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Population densities of the serpentine leaf miner *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and the associated parasitoids *Diglyphus isaea* and *Opius pallipes* on two weed host plants.

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Abstract \

The present study was carried out in Alzawia region. Blackberry nightshade (*Solanum nigrum*) and Sour clover (*Melilotus indicus*) were selected as a summer and winter host plants during the growing seasons 2020 and 2021. *L. trifolii* larvae showed three peaks of abundance on nightshade and sour clover recording (11, 19 and, 29) and (58, 119 and, 38 individuals/ 100 infested leaflets) respectively. On the other hand, *D. isaea* recorded three peaks of abundance on sour clover and nightshade with (27, 55 and, 66) and (9, 11 and, 19 individuals/ 100 infested leaflets) respectively. The percentage of parasitism ranged between (12.9% - 91.3%) on sour clover and (0.0% - 59.7%) on nightshade. The larva pupal endoparasitoid *O.pallipes* recorded three peaks of abundance on sour clover and nightshade with (15, 25 and, 14) and (8, 10 and, 12 individuals/ 100 infested leaflets). The percentage of parasitism ranged between (0.0% - 46.8%)

on sour clover and (0.0% - 72.7%) on nightshade. Correlation coefficient (r) between *D.isaea* and *L. trifolii* recorded (0.91 and 0.75) on Nightshade and Sour clover. On the other hand, r value recorded (0.52 and 0.39) for *O. pallipes* on Nightshade and Sour clover respectively. It could be concluded that, *L. trifolii, D.isaea* and *O. pallipes* recorded the highest average population occurrence on sour clover. On the other hand, The percentage of parasitism of *D.isaea* and Infested leaflets recorded the highest occurrence on sour clover. While The percentage of parasitism of *O. pallipes* was the highest on nightshade. It could be seen that, weed host plants can play a vital role in keeping the populations of *L. trifolii* and its parasitic fauna in the absence of their economic favorite host plants.

Key words: *D. isaea* , *O. pallipes* , *weeds* , *L. trifolii* .

Introduction \

The leaf mining habit has been developed by a group of over 10,000 species of holometabolous insects, concentrated in four orders: *Diptera, Coleoptera, Hymenoptera,* and *Lepidoptera* **[9].** Worldwide, more than 300 species of parasitoids are associated with Agromyzids, and, more than 80 species are known to attack *Liriomyza spp***. [27].** Leafminer adults have developed resistance, going from being secondary pests to becoming primary pests **[7].** Genus *Liriomyza* has more than 300 species, 20 of which are considered economically important. Among them, *L. trifolii* Burgess (Diptera: Agromyzidae), the American serpentine leaf miner is known as one of the most serious pests of many vegetable and horticultural crops worldwide **[34].** Eulophid hymenopterous were the commonest and widely distributed parasitoids such as *D. isaea,* and *Pediobius metalicus* attack *Liriomyza* species such as *L. sativa, L. bryoniae,* and *L. trifoli.* which are very important that attack crops and vegetables in several regions **[29].** *L. trifolii* is perhaps best known as a pest of chrysanthemums and celery, but it has a wide host range. For example, **[35]** recorded 55 hosts from Florida, including bean, beet, carrot, celery, cucumber, eggplant, lettuce, melon, onion, pea, pepper, potato, squash, and tomato. Flower crops that are readily infested and which are known to facilitate spread of this pest include chrysanthemum, gerbera, gypsophila, and marigold. Numerous broad-leaved weed species support larval growth. **Schuster *et al*. [32]** found that the nightshade *Solanum americanum*; Spanish needles, *Bidens alba*; and pilewort, *Erechtites hieracifolia*; were suitable weed hosts in Florida. Parasitic wasps of the families *Braconidae, Eulophidae,* and *Pteromalidae* are important in natural control, and in the absence of insecticides usually keep this insect at low levels of abundance. At least 14 parasitoid species are known from Florida alone.

Species of *Eulophidae* such as *Diglyphus begina* (Ashmead), *Diglyphus intermedius* (Girault), *Diglyphus pulchripes*, and *Chrysocharis parksi* Crawford are generally found to be most important in studies conducted in North America, although their relative importance varies geographically and temporally **[22].** The Eulophids, *D. isaea*, *D. begini*, *D. intermedius* and *D. carlylei* are solitary larval ectoparasitoids of Dipteran leaf miners occurring in North American **[17]**. The female *Diglyphus* adult lays one or more eggs attached to the leaf miner late instar larvae **[11,21].** The parasitoid larvae hatch out of eggs and feed on the leaf miner larva externally, eventually killing the leaf miner larvae. The parasitoid larva develops through three instars and pupates in the mine before emerging as an adult. Development time is temperature dependent. *D. isaea* is one of the most effective 17 biological control agents against *Liriomyza* leaf miner in greenhouse **[5]** and, open fields **[10,11,13]**. *D. isaea* takes about 10 days to complete egg to adult development on *L. trifolii* and *L. huidobrensis* at 25°C **[4]. Zoebisch and Schuster [38]** studied the Ovipositional preference, fecundity, and development of *L. trifolii* under laboratory conditions on tomatoes, American black nightshade, common beggar tick, and downy ground cherry. They found that Fecundity of females was significantly greater on foliage of tomatoes and nightshade than on foliage of beggar-tick and ground cherry. *L. trifolii* reared from nightshade were more fecund on tomato foliage than females reared from beggar-tick. Larval and pupal development times in tomato foliage were similar regardless of the origin of the females. Development for larvae that hatched from eggs laid by tomato-reared females was significantly shorter in foliage of nightshade and tomatoes. A total of 646 *Agromyzid* specimens were reared, representing eight genera and 24 species. The most abundant species were *Chromatomyia horticola* (Goureau), *Phytomyza lappae* Goureau, *Phytomyza ranunculi* Schrank, *Phytomyza plantaginis* Robineau-Desvoidy, *L. trifolii* (Burgess), and *Ophiomyia pulicaria* (Meigen). *C. horticola* was the dominant species accounting for 35.76% of adults reared. Agromyzids mined 25 plant species belonging to nine families **[19].** *D. isaea* is the most common synovigenic idiobiont parasitoids of Agromyzid leaf miner species, parasitizing host larvae and feeding on host hemolymph **[6,37].** *D. isaea* is a biparental, arrhenotokous ectoparasitoid, and a non-concurrent and destructive host feeder **[16].** In various parasitoids, particularly ecto-parasitoids like *D. isaea*, induced host nonreproductive mortality, through host feeding and stinging, have been reported to be very critical in parasitoids' performance measurement **[1,18].** Moreover**,** *Diglyphus* is a cosmopolitan genus that currently includes 39 species as ahost range, and 14 of these are recorded from Europe **[26].**

