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Evaluation of Super parasitism Behavior of the Larval Pupal Endoparasitoid *Opius Pallipes* Wesmael (Hymenoptera: Braconidae) On the Serpentine Leafminer *Liriomyza*.*Trifolii* (Burgess)

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Abstract: Background: Superparasitism refers to the oviposition behavior of parasitoid females who lay their eggs in an already parasitized host. This often yields intense competition among larvae that are sharing the same host. Objectives: the present study aimed to Evaluate the supperparasitism behavior of the larval pupal endoparasitoids O. pallipes as affected by the host population. Methods: Broad bean (Vecia faba), was selected as a host plant. Hundred infested leaves with L. trifolii were taken weekly. Samples were kept in plastic bags and transferred to be examined in the laboratory. To evaluate Superparasitism for the parasitoids O. pallipes, 100 parasitized larvae were collected weekly. Larvae were checked and the number of the parasitoid larvae counted and recorded. Results: out of (1700) parasitized larvae (1224) were described as solitary parasitized and, (476) were described as superparasitised. The total number of the parasitoid individuals recorded (2278) while, the parasitoid host ratio calculated was 1.34: 1. Percentage of solitary parasitism recorded (72%) and, the percentage of superparasitism recorded (28%). On the other hand superparsitised larvae were divided into three groups: the presence of 2 larvae in a single host larva (first type) recorded (385), the presence of 3 larvae in a single host larva (second type) recorded (69), and, the presence of mor than 3 larvae (third type) in a single host larva recorded The highest occurrence of solitary parasitism recorded (88 solitary parasitized larvae) (22).combined with (312 L. trifolii larvae/ 100 infested leaflets) recorded in 11th of February while, the lowest occurrence of solitary parasitism recorded (50 solitary parasitized larvae) combined with (138 L. trifolii larvae / 100 infested leaflets) recorded in 17th of December. The highest occurrence of the first type of superparasitism recorded (33) in 7th of January combined with (144 L. trifolii larvae / 100 infested leaflets) while, the lowest occurrence recorded (10) in 11th of March combined with (261 L. trifolii larvae / 100 infested leaflets). The highest occurrence of the second type of superparasitism recorded (16) in 17th of December combined with (138 L. trifolii larvae / 100 infested leaflets) while, the highest occurrence of the third type of superparasitism recorded (10) in 17th of December while, the lowest occurrence recorded (0) in several weeks of sampling combined with high populations of L. trifolii for the second and the third types. The highest average occurrence of solitary parasitism recorded in February (80.50 ± 6.45) combined with the highest average occurrence of L. trifolii (284.25±37.45) and the lowest average occurrence of superparasitism recorded (19.75±6.07). Moreover, the highest average occurrence of superparasitism recorded in December (36.66 \pm 13.01) combined with an average occurrence of L. trifolii (165.00±25.63). On the other hand, the highest average occurrence of individuals/100 parasitized larvae recorded in December (156.0 \pm 29.30) and the lowest recorded in February (120.5 \pm 6.45). *Conclusion:* the females of the larval pupal endoparasitoid *O. pallipes* prefers the behavior of solitary parasitism than superparasitism and could successfully discriminate between unparasitized larvae and those previously attacked so, superparasitism occurres only under the pressure of competition with other parasitoids and the low density of the insect host. It could also concluded that, the highest occurrence of solitary parasitism recorded during the highest populations of L. trifolii and, the lowest occurrence recorded during the low populations of theinsect host, in contrast the highest occurrence of superparasitism recorded during the low populations of the insect host..

Keywords: Superparasitism - Opius pallipes - L. trifolii.

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INTRODUCTION

Parasitoids occur in six different orders of insects, with a particularly high species richness in Hymenoptera (Pennacchio and Strand, 2006). Parasitoid wasps shows a wide spectrum of interactions with their hosts. There are solitary and gregarious species that can even be congeners (Pexton and Mayhew, 2005). (Godfray, 1994) stated that, numerous species can superparasitize hosts, superparasitism occurs with a deposition of a clutch of eggs (or a single egg) by a female parasitoid on or in a host already parasitized by itself or a conspecific female.

According to (Fiske, 1910), who first used the term (superparasitism), it meant the simultaneous attack of a host either by two or more species of primary

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parasites or by one species more than once. Another definition by (Smith, 1916), superparasitism is the superabundance of individuals of a single parasite species attacking a single host so that there are more parasite larvae than can reach maturity.

