EVALUATION OF SUPERPARASITISM BEHAVIOR OF THE LARVAL PUPAL ENDOPARASITOID CHRYSOCHARIS PARKSI (CRAWFORD) (HYMENOPTERA: EULOPHIDAE) ON THE SERPENTINE LEAF MINER LIRIOMYZA TRIFOLII (DIPTERA: AGROMYZIDAE).

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ABSTRACT

Supperparasitism behavior of the endoparasitoid Chrysocharis parksi was evaluated. 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. Hundred parasitized larvae were collected weekly. Larvae were checked and number of the parasitoid immatures was recorded. Out of (1700) parasitized larvae (1224) were solitary parasitized and, (513) were superparasitized. The total number of the parasitoid individuals recorded (2329) while, the parasitoid host ratio was (1.37: 1). Total percentage of solitary parasitism recorded (69.82%) and, the total percentage of superparasitism recorded (30.17%). Superparsitized larvae were divided as follow: the presence of 2 larvae in a single host larva (first type) (423) (82.45%), the presence of 3 larvae in a single host larva (second type) (75) (14.61 %), and, the presence of 4 larvae in a single host larva (third type) (15) (2.92%).

The highest occurrence of solitary parasitism recorded (87 solitary parasitized larvae) combined with (229 L. trifolii larvae/ 100 infested leaflets) recorded in 2nd of February. The highest occurrence of the first type of superparasitism recorded (41) in 14th of January combined with (194 L. trifolii larvae / 100 infested leaflets). The highest occurrence of the second type of superparasitism recorded (12) in 17th of December combined with (138 L. trifolii larvae / 100 infested leaflets). The highest occurrence of the third type of superparasitism recorded (3) in 21th of January combined with (141 L. trifolii larvae / 100 infested leaflets). 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).

Keywords : Superparasitism - Chrysocharis parksi - L. trifolii

الملخص:

تم تقييم سلوك التطفل المتزايد للطفيل **Chrysocharis parksi** . اختير نبات الفول كعائل نباتي وحشرة نافقة أوراق الفول كعائل حشري . جمعت 100 يرقة متطفل عليها أسبوعيا وذلك من خلال عينات نباتية يجري تجميعها ونقلها للمعمل للفحص. فحصت اليرقات تحت ميكروسكوب ضوئي وفي وسط مائي وتم عد وتسجيل الاطوار غير الكاملة للطفيل. عدد اليرقات التي فحصت هو 1700 يرقة ، 1224 منها سجلت كحالات تطفل انفرادي و 513 سجلت كحالات تطفل متزايد. العدد الكلي لاطوار الطفيل غير الكاملة هو 2329

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طور . فيما كانت نسبة العائل الى الطفيل هي 1: 1.37 . النسبة المؤية للتطفل الانفرادي سجلت 69.82% وللتطفل المتزايد 30.17% . قسمت حالات التطفل المتزايد الى ثلاثة أنماط. النمط الأول (وجود 2 من الاطوار غير الكاملة داخل يرقة العائل) بواقع 423 (82.45%) من اجمالي حالات التطفل المتزايد . النمط الثاني (وجود 3 من الاطوار غير الكاملة داخل يرقة العائل) بواقع 75 (14.61%). النمط الثالث (وجود 4 من الاطوار غير الكاملة داخل يرقة العائل) بواقع 15 . .**(2.92%)**

سجلت اعلى كثافة للتطفل المتزايد بواقع 87 حالة عند كثافة عددية ليرقات العائل بمعدل 229 يرقة / 100 وريقة من نبات الفول في الثاني من فبراير. اما اعلى كثافة للنمط الأول فكانت بواقع 41 حالة عند كثافة عددية ليرقات العائل 194 يرقة/ 100وريقة في 14 فبراير ومن ناحية اخري فقد سجل النمط الثاني اعلى كثافة بواقع 12 حالة عند كثافة عددية ليرقات العائل 138 يرقة /100 وريقة في 17 ديسمبر. سجل النمط الثالث اعلى كثافة بواقع 3 حالات عند كثافة عددية ليرقات العائل 141 يرقة/ 100وريقة في 21 يناير. سجل المتوسط الموسمي للتطفل المتزايد 6.45 ± 80.50 عند كثافة موسمية متوسطة ليرقات العائل 37.45 ± 284.25.

