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Seasonal occurrence of *Liriomyza bryoniae* (Kaltenbach) and their parasitoids on cucumber crop

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Abstract---The results of this study revealed a steady increase in population density of leaf miners *Liriomyza bryoniae* (Kaltenbach) from 1.33 larvae/leaf at the beginning of the season to 3.3, 6, and 7 larvae/leaf with the progression of the agricultural season until 10 November, a slight drop in population occurred at the end of the season. The results revealed that the leaf miner population showed a significant negative correlation (-0.74) with maximum temperature and a non-significant negative correlation with minimum temperature. The maximum percentage of factor role was 55% for maximum temperature and (18 %) for the minimum temperature. Two species of insect parasitoids (*Diglyphus isaea* and *Opius sp*) Hymenoptera order were recorded on the leafminer *Liriomyza bryoniae* larvae and pupae. *Diglyphus isaea* was the most common species. It was active throughout the growing season and increased in number as the leafminer populations increased. The rate of % parasitism was 4.3 % on 7 Aug. increased to 7.8, 12.8, 20.3, and 38.2% on 1 Sept., 7 Sept., 7 Oct., and 21 Oct respectively, while the rate of % parasitism of *Opius sp* ranged from 2.6% to 5.9% from 14 Sept to 21 Oct.

Keywords---Leaf Miners, *Liriomyza* spp., *Liriomyza bryoniae* , *Diglyphus isaea*

Introduction

Genus *Liriomyza* contains more than 300 species, widely distributed in the new and old world but are commonly found in temperate areas. (Patel et al,2005). *L. bryoniae* is a highly polyphagous species, and its primary hosts of economic

importance, include: cabbages (*Brassica oleracea*), cucumbers (*Cucumis sativus*), lettuces (*Lactuca sativa*), courgettes (*Cucurbita pepo*), melons (*Cucumis melo*), tomatoes (*Lycopersicon esculentum*) and watermelons (*Citrullus lanatus*) (Gathage et al., 2016). In the pan-temperate region, *L. bryoniae* has been reported to complete its life cycle on plants from 16 families. (Spencer, 1973).

Diglyphus isaea is a primary parasitoid of agromyzid leaf miners and has been commercialized as biological control agent. (Sha et al. 2007). This species is a primary ectoparasitoid, capable of developing on at least 18 different agromyzid leaf miner species (Boucek and Askew, 1968). Minkenberg and van Lenteren (1989) describe *D. isaea* as being associated with *Liriomyza* hosts in herbaceous plants but are scarce in trees. The average monthly rates of parasitism were the highest on cowpea followed by kidney bean and tomato, respectively. (Awadalla et al., 2009). Johnson and Hara (1987) found that the parasitoids *Diglyphus begini* (Asmead) and *Diglyphus inlermedius* (Gerault) preferred celery, tomato, beans, cucumber, cabbage and spanish as host plants. *D. isaea* has certainly been used as a biological control agent in a wide range of crops, whilst in the wild, populations of *D. isaea* have been found to use at least 14 species of weedy host plants in northern Italy. (Burgio et al., 2007). *Diglyphus isaea* has been used as a biological control against *L. bryoniae* since the mid-1980s (Malais and Ravensberg, 2003). Only a few parasitoid species are actually mass reared for biological control of leaf miners in the genus *Liriomyza*. These include *D. isaea* against *L. trifolii* and *L. bryonia*, which is employed for different vegetable and ornamental crops, especially in Mediterranean greenhouses where temperatures are more suitable for its viability. (Bazzochi et al. 2003). The parasitoid *D. isaea* was effective in controlling *L. trifolii* in tomato greenhouses in spite of high levels of infestation of the pest (which reached 74 mines/ plant). The level of parasitism was up to 100%. (Cabitza et al., 1993). *Diglyphus isaea* Walker was the most dominant parasitoid species against *L. trifolii* of the parasitoid complex which contains *Opius pallipes* Wesmead and *Chrysocharis parksi* Crawford (Hymenoptera: Eulophidae) as endoparasitoids. (Khouly, 2003). Ozawa et al. (2001) found that the dominant parasitoid species emerging from *L. trifolii* larvae in Homaoka tomato greenhouses was *D. isaea*. This parasitoid was released in tomato greenhouses to control *L. trifolii* at different release doses. The percentage of parasitism ranged 94.1-100% by the end of the growing season. Goncalves and Almeida (2005) reported that through a survey started in 1993 in several protected crops, two ectoparasitoids of *Liriomyza* spp., *D. isaea* and *D. poppoe* had been found every year, reaching rate of parasitism of 80-85% with predominance of the first one among the parasitoid. Due to the importance of biological aspects, in determining the time and techniques of control, in addition to the lack of population density studies, this research aimed to study the dispersal and population density of the cucumber leaf miner *L. bryoniae* and their parasitoids.

