

Biocontrol efficiency, population densities and parasite – host synchronization of the larval ectoparasitoid *Didglyphus isaea* (Hymenoptera: Eulophidae) on the tomato leafminer *Liriomyza bryoniae* on six studied host plants.

Elkhouly, A. R.^{1*}, Albasha, M. O.² And, Shelig N.³

¹ Department of Biology, Faculty of Science – Sabratha University, Libya.

² Department of Zoology, Faculty of Science – Zawia University, Libya.

³ Department of Biology, Faculty of Education – Sabratha University, Libya

*1 alanelkpuly@gmail.com

Submitted: 20- Feb – 2024

Accepted: 07- Mar – 2024

Published: 16-Mar – 2024

ABSTRACT:

Background: *L. bryoniae* is a polyphagous species on important primary hosts of economic importance. On the other hand, *D. isaea* appears to be one of the major biological antagonist against *Liriomyza spp.* This species is a primary ectoparasitoid capable of developing on at least 18 different *Agromyzid* species.

Objectives: The present study aimed to investigate the population densities and the Biocontrol efficiency of the larval ectoparasitoids *D. isaea* on the tomato leafminer *L. bryoniae*.

Methods: Six host plants were selected. Three as summer host plants; kidney bean (*Phaseolus vulgaris*) tomatoes (*Solanum lycopersicum*) and pepper, (*Capsicum annum*) and three as winter host plants broad bean (*Vicia faba*), pea (*Pisum sativum*) and, mallow (*Malva sylvestris*). 100 mined leaflets (25 leaflets for each plot) were collected at weekly intervals during the two experimental seasons for each crop. Samples were kept in plastic bags and transferred to be examined in the laboratory. Number of a life *L. bryoniae* larvae, immature stages of the ectoparasitoid *D. isaea* were counted and recorded weekly

Results: On broad bean, the parasitoid *D. isaea* recorded four peaks of abundance (87, 91, 121, and 35 individuals/ 100 infested leaflets) with percentage of parasitism ranged between 27.1 and 66.6%. On pea *D. isaea* recorded four peaks of abundance (98, 103, and 114 individuals/100 infested leaflets), while the percentage of parasitism ranged between 44.1 and 68.8%. On mallow *D. isaea* records three peaks of abundance (62, 87 and 76 individuals/100 infested leaflets), while the percentage of parasitism ranged between 34.5 and 59.2%.

On kidney bean *D. isaea* recorded three peaks of abundance (78, 89, and 114 individuals/ 100 infested leaflets) occurred in 22th of May, 26th of June, and in 24th of July, respectively. While the percentage of parasitism ranged between 23.5 and 68.2%. On tomatoes *D. isaea* recorded three peaks of abundance (49, 79, and 61 individuals/ 100 infested leaflets) while, the percentage of parasitism ranged between 18.65 and 65.2%. On bell pepper *D. isaea* three peaks of abundance (49, 53, and 44 individuals/ 100 infested leaflets). On the other hand, the percentage of parasitism ranged between 18.9 and 65.7%.

The total numbers of *L. bryoniae*, *D. isaea* and the percentage of parasitism recorded in the winter host plants (4623, 2399 and 51.89) respectively while, lower number recorded on the summer ones (4374, 2134 and 48.788) respectively.

Correlation coefficient values were the highest on winter host plants compared with summer ones. The highest Correlation coefficient value ($r = 0.973232$) recorded on broad bean while, the lowest ($r = 0.894386$) recorded on tomatoes

KEYWORDS: *L. bryoniae* - *D. isaea* - population densities– host synchronization

1. INTRODUCTION

There are almost 400 species in the genus *Liriomyza* of which around 140 species are found naturally in Europe [1]. On the other hand, *L. bryoniae* which belongs to the former genera is a Palearctic species which probably originates from southern Europe, where it occurs commonly outdoors but has spread to greenhouses in many parts of central and northern Europe [2]. This species is also reported in North Africa (Morocco and Egypt), as well as in several countries in Asia [3]. *L. bryoniae* was first described on Bryonia (Cucurbitaceae), but it has been rarely reported on that host. It is a highly polyphagous species and infests several hosts of economic importance [4]. Natural control of *Liriomyza* populations by a complex of parasites is regularly occur in fields where the parasites for biological control of *Liriomyza* pests are found. Parasites can be used in different ways in biological control programs [5].

L. bryoniae was reported as a pest in the Europe in 1965 and 1966, but since 1976 it occurs in large numbers on tomato [6]. Biological control programs against *L. bryoniae* existed Since 1980s, parasitoids such as the European native species *D. isaea*, *Dacnusa sibirica* and *Opius pallipes* have been used against *L. bryoniae* on tomatoes and other crops with increasing success in European greenhouses and very good results have been achieved [7]. **The leafminers *L. trifolii* and *L. bryoniae* have more than 40 parasitoid species in the families *Braconidae*, *Eulophidae*, and *Pteromalidae* [8].** Among them, the Eulophid *Diglyphus* which considers an economically important genus of solitary ectoparasitoids against Agromyzid leafminers, with a wide distribution worldwide (39 species) [9] and, is an important parasitoid of Agromyzid leafminers, which includes the economically important genus *Liriomyza* [10]. The females of