الكلمات المفتاحية: التطفل المتزايد - Chrysocharisparksi - نافقة أوراق الفول.

1. INTRODUCTION

C. parksi Crawford 1912 belongs to the *Entedontinae* of the family *Eulophidae.* This subfamily are internal or external parasites. *Chrysocharis spp* are primary, rarely secondary, endoparasites of larvae and pupae (Boucek and askew 1968). The parasitoid *C.parksi* is an internal parasite of *L.trifolii* and was found to be a major species attacking this leaf miner. The female injects her eggs into the larvae; the host larvae are usually able to pupate normally and the adult parasites emerge from the puparia formed by the Agromyzid larva. *C. parksi* parasitized *L. trifolii* pupae can be instantly separated from those unparasitized, because of a difference in color. *Chrysocharis* females feed from the host exudates during oviposition (Parrella *et al*., 1982).

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 a host (Godfray, 1994). 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 parasites or by one species more than once. Subsequently, this term was given a more restricted meaning than the one used by Fiske. According to Smith, (1916), superparasitism meant the superabundance of individuals of a single parasite species attacking a single host so that there are more parasite larvae than can reach maturity. The phenomenon in which there was a simultaneous attack by two or more species of primary parasites in a single individual host was referred to as multiple parasitism. Thus, the original term of Fiske was split in to superparasitism in a restricted sense and multiple parasitism.

Ayala *et al.*, (2021) defined Superparasitism and multiparasitism, as an oviposition in a previously parasitized host by a female of the same or different species. They studied the two former behaviors in three species of native larval−pupal solitary endoparasitoids that attack *Anastrepha shiner(Diptera: Tephritidae)* vin the Neotropical region, and the possible effect on offspring fitness parameters. *Doryctobracon crawfordi* (Viereck), *Utetes anastrephae* (Viereck), and *Opius hirtus* (Fischer) are high abundant parasitoids and currently under consideration for use as biocontrol agents. They concluded that, most females appeared to avoid superparasitism, specifically when acting alone, suggesting a high discrimination ability, which is probably a result of a close relationship and evolutionary history with *Anastrepha* hosts.

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. However, different authors have stated that under specific conditions, superparasitism may be an adaptive strategy (Van Alphen and Visser 1990); resulting from a balance between the benefits and the costs of laying an egg in an already parasitized host. 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).

(Elkhouly, 2009) concluded that, superparasitism by *C parksi* females recorded its highest numbers at the low populations of their host; on the other hand, their lowest numbers were recorded at the high population of their host. *C. parksi* occurred at high populations at the first and the last months of the winter growing seasons, when its host was at low population levels so, *C. parksi* females can't distribute their reproductive output in solitary parasitism. On the other hand, the high competition with the larval ectoparasitoid *D. isaea* may force the females for superparasitism. Correlation values between superparasitized larvae and *L. trifolii* population were significantly negative during the three successive seasons, respectively.

From the available literature very few authors have studied superparasitism behavior of *C. parksi* (Elkhouly, 2009 and, 2018). Therefor the present study was undertaken to evaluate superparasitism behavior of the larval pupal endoparasitoid *C. parksi* under the Libyan conditions.

2.MATERIALS AND METHODS

2.1 Seasonal abundance of the serpentine leaf miner *L. trifolii.*

Broad bean (*Vecia faba*), was chosen as a host plant because it has a heavy infestation by the Serpentine leaf miner *L. trifolii* combined with a good population of *C. parksi*. 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.

2.2Superparasitism of the parasitoid *C. parksi*

To evaluate superparasitism for the parasitoids *C. parksi*, 100 parasitized larvae were collected weekly. Larvae were checked and the number of the parasitoid immature stages was counted and defined 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 eggs or larvae could be counted and recorded. Larvae which contained eggs of the parasitoids and those with gregarious parasitism were excluded, the investigation depended only on the host larvae which contained only the parasitoid larvae so, the discrimination between parasitoids was possible. Samples were taken from the appearance of the emergence of the first leaves and continued weekly until harvest. Normal agricultural practices of fertilizing and irrigation were followed and no chemical control measurements were applied.

