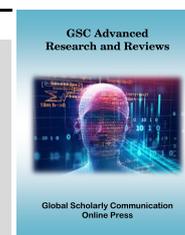


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(RESEARCH ARTICLE)



Seasonal abundance of the serpentine leaf miner *Liriomyza trifolii* and the bio efficiency of the larval ectoparasitoid *Diglyphus isaea* on cowpea in Alejelat region, Libya

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Abstract

The American serpentine leaf miner, *L. trifolii* (Burgess), is one of the most problematic insect pest species attacking a large number of vegetable crops, weeds, and ornamentals. The present study aimed to determine the natural abundance of the larval parasitoid *D. isaea* and the serpentine leaf miner *L. trifolii*. This study was carried out in Alejelat region during two summer seasons of 2018 and 2019 on cowpea. The highest monthly total numbers of *L. trifolii* larvae, *D. isaea*, host fed, and total killed larvae that occurred on August recording (1187, 535, 209, and 744), respectively. While the monthly average numbers recorded (862.5 ± 289.8 , 341.7 ± 139.5 , 123.75 ± 57.3 , and 465.5 ± 194.8) for the former parameters during season 2018, respectively. On the other hand, the highest monthly total numbers of *L. trifolii* larvae, *D. isaea*, host fed, and the total killed larvae occurred on August recording (3211, 1264, 580, and 1844), respectively. While the monthly average numbers recorded (1517.5 ± 1299.6 , 632 ± 438.5 , 285.5 ± 201.8 , and 917.5 ± 640.2) for the former parameters during season 2019, respectively. The percentage of total mortality reached 91.8%, and 87.6 in the first and the second season, respectively.

Keywords: Abundance; Cowpea; Leaf miner; *L. trifolii*; *D. isaea*; Alejelat; Libya

1. Introduction

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 [1]. The leaf miner *L. trifolii* has become an important pest in Ornamentals and vegetables. chemical control of *L. trifolii* is problematic due to the rapid development of resistance against insecticides. Application of biological control may help to overcome both the difficulty of control of *L. trifolii* and integration problems with other biological pest control methods [2]. In their native areas of South America, *Liriomyza* species are naturally controlled by a complex of more than 60 parasitoid species without any lethal interspecific competition occurring among them [3]. In Asia 41 species of parasitoids in four different families were found. In general, and under natural conditions, parasitism is usually low early in crop development and gradually increases as the crop matures [4]. *Eulophid hymenopterous* was the commonest and widely distributed parasitoids such as *D. isaea*, *Pediobius metallicus* attack *Liriomyza* species such as *L. sativa*, *L. bryoniae*, and, *L. trifoli*. which are very important that attack crops and vegetables in several regions [5].

The Eulophids, *D. isaea*, *D. begini*, *D. intermedius*, and *D. carlylei* are solitary larval ectoparasitoids of Dipteran leaf miners occurring in North America [6]. The adult *Diglyphus* female lays one or more eggs attached to the leaf miner late instar

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larvae [7]. 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 of *Liriomyza* leaf miner in greenhouses [8], and open fields [9, 10, 11]. *D. isaea* takes about 10 days to complete maturation from egg to adult development on *L. trifolii* and *L. huidobrensis* at 25°C [12].

D. isaea is the most common synovigenic idiobiont parasitoids of Agromyzid leaf miner species, parasitizing host larvae and feeding on host hemolymph [13, 14]. *D. isaea* is a biparental, arrhenotokous ectoparasitoid, and a non-concurrent and destructive host feeder [15]. In various parasitoids, and particularly ectoparasitoids like *D. isaea*, induced host non-reproductive mortality, through host feeding and stinging, has been reported to be very critical in parasitoids' performance measurement [16, 17].

Nicoli and Pitrelli 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 [18]. 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 [19].

This research was established to assess the 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 measurements against the serpentine leaf miner *L. trifolii* in, Libya.

2. Material and methods

The present study was carried out in Alejelat region. Cowpea (*Vigna unguiculate*) was selected as a summer host plant during the growing seasons of 2018 and 2019. The experimental field was about 400m² (divided into 4equal plots). Hundred leaflets infested with *L. trifolii* (25 from every plot) were collected. Samples were kept in plastic bags and transferred to be examined in the laboratory. Number of living *L. trifolii* larvae, immature stages of the larval ectoparasitoid *D. isaea*, and number of killed larvae according to feeding (no oviposition) were counted and recorded. 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. Infested leaves were dissected under a strew binuclear microscope (64X). Normal agricultural practices of fertilizing and irrigation were followed and no chemical control measurements were applied. Samples took place from the appearance of the emergence of the first leaves and continued weekly until harvest.

