 and 63.0 % on the larvae of *S. littoralis* and *H. armigera*, respectively. The results showed that the sex ratio of the offspring of the parasite originating from that different hosts has varied and the percentage of female individuals exceeded the rate of male individuals and recorded 0.65♀: 0.35 ♂/ P.gossypiella, 0.73♀: 0.27♂ / E. insulana, 0.69: 0.31♂ / *S. littoralis,* while the sexual ratio was nearly balanced on the host, *H. armigera* and recorded 0.56♀: 0.44 ♂. Larval and cocoon duration of the parasitoid *B. brevicornis* reared on four hosts species in a no-choice way were recorded in Table (2). The duration of the larval stage of the parasitoid *B. brevicornis* hosted on *E. insulana, H.armigera and* **S.** *littorals* did not produce significant differences and ranged from 6.3 – 6.5 days, whilst that period was significant longest on the *P.gossypiella* compared to each of the previous hosts and recorded 7.6 days. On the other hand, cocoon duration on *P. gossypiella*, *E. insulana* and *H.armigera* ranged from 5.3 – 5.6 days without significant difference, while this period prolonged significantly with the host, S**.** *littoralis* compared to each one of those three hosts and recorded 6.3 days. The results recorded in Table 3 indicate that the duration pre-oviposition of the parasitoid, *B.brevicornis* hosted on that four different species larvae were not significantly affected by this difference and the period had ranged from 1.6 – 2.4 day. The results (Table 3) showed that the oviposition period was affected by the diversity of the hosts. The shortest period with significant difference occurred with those that were hosted on *S. littoralis* and recorded 9.4 days. In contrast, the parasitization on the host, *P. gossypiella* recorded the longest oviposition period (13.9 days) with a significant difference from the other hosts. The oviposition period for both hosts, *E. insulana* and *H.armigera* recorded 12.7 and 11.3 days, respectively with no significant difference when compared to each other. On the other hand, the females, *B.brevicornis* that were hosted on the *P. gossypiella* and *E. insulana* did not produce significant differences between each of them in terms of the total number of the deposited eggs (fecundity) and recorded 142.0 ± 0.2 and 139.0±0.1, respectively. Female the parasitoid hosted on *H.armigera* and *S.littoralis* showed pronounced fertility (169. ± 0.3 and 155.0 ± 1.1, respectively) with significant differences, and both had recorded superiority with significant difference compared to those recorded on *P gossypiella* and *E. insulana*. In terms of female longevity, the females which hosted on the ***P**. gossypiella*, recorded the prolonged period of life span (17.5±01.4 days) with a significant difference compared to others. Females hosted on *E. insulana* and *H.armigera* were the next in the order of longevity (15.5±0.9 and 15.3.0±1.5 days) with no significant differences between them occurred. By contrast, the female who accompanied by the *S. littoralis* host was distinguished by a short life span (13.9 ± 1.1 days) with a significant difference compared to the others.

Table 2: The times (days) of the immature stages of the parasitoid, *B.brevicornis* on the four hosts under the no-choice situation.

The parasitoid vs. host	Eggs Incubation period(days)*	Larva Period (days)	Cocoon Period (days)	The entire life cycle
Small size host ***B.brevicornis vs. P. gossypilla***	2.00±0.1a	7.6±0.2^{b}	5.4±0.4b	15.0±1.2c
B.brevicornis vs. E. insulana	2.30±0.3b	6.5±0.1^{a}	5.6±0.3b	14.3±1.3b
Large size host ***B.brevicornis vs. H. armegra***	2.40±0.1b	6.3±0.3^{a}	5.3±0.2b	13.0±0.5^{a}
B.brevicornis vs. S. littoralis	± 02 c٢,٦	6.4±1.1^{a}	6.30±0.4a	15.3±1.1c
LSD 0.05	0.213	1.055	0.642	1.226

The mean inside each column followed by the same letter do not differ significantly from each other by the L.S.D at the 0.05 level

Table 3: Duration of pre-oviposition, oviposition, and post-oviposition (days**),** longevity and fecundity of the parasitoid, *B.brevicornis* hosted on four different species under the no-choice situation.

