The Response of Leaf Miner *Liriomyza trifolii* to Cowpea *Vigna* spp Plant
استجابة حفار الاوراق *Liriomyza trifolii* الى نبات اللوبيا *Vigna* spp

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Abstract

This study was conducted in a field located at Department of Biology/College of Sciences/University of Baghdad, from the period between the Mid of August to the second half of October 2015, in order to study the population density of cowpea leaf miner on California Ramshorn cowpea and local varieties (Bayader) and their natural enemies. The result showed fluctuation of the population density for *Liriomyza trifolii* on the American variety. Two peaks for population density were shown and the second was higher than the first. The highest population density was 6.5 leaf miner/ leaf while the lowest was 0.7 leaf miner/leaf at the beginning of sampling. For the local variety, three peaks were recorded. The first was the highest with 2.61leaf miner/leaf and the lowest was 0.2 leaf miner/leaf. Population density of insect was higher on American variety has higherthan the local variety in its protein content which reached to 24.3%. Two hymenopteran parasites *Pediobius matallicus* and *Diglyphus isaaea* belong to the family Eulophidae were recorded on *L. trifolii.* This study, the first was conducted on the leaf miner on this host plant.

Keywords: *Liriomyza trifolii* , Cowpea, Protein, Parasite

الملخص

اجريت الدراسة في حقل تابع الى قسم علوم الحياة/كلية العلوم/ جامعة بغداد للفترة من 8/18 ولغاية 2015/11/2 , واظهرت النتائج عن تذبذب الكثافة السكانية للحافرة *Liriomyza trifolii* على صنف اللوبيا الامريكي (California Ramshorn) وبينت النتائج عن وجود ذروتان للحافرة وتفوقت الذروة الثانية على الاولى، كما اظهرت النتائج ان اعلى كثافة سكانية للحافرة بلغت 6.5 حافرة / ورقة. وتبين من النتائج ايضا أن اقل كثافة سكانية في بداية اخذ العينات أذ كانت 0.7 حافرة/ ورقة، اما صنف اللوبيا المحلي (بيادر) فأظهر وجود ثلاث ذروات للحافرة وتفوقت الذروة الاولى على الثانية والثالثة وان اعلى كثافة سكانية للحافرة قد بلغت 2.61 حافرة / ورقة، فيما بلغت اقل كثافة سكانية 0.2 حافرة / ورقة، ويلاحظ ايضا تفوق الصنف الامريكي على الصنف المحلي بنسب البروتين اذ بلغ 24.3. واظهرت النتائج ايضا عن وجود المتطفلين *Diglyphus isaaea* و*pediobius matallicus* واللذان يعودان الى عائلة Eulophidae والى رتبة Hymenoptera والتي سجلت على حافرة الاوراق *L. trifolii*. وهذه الدراسة تعد الاولى التي اجريت للحافرةعلى هذا العائل النباتي.

الكلمات الدالة: *Liriomyza trifolii* , اللوبيا , بروتين , متطفل

Introduction

Cowpea *Vigna* spp is one of economically important leguminous crop in Iraq and worldwide. It is grown for its edible parts, the green pod and dry seeds, as a pasture plant and to improve soil [1]. Cowpea has a nutritional value, as a source of protein, carbohydrates and minerals. It has been suggested that cowpea was originated in West Africa [2]. Cowpeas are attacked by many pests including aphids, legume worm, bean beetle and cowpea leaf miner *Liriomyza trifolii*.

Leaf miner (*Liriomyza trifolii*) is a phytophagous fly feeding on various vegetable and ornament plants worldwide [3], particularly, those grown in protected culture. The adult feeds on leaves and oviposit between epidermis layers of the leaf. Females avoid ovipositing in immature leaves. Eggs are white, small and oval-shaped, when hatched; larvae immerged and start to feed. They make snake-like tunnels; appear obviously, 3-4 days after oviposition. Tunnels increase in size while larvae grow, resulting in destroying of leaves and poor photosynthesis of infested plants [4]. When growth is complete, larvae emerge from tunnel to pupate in soil [5]. The larva completes growth and development in a single leaf. Females lay about 300 eggs during their short life cycle which is up to 17-19 days at 25°C, and they have 6 generations in tropical climates [6]. Female selects host and food. It has been indicated that females decide whether to oviposit or not based on three major factors: the suitability of the host plant, presence or absence of other plants with a similar structure and the availability of environmental conditions. Results from published

works, show the suitability of host plant depends on many factors like leaf size and thickness, mechanical barriers in the leaf, nutrient levels besides leaf quality, all of which have a major effect on insect performance feed on it [7]. Biological control is one of the natural controls which have a role to keep environment healthy and unpolluted. Natural enemies include parasites feed on adults or larvae in infested leaves as there are over 14 parasite species. However, predators and bacterial disease are less important compared to parasites attack leaf miners [8]. This study aimed to study the population density of cowpea leaf miner and their natural enemies.