Diglyphus species show three types of host-killing behavior, reproductive (parasitism), non-reproductive host killing by feeding (host feeding), and host stinging without oviposition or feeding (host stinging), with the non-reproductive host-killing behavior to significantly increase the biocontrol potential [11]. **Elkhouly et al (2020) [12]** evaluated the host stinging behavior of *D.isaea* on *L.trifolii* on four host plants (2 as winter host plants and 2 as summer ones) and found 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

Musundire et al, (2011) [13] calculated that 90 parasitoids, with species belonging to 10 families and 28 genera, were associated with only 20 Agromyzid species belonging to 10 genera in the tropical region. The comparatively low parasitoid diversity in this region may be due to limited sampling effort and a lack of taxonomic expertise for parasitoid species. **In the entomological literature, many publications on the development, reproduction and population growth of natural enemies are available and this information might be useful in as election procedure. However, data are greatly lacking on the searching efficiency of natural enemies [14].**

Behavioral components, and, population abundance of the parasitoid complex of the leaf mining insects specially Agromyzids that enhance the biological control efficiency of natural enemies need further to be determined. The present study aimed to evaluate the role of the larval ectoparasitoid *D.isaea* as a biocontrol antagonist against the tomato leafminer *L. bryoniae* on six selected host plants.

2. MATERIALS AND METHODS

HOST PLANTS.

SIX HOST PLANTS WERE SELECTED. THREE AS SUMMER HOST PLANTS; KIDNEY BEAN (*PHASULUS VULGARIS*) TOMATOES (*SOLANUM LYCOPERSICUM*) AND PEPPER, (*CAPSICUM ANNUUM*) AND THREE AS WINTER HOST PLANTS BROAD BEAN (*VECIA FABIA*), PEA (*PISUM SATIVUM*) AND, MALLOW (*MALVA SYLVESTRIS*).

EXPERIMENTAL FIELD.

THE PRESENT STUDY WAS CARRIED OUT IN JANZOUR REGION, FOR TWO SUCCESSIVE SEASONS. THE EXPERIMENTAL FIELD WAS ABOUT $\frac{1}{4}$ HECTARE. WHICH DIVIDED TO EQUAL PLOTS (4 PLOTS FOR EACH CROP. EACH PLOT WAS ABOUT 200 M² PLANTED DURING THE THIRD WEEK OF NOVEMBER AS A WINTER CROPS. WHILE SUMMER CROPS WERE PLANTED DURING THE THIRD WEEK OF MAY. THE ROLE AND EFFECT OF HOST PLANTS ON THE PARASITIDS ACTIVITY WAS EVALUATED ACCORDING TO THE COLLECTED SAMPLES FROM ALL HOST PLANTS.

SEASONAL ABUNDANCE OF THE PARASITIDS *D. ISAEA*.

TO DETERMINE THE SEASONAL ABUNDANCE OF *L. BRYONIAE* PARASITIDS, SAMPLES OF 100 MINED LEAFLETS (25 LEAFLETS FOR EACH PLOT) WERE COLLECTED AT WEEKLY INTERVALS DURING THE TWO EXPERIMENTAL SEASONS FOR EACH CROP. SAMPLES WERE KEPT IN PLASTIC BAGS AND TRANSFERRED TO BE EXAMINED IN THE LABORATORY. NUMBER OF A LIFE *L. BRYONIAE* LARVAE, IMMATURE STAGES OF THE ECTOPARASITOID *D. ISAEA* WERE COUNTED AND RECORDED WEEKLY. SAMPLES TOKE PLACE AS SOON AS THE TRUE NEWLY VEGETATIVE GROWTH WAS COMPLETELY APPEARED IN THE EXPERIMENTAL AREA AND WERE CONTINUED WEEKLY

4. RESULTS

4.1. Seasonal abundance of the larval ectoparasitoid *D. isaea* on Winter host plants:

1. On broad bean:

On broad bean, the parasitoid *D. isaea* has been recorded with low numbers at the beginning of the season in early December. Then the population increased, revealing four peaks of abundance (87, 91, 121, and 35 individuals/ 100 infested leaflets) occurred on 27th of December, 17th of January, 14th of February and the 21th of March, respectively. On the other hand, the percentage of parasitism ranged between 27.1 and 66.6%.