Nicoli and Pitrelli, [25] reported that, Besides the mortality induced by larval parasitic activity, *D. isaea* females can also cause host mortality by host feeding behavior. Females of the parasitoid sting host larvae (normally, 1st and 2nd instar larvae) with their ovipositor, feed on the body fluids that come out and kill them**.** On the other hand, *D. isaea* can be seasonal inoculative release, of which control on pest population is obtained over many pest generations and can have a long-term impact if crops are grown for a season-long period **[37]**. **Musundire *et al.*, [24]** studied the effect of Host plant species on the behavior and attributes of the parasitoid *D. isaea* and found that, %parasitism, host feeding, and sex ratios of *D. isaea* on *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard, and *L. trifolii* larvae reared on *Phaseolus vulgaris* L., *Pisum sativum* L., *Solanum lycopersicum* L., and *Vicia faba* L, in no-choice tests, *L. huidobrensis* had the highest rate of parasitism when reared on *P. vulgaris* (46%), *L. sativae* when reared on *V. faba* (59%) and *P. vulgaris* (59%), and *L. trifolii* when reared on *S. lycopersicum* (68%).They concluded that, the efficacy of the parasitoids depends on the ability to locate suitably sized hosts within crop habitats to kill *Liriomyza* larvae through host feeding or parasitism **[28]**. Various attributes of host plant species can affect the biological characters of the parasitoid such as mate location **[20]**, oviposition **[30]**, fecundity **[33]**, rate of parasitism **[31]**, survival and sex ratio **[14],** and body size **[31]. Iqbal *et al.* [15]** evaluated the response of pupae and adults of *L. trifolii* and *L. sativae* to acute cold exposures. *L. trifolii* exhibited stronger cold tolerance than *L. sativae* they concluded that, subtle differences in RCH and basal cold tolerance impact the competitiveness of the two leaf miners. This research was established to assess seasonal abundance of leaf miner *L. trifolii* and the larval ectoparasitoid *D. isaea*. This study will help pest management strategies and enhance the effectiveness of IPM in leaf miner control against the serpentine leaf miner *L. trifolii* in, Libya.

Materials and Methods \

The present study was carried out in Alzawia region. Blackberry nightshade (*Solanum nigrum*) was studied as a summer and Sour clover (*Melilotus indicus*) as a winter host plants during the growing seasons 2020 and 2021. Hundred randomized leaflets were collected weekly from every host plant. Samples were kept in plastic bags and transferred to be examined in the laboratory. Number of infested leaflets, number of living *L. trifolii* larvae, immature stages of the larval ectoparasitoid *D. isaea* were counted and recorded. Infested leaves were dissected under strew binuclear microscope (64X). Examined leaves were arranged over a moistened filter paper in Petri dishes (12 by 1.5 cm). Filter papers were remoistened daily or when necessary to prevent leaflets from drying till the emergence of *L. trifolii* or the larval pupal endoparasitoid *O.pallipes* which were counted and recorded.

Results and discussion \

Seasonal abundance of *L.trifolii* On nightshade: *L. trifolii* larvae recorded low numbers in the beginning of the season in early December, then the population increased recording three peaks of abundance (11, 19 and, 29 individuals/ 100 infested leaflets) occurred in 23th of December, 24th of March and, 5th of May respectively. On the other hand, the number of infested leaflets reached 61/ 100 leaflets).

On Sour clover: *L. trifolii* larvae recorded low numbers in the beginning of the season in early December, then the population increased recording three peaks of abundance (58, 119 and, 38 individuals/ 100 infested leaflets) occurred in 30th of December, 17th of February and, 31th of March respectively. On the other hand, the number of infested leaflets reached 93/ 100 leaflets).

From the previous results, it could be cleared that, *L. trifolii* showed high preference towards sour clover compared with nightshade although the total foliar area of nightshade is larger than that of sour clover. A possible explanation is that, a more preference alternative host plants are available for *L. trifolii* including sour clover among the winter host plants. **Elkhouly *et al*., [12]** studied the population dynamics of *L. trifolii* on four winter host plants [broad bean (*Vecia faba*), pea (*Pisum sativum*), fenugreek (*Trigonella finum gradum*) and snow thistle (*Sonchus oleraceus*)] and concluded that, larvae of *L. trifolii* showed (3- 4) peaks of abundance on all studied host plants, recording low populations in the beginning of the growing season, then reaching its highest peaks in February and March, then the population decreased towards the end of the growing season. *L. trifolii* showed high preference towards broad bean followed by pea, snow thistle and, fenugreek respectively. Moreover, the host plant leaflet size , the foliar plant density and plant morphology could play an important role of the infestation by *L. trifolii*. These results are in line with those of ours. **Murphy and La Salle, [23]** explained the variations of leaf miner densities on different host plants and concluded that, a certain leaf miner could be considered a pests is the increase in monocultivations. Many parasitoids have preference for specific plants. Therefore, if the only crop present is not attractive for the parasitoids, leaf miners may escape parasitoidism in this environment. This finding is supporting our results because a considerable parasitic stress occurring on the studied weed host plants which were growing in the presence of a various preference widespread economic host plants. Finally, the increase of extensive horticulture, lack of weed control and plant commercialization without appropriate quarantine controls has also favored the expansion of leaf miner pest distribution. These results are also in line with those of **[19]** who studied the natural occurrence of the Agromyzed Diptera on the weed canopy in three site locations in Italy and found that, six *Liriomyza* species totally accounted for 14.09% of the emerged Agromyzids. The highly polyphagous *L. bryoniae* (accounting for 2.17%), *Liriomyza strigata* (3.25%), and *L. trifolii* (5.73%) were present in every site. Even though potential host plants for these three species have been recorded in 30 botanical families.

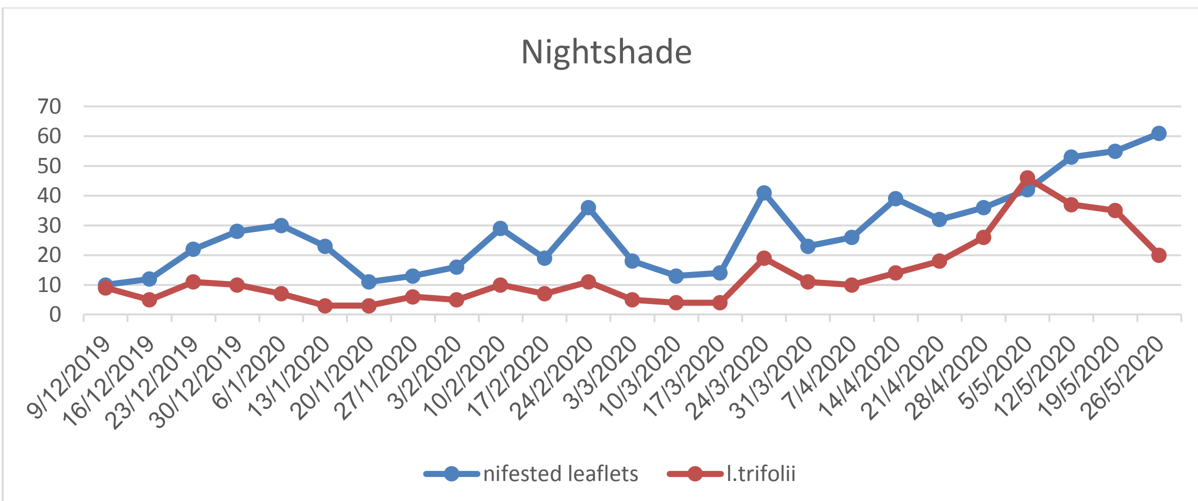

Figure (1) Population densities of the serpentine leaf miner *L. trifolii* and numbers of infested leaflets on nightshade.