Materials and Methods

Field sampling

Leaf samples were collected from California Ramshorn cowpea and local (Bayader) varieties grown in 400m^{2}field located at Botanical garden in College of Science/ University of Baghdad in 15 th of September. Plants were grown in 10 rows, each row length is 10 meters. Two seeds per hole were sown in the cowpea growing season started in the 1st of August, 2016, then one cowpea plant per hole was kept when plants were at the five leaves growth stage. Fifteen leaf samples per week were collected randomly, starting from the 18th of August, up to the last week of November, 2016. Each samples was placed in a clear polyethylene bag, then transferred to the laboratory. Population density was measured for each leaf based on the number of infested tunnels, then leaves stored in plastic boxes sealed with organza fabric by a rubber band. Extra samples were collected and placed in (7x15) plastic boxes with a piece of wet cotton, then used for further characterization. When emerged, leaf miner adults and their parasites emerged were collected, placed in small plastic containers contain preservative solution and sent to the Iraqi Natural History Museum/University of Baghdad for identification.

Estimation of protein

The amount of protein was measured in leaves as follows: ten leaves randomly collected from the field were mixed and dried in the oven for 24 h at 40°C. Protein percentage was estimated using Micro-Kjeldahl device as follows: up to 0.2 gm of grounded leaves was mixed with 3.5 ml of concentrated H_2SO_4 and stored for 24h at room temperature. The reactant mixture was placed on a hot plate, then 3 ml of 1:1($HCLO_3$: H_2SO_4) mixture was added at a controlled temperature. When changed to a clear color, the solution mixture was cooled, then adjusted to 50 ml using distilled water. Dye was prepared using 20 g boric acid diluted in 1L distilled water and 20 ml of dye, pH adjusted to 3.8-4.5. Dye contained 0.066 ml methyl red, 0.044 ml bromoresol green diluted in 100 ml ethanol. About 10 ml of dye was added into Micro-Kjeldahl tube, 4ml of 10% NaOH was added and placed in the apparatus. When solution volume was 35 ml, it was tittered using HCl [9].

Nitrogen was estimated using the followed equation:

$$\mathbf{N\%==\frac{1.401\ X\ 0.05\ (Molarity) X\ 10\ (Dilution) X[Volume\ of\ Acid\ used\ in\ titeration-(02) Blank]}{Sample\ wieght\ (g)}}$$

Protein percentage was calculated as follows:

Protein%=N%x 6.25 (factor) (AOAC, 1984)

Results and Discussion

The population density of cowpea leaf miner *L. trifolii* on the California Ramshorn cowpea variety in the field for the period from 18/8/2016 to 24/11/2016 was shown in (Fig1). Results revealed that the leaf miner was observed in the cowpea field during the growing season with fluctuated population densities. The presence of insect started at the beginning of seedling growth, continued but fluctuated throughout to the end of the season. Two peaks for the population density were shown; the second was higher to score 6.5 miner/leaf in the second week of November. While the first was in mid-September and scored 4.3 miner/leaf. The lowest population density was at the beginning of sampling which was 0.7 miner/leaf. At the end of experiment, the number of pest individuals decreased due to the presence of natural enemies as numbers of parasites increases whenever the pest increases and vis versa [10]. This fluctuation of cowpea leaf miner density may be attributed to environmental conditions and the presence of natural enemies. Thus, the miner crop preference depends on the suitability of plant properties to insect feeding habits,

oviposition and the protection against external condition [11], as [12] revealed that chemical and morphological characterizations determine the infection severity.

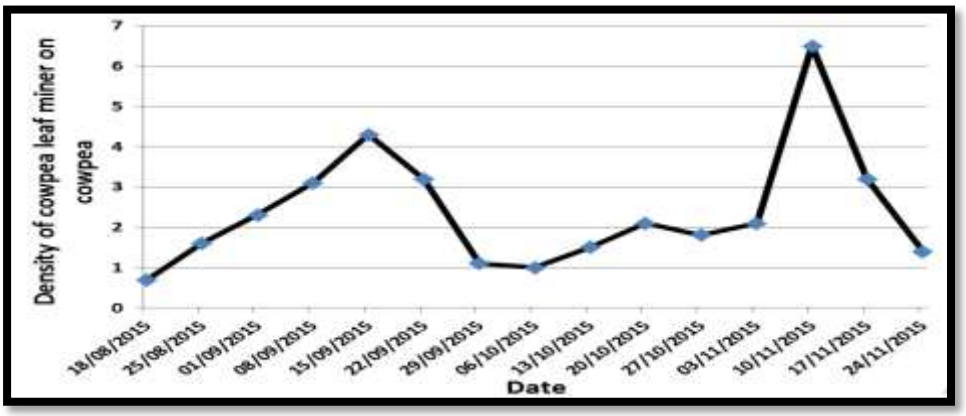


Fig.(1): Population density of cowpea leaf miner *Liriomyza trifolii* on the California Ramshorn cowpea variety