Parasitoids are classified as solitary if at most one offspring is able to complete development on or in a single host, and as gregarious when more than one offspring can successfully complete development on or in <u>a</u> host (Godfray, 1994). Theoretical models predict that whether a female parasitoid should avoid or accept an already-parasitized host depends on the relative costs and benefits of oviposition (Visser, 1994 and Godfray, 1994).

In the past, superparasitism has been attributed to the inability of females to discriminate between parasitized and non-parasitized hosts, and has been interpreted as an error by the ovipositing female (Van Alphen and Visser 1990). The advantages of superparasitism are an increased possibility of producing offspring from a host and the stabilization of host parasitoid interactions in solitary and gregarious parasitoids (Van Alphen, 1988).

(Kaya and Nishida, 1968) found that, superparasitism of *Opius oophilus* was affected to a greater extent by host egg density than by the host plant density. They also found that there was no significant difference in the rat e of superparasitism between trees with low fruit abundance and those with high fruit abundance, suggesting that *O. oophilus* tends to remain in areas where insect host eggs are present rather than in areas where host plant fruits are abundant.

(Hendrikse, 1980) concluded that, O. pallipes is a solitary endoparasite of L. bryoniae. It oviposits in all instars. The adult parasite emerges in the puparium of the host and escapes by making a hole with its mandibles.

(Hendrikse and Zucchi 1979) studied the searching behaviour of *O. pallipes* and described it as follows: the female hovers around the leaves. After landing on a leaf, she starts scanning the leaf surface with her antennae and stings it rhythmically with her ovipositor. If a mine is encountered, the wasp follows it; again, the antennae and ovipositor are used for scanning. The female tries to determine the position of the larva. When the host is found, she inserts her ovipositor into the larva. She may reject it or will lay an egg in it.

(Elkhouly, 2009) studied the biological aspects of O. pallipes on L. trifolii and, found that, superparasitized larvae were significantly higher (P=0.1%) recording (2.6 \pm 1.1 super parasitized larvae/female) when 5 host larvae were presented for every parasitoid female compared with the presence of (10, 20, and 30 host larvae). (Elkhouly, 2018) studied superparasitism behavior of O. pallipes as affected by the insect host and concluded that, O. pallipes females reached its highest numbers at the low population levels of the insect host on either L. trifolii or L. bryoniae with low preference towards L. trifolii so, superparasitism by O. pallipes recorded slightly high numbers on L. trifolii larvae compared with L. bryoniae.

From the available literature very, few authors have studied superparasitism behavior of *O. pallipes* under Libyan conditions (Elkhouly, 2018 and Albasha and, Elkhouly 2020). Therefor the present study was undertaken to evaluate superparasitism behavior of the larval pupal endoparasitoid *O. pallipes* under the Libyan conditions.

MATERIALS AND METHODS

Seasonal Abundance of the Serpentine Leaf Miner L. trifolii

Broad bean (*Vecia faba*), was selected as a host plant because it has a heavy infestation by the Serpentine Leaf Miner *L. trifolii* combined with a good population of *O. pallipes*. Hundred infested leaves with *L. trifolii* were taken weekly. Samples were kept in plastic bags and transferred to be examined in the laboratory. Number of *L. trifolii* larvae were counted and recorded.

Superparasitism of the Parasitoid O. pallipes

To evaluate superparasitism of O. pallipes, 100 parasitized larvae were collected weekly. Larvae were checked and the number of the parasitoid larvae was counted according to (Linden and Achterberg, 1989). The 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 64x, the larvae were opened with a pair of minute tweezers. The contents of the larvae and the parasitoid immature stages spread in the droplet of water. The parasitoid larvae could be counted and recorded. Normal agricultural practices of fertilizing and irrigation were followed and no chemical control measurements were applied. Samples were taken from the appearance of the emergence of the first leaves and continued weekly until harvest.

Results

Data presented in table (1) showed that, out of (1700) parasitized larvae (1224) were described as solitary parasitized and, (476) were described as superparasitized. The total number of the parasitoid individuals recorded (2278) while, the parasitoid host ratio calculated was 1.34: 1. Percentage of solitary parasitism recorded (72.00%) and, the percentage of superparasitism recorded (28.00%).

Superparsitised larvae were divided as follow: the presence of 2 larvae in a single host larva (first type) (385), the presence of 3 larvae in a single host larva (second type) (69), and, the presence of mor than 3 larvae in a single host larva (third type) (22). With regard to data presented in table (1), it could be seen that, the females of the larval pupal endoparasitoids *O. pallipes* prefers the solitary type of parasitism. (72.00%) of the parasitized larvae were solitary while, only (28.00%) were superparasitised.