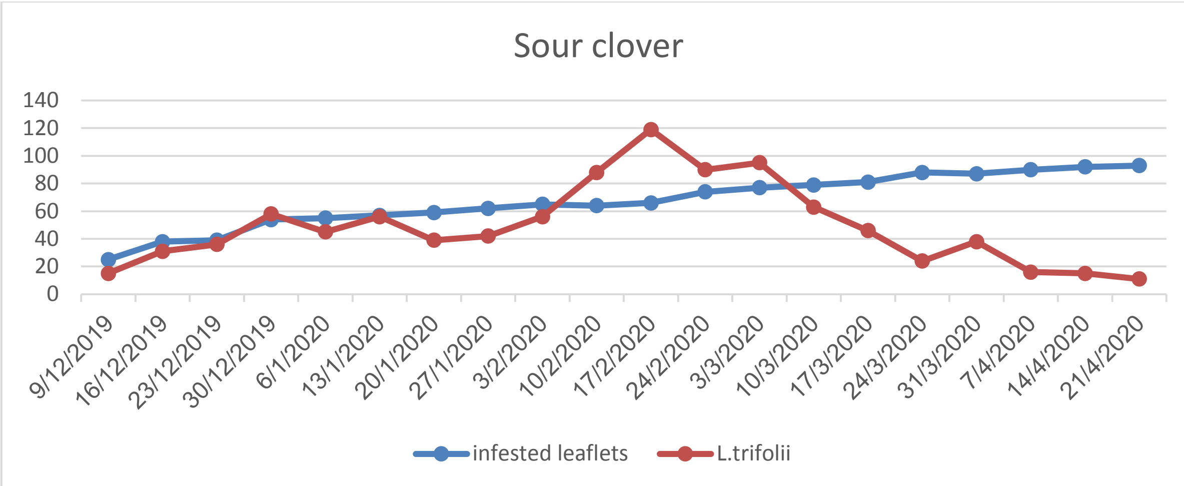

Figure (2) Population densities of the serpentine leaf miner *L. trifolii* and numbers of infested leaflets on sour clover.

Seasonal abundance of *D. isaea*: On Sour clover *D. isaea* recorded low numbers in the beginning of the season in early December, then the population increased recording three peaks of abundance (27, 55 and, 66 individuals/ 100 infested leaflets) occurred in 30th of December, 17th of February and, 3rd of March respectively Fig (3). On the other hand, the percentage of parasitism of *D. isaea* ranged between (12.9% - 91.3%) figure (5). Synchronization between the larval ectoparasitoid *D. isaea* and its insect host the serpentine leaf miner *L. trifolii* was very good on sour clover with (r = 0.75) figure (4) and table (2). The present data shows that, *D. isaea* recorded a relatively high population on sour clover compared with night shade, according to our studies on *D. isaea*_for several seasons and hosts, *D. isaea* and its insect host occurring in higher densities on the winter than summer seasons. The relatively high population on sour clover could be explained by the absence of the more preferred host plants of its insect host. These results could be explained by those of **[10]** who concluded that *D. isaea* showed high preference towards broad bean than fenugreek and sour clover during three seasons of the study. Moreover, **[2]** reported that, the average monthly rates of parasitism were the highest on broad bean followed by lentil and chickpea during the three successive seasons. These results are also in agreement with those of **[3,8].**

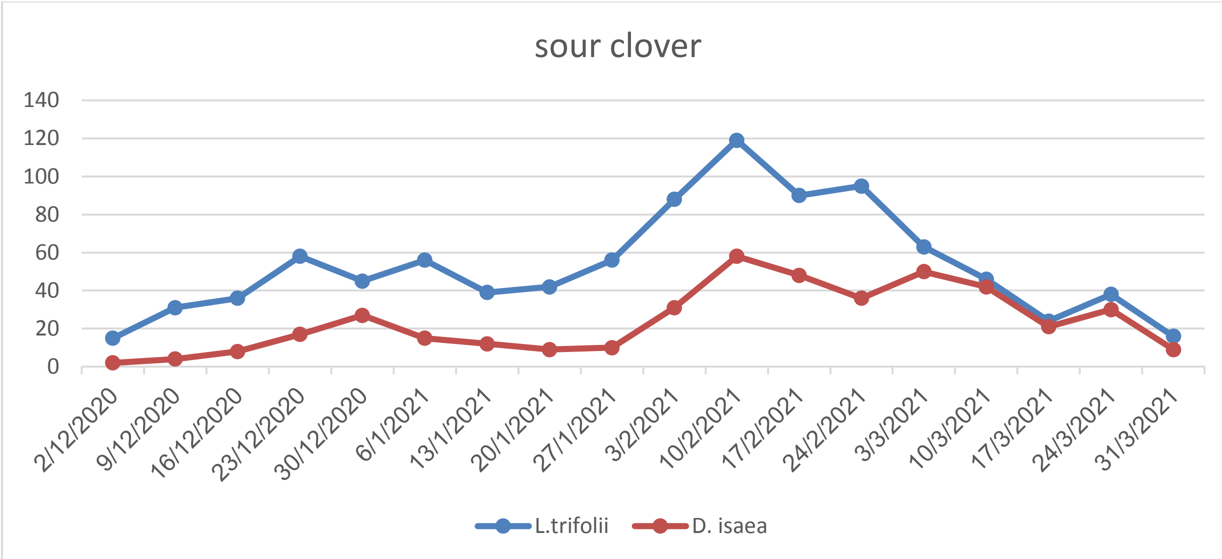

Figure (3) Population densities of the serpentine leaf miner *L. trifolii* and the larval ectoparasitoid *D. isaea* on sour clover.

