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BIOLOGICAL STUDIES ON THE LARVAL PUPAL ENDOPARASITOID *Opius pallipes* WESMAEL (HYMENOPTERA: BRACONIDAE).

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ABSTRACT

Under laboratory conditions, some biological aspects of the parasitoid *O. pallipes* were studied. Results obtained revealed that ovipositrion, postoviposition periods and adult longevity were significantly higher when *O. pallipes* female reared on the 3rd *Liriomyza trifolii* (Burgess) larval instar recording (3.0±1.1, 3.1±1.1 and 6.8±1.8 days, respectively), while the immature stage was significantly shorter (27.1±3.3 days) if compared with the 2nd instar larvae. Moreover, number of eggs/female, number of parasitized larvae were higher on the 3rd *L. trifolii* larval instar than on the 2nd instar larvae with insignificant differences.

Number of produced eggs in the females ovaries was significantly higher when the parasitoid females were fed on the host exudates (43.7±11.6 eggs/female) in comparison with those fed on honey or sugar solution 10% (35.6±12.3 and 28.2±5.4 eggs/female, respectively.It is interesting to mention that the starved females absorbed ≈ 40% of the eggs in their ovaries in comparison with the newly emerged ones.

Number of eggs and number of parasitized larvae per female were significantly higher (13.9±3.7 eggs/female and 9.2±2.3 host larvae/female) when 30 *L. trifolii* larvae were presented to *O. pallipes* females in comparison with 5, 10 and 20 host larvae, while number of super-parasitized larvae was significantly higher (2.6±1.1) when 5 host larvae were presented.

Number of eggs and parasitized larvae per parasitoid female were higher on *L. trifolii* than on *L. bryonia* (Blanchard) with significant differences in the choice test recording (9.2±3.9 eggs/female and 7.0±2.9 host larvae/female, respectively), while only number of parasitized larvae per female was significantly higher and also number of eggs per female was higher on *L. trifolii* than on *L.bryonia* in no–choice test with insignificant differences.

Keywords: *Liriomyza trifolii*, *O. pallipes*, biological studies.

INTRODUCTION

The largest number of adult parasitoids were reared from foliage collected from lettuce plantings were *Opius* spp. in Arizona (Palumbo *et al.*, 1994). Shuster *et al.*, 1991 added that *Opius* spp. accounted nearly 42% of the parasitoid complex reared from tomatoes foliage and some associated weeds in Florida.

O. pallipes, *Dacnusa sibirica* (Telenga) and *Diglyphus isaea* (Walker) were the most dominant parasitoid species in Russian tomatoes and cucumber greenhouses as an effective biological antagonists against *Liriomyza* leaf miners (Ushchekov, 2002). *O. pallipes* and *O. dimidiatus* established well and kept *L. trifolii* and *L. bryonia* at very low populations in

Dutch greenhouses and reached in combination with *D. sibirica* 100% parasitism (Linden, 1986). The endoparasitoid *Opius* sp. was the key parasitoid of *L. trifolii* pupae caused the highest percentage of parasitism during the two successive seasons in Mansoura region (Awadalla and Fathy, 1998). The percentage of parasitism by *O. pallipes* reached 29.6% and 55.0% on *L. trifolii* pupae on broad bean and cowpea, so it considered as the most effective larval-pupal parasitoid. On the other hand, *O. pallipes* female could successfully put eggs on either the 2nd or the 3rd *L. trifolii* larval instars (El-Khouly, 2003). According to Linden and Achterberg (1989), the eggs of *O. pallipes* are elongated and the larvae has a blunt heads with large red mandibles, their length is nearly 0.55 mm.

From the available literature a very few authors have studied the biological characters of *O. pallipes* (Linden, 1986; Linden and Achterberg, 1989; Ushchekov, 2002 and Awadalla *et al.*, 2003). Therefore, the present investigation was undertaken to study some biological characters of the parasitoid *O. pallipes*.

MATERIALS AND METHODS

1- Effect of host size on the endoparasitoid *O. pallipes*.