3.RESULTS AND DISCUSSION

Data presented in table (1) showed that, out of (1700) parasitized larvae (1224) were described as solitary parasitized and, (513) were described as superparasitized. The total number of the parasitoid individuals recorded (2329) while, the parasitoid host ratio calculated was (1.37: 1). Total percentage of solitary parasitism recorded (69.82%) and, the total percentage of superparasitism recorded (30.17%).

Superparsitised larvae were divided as follow: the presence of 2 larvae in a single host larva (first type) (423), the presence of 3 larvae in a single host larva (second type) (75), and, the presence of 4 larvae in a single host larva (third type) (15). A very few larvae contained more than 4 immatures so, they were excluded.

With regard to data presented in table (1), it could be seen that, the females of the larval pupal endoparasitoid *C. parksi* prefers the solitary type of parasitism. (69.82%) of the parasitized larvae were solitary while, only (31.17%) were superparasitised. Moreover, the first type of superparasitism recorded the highest abundance with (82.45%) while, the second and the third types recorded (14.61 % and, 2.92%) respectively. According to (**Kaya and Nishida, 1968)**, there was a dramatic decrease in superparasitism with an increase in the host egg density. However, this relationship was nonlinear. Ingeneral, there was an increase in percent parasitism with increased superparasitism.

Table (1) Total numbers of examined larvae, solitary parasitized larvae, percentage of solitary parasitism, superparasitized larvae, percentage of superparasitism, number of parasite individuals and, Parasite: Host ratio.

Examined larvae	**Solitary parasitized**	**% solitary parasitism**	**Supper parasitized larvae 513**			**% supperparasitism**	**No. of parasite individuals.**	**Parasite: Host ratio**
			2larvae	3larvae	4larvae			
1700	1187	69.82	423	75	15	30.17	2329	1.37: 1

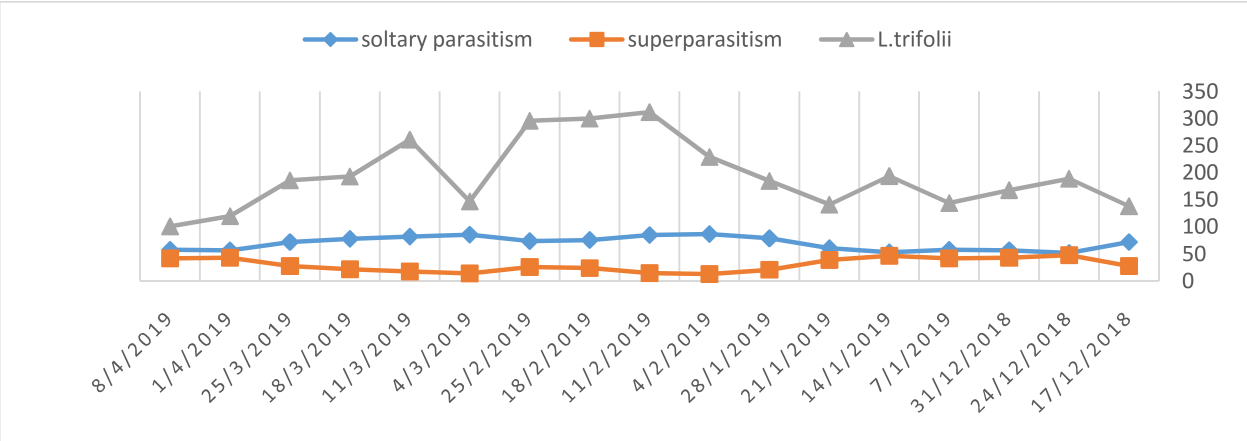

Fig.1: population abundance of *Liriomyza trifolii* combined with numbers of solitary parasitized and superparasitised larvae on broad bean as a selected host plant.