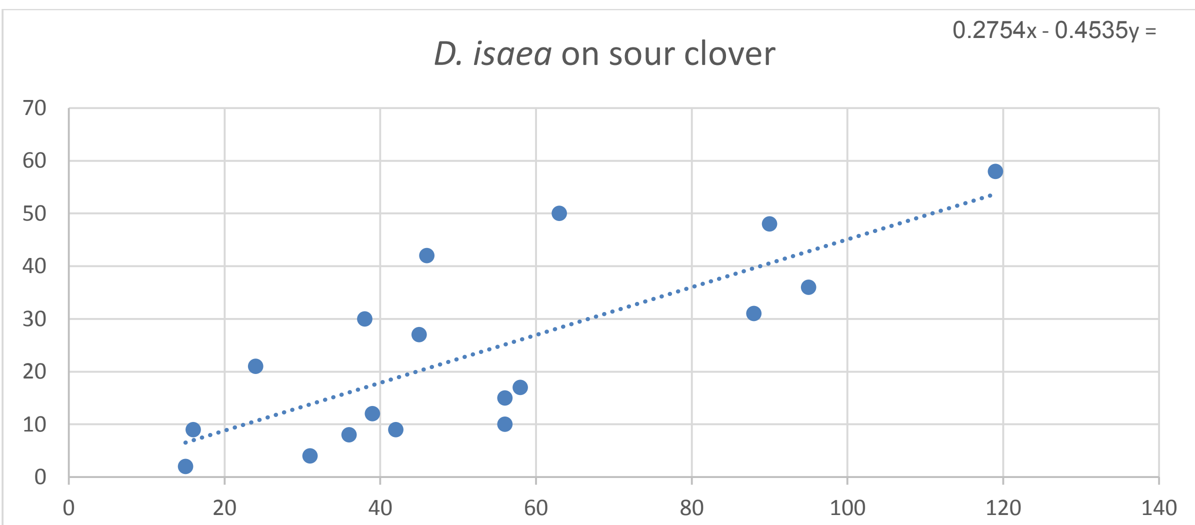

Figure (4) Regression analysis of *D. isaea* population vs. *L. trifolii* population on sour clover.

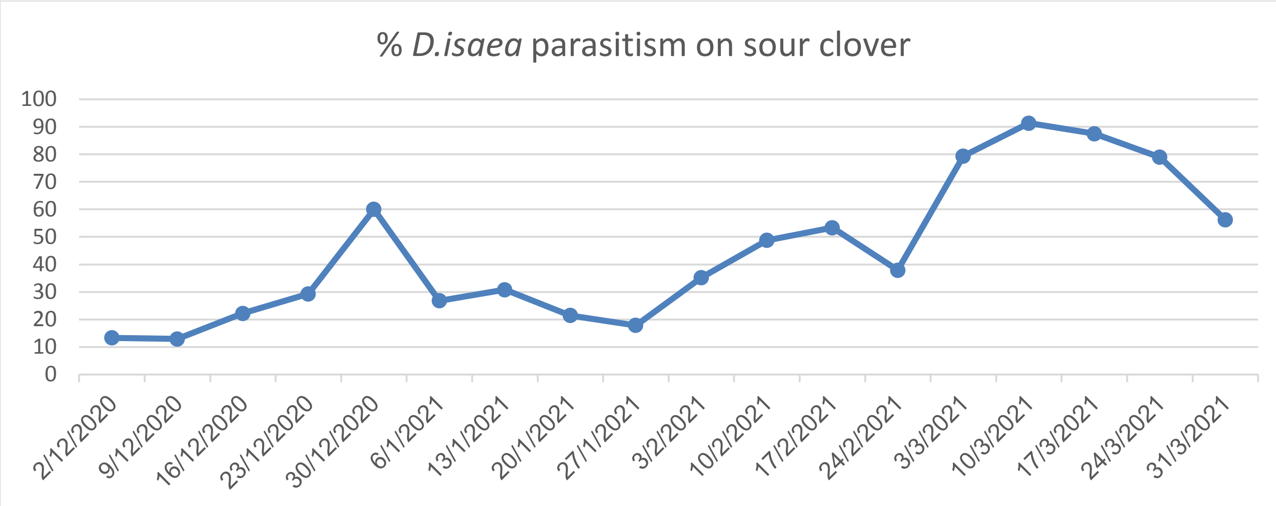

Figure (5) percentages of parasitism of the larval ectoparasitoid *D. isaea* on nightshade on the serpentine leaf miner *L. trifolii* on sour clover.

Seasonal abundance of *D. isaea:* On nightshade *D. isaea* recorded low numbers in the beginning of the season in early December, then the population slightly increased recording three small peaks of abundance (9, 11 and, 19 individuals/ 100 infested leaflets) occurred in 30th of December, 19th of March and, 5th of May respectively Fig (6). On the other hand, the percentage of parasitism by *D. isaea* ranged between (0.0% - 57.9%) figure (8). Synchronization between the larval ectoparasitoid *D. isaea* and its insect host the serpentine leaf miner *L. trifolii* was very good on night shade with (r = 0.91) figure (7) and table (2). The previous data shows that, *D. isaea* recorded low population on night shade. The morphological characters of the plant and the low densities of the insect host on the summer host plants may be a comprehensive reason. Similar results on tomatoes from the same plant family were found by **[11]** who concluded that, the average monthly rates of parasitism were the highest on cowpea followed by kidney bean and tomatoes during three successive seasons. Moreover, *D. isaea* has been reported to host feed, lay eggs on hosts (parasitize), and allocate sex based on the quality of larval hosts **[28].** Therefore, host feeding behavior, parasitism, and sex allocation of parasitoids can indirectly be influenced by the hostplant, *Liriomyza* species, and the larval size of the host **[31].** Our results could be supported by those of **[24]** who suggest that the rate of parasitism may not necessarily depend on insect host size alone but also on host plant characteristics. *L. huidobrensis*, was the largest of the three studied *Liriomyza* species did not have the highest rate of parasitism in the no-choice and choice experiments. In addition, using the same host plant species and cultivars, *V. faba* seemed a more suitable host plant for *L. trifolii* than *P. vulgaris* or *S. lycopersicum* based on adult size as a measure of performance, whereas parasitism was highest on *S. lycopersicum*.

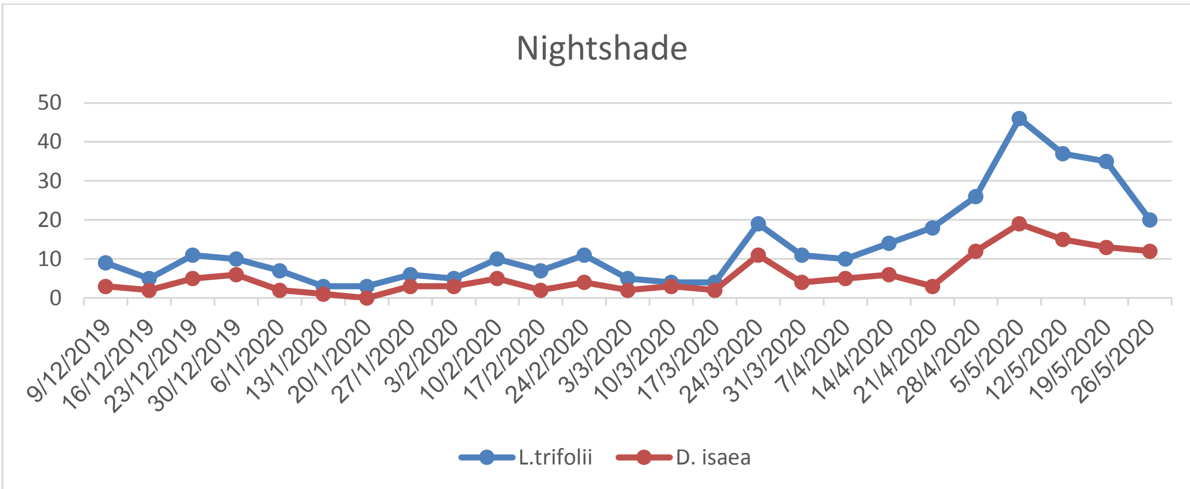

Figure (6) Population densities of the serpentine leaf miner *L. trifolii* and the larval ectoparasitoid *D. isaea* on nightshade.