To study the effect of host size (second or third instar larvae) of the host *Liriomyza trifolii* on some biological characteristics of the parasitoid (the immature stage, preoviposition, oviposition and postoviposition periods as well as adult longevity, number of eggs laid, number of parasitized larvae/female), infested leaflets were collected from the field and transferred to the laboratory and arranged on a plastic sheet over a bench. Formed pupae were collected and kept in Petri dishes over a moistened filter paper which was remoistened when necessary to prevent them from drying till the emergence of *O. pallipes* adults.

A newly emerged pairs (male and female) of the parasitoid were transferred in Petri dishes (12.0 by 1.5 cm). Irregular sections (≈3 cm^2) were cut from broad bean leaflets. Each section contained a single *L. trifolii* second or third instar larvae and then arranged over a moistened filter paper in each dish and remoistened when necessary, 20 replicates were done. After 24 hours parasitoids were transferred from Petri dishes. Formed pupae were collected after the death of females and dates of exposure were recorded to evaluate the immature stages of the parasitoid.

Emerged parasitoids from second *L. trifolii* larvae exposure as well as those of the third instars were collected and transferred to Petri dishes (12.0 by 1.5 cm) where 30 sections of broad bean leaflets (≈3 cm^2) were arranged over a moistened filter paper, each section contained a single *L. trifolii* larva.

Recognition of the parasitoid eggs or larvae was described by Linden and Achterberg (1989). Leaf miner larvae were dissected under the microscope. Each leaf miner larva was removed from the leaf and put in a droplet of water. At a magnification of 48 x, the larva was opened with a pair of minute tweezers. The contents of the larva with or without parasitoid eggs or larvae spread in the droplet of water. The parasitoids can be recognized by

the shape of their eggs or larvae according to the previous authers. The experiment was replicated 20 times for every treatment.

2- Effect of feeding diet on *O. pallipes*.

To study the effect of feeding diet on the total formed eggs of females ovary and its longevity, females of *O. pallipes* were kept in Petri dishes (12.0 by 1.5 cm) and treated with different diets (honey, sugar solution 10% and insect host in addition to starving females). Females longevity were recorded, then they were dissected under the microscope. Each female was put in a droplet of water,at a magnification of 48 x, the female's abdomen was opened and its ovary was removed, eggs which were brightly white in their ovarioles were counted and recorded. Females fed on the insect host exudates were transferred to Petri dishes contained *L. trifolii* larvae as described in the previously mentioned experiments and the parasitoid eggs or larvae were recognized and described as reported by Linden and Achterberg (1989). Eggs which recorded in the host insect were added to those counted in the females ovary. The experiment was replicated 20 times for every treatment.

3-. Effect of host density on *O. pallipes*.

To study the effect of insect host density on some biological characters (number of eggs, parasitized larvae and superparasitized larvae/female) of *O. pallipes*, a newly emerged pairs of the parasitoid were transferred to Petri dishes (12.0 by 1.5 cm) contained 5, 10, 20 and 30 leaflet sections (≈3 cm^2), a single *L. trifolii* larva was presented in each section. Arrangement of leaf sections are remoistened when necessary as mentioned befor. Recognition of the parasitoid eggs or larvae as described by Linden and Achterberg (1989) was followed and the tested parameters were recorded. The experiment was replicated 20 times for each treatment.

4- Influence of insect host on the endoparasitoid *O. pallipes*.

- No choice test:

To study the influence of insect host on some biological characters (number of eggs and parasitized larvae/female) of *O. pallipes*, a newly emerged pairs of the parasitoid were transferred to Petri dishes contained an irregular sections of broad bean leaflets (≈3 cm^2), each section contained a single *L. trifolii* or *L. bryonia* larva. Each dish contained 30 sections containing *L. trifolii* or *L. bryonia* larvae. Each treatment was replicated 20 times.

The statistical analysis was carried out by using one way ANOVA. The correlation coefficients and regressions were estimated.

- Choice test:

Fifteen irregular sections (≈3 cm^2) contained *L. trifolii* larvae and another similar numbers contained *L. bryonia* larvae were kept in Petri dishes (12.0 by 1.5 cm) and a newly emerged pairs of *O. pallipes* were transferred to each dish. The treatment was replicated 20 times. Recognition of the parasitoid eggs or larvae was followed as described before.

RESULTS AND DISCUSSION

1- Effect of host size on the biological characters of *O. pallipes*.