Material and Methods

Seasonal occurrence of leaf miner *Liriomyza bryoniae* on cucumber:

The study was conducted to determine the role of weather in the fluctuation of Leaf miner *Liriomyza bryoniae* (Kaltenbach) on cucumber during autumn 2021. Seeds of cucumber BEITH ALPHA (Akgen, Green South) variety were sown in the

experimental area in august, 2021. Experiments were laid out in a Randomized Complete Block Design (RCBD) with three replications. The row-to-row distance was kept to be 75 cm and plant to plant to be 50 cm. during the study seasons, no plant protection measure was applied. All the recommended agronomic practices were adopted during the experiment. The leaf miner population was recorded 20 days after sowing early in the morning weekly. For the counts of the leaf miner population, 15 plants in each replication were selected at random. The leaves were observed in such a way that one leaf of the upper part of the first plant, one leaf of the middle part of the second plant, and one leaf of the bottom part of the third plant was taken into account. Metrological data related to the temperature and was recorded. The effect of abiotic factors on the leafminer population densities was determined by working out a simple correlation (Pedigo, 1996). The combined effect of the factors like temperatures, by using a Multiple Linear Regression Equation.

Seasonal occurrence of parasitoids

The survey on parasitoids of leafminer was carried out from September to December 2021 The infested leaves of cucumber, *Cucumis sativus L.*, were collected from the field weekly and transferred in plastic bags to the laboratory. The infested leaves were then placed in Petri dishes and covered with a layer of filter paper. The Petri-dishes were placed inside the incubator under constant conditions (temperature of $25\pm 1^{\circ}\text{C}$, relative humidity $0\pm 5\%$, and under 16 Light: 8 Dark photoperiod). The wasps that emerged were collected daily using an aspirator and stored in 75% ethyl alcohol for future work. The rates of parasitism were calculated using the formula:

$$\% \text{ parasitism} = (\text{no. of parasitoids} / \text{no. of leafminers} + \text{parasitoids}) \times 100$$

Results and discussion

population density of *Liriomyza bryoniae* on cucumber

The results of this study revealed a steady increase in population density of leaf miners from 1.33 larvae/leaf at the beginning of the season to 3.3, 6, and 7 larvae/leaf with the progression of the agricultural season until 10 November, a slight drop in population occurred at the end of the season. Fig (1), Fig (2).

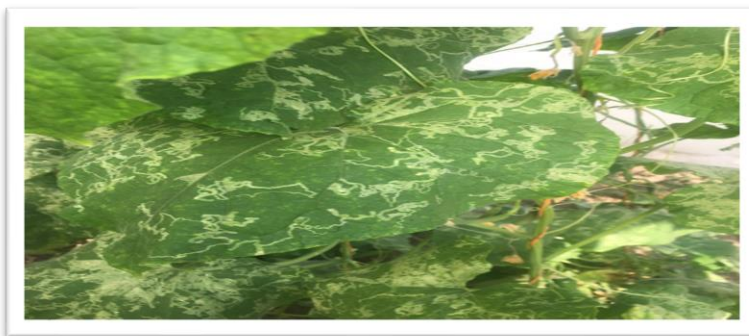


Fig.(1) Infection of cucumber leaves by *Liriomyza bryoniae*

The maximum population was recorded to be 7 per leaf at the beginning of November when the maximum and minimum temperatures were 33°C, and 20°C respectively. The results revealed that the leaf miner population showed a significant negative correlation (-0.74) with maximum temperature and a non-significant negative correlation with minimum temperature Fig (3), Fig(4). This result was confirmed in Table(1) which explains the role of these factors, in the fluctuation of the population the maximum percent of factor role was 55% for maximum temperature and then minimum temperature (18 %).

In a similar study, Abbas (2018) diagnosis of Leaf Miners *Liriomyza* spp seasonal occurrence, Economic Importance on Cucumber in Field and greenhouse, the results indicated that the percentage of plant infestation was 33.3% and leaves 50% when the maximum temperatures were 30.7 m and the smallest 11.5 m and the relative humidity is 45%, explains it decreased with decreasing temperatures and gradually increased with increasing temperatures until it reached its highest level 2.83 This means that 80% of the leaves bear at least 6-10 infected leaf tunnels.