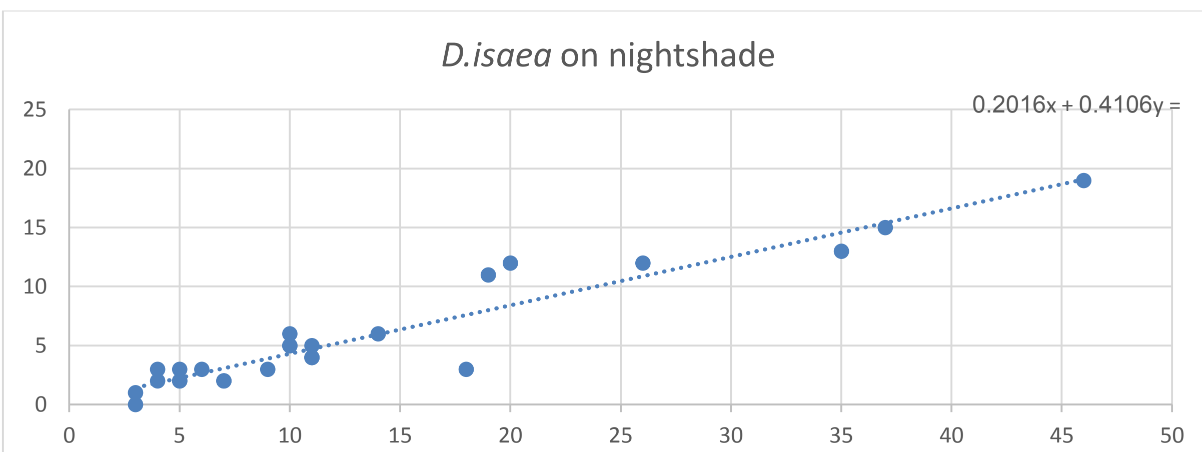

Figure (7) Regression analysis of *D.isaea* population vs. *L. trifolii* population on nightshade.

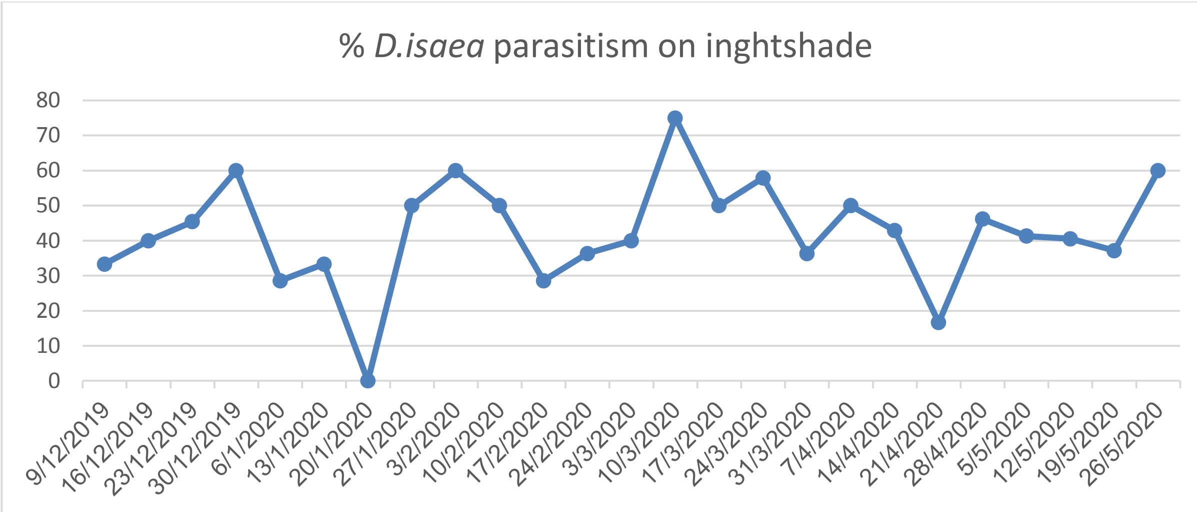

Figure (8) percentages of parasitism of the larval ectoparasitoid *D. isaea* on nightshade on the serpentine leaf miner *L. trifolii* on nightshade.

Seasonal abundance of *O.pallipes* : On sour clover The larval pupal endoparasitoid *O.pallipes* recorded low numbers in the beginning of the season in early December, then the population increased recording three peaks of abundance (15, 25 and, 14 individuals/ 100 infested leaflets) occurred in 23th of December, 3rd of February and, 10th of March respectively Fig (9). On the other hand, the percentage of parasitism of *O.pallipes* ranged between (0.0% - 46.8%) figure (11). Synchronization between the larval pupal endoparasitoid *O.pallipes* and its insect host the serpentine leaf miner *L. trifolii* was low on sour clover with (r = 0.39) figure (10) and table (2). The present data shows that, *O.pallipes* recorded a slightly high population on sour clover compared with night shade, according to our studies on *O.pallipes* for several seasons and hosts, *O.pallipes* preferring winter host plants than summer ones. The low abundance of *O.pallipes* could be explained by the high competition and parasitic the capacity of the larval ectoparasitoid *D. isaea* . These results could be explained by those of **[10]** who concluded that, *O.pallipes* showed high preference towards broad bean than fenugreek and sour clover during three seasons of the study. These results are also in agreement with those of **[3,11]**.

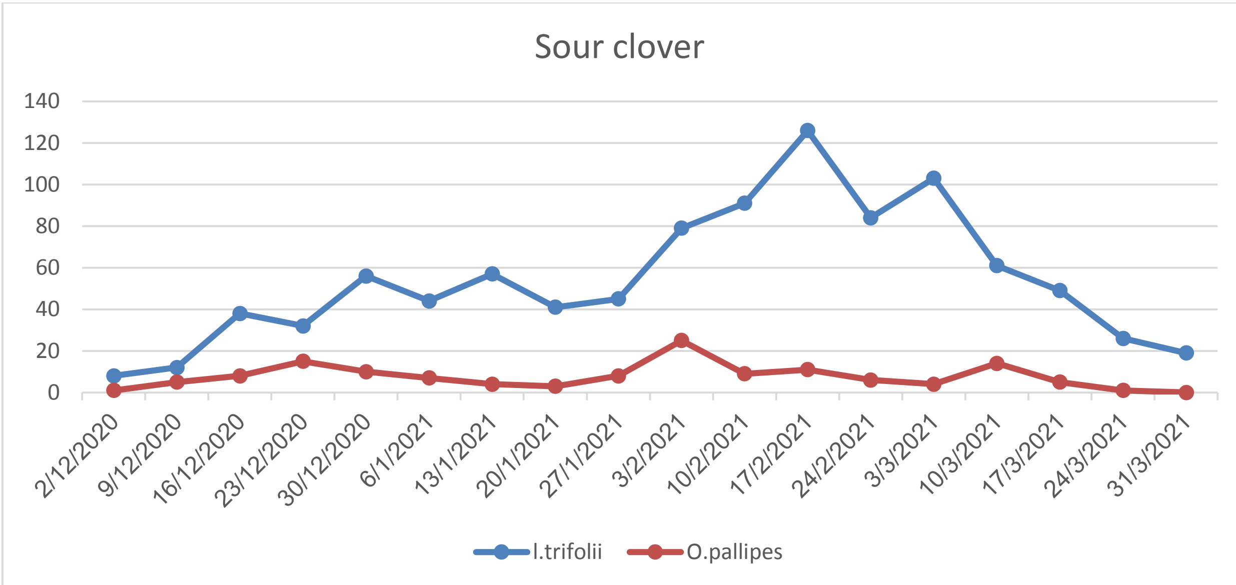

Figure (9) Population densities of the serpentine leaf miner *L. trifolii* and the larval pupal endoparasitoid *O.pallipes* on sour clover.