The parasitoid vs. host	Pre-oviposition	oviposition	Post - oviposition	Longevity	Fecundity
Small size host ***B.brevicornis vs. P. gossypilla***	1.6±0.2a	13.9±0.8c	2.0±0.2b	17.5±1.4 c	142.0±0.2 a
B.brevicornis vs. E. insulana	1.9±0.1a	**12.7**±1.1b	0.9±0.2a	15.5±0.9 b	139.0±0.1a
Large size host ***B.brevicornis vs. H. armegra***	2.2±0.3a	**11.3**±1.3b	1.8±0.2b	15.3±1.5 b	169.±0.3c
B.brevicornis vs. S. littoralis	2.4±0.4a	**9.4**±0.6a	2.1±0.2b	13.9±1.1 a	155.0±1.1b
LSD 0.05	0,336	**1.314**	0.420	0.551	6,511

*The mean inside each column followed by the same letter do not differ significantly from each other by the L.S.D at the 0.05 level

2. Total soluble Protein, Lipid, and Carbohydrate in the Haemolymph of the Four Tested Hosts:

Data presented in Table (4) revealed that the four tested hosts clearly differ in total soluble protein, lipid, and carbohydrate. The total soluble lipid was 33.0, 31.4 and 27.93 in *H. armigera, S. littoralis* and *P. gossypiella,* while, it decreased to 19.77 mg/ml in *E. insulana*. The total lipid increased approximately by 6 to 5 times (46.0 to 57.3mg/m.) in *H. armigera* and *S. littoralis* compared to 8.59 and 35.60 mg/m in *P. gossypiella* and *E. insulana*. The highest value of the total soluble carbohydrate recorded / *P. gossypiella*, 51.66 mg/m. whilst, the lowest value was recorded / *E. insulana*, 28.27 mg/m.

Numerous examples of biological control studies indicated that to produce effective control methods, detailed knowledge of the biology, ecology, and biochemistry of parasites and predators is important and necessary to achieve success and produce effective control methods. In this context, the group of braconidae parasites is defined as a biological control agent for the management of different lepidopteran larvae (Abbas, 1980; Temirak, 1981a, and b; Temirak, 1983; Jackson and Butler, 1984; Tanafandan and Jayarani, 2010 Shalaby *et al.*, 2015). The present study comes in the context of more studies on the behaviour of *B. brevicornis* on *p. gossypiella, E. insulana, H. armegra* and *S.* ***littoralis*** which many studies have indicated response and suitability of these pests and others to the development of parasitoid (Yu *et al.*, 2002; Darwish *et al.*, 2003 and Thanavendan and Jeyarani, 2010). The data obtained in the present investigations were generated under laboratory no-choice conditions in which the host was available to the adults and that the parasite and its host were artificially brought together.

Table 4: The total soluble protein, lipid and carbohydrate in the haemolymph of *P. gossypiella, E. insulana, H. armigera* and *S. littoralis.*

Hosts	Total Protein and (rang) mg/m		Total lipid and (rang) mg/m		Total Carbohydrate and (rang)mg/m	
	mean	Range	mean	Range	mean	Range
Small size host *P. gossypiella*	27.93± 0.7b	21 -54	8.59 ±0.19a	6 -12	51.66± 2.6d	32 -46
E. insulana	19.77±0.29a	17 -26	35.60±0.95b	28 -41	28.27±1.5a	23 - 37
Large size host *H. armigera*	33.2.1c	21-37	46.0±2.4c	31-52	49.0±1.7c	38-53
S. littoralis	31.4±1.3c	23 -39	57.3±20.95d	45 -63	34.6±1.9b	29- 41
LSD	1.487	-	7.211	-	5.316	-

*The mean inside each column followed by the same letter do not differ significantly from each other by the L.S.D at the 0.05 level

The results indicated that the host's species had an effect on the behavioural and ovipositional responses of *B.brevicornis.* Temerak (1983) reported that *B. brevicornis* preferred to parasitize and complete its life cycle on *Earias spp., H. armigera, P. gossypiella* than other host insects offered. The results showed that the duration and viability of the parasitoid biological cycle (egg-adult) were affected by the host species with seemingly inconsistent effects. Although many studies (Xie *et al.* 1989, Yu *et al.* 2003 and Khalil *et al.* 2016) reported that the host size plays an important role on the oviposition behaviour of braconid ectoparasitoid and the large-sized larvae like noctuids receives a significantly higher number of eggs when compared to the smallest host larvae, the biological parameters of the parasitoid on