A fundamental step in securing proper IPM is to adopt the method of monitoring. Monitoring can give the farmer the necessary information on the basis of which to make decisions pertaining to which management practices to follow (Dara 2019; Sharma et al. 2020). Effective monitoring practices help predict the presence of the pest, the pest numbers, and the weather conditions that favour its presence (Dreistadt et al. 1998).

Sarade et al. (2021) found the Seasonal incidence of leaf miner *L. trifolii* on cucumber was noticed from 0.2 to 3.84 percent. The incidence of leaf infestation was noticed during the 3rd week of December at 0.2 percent. The leaf infestation was recorded at 3.84 percent in 1st MW corresponding to the January first week which was higher than the rest of the weeks when the maximum and minimum temperature was 29.2 0C and 10.5 0C respectively along with 58.85 percent average relative humidity.

However, the least leaf infestation was observed at 0.05 percent was observed in the 5th MW. American leaf miner population were negatively non-significant with maximum temperature ($r = -0.66668$), minimum temperature ($r = -0.31272$) and relative humidity ($r = -0.8244$) , During the survey of Rauf et al.(2000), *Liriomyza* infestations usually occurred at the end of the dry season (i.e. June ± July) and continued to the initial phases of the rainy season (August± November). Infestations during the intense rainy season (January± March) were generally low. Within-field infestations were associated with crop phenology. For example, in potatoes, leafminer populations increased slowly during vegetative growth and increased rapidly during generative growth.

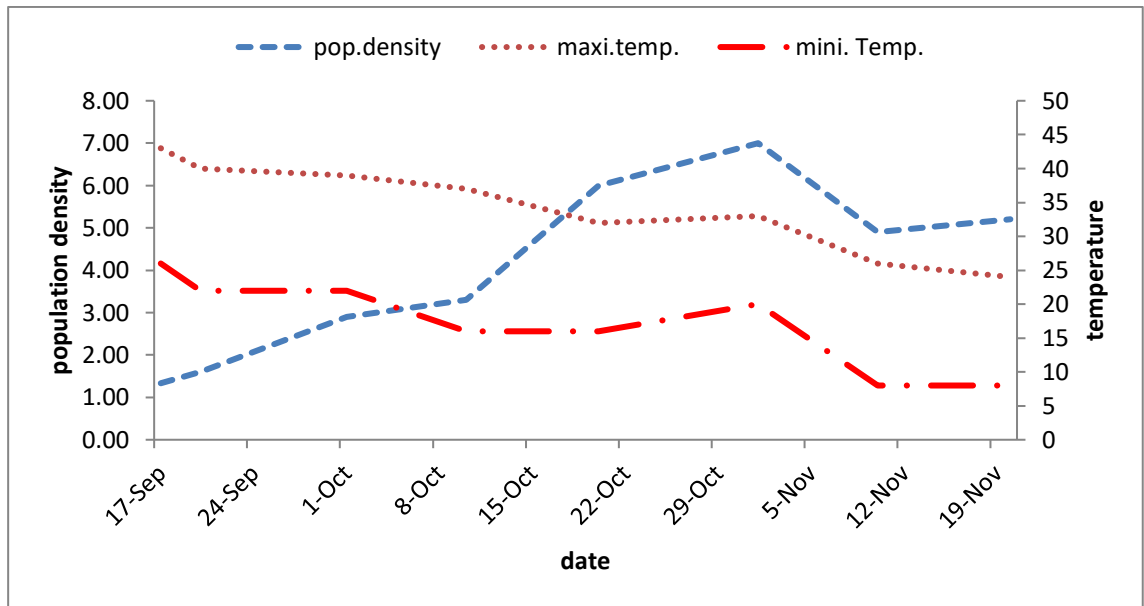
The influence of weather factors on the population density of leaf miners was analyzed (Lad et. al 2021) by a simple correlation study and coefficients were worked out. Results indicated that there were marked differences observed in the infestation of leaf miners. The population of leaf miners (0.32 ± 0.22) was observed in the 13th SMW (26th March - 1 st April). Minimum leaf miner infestation (0.01 ± 0.22) was recorded in 23rd SMW (5th - 11th June), while

maximum (0.50 ± 0.22) infestation was recorded during 15th SMW (9th - 15th April) and 16th SMW (23rd - 29th April). The data on the correlation between the mean population of leaf miner infesting bitter gourd and different weather parameters revealed that all the meteorological parameters viz., maximum temperature, morning relative humidity, and evening relative humidity were found to be non-significant with the mean population of leaf miner. The minimum temperature was negatively significant with the leaf miner population. Aawathanarayana Reddy and Ashok Kumar (2004) found that the peak infestation of leaf miners on melons was noticed during March - April.