Moreover, the presence of 2 larvae in a single host larva recorded the highest abundance with (80.88%) while, the presence of 3 larvae and more than 3 larvae in a single host larva recorded (14.49 % and, 4.62%) respectively.





Figure (1) population abundance of *Liriomyza trifolii* combined with numbers of solitary parasitized and superparasitised larvae on broad bean as a selected host plant.

February while, the lowest occurrence of solitary parasitism recorded (50 solitary parasitized larvae) combined with (138 *L. trifolii* larvae / 100 infested leaflets) recorded in 17th of December.

Data presented in figure (1) showed that, the highest occurrence of solitary parasitism recorded (88 solitary parasitized larvae) combined with (312 *L. trifolii* larvae/ 100 infested leaflets) recorded in 11th of



Figure (2) occurrence of solitary parasitism and three types superparasitism (2 larvae, 3 larvae and, mor than 3 larvae in a single host larva) during the period of the study.

Data presented in figure (2) showed that, the highest occurrence of the first type of superparasitism (2 larvae in a single host larva) recorded (33) in 7th of January combined with (144 *L. trifolii* larvae / 100 infested leaflets) while the lowest occurrence recorded (10) in 11th of March combined with (261 *L. trifolii* larvae / 100 infested leaflets). recorded (50 solitary parasitized larvae) combined with (138 *L. trifolii* larvae / 100 infested leaflets) recorded in 17th of December.

The highest occurrence of the second type of superparasitism (3 larvae in a single host larva)

recorded (16) in 17^{th} of December combined with (138 *L. trifolii* larvae / 100 infested leaflets) while the lowest occurrence recorded (0) in several weeks of sampling combined with high populations of *L. trifolii*.

The highest occurrence of the third type of superparasitism (more than 3 larvae in a single host larva) recorded (10) in 17th of December combined with (138 *L. trifolii* larvae / 100 infested leaflets) while the lowest occurrence recorded (0) in several weeks of sampling combined with high populations of *L. trifolii*.

 Table (2) Monthly mean values of superparasitism, solitary parasitism, number of individuals/ 100 parasitized larvae and numbers of L. trifolii.

Months	Superparasitism				_	No.	
	2 larvae	3 larvae	More than 3	Total	Solitary Parasitism	individuals/100 parasitized larvae	No. L. trifolii
December	23.33 ± 6.02	8.33 ± 6.02	5.00 ± 4.35	36.66 ± 13.01	63.33 ± 13.01	156.00 ± 29.30	165.00 ± 25.63
January	24.25 ± 6.99	3.75 ± 1.25	1.25 ± 0.95	29.00 ± 8.60	71.00 ± 8.61	135.25 ± 10.81	166.00 ± 27.40
February	17.75 ± 5.30	1.75 ± 1.25	0.00 ± 0.00	19.75 ± 6.07	80.50 ± 6.45	120.50 ± 6.45	284.25 ± 37.45
March	21.75 ± 8.50	3.25 ± 1.25	0.25 ± 0.50	25.25 ± 9.46	74.90 ± 9.00	127.50 ± 10.75	196.75 ± 47.37
Aprille	$29.50\pm.070$	4.50 ± 0.70	0.00 ± 0.00	35.00 ± 2.82	65.00 ± 2.82	137.00 ± 1.40	110.50 ± 13.43
Mean± S. D	23.32 ± 4.25	4.32 ± 2.46	1.30 ± 2.13	29.13 ± 6.97	70.76 ± 6.96	135.25 ± 13.33	184.5 ± 13.33

As shown in table (2) the highest average occurrence of solitary parasitism recorded in February (80.50 ± 6.45) combined with the highest average occurrence of L. trifolii (284.25 \pm 37.45) and the lowest average occurrence of superparasitism recorded (19.75 \pm 6.07). Moreover, the highest average occurrence of superparasitism recorded in December (36.66 ± 13.01) combined with average occurrence (165.00 \pm 25.63) of L. trifolii. On the other hand, the highest average occurrence of individuals/100 parasitized larvae recorded in December (156.00 \pm 29.30) and the lowest recorded in February (120.50 \pm 6.45). on the other hand, the monthly average occurrence of the three types of superparasitism recorded (23.32 \pm 4.25, 4.32 \pm 2.46 and, 1.30 ± 2.13) for the first, the second and the third type of superparasitism respectively.