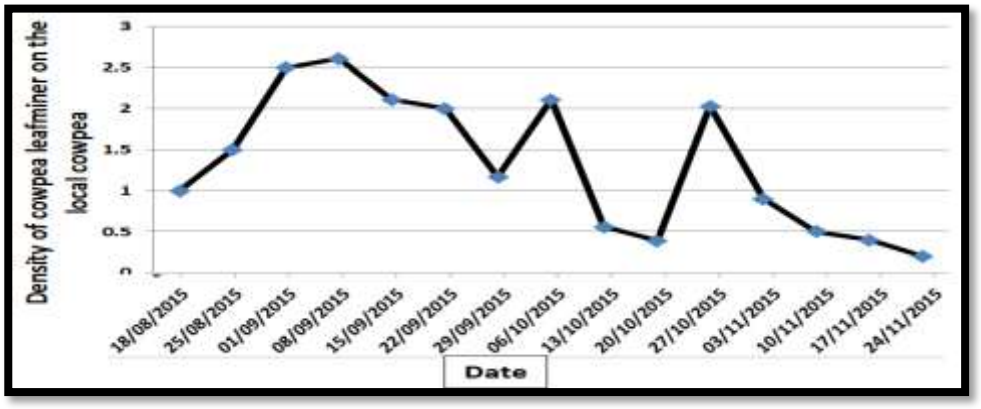


Fig. (2): Population density of cowpea leaf miner *L. trifolii* on the local cowpea variety (Bayader)

Three peaks of *L. rifolii* on local cowpea variety (Bayader) were observed, the first was the highest (Fig 2). Results obtained from the pest population density showed the highest was 2.61 miner/leaf in 8th of September, while it was 2.11 miner/leaf in 6th of October and 2.03 miner/leaf in 27th October for the second and third peaks respectively. Whereas, the lowest was 0.2 miner per leaf in 24th November, 2016 cowpea growing season. Similarly, [13] indicated that the population densities of whitefly and cowpea leaf miner were quit high during 2011-2012 bean growing season, but they increased for the next season. They suggested presence of two peaks for the pests during the season based on temperature and relative humidity, as they found relative humidity had a positive effect on cowpea leaf miner.

Table (1): Natural enemies of leaf minor *L. trifolii*

Family	Order	Parasite
Eulophidae	**Hymenoptera**	***Diglyphus isaaea* (Walker)**
Eulophidae	**Hymenoptera**	***Pediobius matallicus***

Two parasites, *Diglyphus isaaea* and *Pediobius matallicus* belong to the family Eulophidae and the Order Hymenoptera were identified. Conceivably, they have a role in lowering *L. trifolii* population density as a part of natural balance [10]. In Antalya, The population density of cowpea leaf miner *L. trifolii* was investigated on cowpea and tomato grown in protected culture. The study indicated the occurrence of *Neochrysocharis `ormosa*, *Diglyphus isaea* and *D. carssinervis* parasitized on *L. trifolii* by 67%. The study indicated that *L. trifolii* infestation was below economic threshold due to natural enemy activity

during spring season [14]. In Florida, there are at least 14 species of *L. trifolii* parasitoids occur [15]. Yellow, sticky traps were used to monitor and observe *L. trifolii* and its parasites activities. This approach could identify three different species of *Diglyphus* spp, namely, *D. begini*, *D. intermedius* and *D. iseae* [16]

Protein percentages were 24.3% and 13.4 for the California Ramshorn cowpea and local verities (Bayader), respectively. Despite they used higher numbers of leaves for protein analysis, our findings approached [17] results. They showed that protein percentages of cowpea cultivar IT84E-124 leaves were 43% and 30.5% for 7-10 and 22-25 days growth stage leaves. [18] Indicated that chemical and morphological characterizations determine the infection severity. Therefore, higher protein concentration in American variety than the local variety lead to increased numbers of leafminers to American variety than the local variety. Larbat et al. evaluated the influence of different levels of nitrogen fertilization on the tomato and the leafminer (*Tuta absoluta*), they concluded that from this study provide a comprehensive dataset concerning interrelated responses of tomato plants and *Tuta absoluta* to nitrogen nutrition [19].

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