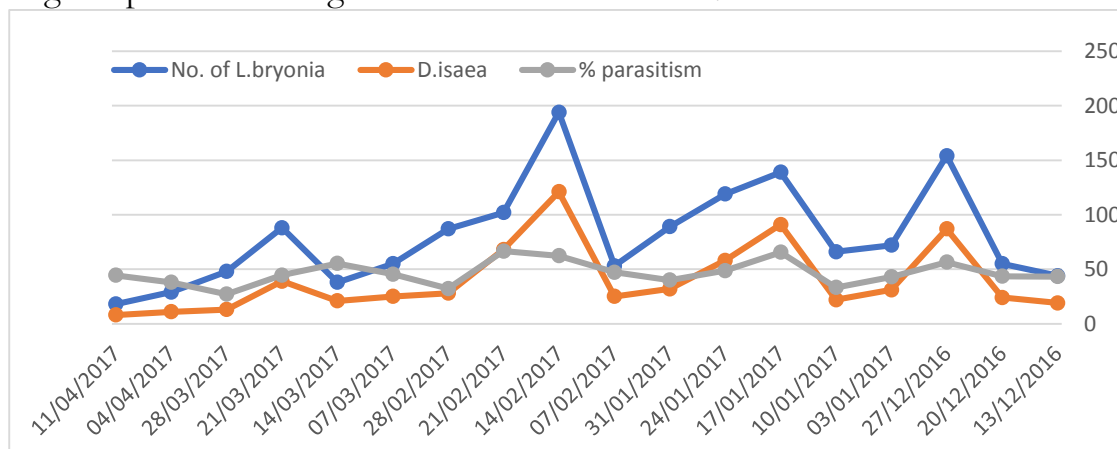


Fig (1) Population abundance and percentage of parasitism of the larval ectoparasitoid *D. isaea* on Broad bean during the growing season 2016/2017.

2. On pea.

The population of *D. isaea* recorded four peaks of abundance (98, 103, and 114 individuals/100infested leaflets) occurred on 3rd and 31st of January, and 7th of March, respectively, while the percentage of parasitism ranged between 44.1 and 68.8%

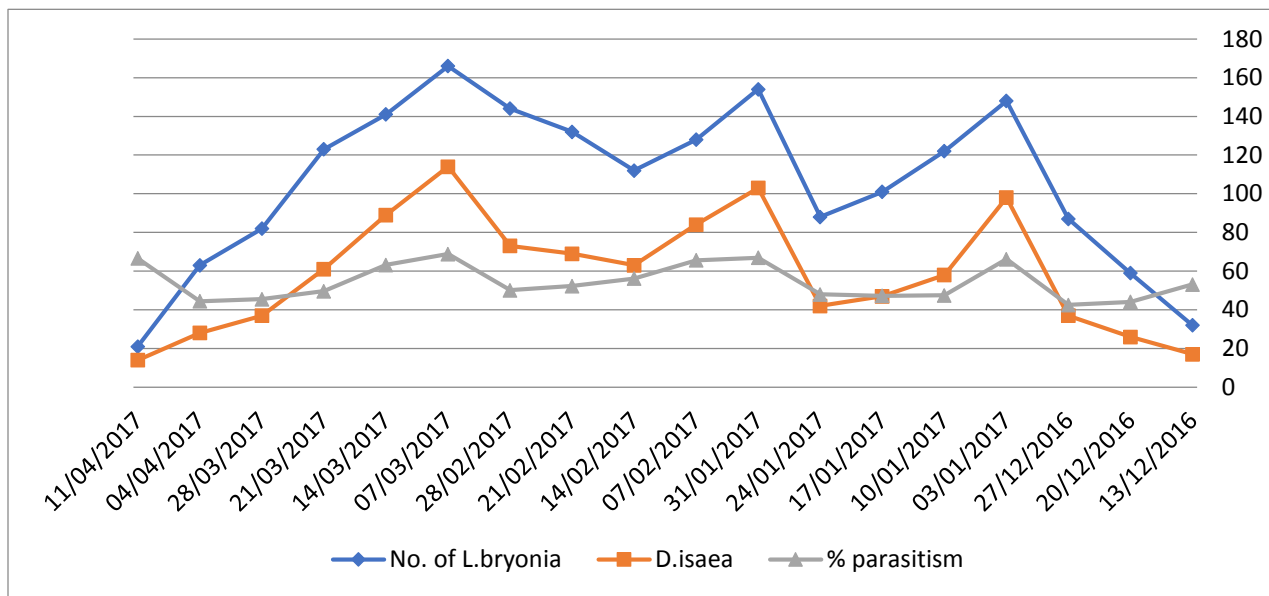


Fig (2) Population abundance and percentage of parasitism of The larval ectoparasitoid *D. isaea* on Pea during the growing season 2016/2017.

3. On Mallow

The population of *D. isaea* records three peaks of abundance (62, 87 and 76 individuals/100 infested leaflets) occurred on 3rd of January, 14th of February and 14th of March respectively, while the percentage of parasitism ranged between 34.5 and 59.2%