DISCUSSION

The females of the larval pupal endoparasitoids *O. pallipes* prefers the solitary type of parasitism, (72.00%) of the parasitized larvae were solitary while, only (28.00%) were superparasitised. Moreover, the first type of superparasitism recorded the highest abundance with (80.88%) while, the second and the third types of superparasitism recorded (14.49 % and, 4.62%) respectively table (1). (Kaya and Nishida, 1968) concluded that, superparasitism in one sense might be considered as an index of thesuitability of the habitat to a parasite. Since by definition superparasitism means a superabundance of reproductive units within a host, it is reasonableto expect a high degree of superparasitism in areas suitable to a particularspecies of parasite. With regard to their proposal, the serpentine leaf miner L.trifolii is a suitable insect host for endoparasitoids O. pallipes. Furthermore the presence of superparasitism behavior occurred only during the high activity periods of the parasitoid which combined with the low or moderate densities of L. trifolii or the high abundance of the larval ectoparasitoid Diglyphus isaea. The females of O. pallipes could successfully distribute their out put according to its ecological needs so, the first type of superparasitism was the highest compared with the second and the third types. The parasitoid host ratio calculated was 1.34: 1 so, it is clear that the females of O. pallipes have the capability to deposit eggs in all available insect host larvae but, they can not discriminate between unparasitized host larvae and those previously parasitized (Linden, 1986). On the other hand, low rates of parasitism were observed in the field studies of O. pallipes populations on different host plants either in open fields and greenhouses (Elkhouly et al., 2018 and, Elkhouly et al., 2019).

Field studies of *O. pallipes* superparasitism behavior showed that, the highest occurrence of solitary parasitism recorded during the highest peak of *L. trifolii* in 11^{th} of February while, the lowest occurrence of

solitary parasitism occurred during the lowest peak of L. trifolii in 17th of December. According to (Kaya and Nishida, 1968), there was a decrease in superparasitism with an increase in the host egg density. However, this relationship was nonlinear. In general, there was an increase in percent parasitism with increased superparasitism. (Montoya et al., 2012) Studied superparasitism of the Braconid Diachasmimorpha longicaudata on the Mexican fruit fly Anastrepha *ludens* they found that, this species showed a moderate levels of superparasitism result in a female-biased sex ratio and that both mass reared and wild females superparasitize their hosts without detrimental effects on offspring demographic parameters, including longevity and fecundity. they suggested that, superparasitism in this species is advantageous. These results could be on line with ours because the females of O. pallipes are also superparasitize irrespective to the population abundance of their host with an expiation is that O. pallipes females had low superparasitism rates during the high abundance of their host.

The highest average occurrence of solitary parasitism recorded in February while, highest average occurrence of superparasitism recorded in December. On the other hand, the highest average occurrence of individuals/100 parasitized larvae recorded in December and the lowest recorded in February. Furthermore, the monthly average occurrence of the three types of superparasitism recorded (23.32 \pm 4.25, 4.32 ± 2.46 and, 1.30 ± 2.13) for the first, the second and the third type of superparasitism respectively during the season of the study. With regard to the previous data we should take superparasitism of O. pallipes into account when biological control programs are established to avoid the probable loss of its reproductive output due to superparasitism behavior because a competition behavior may occur among O. pallipes larvae when sharing the same host.

Ideally, parasitoids used as biocontrol agents are expected to be highly efficient in finding hosts and able to discriminate between parasitized and nonparasitized hosts (Van Lenteren, 1981), which avoids superparasitism and minimizes the loss of eggs, time and energy associated with searching behavior (Godfray, 1994). The ability to recognize hosts that are parasitized by conspecifics (host discrimination) has been documented in representatives of most major families of the parasitic Hymenoptera (Mackauer, 1990). Empirical studies have shown that the consequences of superparasitism in parasitoids can vary among species. In solitary parasitoid wasps for example, the duration of immature developmental stages increased in Microplitis croceipes (Cresson) (Braconidae) (Eller et 1990) and Venturia canescens (Gravenhorst) (Ichneumonidae) (Harvey et al., 1993) but not in Aphidius ervi Haliday (Braconidae) (Bai and, Mackauer 1992).

Older larvae are found faster than younger ones. Host feeding is never observed. Experiments indicated that *O. pallipes* is able to distinguish plants infested with *L. bryoniae* from uninfested plants. *O.* *pallipes* accepts all larval stages of *L. bryoniae* for oviposition and can discriminate between parasitized and unparasitized hosts (Hendrikse *et al.*, 1980).

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