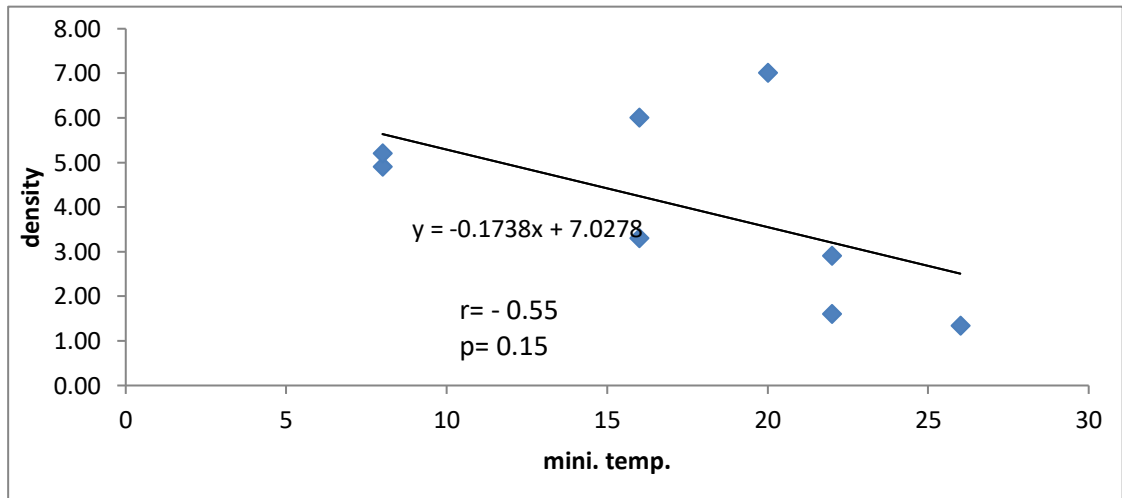
Saha et al. (2018) noticed that the leaf damage by leaf miner (*Liriomyza trifolii* Burgess) on cucumber was maximum (3.30 to 4.20 mines/ vine) during the last week of April to the third week of May, the correlation studies of leaf miner showed a significant positive correlation with maximum and minimum temperature, whereas negative and no significant correlation with relative humidity and rainfall.

Sunil (2015) reported that during Kharif season positive correlation existed between leaf miner infesting bitter gourd with maximum temperature ($r = 0.07$), minimum temperature ($r = 0.43$), maximum RH ($r = 0.02$), minimum RH ($r = 0.10$) and negative correlation with rainfall ($r = -0.26$). Dubale et al., (2018) reported that the various meteorological parameters like maximum temperature, minimum temperature, morning relative humidity, evening relative humidity, Bright Sun Shine Hours (BSS), and rainfall were found to be non-significant. The results of the investigation on population dynamics of leaf miner, *Liriomyza bryoniae* in relation to abiotic factors revealed that the pest commenced from the 10th standard week, which remained till the 26th standard week with its peak activity during the 15th standard week. The correlation studies indicated a highly significant and positive association between the population of leaf miners and maximum temperature (0.120), while, significant and negative correlation with relative humidity in the evening (-0.488) and rainfall (-0.538). The overall impact of abiotic factors on the population build-up of leaf miners was 80.30 percent (Khaliq and Shankar 2020).