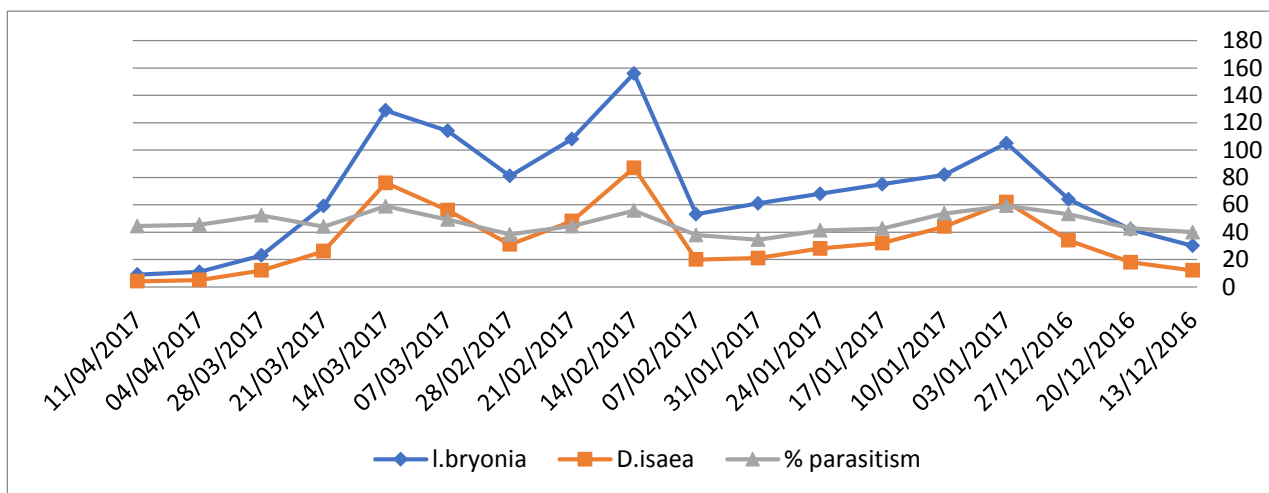


Fig (3) Population abundance and percentage of parasitism of The larval ectoparasitoid *D. isaea* on Mallow during the growing season 2016/2017.

4.2. Seasonal abundance the larval ectoparasitoid *D. isaea* on summer host plants.

1. On kidney bean.

The parasitoid *D. isaea* has been recorded with low numbers at the beginning of the season in early May. Then the population increased, revealing three peaks of abundance (78, 89, and 114 individuals/ 100 infested leaflets) occurred in 22th of May, 26th of June, and in 24th of July, respectively. On the other hand, the percentage of parasitism ranged between 23.5 and 68.2%.

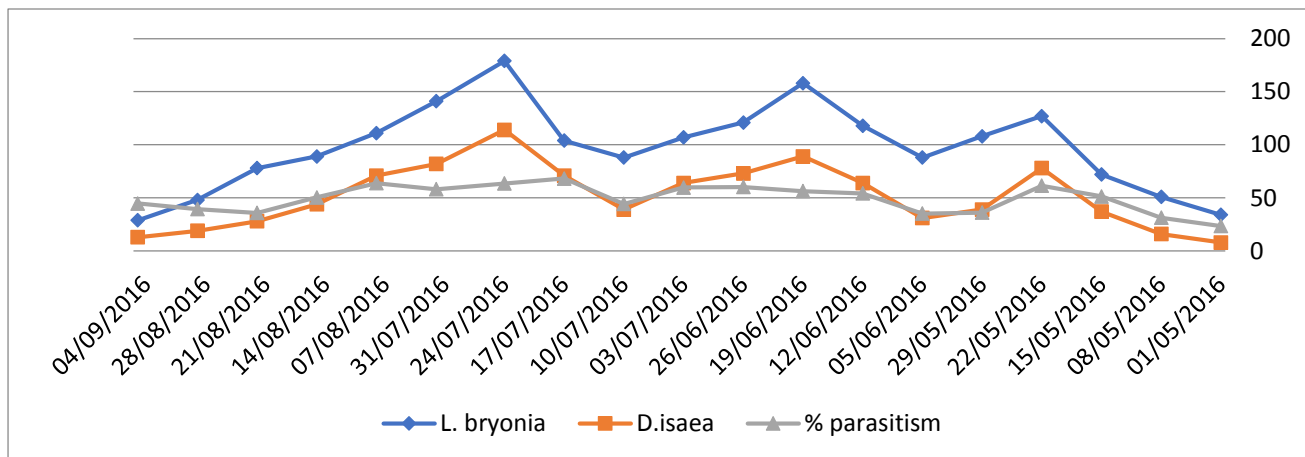


Fig (4) Seasonal abundance of *D. isaea* on kidney bean during the growing season (2016).

2. On tomatoes.

The parasitoid *D. isaea* has been recorded with low numbers at the beginning of the season in early May. Then the population increased, revealing three peaks of abundance (49, 79, and 61 individuals/ 100 infested leaflets) occurred in 29th of May, 3rd of July, and in 31th of July, respectively. On the other hand, the percentage of parasitism ranged between 18.6.5 and 65.2%.

