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**Effect of Temperature and Host Density on Some Characteristics of The
Ectoparasitoid *Bracon brevicornis* (Walker) (Hymenoptera: Braconidae)**

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ABSTRACT

The present work was carried out to study the effect of exposure to different temperatures of 15, 20,25,30 and 35°C and different host densities of 2, 4, 6, 8 and 10 larvae of the host on some biological aspect of *Bracon brevicornis* (Walker) (Hymenoptera: Braconidae) adult females when reared on *Ephestia kuehniella* full-grown larvae. Results indicated that exposure to different temperatures had a significant effect on pre-oviposition, oviposition, post-oviposition and longevity periods of an adult female, As the shortening of these periods was observed with a high temperature and vice versa. Also, a significant effect of temperatures on parasitism % and eggs laid / female were found. The obtained results showed an insignificant effect of host densities on biological aspects of an adult female of *B. brevicornis*, except in the case of Parasitism % and eggs laid /female.

INTRODUCTION

Pest control is one of the important measures that help increase the productivity of various crops. Chemical control is an effective method, but it is expensive and has resulted in severe damage to the environment and to humans. And biological control is considered one of the important methods of pest control and at the same time limiting the use of pesticides and chemicals, thus reducing environmental damage. Therefore, the general trend in the world today has been to maximize the role of the enemies of nature. (Reda *et al.*, 2014).

Predators and insect parasitoids are the most important pillars of the biological control of insect pests. Insect parasitoids include many ranks, such as the order Hymenoptera, which includes the family of Braconidae, which is considered one of the most important insect parasitoids as they parasitize many pests. (Greathead, 1986, Wharton *et al.*, 1997, and Sharma *et al.*, 2000)

Bracon brevicornis (Wesmael) (Hymenoptera: Braconidae) is a gregarious, larval ectoparasitoid that attacks many Lepidoptera, Family: Pyralidae including some serious stored products pests such as Mediterranean flour moth *Ephestia kuehniella* (Zeller) and *Plodia interpunctella* (Hübner) (Malesios, G.A. ;D.A. Prophetou-Athanasidou 2014 and Taweel, A.A. Al-, *et al.*, 2014), it is an important potential biological control agent of stored product moths (Reda F. A. Bakr *et al.*, 2014 and Landge *et al.*, 2009). *B. brevicornis* female first paralyzes its host by stinging and then lays a variable number of

eggs on the ventral surface of the larva (Dabhi (2011). Gregarious parasitoid species laid several eggs in, or on a single host. (Reda F. A. Bakr *et al.*, 2014; Kandil, Mervat, 2001; and Brower *et al.*, 1996) and many pests of stored materials (LaSalle and Gauld 1993). Temperature is considered one of the important environmental factors that affect the life of the parasitoid and greatly affects various biological factors as well as affects feeding behavior and thus affects the efficiency of parasitism in various hosts, as well as the density of the host, affects the rates of parasitism and the rate of laying eggs. In this context, we studied the effect of temperature and host density on parasitism efficiency and reproductive potential of *B. brevicornis* to benefit in laboratory mass rearing.

MATERIALS AND METHODS

Stock Culture of *Ephestia kuehniella* (Mediterranean flour moth):

The stock culture of *Ephestia kuehniella* has been raised for several generations from infested dead flour in the biological laboratory of the Faculty of Agriculture, Menoufia University. One – day old moths were collected and transferred to glass jars (1/2 kg) containing dried flour for oviposition.

Stock Culture of *Bracon brevicornis*.

Five couples (female and male) of *B. brevicornis* were placed in cylindrical glass tube (2.0 x 10 cm) maintained on the full-grown larvae of, *Galleria mellonella* and covered with muslin cloth then fitted with rubber lids. After 24h, parasitized larvae were removed from the tube, transferred to Petri dishes and kept incubated at 25±2°C and 60–65% RH until adult emergence. Freshly emerged adults (within 24 hours) were used in the current experiments.