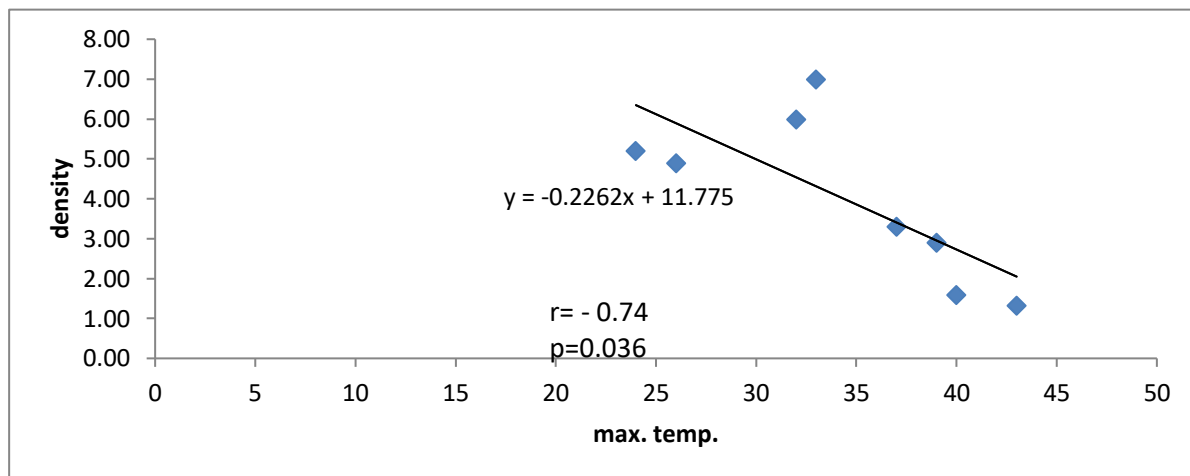
Ashlata (2002) recorded that leaf infestation (%) by leaf miners and pest population on leaf miners correlated with meteorological parameters; she found that both positively correlated with maximum temperature and sunshine hours and negatively correlated with relative humidity and minimum temperature on tomato crop. Whereas, Bagmare et al. (1995) reported that the maximum temperature and sunshine hours had a positive correlation with the population of *Liriomyza bryoniae* on tomatoes, whereas rainfall and relative humidity had a negative association with the population of leaf miners.



Fig(2) population density of leafminer on cucumber



Fig(3) Correlation coefficients (r) between population of leaf miner on cucumber and minimum temperature.



Fig(4) Correlation coefficients (r) between population of leaf miner on cucumber and maximum temperature.

Table(1) Impact of weather factors on the population of leaf miner on cucumber

Factor	R ²	100 × R ²	Role of factor (%)
$Y=11.78 -1.15x_1$	0.55	55	55
$Y=17.49 -0.595x_1 + 0.402 x_2$	0.73	73	18
x1= Maximum temperatures x2 = minimum temperatures			

parasitoids of the genus *Liriomyza bryoniae*

Two species of insect parasitoids (*Diglyphus isaea* and *Opius sp*) Hymenoptera order were recorded on the leafminer *Liriomyza bryoniae* larvae and pupae. Table (2). Adults of parasitoids were diagnosed at the Research Center and Museum of Natural History / University of Baghdad by Dr. Razak Shaalan Akl Dr. Hana Hani Abdel Hussein.

Table (2) Diagnosis two types of insect parasitoids isolated from leafminer *Liriomyza bryoniae*

	Parasitoid	Order	Family	Type of parasitism	Favorite phase
1	<i>Diglyphus isaea</i> (walker ,1838)	Hymenoptera	Eulophidae	external	Larva
2	<i>Opius</i> sp (wesmael ,1835)	Hymenoptera	Braconidae	internal	larva-pupa

The parasitoid *Opius sp* is a larva - pupa, that is, the female lays her egg inside the larva making leaf tunnels without leading to its death, which develops and molts until it reaches the third larval age and then becomes a pupa. Inside the pupa of the leafminer, the larva of the last larval age of the parasitoid begins to attack and feed on the tissues and organs of the pupa and kill it, then turns into a pupa inside the pupa of the leafminer, of which only the transparent outer cuticle remains until the emergence of an adult parasitoid. (Mahmood, 2020).

Seasonal occurrence of parasitoids

The leafminer *L. bryoniae* was attacked by a number of parasitoids that included *Diglyphus isaea* (Walker,), (Hymenoptera: Eulophidae), *Opius sp.* (Hymenoptera: Braconidae). The average numbers of adults of each parasitoid species during the growing season is given in Figure (5). *Diglyphus isaea* was the most common species. It was active throughout the growing season and increased in number as the leafminer populations increased. The rate of % parasitism was 4.3 adults in 7 Aug. increased to 7.8, 12.8 , 20.3 and 38.2 % in 1 Sept., 7 Sept. , 7 Oct., and 21 Oct respectively ,while rate of % parasitism of *Opius sp* was ranged from 2.6 % to 5.9 % from 14 Sept to 21 Oct. It could be concluded that, the *D. isaea*, seems to be effective natural antagonist on the leaf miner, *D. isaea* recorded highest average numbers in December when its host reached high population because *D. isaea* prefers the high population of its host (Linden,1993).