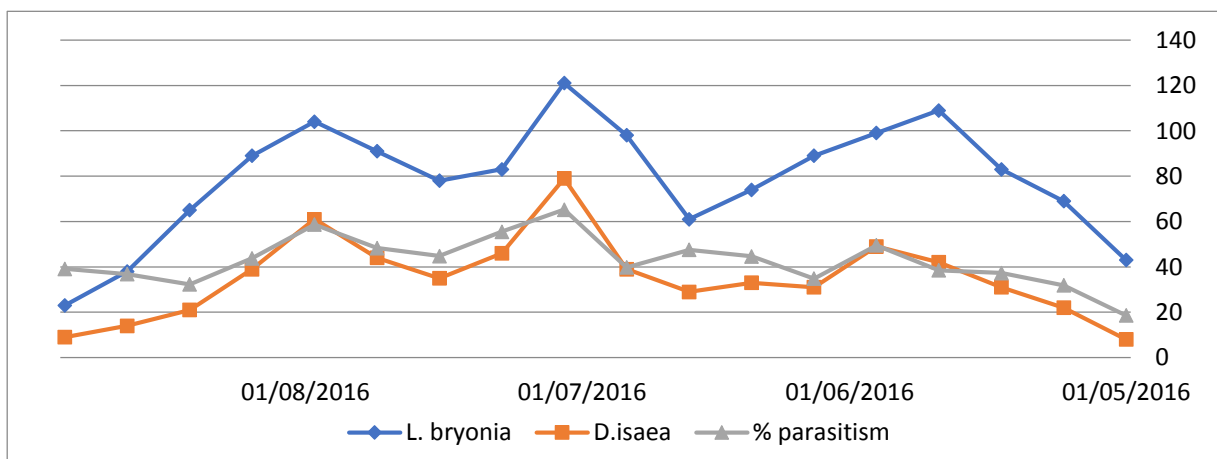


Fig (5) Seasonal abundance of *D. isaea* on tomatoes during the growing season (2016).

3. On bell pepper

The parasitoid *D. isaea* has been recorded with low numbers at the beginning of the season in early May. Then the population increased, revealing three peaks of abundance (49, 53, and 44 individuals/ 100 infested leaflets) occurred in 29th of May, 5th of June, and in 7th of August, respectively. On the other hand, the percentage of parasitism ranged between 18.9 and 65.7%.

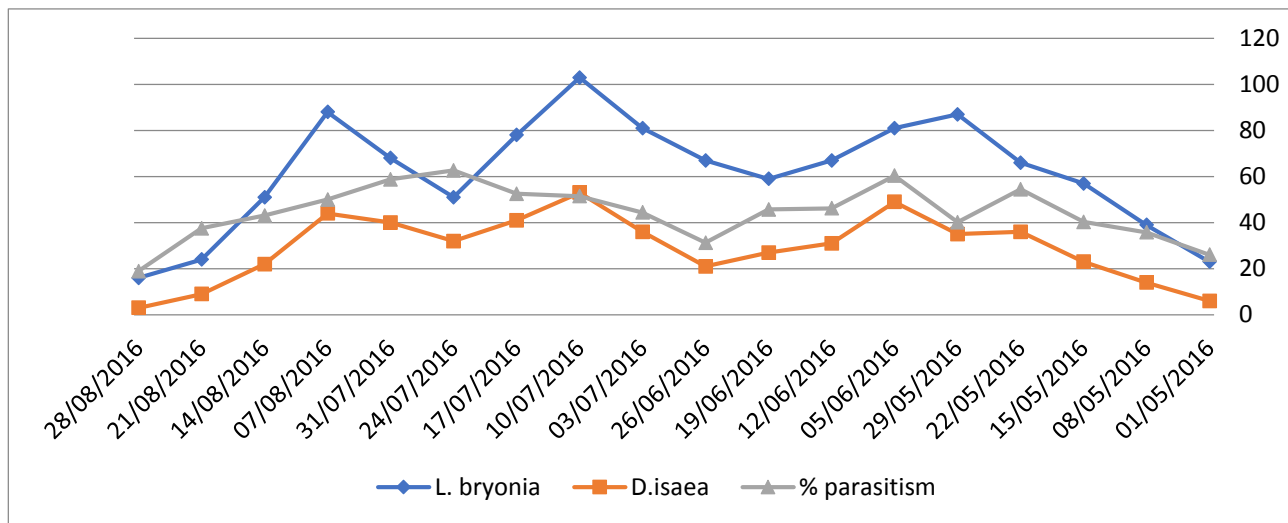


Fig (6) Seasonal abundance of *D. isaea* On bell peppers during the growing season (2016).

Table (1) total numbers of *L. bryoniae*, *D. isaea* and percentage of parasitism on six host plants during two successive seasons

Host plants	Season	<i>L. bryoniae</i>	<i>D. isaea</i>	% parasitism
Broad bean	Winter	1450	723	49.86
Pea	Winter	1903	1060	55.70
Mallow	Winter	1270	616	48.50
Total		4623	2399	51.89
Kidney bean	summer	1851	980	52.94
Tomatoes	summer	1417	632	44.60
Bell pepper	summer	1106	522	47.19
Total		4374	2134	48.788