Data presented in figure (1) showed that, the highest occurrence of solitary parasitism recorded (87 solitary parasitized larvae) combined with (229 *L. trifolii* larvae/ 100 infested leaflets) recorded in 2^{nd} of February while, the lowest occurrence of solitary parasitism recorded (52 solitary parasitized larvae) combined with (189 *L. trifolii* larvae / 100 infested leaflets) recorded in 24^{th} of December.

With regard to the previous data superparasitism recorded its highest numbers during the low populations of its host, the females intending to distribute their output in the first type of superparasitism, then the second and the third types take place. Also, we cannot rule out the low populations of *L. trifolii* that occurring under the high computation of ectoparasitoid *D.*

isaea. With the increase of the host population, superparasitism decreases and, the females of the parasitoid can distribute their output in a solitary parasitism so, the parasitoid could earn a good population abundance and successfully tolerate the high competition of larval ectoparasitoid *D. isaea*. These results are in line with those of **(Jensen and Koehler 1970)** who concluded that, *C.parksi* occurred in moderate numbers on *Liriomyza spp* during the early part of the season on alfalfa in California. (**Linden 1986)** estimated the release of *C.parksi* in tomato glass house and found that an early infestation of *L.trifolii* decreased and even disappeared during the first months of the year. *C.parksi* reached 30% parasitism and no other parasite species was found. He also concluded that *C.parksi* might become a permanent member of the native parasite complex in glasshouse populations. These results are also in agreement with those of **(Elkhouly, 2009)** who reported that, correlation values between *C. parksi* and its host (*L. trifolii*) on broad bean were 0.19, 0.52 and 0.22 during three successive seasons of the study and synchronization between this parasitoid and its host was less in comparison with *D. isaea*.

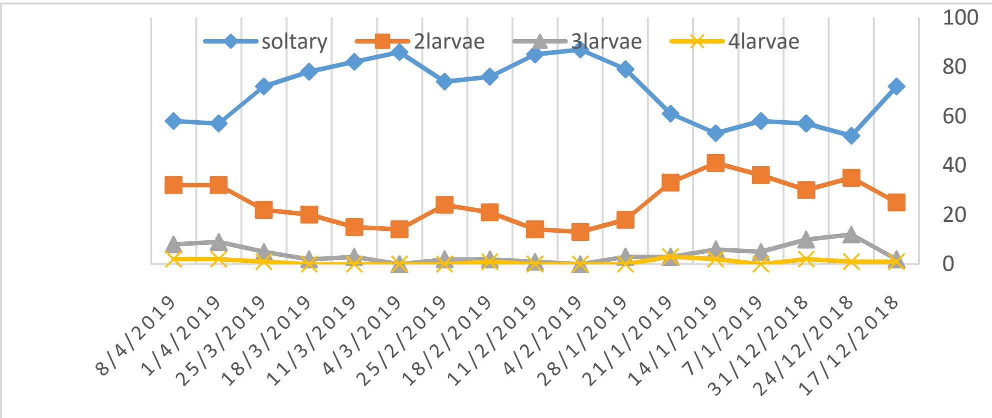

Fig.2: Natural occurrence of solitary parasitism and three types superparasitism (2 larvae, 3 larvae and, 4 larvae in a single host larva during the period of the study.

Data presented in figure (2) showed that, the highest occurrence of the first type of superparasitism recorded (41) in 14th of January combined with (194 *L. trifolii* larvae / 100 infested leaflets) while the lowest occurrence recorded (13) in 4th of February combined with (229 *L. trifolii* larvae / 100 infested leaflets).

The highest occurrence of the second type of superparasitism recorded (12) in 17th of December combined with (138 *L. trifolii* larvae / 100 infested leaflets) while the lowest occurrence recorded (0) combined with (229 *L. trifolii* larvae / 100 infested leaflets) in 4th of February.