P. gossypiella or E. insulana (Small size) in the most case proved to have a significant effect on their egg-adult developmental stages compare to its interaction vs. H. armegra or *S. littoralis* host (large size). The results indicated that *P. gossypiella* and *E. insulana* were similar hosts for *B.brevicornis*, this is similar to that reported by Kandil, 2001; Kandil *et al.* 2013 and Alam *et al.* (2016). The hosting on *P. gossypiella* or *E. insulana* the parasitoid produced excellent paralyzing and parasitism capacities but this capacity was less efficient on large size hosts but they received a significantly lower number of eggs as compared to *H. armegra or S. littoralis* which they received a good number of eggs. Xie *et al.* (1989) and *Khalil et al.* (2016) revealed that *B. hebetor* prefers to lay more eggs on large larvae as compared to smaller ones. Khalil *et al.*, (2016) mentioned more hosts can be paralyzed than the number that is needed for oviposition; even when no egg is deposited, Doten (1911) and Richards and Thomson (1932) reported females of the parasitoid *H. hebetor* to paralyze many more hosts than needed for oviposition and only a small proportion of the paralyzed larvae are actually used for oviposition. No variation was observed considering the egg-to-adult development time of *B. brevincornis* parasitizing *P.gossypiella* (small size) and *S. littoralis* (large size), but the lowest egg-to-adult development time was observed on H. armegra (large size). Considering adult production related to hosting species, a difference was observed in the parasitoid sex ratio. The highest percentage of female progeny came from *E. insulana* (small size) followed by *S. litorallis* (large size) and *P.gossypiella* (small size) followed by *H. armigera* (large size). Our results are opposite to what Godfray’s (1994) found that under the choice conditions that small hosts received more male eggs and large hosts more female eggs. Thus our results showed that the parasite's behaviour of *B.brevicornis* did not match when it hosted on different hosts under the no-choice condition in terms of using its ability to cause the paralysis, parasitism, longevity, sex ratio of the progeny and life cycle. Ghimire and Phillips (2010) reported *H. hebetor* females can paralyze and lay eggs on several host species, but it cannot necessarily develop and reproduce optimally on all host species that it can paralyze and parasitize. On the other hand, our results for the biochemical analysis of the natural total content of proteins, fats, and carbohydrates in the tissues of the hosts' body tissue before parasitism occurred showed that it is proportional to the host's size as its content was high in large compared to small size. In the current study, the biological parameters of the parasitoid interaction on these hosts were inconsistent and contradicted with this trend. Although the larger size hosts contained more resources for the growing the parasitoid, this was clearly reflected only on fecundity. The other biological parameters: hatchability, longevity, lifetime and the percentage of hosts producing parasitoid offspring and sex ratio were not dependent on host size regardless of parasitoid generation. Thus, our results indicate that the parasite's behaviour of *B.brevicornis* can develop under no-choice situation equally successfully in both small and large. The results indicate that *B.brevicornis* can parasitize a wide range of hosts of different sizes.

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ARABIC SUMMARY

تقييم المعالم البيولوجية لمفاعلة طفيل براكون بريفينكورنيس (عائلة براكونيدى-رتبة هيمنوبترا) على اربع عوائل مختلفة بدون إتاحة الإختيار

ميرفت عبد السميع قنديل، أيمن محمد محى الدين عدلى، أشرف فايز أحمد، مصطفى عبد الحكيم محمد شلبى
معهد بحوث وقاية النباتات

الطفيل براكون بريفينكورنيس (عائلة براكونيدى- رتبة هيمنوبترا) طفيل خارجى يتطفل له عوائل عديدة من يرقات حرشفية الاجنحة وفى هذا السياق تستهدف هذة الدراسة إعالة هذا الطفيل معملياً على اربعة عوائل من يرقات حرشفيات الاجنحة تتميز طبيعياً بتباين احجامها وهى يرقات اللوز القرنفلية بكتينوفورا جوسيبيلا ويرقات اللوز الشوكية ايرياس انسولانا وكلاهما اصغر حجماً من كل من يرقات اللوز الآمريكيه هليكوفيربا ارميجرا ويرقات دودة ورق القطن سبدوبترا ليتوراليس وكلاهما طبيعياً ذوى احجام كبيرة وذلك فى تجريب لايتيح للطفيل اختيار عائلة بهدف اختبار مدى تأثير العوائل متباينة الاحجام على المعايير البيولوجية للطفيل . وقد اظهرت النتائج تأثيرات معنوية متباينة بين العوائل المختبرة حيث تميزت قدرات الطفيل على احداث الشلل والتطفل فى تفاعلة مع اليرقات صغيرة الحجم مقارنة بالاكبر حجماً فى حين ان العوائل الاكبر حجماً استقبلت من بيض الطفيل اعداد تفوق ما وضع على العوائل الاصغر حجماً وعلى جانب اخر لم تتباين مدة تطور الطفيل من البيض الى الطور اليافع على مختلف احجام العوائل المختبرة لكن اقل مدة تطور سجلت على العائل كبير الحجم. وقد اظهرت نتائج الحليل البيوكميائى لمكونات البروتين والدهون والكربوهيدرات للعوائل الطبيعية المختبرة تفوق محتوى هذة المكونات بالعوائل الكبيرة الحجم مقارنة بالاصغر إلا ان المعايير البيولوجية للطفيل من حيث نسب الفقس وطول عمر الإناث ومدة التطور والنسب الجنسية للذرية الناتجة لم تتباين بين العوائل الاصغر والاكبر حجماً تبعاً لهذا المحتوى . استدل من نتائج التجريب ان الطفيل براكون بريفينكورنيس تحت الظروف المعملية استطاع التطور بنجاح على العوائل المختبرة