Effect of Temperature:

To study the effect of temperature on parasitism % (parasitism efficiency), the longevity of adult females (pre- oviposition - oviposition and post- oviposition periods) and the daily eggs laid by females, one fresh pair (male and female) of parasitoid were kept for mating purposes, in tubes and fed with drops of 10% honey solution. After 48 hours the pairs were released into glass vials (1x10 cm) for experiments. These vials containing two full-grown larvae of *E. Kunella*. The vials were covered with a muslin cloth and tightened with a rubber band. Then these vials were incubated at 15, 20, 25, and 30 °C. After 24 hours, the pairs of parasitoids were transferred to new tubes containing new full-grown larvae of the host, and the paralyzed larvae were incubated at the same previous temperatures. The number of daily and eggs and adult female longevities were recorded. The experiments contained 3 replicates.

Effect of Host Density:

One- old day pairs of parasitoids were kept for mating purposes in tubes and fed with 10% honey solution. After 48 hours the pairs were released into glass vials (1x10 cm). These vials containing full-grown larvae of *E. Kunella* at densities of 2,4,6,8 and 10 larvae/vial. The vials were covered with a muslin cloth and tightened with a rubber band then incubated at 25±2°C and 70–75% RH. The parasitism %, the longevity of adult females includes (pre-oviposition - oviposition and post-oviposition periods) and eggs laid by females were recorded. The experiments contained 3 replicates

Statistical Analysis:

The results were analyzed by one–way analysis of variance (ANOVA) using COSTAT statistical software (Cohort Software, Berkeley). When the ANOVA statistics were significant ($P < 0.01$), the means were compared by Duncan's multiple range test (1957). also, the regression line equation was done using Microsoft office excel worksheet (version7).

RESULTS AND DISCUSSION

Effect of Temperature on Parasitism %, Longevity and Fecundity of Adult Parasitoid *Bracno brevecornis*:

The effect of the exposure to different temperatures on mating females was studied as follows in Table (1).

Pre- Oviposition Period:

The obtained results showed that the pre-oviposition was short at all tested temperatures, Statistical analysis showed insignificant differences in case of 15, 20 °C and 25 where it was 4.1, 1.81 and 1.62 days, also the same relationship between 30 and 35 °C where it was 1.23 and 1.14 days. But there were significant differences between low temperatures (15, 20 and 25°C) and high temperatures (30 and 35°C).

Oviposition Period:

The results in table (1) showed that oviposition periods of paraboloids females were long at low temperatures and vice versa in high temperatures. Statistical analysis showed significant differences in case of all tested temperatures except in case of 30 and 35°C. The means of the oviposition period were 19.35, 15.30, 11.34, 5.31 and 3.12 days at 15, 20, 25, 30 and 35°C, respectively.

Post Oviposition Period:

Also results in Table (1) showed the post oviposition periods of *B. brevecornis* females where these periods were 3.5, 1.1, 1.3, 1.99 and 1.06 days when exposed to 15, 20, 25, 30 and 35 °C, respectively.

Thus, the tested temperatures affected the longevity of the females, these longevities were 26.95, 18.22, 14.33, 8.53 and 5.32 days at 15, 20, 25, 30 and 35 °C. The equation of the regression line was ($y=1.031x+40.299$) and a strong R-squared value ($R^2=0.9775$) indicated that there was a strong relationship between the independent variable (temperature) and dependent variable (Longevity of adult female) - regardless of the variable significance, this is letting to know that the identified independent variable, even though significant, is not accounting for much of the mean of the dependent variable. This relationship was presented in figure 1.

Parasitism %:

Percentages of parasitism were significant at all tested temperatures, percentages of parasitism were 33.3, 77.8, 92.85, 88.89 and 66.67 when females were exposed to 15, 20, 25, 30 and 35 °C, respectively. The equation of the regression line was ($y=1.55x+33.6$) and a low R-squared value ($R^2=0.2656$) was presented in figure 2, this value indicated that there was a weak relationship between the independent variable (Temperature) and dependent variable (Parasitism %).