3. Results

Results obtained in fig (1) showed the seasonal occurrence of *L. trifolii*, *D. isaea*, %of parasitism, host fed, the total killed larvae, and % of the total killed larvae during the growing season 2018.

3.1. Seasonal abundance of *L. trifolii*

L. trifolii larvae recorded low numbers at the beginning of the season in early June, then the population increased recording three peaks of abundance (256, 282, and 368 individuals/100 infested leaflets) that occurred on the 15th of June, 13th of July, and 24th of August, respectively.

3.2. Seasonal abundance of *D. isaea*

D. isaea recorded low numbers in the beginning of the season in early June, then the population increased recording three peaks of abundance (109, 129, and 169 individuals/100 infested leaflets) that occurred on the 22th of June, 13th of July, and 24th of August, respectively.

3.3. Seasonal occurrence of host fed larvae

Host fed larvae (killed larvae according to the parasitoid female feeding with no oviposition) recorded three peaks of occurrence (34, 33, and 61 individuals/100 infested leaflets) that occurred on the 22th of June, 27th of July, and 24th of August, respectively.

3.4. Seasonal occurrence of % parasitism

Seasonal occurrence of % parasitism recorded three peaks of occurrence (47.6, 45.7, and 63.6) that occurred on the 22th of June, 13th of July, and 17th of August, respectively.

3.5. Seasonal occurrence of the total killed larvae

Seasonal occurrence of the total killed larvae recorded three peaks of occurrence 143, 145, and 230 individuals/100 infested leaflets) that occurred on the 22th of June, 13th of July, and 24th of August, respectively.

3.6. Seasonal occurrence of % the total killed larvae

Seasonal occurrence of % the total killed larvae recorded three peaks of occurrence (62.4, 91.8, and 71.3) that occurred on the 22th of June, 27th of July, and 21th of September, respectively.

Table 1 Monthly total numbers of *L. trifolii*, *D. isaea*, host fed and the total killed larvae during the growing season Summer 2018.

Months	<i>L. trifolii</i>	<i>D. isaea</i>	Host fed	The total killed larvae
June	979	322	105	427
July	776	308	92	400
August	1187	535	209	744
September	508	202	89	291
Mean ± S. D	862.5 ± 289.8	341.7 ± 139.5	123.75 ± 57.3	465.5 ± 194.8

Results obtained in Table (1) showed that the highest monthly total numbers of *L. trifolii* larvae, *D. isaea*, host fed and the total killed larvae occurred on August recording (1187, 535, 209, and 744), respectively. While the monthly average numbers recorded (862.5 ± 289.8, 341.7 ± 139.5, 123.75 ± 57.3, and 465.5 ± 194.8) for the former parameters, respectively.

Table 2 Monthly average numbers of % parasitism, and % the total killed larvae during the growing season Summer 2018

Months	% Parasitism	%Host fed	% The total killed larvae
June	32.28 ± 9.9	10.75 ± 2.64	43.06 ± 12.33
July	38.75 ± 4.7	13.32 ± 6.99	52.05 ± 4.9
August	43.96 ± 13.07	17.70 ± 6.27	61.58 ± 18.4
September	41.87 ± 6.7	17.68 ± 2.85	59.79 ± 8.5
Mean ± S. D	39.215 ± 5.01	4.88 ± 3.43	54.12 ± 8.5

Results obtained in Table (2) showed that the highest average percentages of parasitism, % host fed and the total killed larvae recorded on August with (43.96 ± 13.07, 17.70 ± 6.27, and 61.58 ± 18.4), respectively. While the monthly average numbers recorded (39.215 ± 5.01, 4.88 ± 3.43, and 54.12 ± 8.5) during the growing season 2018, respectively.

Results obtained in figure (1) showed the seasonal occurrence of *L. trifolii*, *D. isaea*, %of parasitism, host fed, the total killed larvae, and % of the total killed larvae during the growing season 2019.