The highest occurrence of the third type of superparasitism recorded (3) in 21th of January combined with (141 *L. trifolii* larvae / 100 infested leaflets) while, the lowest occurrence recorded (0) in several weeks of sampling combined with high populations of *L. trifolii*. These results are in agreement with those of (**El-Khouly .2009)** who studied the population of the parasitoid *C. parksi* on three winter host plants and found that, C. *parksi* recorded three peaks of abundance on broad bean, lentil and chickpea during the winter season, the highest peaks recorded (55.0, 15.0 and 6.0 individuals/100 mined leaflets) occurred in the 11th of March on broad bean and lentil and the 4th of February on chickpea. furthermore, the high populations of *C. parksi* combined with high occurrence of solitary parasitism and the low populations were associated with high occurrence of superparasitism. **Eller *et al.*, 1990** reported that,the tendency to superparasitize hosts has been observed in several species of parasitoid wasps used in biocontrol programs. 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*)

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

Months	Superparasitism				Solitary Parasitism	No. individuals/100 parasitized larvae	No. *L. trifolii*
	2 larvae	3 larvae	4 larvae	Total			
December	**30.0 ± 5.00**	**8.0 ± 5.29**	**1.33 ± 0.57**	**39.66 ± 10.4**	**60.33 ± 10.4**	**149.66 ± 15.70**	**165.00 ± 25.63**
January	**32.0 ± 9.89**	**4.25 ± 1.5**	**1.25 ± 1.50**	**37.25 ± 11.3**	**62.75 ± 11.32**	**144.50 ± 15.32**	**166.00 ± 27.40**
February	**17.75 ± 5.35**	**1.25 ± 0.95**	**0.25 ± 0.50**	**19.50 ± 6.45**	**80.50 ± 6.45**	**121.75 ± 8.50**	**284.25 ± 37.45**
March	**18.0 ± 3.9**	**2.50 ± 2.1**	**0.25 ± 0.50**	**20.50 ± 5.97**	**79.50 ± 5.79**	**123.50 ± 8.73**	**196.75 ± 47.37**
Aprille	**32.0 ± 0.0**	**8.50 ± 0.7**	**2.00 ± 0.0**	**42.50 ± 0.70**	**57.50 ± 0.70**	**159.50 ± 7.77**	**110.50 ± 13.43**
Mean±S. D	**25.95 ± 7.4**	**4.9 ± 3.24**	**1.01 ± 0.75**	**31.88 ± 11.0**	**68.11± 11.57**	**139.78 ± 16.57**	**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 ± 37.45) and the lowest average occurrence of superparasitism recorded (19.50 ± 6.45) in the same month. Moreover, the highest average occurrence of superparasitism recorded in April (42.50 ± 0.70) combined with the lowest average occurrence of *L. trifolii* (110.50 ± 13.43). Furthermore, the highest average occurrence of individuals/100 parasitized larvae recorded in April (159.50 ± 7.77) and the lowest recorded in February (121.75± 8.50). On the other hand, the total monthly average occurrence of the three types of superparasitism recorded (25.95 ± 7.4, 4.9 ± 3.24 and, 1.01 ± 0.75) for the first, the second and the third type of superparasitism respectively.

(**Kaya and Nishida, 1968)** concluded that, superparasitism in one sense might be considered as an index of the suitability of the habitat to a parasite. Since by definition superparasitism means a superabundance of reproductive units within a host, it is reasonable to expect a high degree of superparasitism in areas suitable to a particular species of parasite. With regard to their proposal, the serpentine leaf miner *L.trifolii* is a suitable insect host for the endoparasitoids *C. parksi*. Furthermore the presence of superparasitism behavior occurred only during the high activity periods of the parasitoid which combined with the low or the moderate densities of *L. trifolii* or the high abundance of the larval ectoparasitoid *D. isaea*. The females of *C. parksi* could successfully distribute their output according to their 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 1.37: 1 so, it is clear that the females of *C. parksi* have the capability to deposit eggs in all available insect host larvae but, they cannot discriminate between unparasitized host larvae and those previously attacked **(Linden, 1986)**. On the other hand, low rates of parasitism were observed in the field studies of *C. parksi* populationson different host plants either in open fields and greenhouses **(Elkhouly *et al.*, 2018 and, Elkhouly *et al.*, 2019)**.

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 ± 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.

4.CONCLUSION: the females of the larval pupal endoparasitoid *C. parksi* prefers the behavior of solitary parasitism than superparasitism. It could be 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 the insect host.

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