Data in table (1) presented the influence of host size (2^{nd} or 3^{rd} instar larvae of the insect host *L. trifolii*) on the biological characters of *O.pallipes*. It

could be seen that the immature stages of the parasitoid durated 29.6 ±2.6 and 27.1 ±3.3 days with significant differences when the parasitoid was reared on the second and third instars of the host larvae, respectively. Preoviposition period was 0.8 ±0.2 and 0.7 ±0.3 days with insignificant differences on the 2nd and 3rd instars of the host larvae, while the oviposition period (2.2±0.9 and 3.0 ±1.1 days) and the postoviposition period was (2.4±1.2 and 3.1 ±1.1 days) with significant differences when the parasitoid reared on the 2nd and 3rd insect larval instars of the host , respectively.

Table (1): Effect of host size on the biological characters of *O. pallipes*.

Biological characters	Second instar	Third instar	LSD 0.05 0.01
Immature stage	29.6±2.6*	27.1±3.3	1.90 2.55
Pre oviposition period(days)	0.8±0.2 ns	0.7±0.3	0.16 0.22
Oviposition period (days)	2.2±0.9	3.0±1.1 *	0.67 0.89
Post oviposition period (days)	2.4±1.2	3.1±1.1 *	0.70 0.96
Adult longevity (days)	5.4±1.4	6.8±1.8 **	1.03 1.39
No.eggs/ female	10.8±4.9	13.3±5.9 ns	3.46 4.63
No. parasitized larvae/ female	7.8±3.3	9.6±3.0 ns	2.02 2.70

As a conclusion, the obtained results revealed that *O. pallipes* progeny reared in *L. trifolii* third larval instar had the chance to develop in a large host size larvae in comparison with those reared in *L. trifolii* second instar larvae, so they resulted more effective parasitoid females in a significantly shorter development time (27.1±3.3 days). On the other hand, the females reared in the 3rd instar host larvae had a longer longevity and a large reproductive output that resulted in a larger number of *L. trifolii* parasitized larvae comparing to those reared in the 2nd instar larvae.

2- Effect of feeding diet on the produced eggs in the females ovary.

Data presented in table (2) showed the effect of feeding diet on the produced eggs in *O. pallipes* females ovaries and their longevity. The number of eggs developed in the females ovaries was significantly higher (P=0.1%) when they were fed on the heomolemph that exceeds from the attacked larvae after the insertion of the females ovipositor recording (43.7±11.6 eggs/female), 14.6±5.8 in the host larva and 29.1±8.6 remained in the ovary.

When the parasitoid females fed on honey and sugar solution 10% produced a significant less eggs (P=0.1%) in their ovaries (35.6±12.3 and 28.2±5.4 eggs/parasitoid female, respectively). The remained eggs in the starved females ovaries were (14.4±5.6) and significantly less than the number of eggs in the newly emerged females (P=0.1%), this means that the females digested a number of eggs that developed in their ovaries when no nutrition source was available. These findings are ilucidating the important

role of the flowering plants such as herbs that gives the females a carbohydrate source when their insect host occurs at very low populations. This finding also indicates that *O. pallipes* females are synovigenic because they produced more eggs in their ovaries when a nutrition source is available (either the host exudates or carbohydrate source). Correlation value between the number of eggs in the ovary and the starvation time was r = -0.38 (Fig. 36). Shuster *et al.* (1991) found that the larval pupal braconid and pteromalid parasitoids *Opius* spp. and *Halticoptera* spp. accounted ≈ 74% of the reared adults and 42% of them are *Opius* spp. on seven weeds associated with tomatoes in Florida despite these weeds accounted only 13-43% of the weed canopy. So, these weeds providing a carbohydrate source for these parasitoids

Table (2): Effect of feeding diet on egg production of *O. pallipes* females.