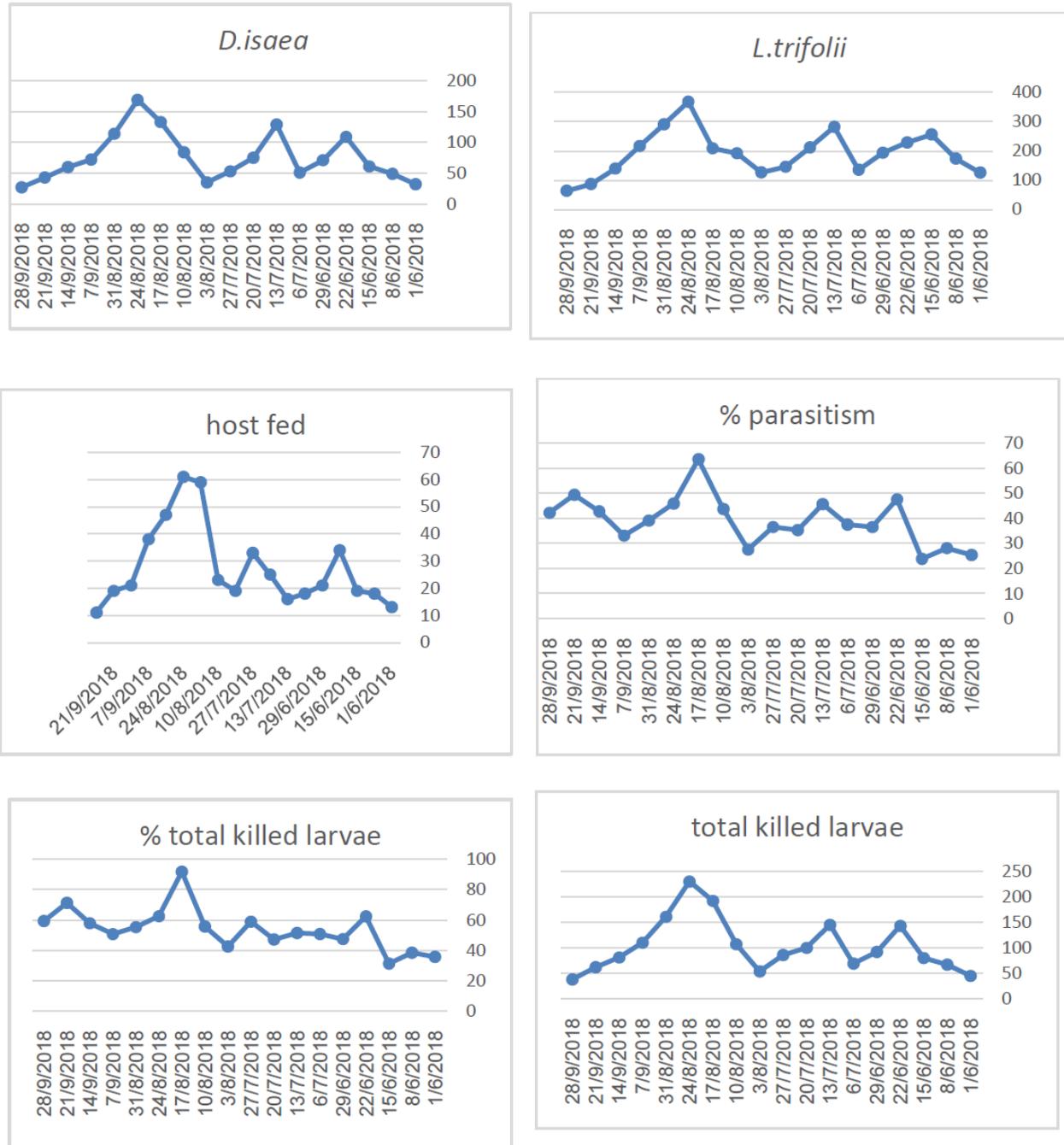


Figure 1 Seasonal occurrence of *L. trifolii*, *D. isaea*, %of parasitism, host fed, total killed larvae, and % of the total killed larvae during the growing season 2018.

3.7. Seasonal abundance of *L. trifolii*

L. trifolii larvae recorded low numbers at the beginning of the season in early June, then the population increased recording three peaks of abundance (289, 381, and 739 individuals/100 infested leaflets) that occurred on the 15th of June, 13th of July, and 17th of August, respectively.

3.8. Seasonal abundance of *D. isaea*

D. isaea recorded low numbers at the beginning of the season in early June, then the population increased recording three peaks of abundance (126, 232, and 343 individuals/ 100 infested leaflets) that occurred on the 15th of June, 13th of July, and 10th of August, respectively.

3.9. Seasonal occurrence of host fed larvae

Host fed larvae (killed larvae according to the parasitoid female feeding with no oviposition) recorded three peaks of occurrence (54, 83, and 156 individuals/ 100 infested leaflets) that occurred on the 15th of June, 13th of July, and 10th of August, respectively.

3.10. Seasonal occurrence of % parasitism

Seasonal occurrence of % parasitism recorded three peaks of occurrence (62.8, 46.9, and 48.8) that occurred on the 6th of June, 27th of July, and 21th of September, respectively.