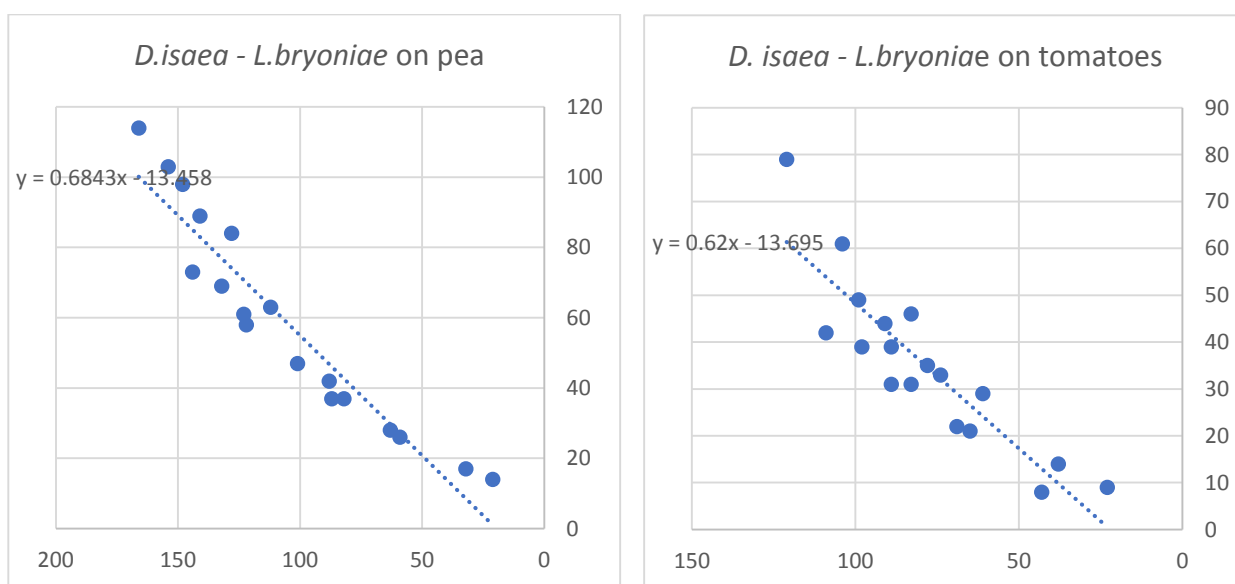
As shown in table (1) the highest population of *L. bryoniae* , *D. isaea* and the percentage of parasitism recorded on pea (1903 , 1060 and 55.70%) respectively. On the other hand, the lowest

population of *L. bryoniae*, *D. isaea* recorded on bell pepper (1106 and 522) respectively while, the lowest percentage of parasitism recorded (44.60%) on tomatoes. Moreover the total numbers of *L. bryoniae*, *D. isaea* and the percentage of parasitism recorded in the winter host plants (4623, 2399 and 51.89) respectively while, lower number recorded on the summer ones (4374, 2134 and 48.788) respectively.

Table (2) Correlation coefficient values and regression equations between *D. isaea* and *L. bryoniae* populations on six host plants during two successive seasons.

Host plants	Season	<i>D. isaea</i>	
		r	regression equations
Broad bean	Winter	0.973232	$y = 0.6572x - 12.77$
Pea	Winter	0.951991	$y = 0.6843x - 13.458$
Mallow	Winter	0.972232	$y = 0.5677x - 5.8339$
Kidney bean	Summer	0.950483	$y = 0.704x - 17.006$
Tomatoes	Summer	0.894386	$y = 0.62x - 13.695$
Bell pepper	Summer	0.931701	$y = 0.5603x - 5.4289$

As shown in table (2) Correlation coefficient values were the highest on winter host plants compared with summer ones. The highest Correlation coefficient value ($r = 0.973232$) recorded on broad bean while, the lowest ($r = 0.894386$) recorded on tomatoes.