Feeding diet	No. total produced eggs/parasitoid female	Female longevity (days)
Newly emerged females	24±5.6	--
Starvation	14.4±5.6	3.9±2.0
Honey	35.6±12.3	5.5±1.2
Sugar solution 10%	28.2±5.4	4.8±2.2
Host exudates Ovary	29.1±8.6**	6.7±1.3**
Host larvae	14.6±5.8	
LSD 0.05	5.46	0.99
0.01	7.23	1.32

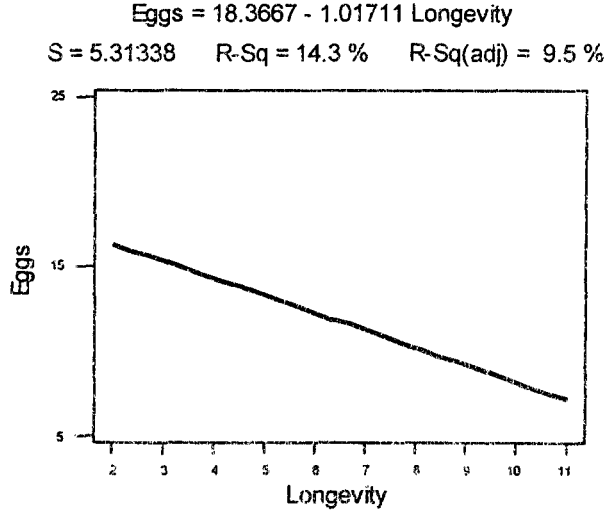

Fig (1): Relation between number of eggs in the ovary and the starvation period

Nevertheless, the large number of eggs remained in the females ovary (nearly twice the number laid in the host larvae) despite the presence of the

host larvae is still uncomprehensive. On the other hand, the females longevity were significantly longer (P=0.5%) when the females fed on the host exudates (6.7 ±1.3 days) because it has a high nutrition value that enabled the female to survive a longer time followed by those fed on honey (5.5 ±1.2 days) and then sugar solution 10% (4.8 ±2.2 days), while the starved females survived only (3.9 ±2.0 days) because no nutrition source was available.

3- Effect of insect host density on *O. pallipes*.

Table (3) illustrated the effect of insect host density on some biological aspects of *O. pallipes*. It could be seen that number of eggs and number of parasitized larvae per female were significantly higher (P=0.1%) when a large number of the host larvae was available (13.9±3.7 eggs/female) and (9.2±2.3 parasitized larvae/female) when 30 *L. trifolii* larvae were presented for every parasitoid female. Super parasitized larvae were also significantly higher (P=0.1%) recording (2.6 ±1.1 super- parasitized larvae/female) when 5 host larvae were presented for every parasitoid female.

Table (3): Effect of insect host density on some biological characters of *O. pallipes*.

Biological characters	No. host larvae/parasitoid female				
	5 Larvae	10 larvae	20 Larvae	30 Larvae	LSD 0.05 0.01
No. eggs/ female	9.1±3.5	10.1±4.7	11.2±4.0	13.9±3.7**	2.57 3.40
No. parasitized larvae/ female	3.9±0.1	6.1±1.9	8.3±2.9	9.2±2.3**	1.35 1.79
No. super parasitized larvae/ female	2.6±1.1**	2.0±1.1	1.6±1.0	1.5±0.6	0.62 0.82

Therefore, it could be concluded that the high densities of the insect host encouraged *O. pallipes* females to lay more eggs and to attack more host larvae in comparison with the low host densities. This finding differs from the field observations that exhibited a preference towards the low host densities, this difference may be due to the absence of the other biological antagonists, especially presence of the ectoparasitoid *D. isaea* that shows a very high activity at the high populations of its host. On the other hand, super parasitism was higher when a few host larvae were presented for every parasitoid female. This may be explained by the shortage of the available host larvae that the females had to lay more than one egg in the same host.

4- Effect of insect host species on *O. pallipes*.

As shown in table (4), in no-choice test the number of eggs per female was higher in *L. trifolii* larvae (13.9±3.7 eggs/female) comparing with that recorded in *L. bryonia* larvae (10.3±7.0 eggs/female) with insignificant difference. The number of parasitized larvae per female was significantly higher (P=0.5%) recording (9.2±2.3 host larvae/parasitoid female) in *L. trifolii* in comparison with (7.1±3.0 host larvae/parasitoid female) recorded in *L. bryonia*.

Table (4): Effect of insect host on some biological characters of *O. pallipes*.