3.11. Seasonal occurrence of total killed larvae

Seasonal occurrence of the total killed larvae recorded three peaks of occurrence (180, 315, and 499 individuals/100 infested leaflets) that occurred on the 15th of June, 13th of July, and 10th of August, respectively.

3.12. Seasonal occurrence of % total killed larvae

Seasonal occurrence of % the total killed larvae recorded three peaks of occurrence (87.6, 73.4, and 68.2) that occurred on the 6th of July, 27th of July, and 10th of August, respectively.

Table 3 Monthly total numbers of *L. trifolii*, *D. isaea*, host fed, and the total killed larvae during the growing season Summer 2019.

Months	<i>L. trifolii</i>	<i>D. isaea</i>	Host fed	The total killed larvae
June	929	379	167	546
July	1112	587	252	839
August	3241	1264	580	1844
September	788	298	143	441
Mean ± S. D	1517.5 ± 1299.6	632 ± 438.5	285.5 ± 201.8	917.5 ± 640.2

Results obtained in Table (3) showed that the highest monthly total numbers of *L. trifolii* larvae, *D. isaea*, host fed and the total killed larvae occurred on August recording (3211, 1264, 580, and 1844), respectively. While the monthly average numbers recorded (1517.5 ± 1299.6, 632 ± 438.5, 285.5, ± 201.8, and 917.5 ± 640.2) for the former parameters, respectively.

Table 4 Monthly average numbers of % parasitism and % the total killed larvae during the growing season Summer 2019.

Months	% Parasitism	% Host fed	% The total killed larvae
June	39.48 ± 6.37	17.92 ± 2.5	57.46 ± 7.9
July	51.8 ± 12.4	22.07 ± 4.8	73.94 ± 15.7
August	38.76 ± 4.7	18.0 ± 3.1	56.8 ± 6.9
September	39.275 ± 7.3	17.05 ± 3.5	56.38 ± 5.8
Mean ± S. D	42.32 ± 6.3	18.77 ± 2.25	61.15 ± 8.5

Results obtained in Table (4) showed that the highest average percentages of parasitism, host fed, and total killed larvae recorded in July with (51.8 ± 12.4, 22.07 ± 4.8, and 73.94 ± 15.7), respectively. While the monthly average numbers recorded (42.32 ± 6.3, 18.77 ± 2.25, and 61.15 ± 8.5) during the growing season 2019, respectively.