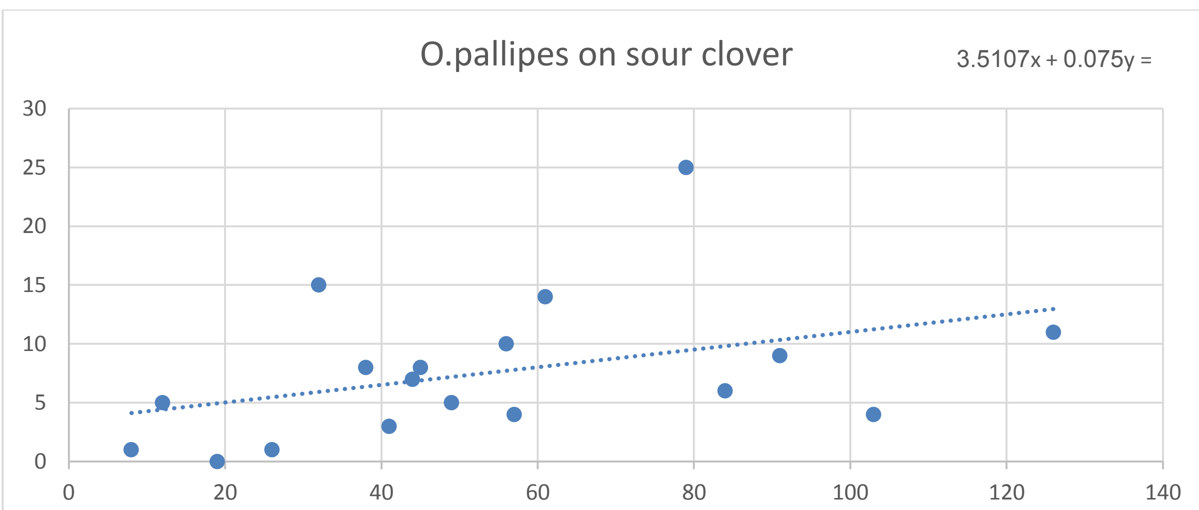

Figure (10) Regression analysis of *O.pallipes* population vs. *L. trifolii* population on sour clover.

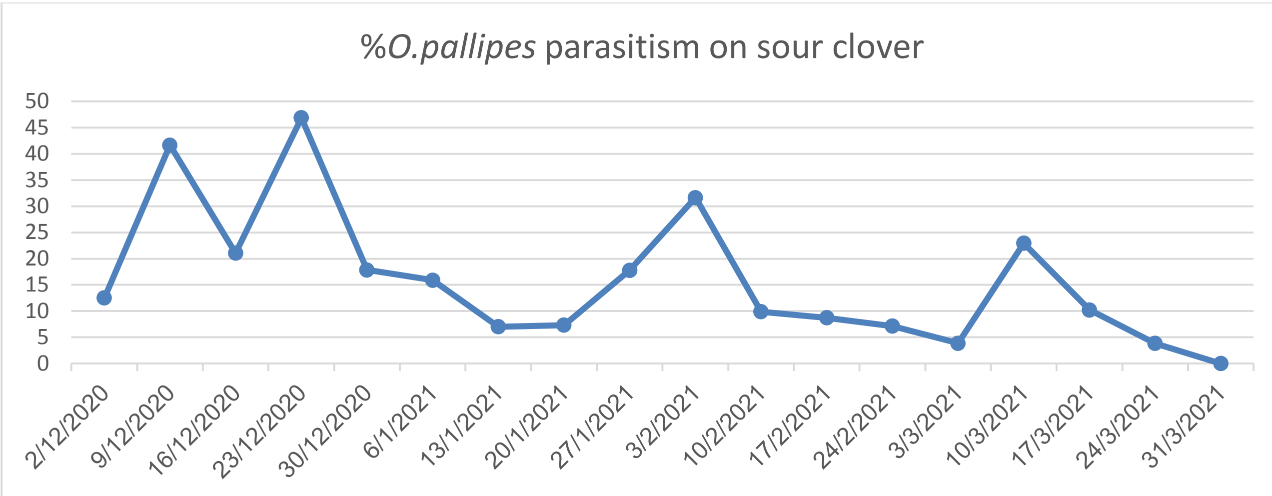

Figure (11) percentages of parasitism of the larval pupal endoparasitoid *O.pallipes* on the serpentine leaf miner *L. trifolii* on sour clover.

Seasonal abundance of *O.pallipes* : On Nightshade: *O.pallipes* recorded low numbers in the beginning of the season in early December, then the population slightly increased recording three small peaks of abundance (8, 10 and, 12 individuals/ 100 infested leaflets) occurred in 10th of October , 21th of April and, 19th of May respectively Fig (12). On the other hand, the percentage of parasitism by *O.pallipes* ranged between (0.0% - 72.7%) figure (14). Synchronization between the larval ectoparasitoid *O.pallipes* and its insect host the serpentine leaf miner *L. trifolii* was moderate on night shade with (r = 0.52) figure (13) and table (2). The present data shows that, *O.pallipes* recorded a very low population on night shade. The low abundance of *O.pallipes* could be explained by the high competition and the parasitic capacity of the larval ectoparasitoid *D. isaea* . These results could be explained by those of **[3,10,11].**