Fecundity of Females:

The obtained numbers of eggs laid per female of *B. brevecornis* were significant in case of all tested temperatures, but insignificant differences were found in case of 15, 20 and 30°C. The average numbers were 32.51, 51.31, 95.52, 63.42 and 44.11 eggs per female at 15, 20, 25, 30 and 35 °C. The equation of the regression line was ($y=0.7062x+39.719$) and R2-squared value =0.0537. This means that there is no relationship between the independent factor (temperature) and the dependent factor (Fecundity), although there was a significant relationship between variables (Fig. 3).

Temperature is a critical abiotic factor that affected the population dynamics of insect pests and their natural enemies. Knowledge on the adaptation of the natural enemies to climatic conditions plays an essential role in pest management, the egg-to-adult developmental periods for *B. hebetor* varied significantly with the temperature (Thanavendan and Jeyarani, 2010, El-Basha 2015, Obrycki and Kring, 1998).

(Thanavendan and Jeyarani, 2010) reported that the preimaginal development of *B.hebetor* was temperature-dependent, with development being significantly faster at 35 °C than at the lower tested temperatures. Similar findings were reported in the present research for *B. brevecornis* on different host larval ages and Engroff and Watson,1975 stated that *B. kirkpatricki* on *Spodoptera exiguae* life cycle is very short at 35 °C, while at 20 °C it was prolonged. This conclusion goes with that observed in the present study, where the life cycle of *B. hebetor* was greatly influenced by the change in temperature regimes.

Table 1: Parasitism %, adult longevity and average eggs laid / female of *B. brevecornis* on different tested temperatures.

Temperature	Parasitism %	Average No. of eggs laid/ female	Ovipositional Periods/ days			Adult female longevity
			Pre	Ovi	Post	
15	33.33±1.31d	32.51±2.54d	4.1±0.34a	19.35±0.93a	3.5±0.14a	26.95±0.52a
20	77.8 ±3.45b	51.31±3.78b	1.81±0.21b	15.3±0.68b	1.10±0.32 b	18.22±0.64b
25	92.85± 3.47a	95.52±3.89a	1.62±0.34b	11.34±0.87c	1.34±0.24b	14.3±0.82c
30	88.89 ±4.41a	63.42±4.35b	1.24±0.15b	5.31±0.31d	1.99±0.31b	8.53±0.45d
35	66.67±2.82 c	44.11±1.69b	1.14±0.12b	3.12±0.17c	1.06±0.44b	5.32±0.42 e
LSD	14.51	13.52	1.31	4.51	3.66	22.35

Means ± SE in a column followed with the same letter(s) are not significantly different at 5% level of probability