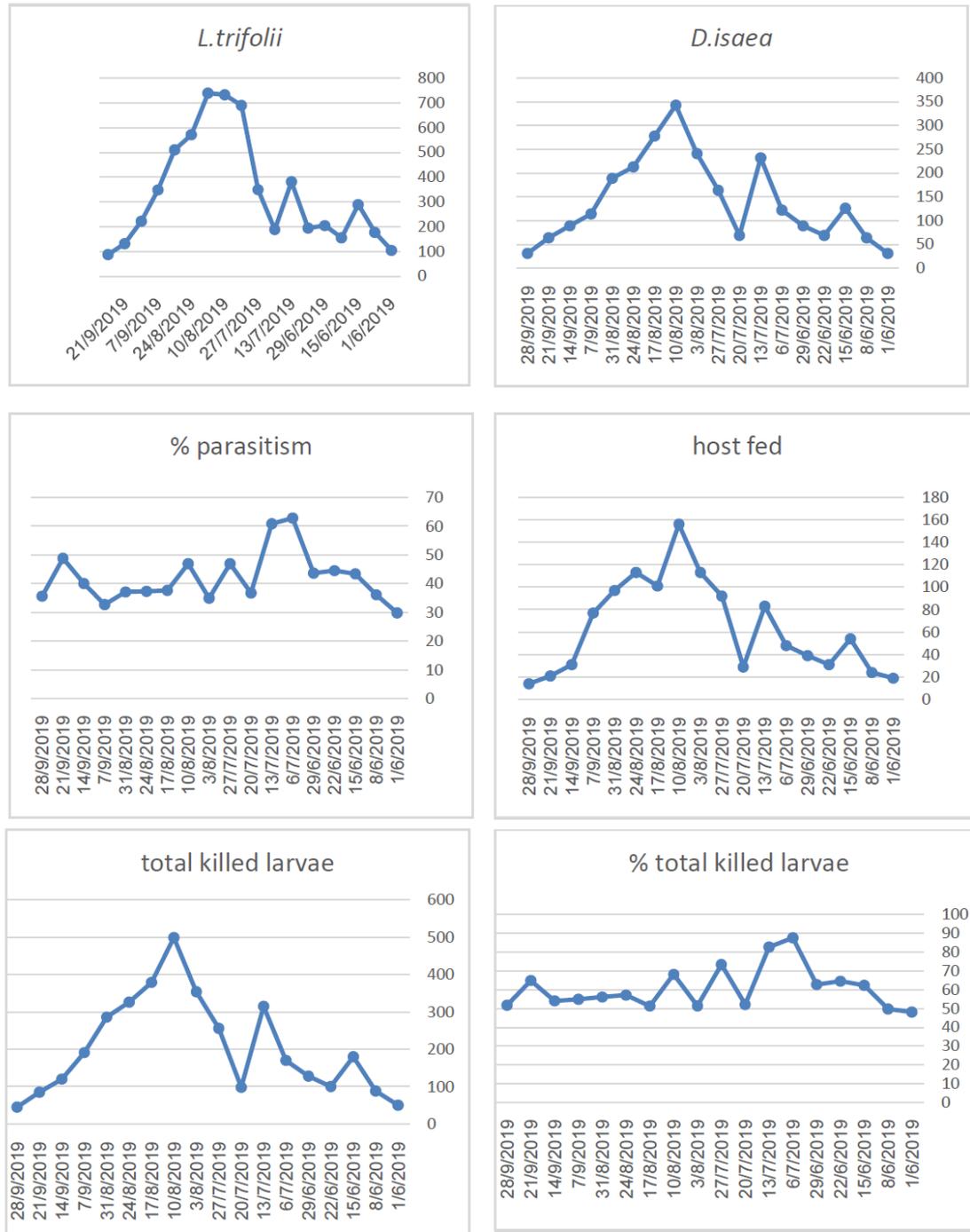


Figure 2 Seasonal abundance of *L. trifolii*, *D. isaea*, %of parasitism, host fed, the total killed larvae, and % of the total killed larvae during the growing season 2018

4. Discussion

The population of *L. trifolii* larvae and *D. isaea* showed 3 peaks of abundance during the two seasons of the study, recording low populations at the beginning of the growing season, then reaching its highest peaks in July and August, then the population decreased towards the end of the growing season. Similar results for *L. trifolii* were obtained by Khoully, who found that the *L. trifolii* recorded the same number of population peaks on cowpea, kidney bean, and tomatoes as summer host plants under Egyptian climatic conditions [10]. These results are also in line with those of Mujica, *et al.* who found that, *L. trifolii* could be developed, 12–20 generations per year in the Mediterranean region [20]. It is evident from the present study that *D. isaea* has proved an effective antagonism in reducing the population of

leaf miner *L. trifolii*. The results were further confirmed by the mortality of leaf miner larvae due to *D. isaea* parasitization and presence of active *D. isaea* larvae feeding on *L. trifolii* larvae under field conditions. Furthermore, the percentage of parasitism ranged between (45.7 – 63.6) over the two growing seasons this finding supporting our proposal of the high activity of this parasitoid in the biological control of *L. trifolii* these results are supported by those of Nicoli, and Pitrelli, [18]. The average monthly percentages of host fed recorded (14.88 ± 3.43 and 18.77 ± 2.25) during 2018 and 2019, respectively. Benuzzi and Raboni, [21]. reported that, Besides the mortality induced by larval parasitic activity, *D. isaea* can also cause host mortality by the adult activity of host feeding. 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. This behavior could occur whether the female deposit eggs or not and could successfully keep the population of *L. trifolii* at low densities these results are also in agreement with those of Malais and Ravensberg, [22] who mentioned that female with immature eggs or which has laid many of her eggs can gain more from host feeding. Patel *et al*, [23] concluded that, the host stinging-to-total mortality of *D. isaea* attained 39.9% suggested that the proportions of different host-killing events depended on the density of leaf miner larvae on individual leaflets. Concerning such data, it could be noticed that, *D. isaea* could successfully keep the population of leaf miner at low densities and no further releases will be needed when the population of *D. isaea* is higher.

5. Conclusion

It can be concluded that *L. trifolii* larvae and the associated larval ectoparasitoid *D. isaea* recorded 3 peaks of abundance on cowpea during the two growing seasons, recording low populations at the beginning of the growing season, then reaching their highest peaks in August. *D. isaea* could successfully keep *L. trifolii* population at moderate or low densities without any chemical control measurements. Concerning the harvested data, the present study could support the efforts of biological measurements on *L. trifolii* under Libyan climatic conditions.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

This work was carried out in collaboration between all authors. Author Elkhouly. A.R, designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Albasha. M. O managed the analyses of the study, reviewed the manuscript and, the literature searches. All authors read and approved the final manuscript.

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