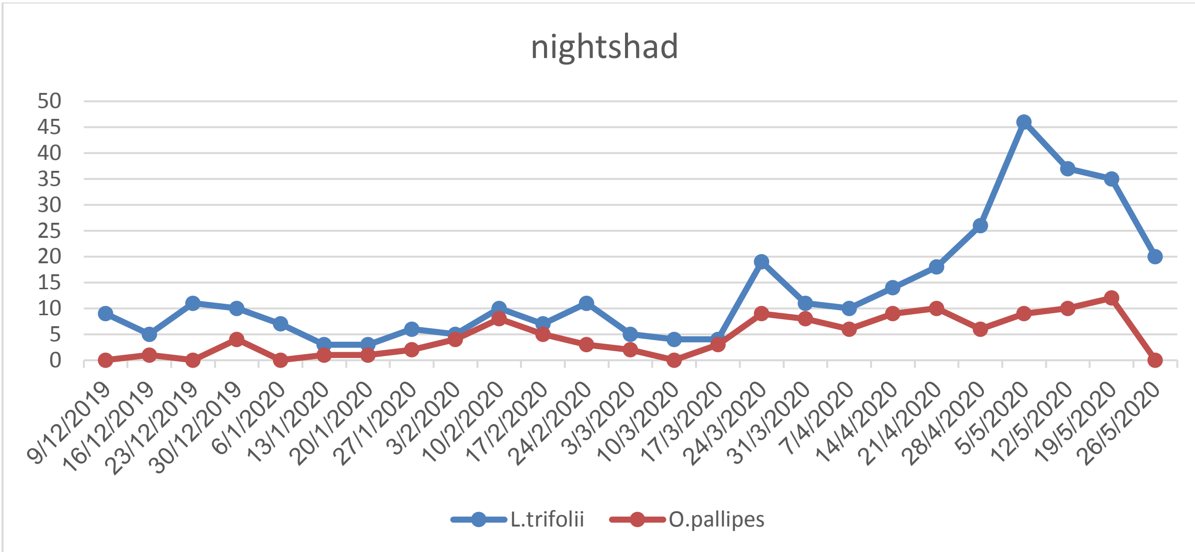

Figure (12) Population densities of the serpentine leaf miner *L. trifolii* and the larval pupal endoparasitoid *O. pallipes* on nightshade.

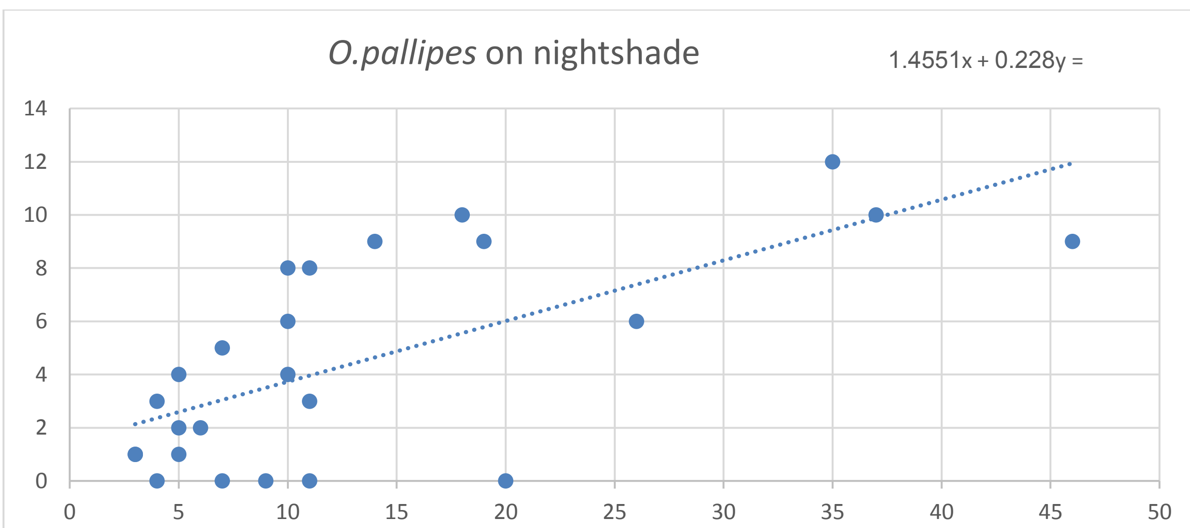

Figure (13) Regression analysis of *O.pallipes* population vs. *L. trifolii* population on nightshade.

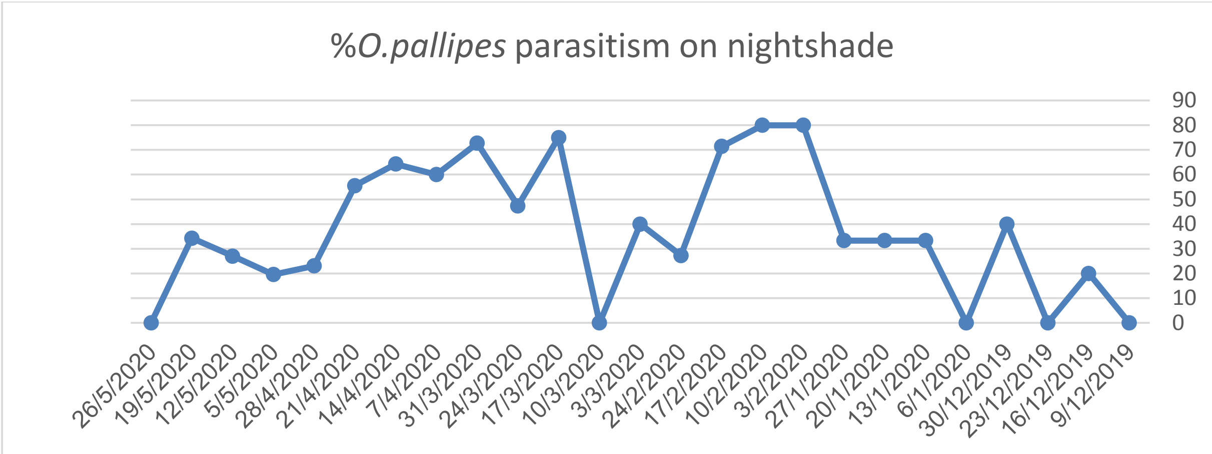

Figure (14) percentages of parasitism of the larval pupal endoparasitoid *O.pallipes* on the serpentine leaf miner *L. trifolii* on nightshade.

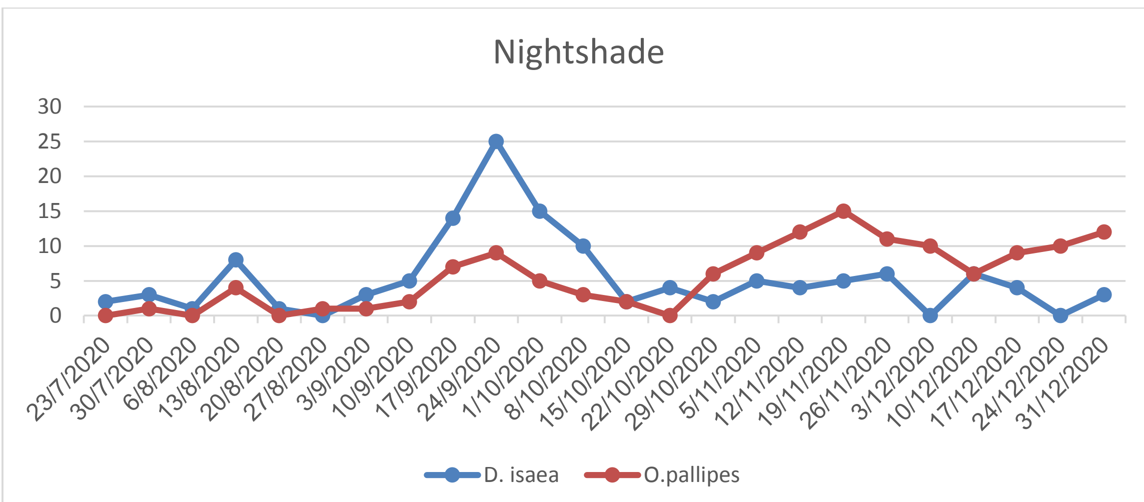

Figure (15) Population densities of the larval ectoparasitoid *D. isaea* and the larval pupal endoparasitoid *O. pallipes* on nightshade.