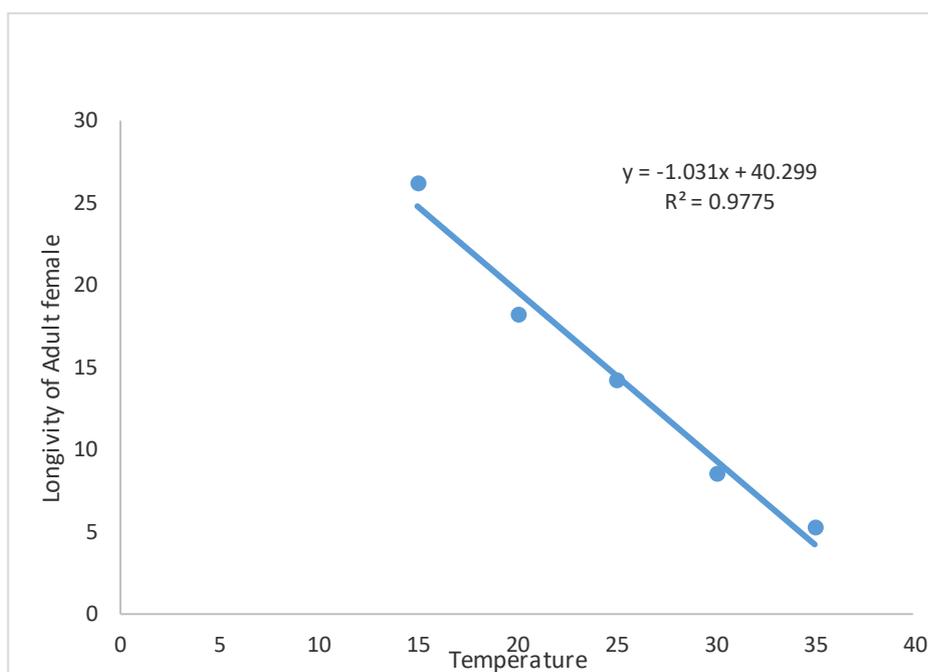


Fig.1: Regression line of relationship between longevity of adult female of *B. brevecornis* at different temperatures.

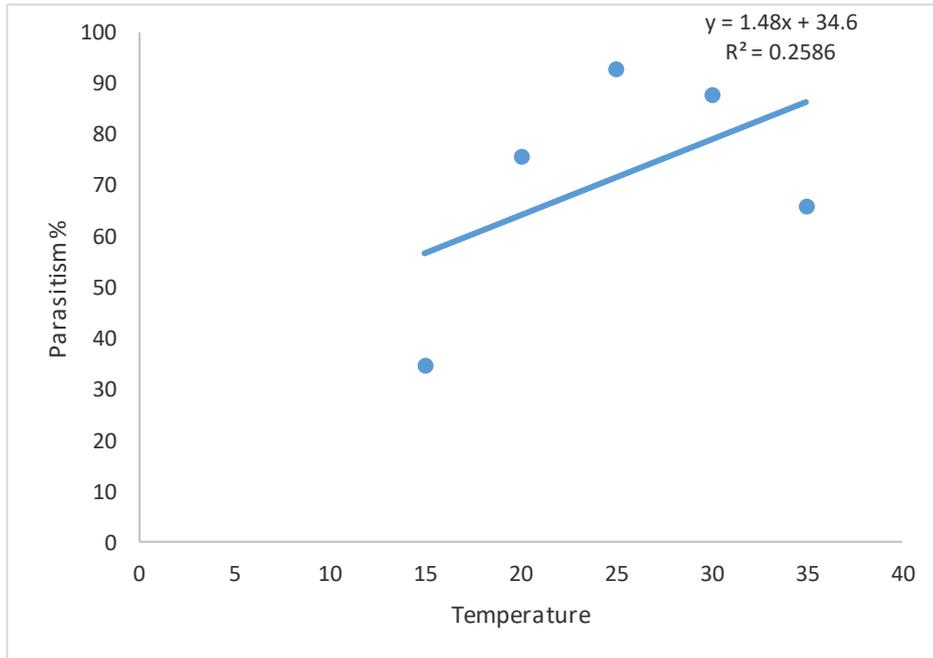


Fig.2: Regression line of relationship between Parasitism % of *B. brevecornis* at different temperatures.