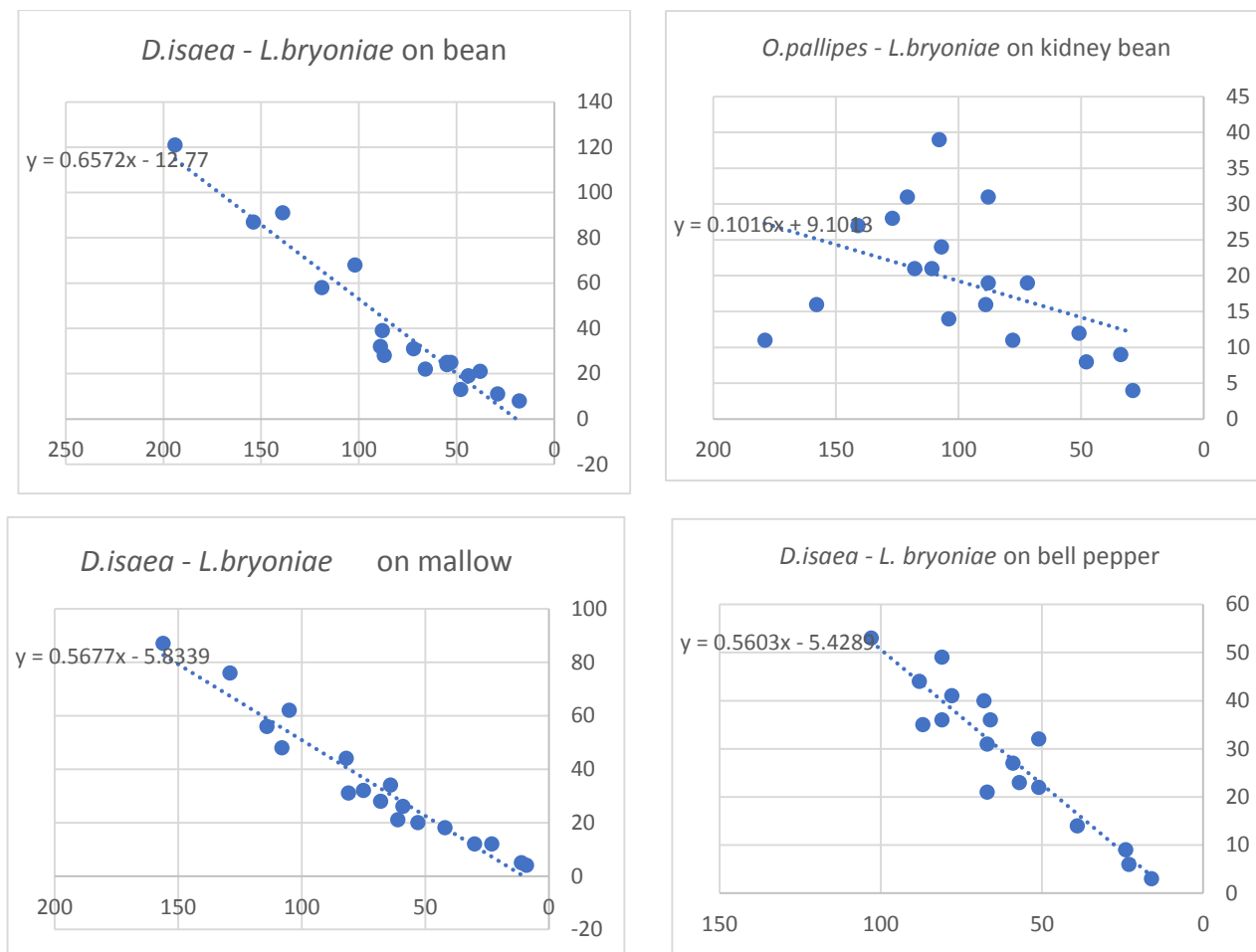


Fig (7): The liner regression showing the relation between *D. isaea* and *L. bryoniae* populations on six host plants during two successive seasons.

5. Discussion:

The parasitoid *D. isaea* showed low abundance from the beginning of the season till the second half of January, may be due to that, this parasitoid needs high densities of its host (*L. bryoniae*) [15], which records low abundance at the same period, another possible explanation is that the parasitoid females kill a large number of *L. bryoniae* larvae for oviposition and feeding, so this sort of behavior requires a high densities of the host. By the second half of January the population of *D. isaea* increased gradually reaching its highest densities in February and March in the three successive seasons depending on the high abundance its host occurred at the same period. By the beginning of April, the parasitoid and its host occurred at low densities because

the parasitoid could successfully keep its host at low abundance. Data suggested by [16] concluded that *D. isaea* had a very good synchronization with its host with high correlation values of (0.88, 0.97 and 0.65) during the three successive seasons of the study on broad bean as a host plant. These results are also in agreement with those of [12], and [17].

The parasitoid *D. isaea* was represented in low or moderate abundance in June on the three studied summer host plants. By the beginning of July, the population increased gradually reaching its peak during July and August depending on the high population of its host, and then the population of *D. isaea* decreased with the population decrease of its host keeping the last at low levels. Data represented by [11] and [18] concluded that *D. isaea* had a very good synchronization with its host with correlation values (r) of 0.96, 0.98 and 0.85 during three seasons of the study on cowpea as host plant. These results are in agreement with those of [19] and [20].

On the other hand, our results could be explained by those of [21] who suggested that, the Parasitoids *D. isaea* and *D. sibirica* preferred to land initially on plants with high rather than low densities of hosts. Furthermore, parasitoids spend more time searching on the high host density plants and oviposition rates were enhanced with increasing host densities. Consequently, directly density dependent parasitism occurred at the plant level. This is probably possible because these parasitoids are informed about the distribution of the leafminers at that spatial level. They also concluded that, Generation Synchrony and Population Growth appear to explain, at least in part, the effectiveness of these parasitoids in the different regions and hosts. Therefore, a great synchronization between the Parasitoids *D. isaea* and its host was recorded with r values ranged (0.894386 and 0.973232) on the six studied host plants. These results are also in agreement with those of [22].

6. Conclusion:

The Parasitoids *D. isaea* recorded three peaks of abundance on either summer and winter host plants with relatively high populations on the winter crops. *D. isaea* showed a very high

synchronization with its insect host on all studied host plants. Moreover, *D. isaea* populations were highly affected by the population densities of *L. bryoniae*.