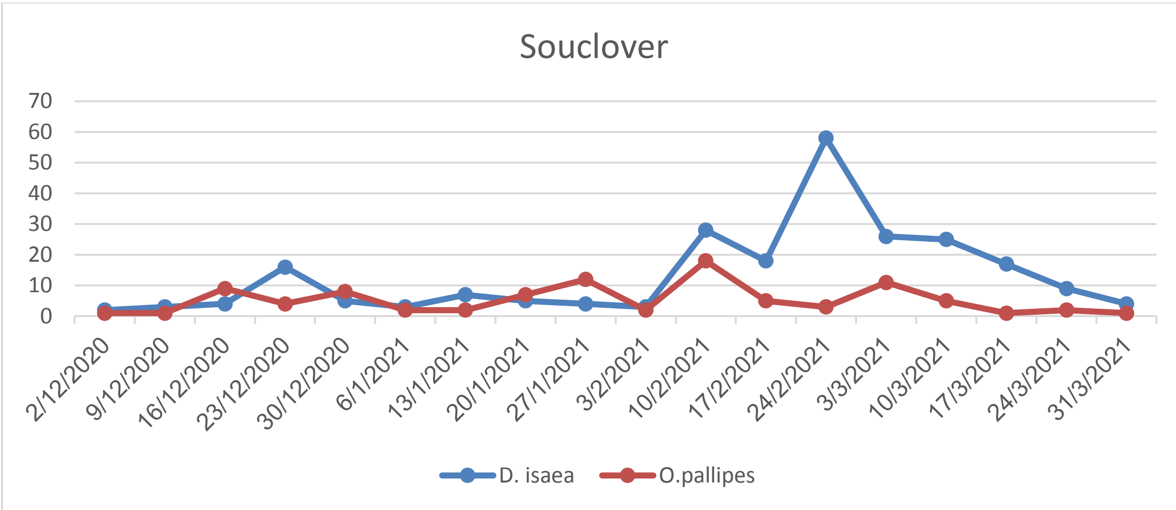

Figure (16) Population densities of the larval ectoparasitoid *D. isaea* and the larval pupal endoparasitoid *O. pallipes* on sour clover .

Table (1) Total average numbers numbers ± SD of *L. trifolii, D.isaea* , *O. pallipes and* Infested leaflets during the period of the study

Abundance	**Host plant**	
	Nightshade (*Solanum nigrum*) Mean ± SD	Sour clover (*Melilotus indicus*). Mean ± SD
L. trifolii	25.84 ± 11.46	91.61 ± 30.07
D.isaea	10.76 ± 5.06	23.83 ± 17.25
% parasitism (*D.isaea)*	42.38 ± 16.61	44.63± 12.90
O. pallipes	4.52 ± 3.38	8.95 ± 4.79
% parasitism (*O. pallipes)*	37.50 ± 28.76	15.90 ± 12.96
Infested leaflets	54.00 ± 14.44	128.09 ± 19.28

Data presented in table (2) shows that, *L. trifolii, D.isaea* and *O. pallipes* recorded the highest average population occurrence on sour clover. On the other hand, % parasitism (*D.isaea*) and Infested leaflets recorded the highest occurrence on sour clover. While % parasitism of (*O. pallipes*) was the highest on nightshade

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Table (2) Correlation coefficient values and regression equations between *D. isaea* and *L. trifolii* populations on two weed host plants.

Parasites	**Host plant**			
	Nightshade (*Solanum nigrum*) Mean ± SD		Sour clover (*Melilotus indicus*). Mean ± SD	
	Regression equation	r	Regression equation	r
D.isaea	y = 0.4106x + 0.2016	0.91	y = 0.4535x - 0.2754	0.75
O. pallipes	y = 0.228x + 1.4551	0.52	y = 0.075x + 3.5107	0.39

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الكثافات العددية لحشرة نافقة أوراق الفول و الطفيليات المصاحبة لها *Diglyphus isaea* و *Opius pallipes* على اثنين من العوائل العشبية في منطقة الزاوية.

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الملخص /

استهدفت هذه الدراسة تقدير الكثافات الموسمية لحشرة نافقة أوراق الفول *Liriomyza trifolii* واثنين من الطفيليات الغشائية الاجنحة المصاحبة لها هما النوع الخارجي *D. isaea* والنوع الداخلي *O. pallipes* على نوعين من النباتات العشبية غير الاقتصادية بمنطقة الزاوية هما نبات عنب الذيب (*Solanum nigrum*) كعائل عشبي صيفي ونبات الحندقوق (*Melilotus indicus*) كعائل عشبي شتوي . جمعت العينات من مناطق مختلفة بجمع 100 ورقة نباتية عشوائيا من كل عائل نباتي والتي وضعت في أكياس بلاستكية ثم حفظت ونقلت الى المعمل لحين فحصها . تم عد الأوراق المصابة وكذلك يرقات الحشرة العائل والطفيليات المصاحبة لها و كانت النتائج كالتالي : سجل تعداد الحشرة ثلاث ذروات على عنب الذيب بواقع (11 , 19 و 29 يرقة / 100 وريقة) وعلى الحندقوق بواقع (58, 119 و 38 يرقة / 100 وريقة) . اما الطفيل الخارجي D. isaea فقد سجل ثلاث ذروات على الحندقوق بواقع (27 , 55 و 66 يرقة / 100 وريقة) وعلى عنب الذيب (9, 11 و19 يرقة / 100 وريقة) كما ترواحت النسبة المؤية للتطفل بين (12.9- 91.3 %) على الحندقوق و (0.0 -59.7 %) على عنب الذيب . اما الطفيل الداخلي *O. pallipes* فقد سجل ثلاث ذروات على الحندقوق بواقع (15 , 25 و 14 يرقة / 100 وريقة) وعلى عنب الذيب بواقع (8 , 10 و 12 يرقة / 100 وريقة) كما ترواحت النسبة المؤية للتطفل بين (0.0 – 46.8%) على الحندقوق و (0.0 -72.7 %) على عنب الذيب. كانت قيمة معامل الارتباط r بين الطفيل الخارجي *D. isaea* وعائله الحشري *L. trifolii* (0.91 و 0.75) على عنب الذيب والحندقوق على الترتيب فيما كانت قيمة معامل الارتباط بين الطفيل الداخلي *O. pallipes* وعائله الحشري هي (0.52 و 0.39)) على عنب الذيب والحندقوق على الترتيب .