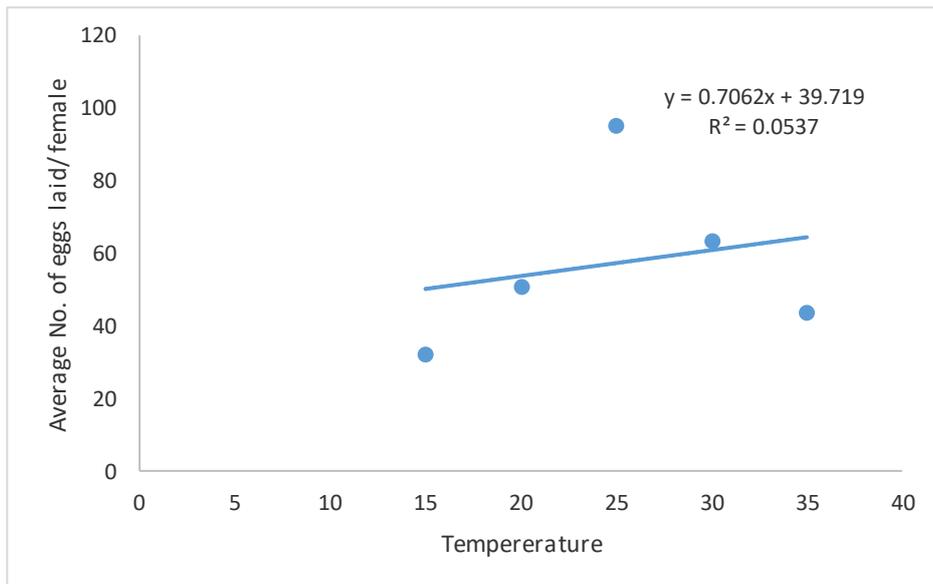


Fig.3: Regression line of relationship between average No. of eggs laid/ female of *B. brevecornis* at different temperatures.

Effect of Host Density on Parasitism %, Longevity and Fecundity of Adult Parasitoid *Bracno brevecornis*:

The ovipositional periods, longevity and fecundity of the females' parasitoid *B. brevecornis* were investigated under the different tested host densities and the obtained data were summarized in table (2).

Pre- Oviposition Period:

The obtained results in Table (2) showed insignificant differences in case of all tested host densities. Pre-oviposition period means were 1.1, 1.3, 1.3, 1.2 and 1.4 at 2, 4, 6, 8 and 10 host densities respectively.

Oviposition Period:

From the recorded results, significant differences were obtained between the treatment of 8 host densities and all other treatments. Where the means of oviposition period were 12.34, 12.71, 12.51, 13.10 and 12.33 days at treatments of host densities 2, 4, 6, 8 and 10 larvae of host.

Post Oviposition Period:

As noticed from Table (2) Statistical analysis showed that means of the post-oviposition period for female parasitoids was insignificant ($P < 0.05$) when exposed to different tested host densities. These means were 1.68, 1.21, 1.51, 1.01 and 1.69 days at 2, 4, 6, 8 and 10 larvae of host.

Also, means of adult longevity showed significant statistical relationships between some treatments of host densities. In general, the treatments can be divided into two groups, the first group was (916.31 and 15.96 days) at 8 and 6 host densities, respectively. The second group was (15.12, 15.23 and 15.42 days) at 2, 4 and 10 host densities, respectively. The equation of the regression line was ($y = 0.084x + 15.105$) and a medium R-squared value ($R^2 = 0.4743$) indicated that there was no strong relationship between the independent variable (host density) and dependent variable (Longevity of adult female), this relationship was presented in figure 4.

Parasitism %:

Statistical analysis of observed results for parasitism percentages showed significant differences between all treatments but showed an insignificant relationship in case of 6 and 8 host densities. In general, it was noted that the parasitism rate increased with an increase in host densities. The mean parasitism percentages were 78.53, 82.33, 91.61, 93.51 and 86.31 % at 2, 4, 6, 8 and 10 larvae of host densities, respectively. The equation of the regression line was ($y = 0.135x + 77.7$) and a medium R-squared value ($R^2 = 0.4714$) indicated that there was no strong relationship between the independent variable host density and dependent variable Parasitism % (Fig. 5).

Fecundity of Females:

The average numbers of eggs laid / female were statistically varied between all treatments, also it was noticed that the number of eggs was increased by increasing host densities. The means were 85.41, 96.35, 110.52, 118.81 and 105.65 eggs at treatments of 2, 4, 6, 8 and 10 host densities, respectively. The equation of the regression line was ($y = 2.797x + 85.166$) and a high R-squared value ($R^2 = 0.6396$) indicated that there was a strong relationship between the independent variable host density and dependent variable Fecundity of females (Fig. 6).