Natural abundance of the ectoparasitoid *Diglyphus isaea* walker was studied on four winter host plants, broad bean (*Vecia faba*), pea (*Pisum sativum*), mallow (*Malva sylvestris*), and snow thistle (*Sonchus oleraceus*), in Alejelat region (Elkhoully et al. 2015), *D. isaea* recorded 3-4 peaks of abundance on all studied host plants, the highest peak recorded 64, 198, 70 and 103 individuals/ 100 infested leaflets on broad bean, pea, mallow, and snow thistle, respectively. The highest average numbers occurred in December on broad bean recording 41.0±20.7, in March on pea recording 127.0±74.0, and in February on mallow and snow thistle recording 37.8±19.1 and 87.5±12.7 individuals/ 100 infested. On the other hand, monthly average percentages of parasitism recorded its highest numbers in January, 66.7±5.6%, 69.5±13.4%, and 57.9±6.7%, on brad bean, pea, and mallow, respectively and 68.8±6.9% on snow thistle in March. The percentage of killed larvae, according to host-feeding recorded, its highest

monthly average numbers January on broad bean, pea, mallow and snow thistle recording, 21.7 ± 17.7 , 17.3 ± 4.3 , 23.7 ± 2.1 and 14.4 ± 5.5 , respectively.

In a study carried out in cucumber greenhouses during the spring and autumn seasons of 1999 and 2000 (C'ivelek, et al. 2002), Five parasitoid species were collected: the braconids *Bracon intercessor* Nees von Esenbeck and *Opius meracus* Fischer, which occurred only in the spring; and the eulophids *Diglyphus crassinervis* Erdos, "*Diglyphus isaea* (Walker) and *Neochrysocharis formosa* (Westwood), which occurred in both the spring and autumn seasons. *D. isaea* and *N. formosa* were the predominant parasitoid species found. *O. meracus* was recorded for the first time as a Turkish species and *B. intercessor* was recorded for the first time in *L. huidobrensis*.

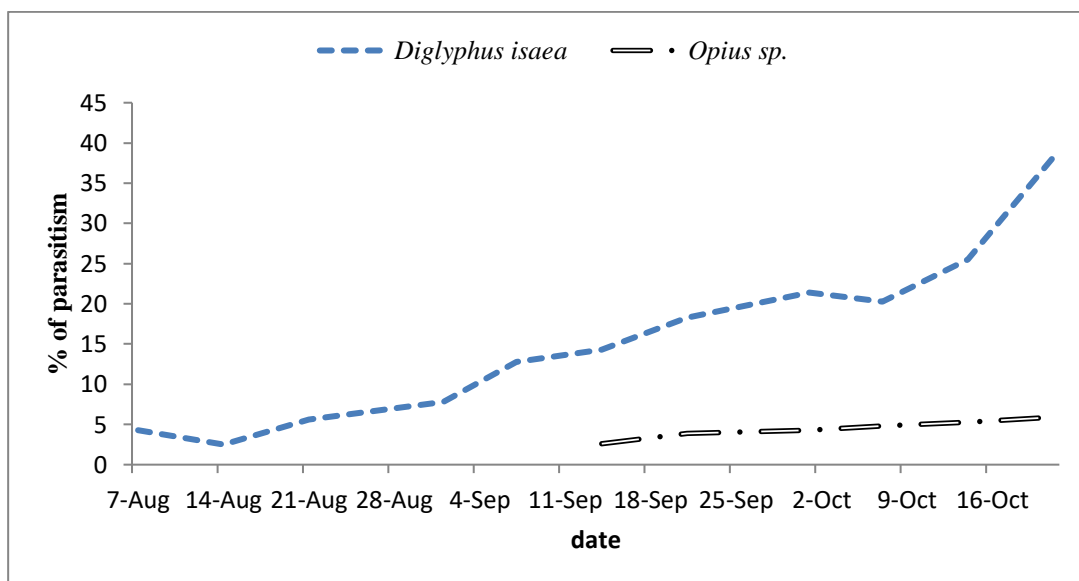


Fig. (5) Seasonal abundance of larval ectoparasitoids *Diglyphus isaea* and larval-pupal endoparasitoid *Opius sp.* on *L. bryoniae*

Conclusion

1. The highest population density of *Liriomyza bryoniae* was recorded in the beginning of November with a negative correlation a high temperature.
2. Two types of insect parasites have been identified on *Liriomyza bryoniae* larvae and pupae
3. *Diglyphus isaea* was the most common species. It was active throughout the growing season and increased in number with increasing numbers of leaf insects.

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