Type of exposure	*L. trifolli*	*L. bryonia*	LSD 0.05 0.01
No choice test			
No. eggs/female	13.9±3.7 ns	10.3±7.0	3.59 4.81
No. parasitized larvae/ female	9.2±2.3 *	7.1±3.0	1.72 2.30
Choice test			
No. eggs/female	9.2±3.9 **	5.3±1.9	1.99 2.66
No. parasitized larvae/ female	7.0±2.9 **	4.0±1.1	1.42 1.90

In the choice test, the number of eggs and the number of parasitized larvae per female were highly significant (P=0.1%) in *L. trifolii* larvae recording (9.2±3.9 eggs/female) and (7.0±2.9 larvae/female) in comparison with (5.3 ±1.9 eggs/female) and (4.0±1.1 larvae/female) recorded in *L. bryonia* larvae, respectively. *O. pallipes* females showed highly preference towards *L. trifolii* larvae than *L. bryonia* in the choice test and less preference towards *L. trifolii* in no choice test. A possible explanation is that in no choice test either *L. trifolii* or *L. bryonia* larvae were the only available host so *O. pallipes* females had to lay eggs and feed on the available insect host, while in the choice test the parasitoid females had the chance to choose their preferred host. The preference of *L. trifolii* may be due to mining behavior of its larvae that mines the upper palisad mesophyll of the leaflets, while *L. bryonia* larvae mines the spongy mesophyll (Hannou and Hegazi, 1996), another possible explanation is that the nutrition contents of *L. trifolii* larvae may be more preferred to *O. pallipes* females. Linden (1986) used *O. pallipes* which thought to be the promising parasitoid against *L. bryonia* in Dutch greenhouses but *O. pallipes* failed to control *L. bryonia*. Dissection of the leaf miner larvae showed that *O. pallipes* females could successfully put the eggs but the eggs were encapsulated.

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دراسات بيولوجية علي طفيل اليرقات- العذاري الداخلي أوبيس باليبس (براكوندي: غشائية الاجنحة)

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تمت دراسة بعض النواحي البيولوجية للطفيل الداخلي *O. pallipes* واوضحت النتائج أن فترة وضع البيض وفترة ما بعد وضع البيض وفترة حياة الانثي كانت أعلي معنويا (٣,٠±١,١و ٣,١±١,١و ٦,٨±١,٨ يوم) علي الترتيب عندما ربيت الاناث علي يرقات العمر الثالث لنافقة أوراق البفول بينما كانت فترة حياة الاطوار غير الكاملة (٢٧,١±٣,٣ يوم) علاوه علي ذلك عدد البيض وعدد اليرقات المتطفل عليها لكل أنثي وكذلك فترة ما قبل وضع البيض كانت عالية عند تربية الطفيل علي العمر الثالث ليرقات العائل بدون فروق معنوية.

أحتوت مبايض الاناث التي غذيت طبيعياً علي العائل عدد اكبرمن البيض (٤٣,٧±١١,٦ بيضة/أنثي) مقارنة بتلك التي غذيت علي العسل (٣٥,٦±١٢,٣ بيضة/انثي) أو محلول سكري ١٠% (٢٨,٢±٥,٤ بيضة/أنثي) أو تلك التي جوعت (١٤,٤±٥,٦ بيضة/أنثي) بينما أحتوت مبايض الاناث الحديثة الخروج من العذراء علي (٢٤,٠±٥,٦ بيضة) مع وجود فروق معنويه.

وضعت الاناث عدد اكبرّ من البيض (١٣,٩±٣,٧) عندما قدم لها ٣٠ يرقة من يرقات العائل *L. trifolii*

بينما كان عدد اليرقات ذات التطفل المتزايد اكبر(٢,٦±١,١) عندما قدم للاناث ٥ من يرقات العائل مع وجود فروق معنويه فى الحالتين

سجلت اناث الطفيل *O. pallipes* عدداً اكبر من البيض واليرقات المتطفل عليها (٩,٢± ٣,٩بيضه / انثى) و (٧,٠±٢,٩يرقه / انثى) على النوع *L.trifolii* عندما اتيح لها الاختيار بين النوعين *L. trifolii* و *L. bryonia*بينما كان فقط عدد اليرقات المتطفل عليها اكبر (٩,٢±٣,٢يرقه / انثى) على النوع *L. trifolii* مقارنة ب (٧,١ ± ٣,٠يرقه / انثى) على النوع *L.bryonia* عندما لم تتح لها الفرصه للاختيار مع وجود فروق معنويه فى الحالتين.