In general, the host density affects some biological aspects, especially the parasitism% and fecundity of adult female, the host density affects the increase in the percentage of parasitism as well as the number of eggs per female. These parameters increased by increasing the number of the exposed host. The same findings were obtained by Vorgas (1982) who studied the effect of host densities on parasitism percentages in *Tichogramma sp*, which found the percentage of parasitized eggs was independent of host density at low densities of parasitoids and was inversely dependent at high densities. Also, Thorpe (1985) showed that the host density did not affect the parasitism percentages and differences were statistically insignificant. Subba (1960) and Ulliyett (1945) observed that in case of *Microbracon hebetor*, in the high density of host, the parasitoid firstly paralyzes all the hosted larvae in the environment and then lays eggs and this takes a long time, but at a low density the parasite fights by paralyzing the host and laying eggs and thus saves time spent laying eggs (Hardy and Blackburn, 1991). and Goubault *et al.* (2007). Generally, oviposition began within 24 hours after paralysis and was completed within 48 hours on a single host Venkatesan *et al.* (2003).

Table 2: Parasitism %, adult longevity and average eggs lid/female of *B. brevecornis* on different tested host densities.

Host density	Parasitism %	Average No. of eggs laid	Ovipositional Periods/ days			Adult longevity
			Pre	Ovi	Post	
2	77.78±3.54b	85.41±3.81b	1.10±0.11a	12.34±1.98a	1.68±0.14a	15.12±2.54a
4	82.22±4.21b	96.35±1.56b	1.30±0.41a	12.71±2.41a	1.21±0.14a	15.23±2.7a
6	90.6±4.61a	110.52±2.52a	1.30±0.24a	13.51±1.74a	1.15±0.13a	15.96±1.89a
8	92.89±3.69a	111.81±1.45a	1.20±0.17a	14.10±1.63a	1.01±0.18a	16.31±2.45a
10	86.04±4.75b	105.65±1.45a	1.40±0.37a	12.33±1.94a	1.69±0.16a	15.42±1.65a
LSD	5.9	7.851	0.41	1.91	1.62	1.12

Means ± SE in a column followed with the same letter(s) are not significantly different at 5% level of probability.

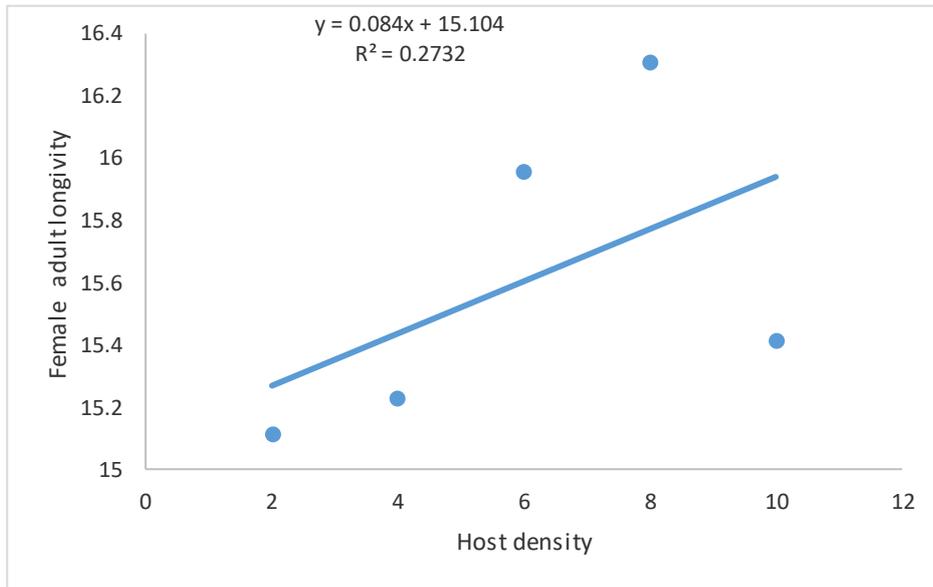


Fig.4: Regression line of relationship between longevity of adult female (days) of *B. brevecornis* at different host densities.