REFERENCES

- [1] EPPO (European and Mediterranean Plant Protection Organization) (2019). EPPO codes. Available online: https://www.eppo.int/RESOURCES/eppo_databases/eppo_codes.
- [2] Minkenberg OPJM and van Lenteren JC, (1986). The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. Agricultural University Wageningen Papers 86-2: 50 pp. Wageningen.
- [3] CABI Knowledge bank (2019). *Liriomyza bryoniae*, available online <https://www.plantwise.org/knowledgebank/datasheet/> 30950 [Accessed in November 2019].
- [4] Spencer KA, (1990). Host specialization in the world Agromyzidae (Diptera). Kluwer Academic Publishers, Dordrecht, Netherlands. 444 pp.
- [5] Van Lenteren J.C (1993). Biological control of pests. In: Zadoks J.C, editor. Modern crop protection: developments and perspectives. Wageningen Press; Wageningen, The Netherlands: 1993a. pp. 179–187.
- [6] De Brouwer, W.M.Th. J .and, Van Offeren, A.L. (1967). Demineervlieg *Liriomyza solani* Her. bij tomaat [inDutch]. Annu. Rep.GCRES,Naaldwijk, the Netherlands, for 1966: 150-155.
- [7] Hendriske A, Zucchi R, Van Lenteren JC and Woets J, (1980). *Daenusa sibirica* Telenga and *Opius pallipes* Wesmael (Hym., Braconidae) in the control of the tomato leaf miner *Liriomyza bryoniae* Kalt. Bull IOBC/wprs, 6, 181–185.
- [8] Heinz, KM. and Parrella, M.P.(1990). Biological control of insect pests on greenhouse marigolds. Envir. Entomol. 19:825-835.
- [9] Hansson, C. and Navone, P (2017). Review of the European species of *Diglyphus* Walker (Hymenoptera: Eulophidae) including the description of a new species. Zootaxa 4269, 197–229 .
- [10] Hendrickson Jr., R.M., Barth, S.E., (1979). Introduced parasites of *Agromyza frontella* (Rondani) in the USA. J. New York Entomol. Soc. 87 (2), 167–174.
- [11] Liu, W. X., Wang, W. X., Wang, W., Zhang, Y. B. & Wan, F. H (2013). Characteristics and application of *Diglyphus* parasitoids (Hymenoptera: Eulophidae) in controlling the agromyzid leafminers. Acta. Entomol. Sin. 56, 427–437.
- [12] Elkhoully.A.R, Husen A. Shafsha and Almabruk A. Al Hireereq (2020). Evaluation of Host Stinging Behavior of the Larval Ectoparasitoid *Diglyphus isaea* on Some Selected Host Plants. *AJAAR*, 14(3): 27-33, 2020.
- [13] Musundire, R., Chabi-Olaye, A., Loehr, B. & Krueger, K (2011). Diversity of agromyzidae and associated hymenopteran parasitoid species in the afrotropical region: implications for biological control. Biol. Control 56, 1–9 .

- [14] Hopper, KR and King, E.D. (1986). Linear functional response of *Microplitis croceipes* (Hymenoptera: Braconidae) to variation in *Heliothis* spp. (Lepidoptera: Noctuidae) density in the field. *Environ. Entomol.* 15:476-480.
- [15] Linden A (1993). Biological control of leaf miners in glasshouse lettuce. "Liriomyza". Conference on leaf mining flies in cultivated plants. Montpellier- France. 1993.
- [16] El Khouly AR. (2003). Studies on some natural enemies associated with the serpentine leaf miner *Liriomyza trifolii* (Burgess) (Doctoral dissertation, M. Sc. Thesis, Fac., Agric., Mansoura Univ pp.116).
- [17] Goncalves, M.A. and M.L. Anunciada (2001). Population dynamics of *Diglyphus isaea* and *D. poppea* (Hymenoptera :Eulophidae) leaf miner parasitoids on greenhouse grown green bean in the Algarve (Portugal). *Zapateri. Revta. Aragon .Entomol.* 9:75-83
- [18] El Khouly AR. (2009). Efficiency of some hymenopterous parasitoids on serpentine leaf miner *Liriomyza trifolii* (Burgess) (Doctoral dissertation, PhD. Thesis, Fac., Agric., Mansoura Univ pp.185).
- [19] Elkhoully AR, Albasha MO, Alhririg MA. (2015) Population abundance of the ectoparasitoid *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) on the tomato leaf miner *Liriomyza bryoniae*. (Kaltenbach) (Diptera: Agromyzidae) On some winter host plants in Ojilate region, Libya. *J. Agric. Engen. Biotec.*;3(1):41–45
- [20] Asadi R, Talebi AA, Fathipour Y, Moharrampour S, Rakhshani E. (2006) Identification of parasitoids and seasonal parasitism of Agromyzid leaf miners genus *Liriomyza* (Dip: Agromyzidae) in Varamin. Iran. *J. Agric. Sci. and Tech.*;8(4): 293-303
- [21] Bragard C, Dehnen-Schmutz K, Di Serio F, Gonthier P, Jacques M-A, Jaques Miret JA, Justesen AF, Magnusson CS, Milonas P, NavasCortes JA, Parnell S, Potting R, Reignault PL, Thulke H-H, Van der Werf W, Vicent Civera A, Yuen J, Zappala L, Czwienczek E, Streissl F and MacLeod A (2020). Scientific Opinion on the pest categorization of *Liriomyza bryoniae*., *EFSA Journal* 2020;18(3):6038, 31 pp. <https://doi.org/10.2903/j.efsa.2020.6038>
- [22] Abul-Nasr S, Assem AH (1961). A leaf miner, *Liriomyza bryoniae* Kalt), attacking cucurbitaceous plants in Egypt. *Bulletin of the Entomological Society of Egypt*;45:401-403