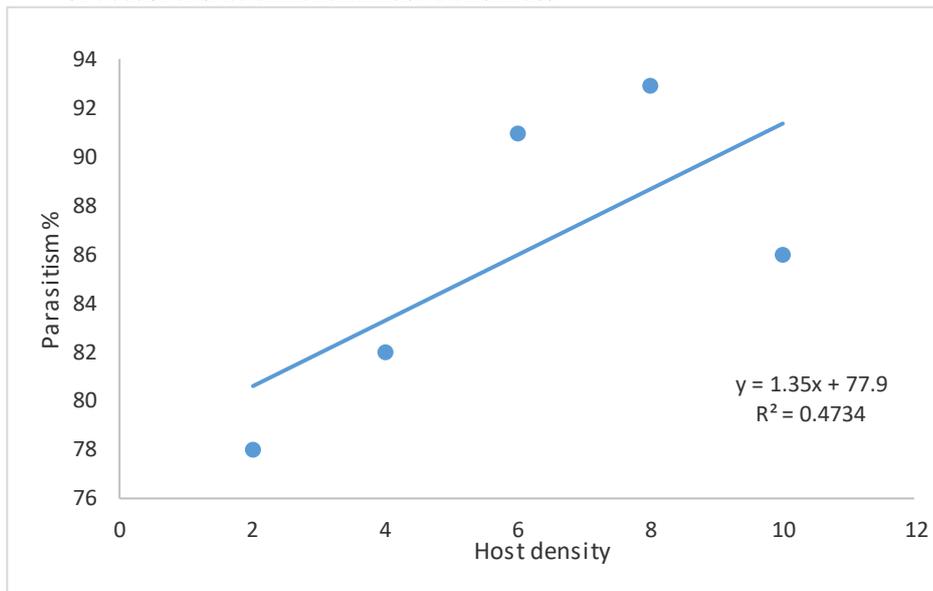


Fig.5: Regression line of relationship between parasitism % of *B. brevecornis* at different host densities.

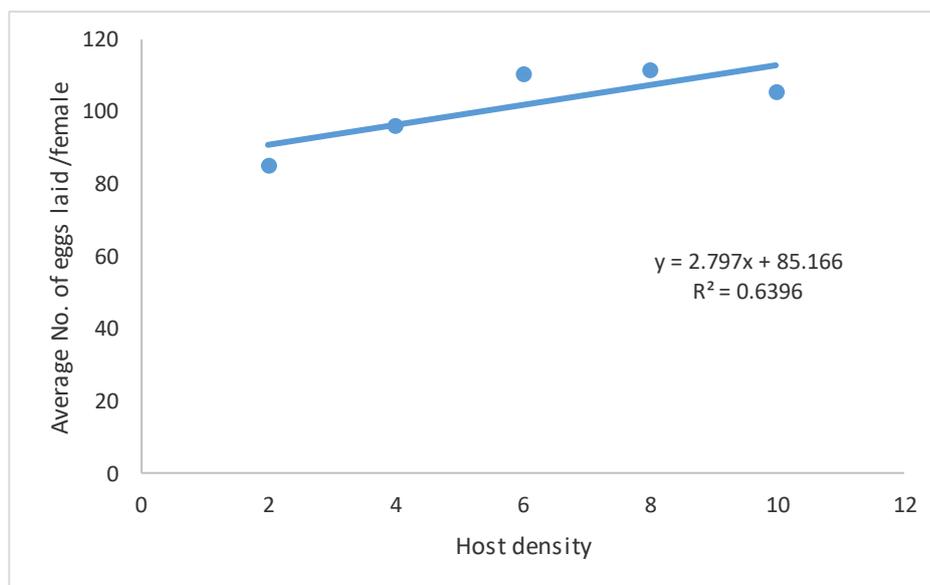


Fig.6: Regression line of relationship between average No. of eggs laid/ female of *B. brevicorins* at different host densities.

Conclusion

The previous study shows that the previous ectoparasitoid of *Bracon brevicorins* grows rapidly with high temperatures, as it is considered one of the most important elements of biological control, but the optimum temperatures are between 25 to 30 °C for laboratory mass rearing, and the host's density does not have a significant effect on the life span of the adult female but affects the number of eggs laid/female. Also, extensive studies will be made on this important parasitoid in order to study some other environmental factors and provide useful information in the field of mass rearing and controlling grain pests and stored products.

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