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THE HOST PLANT PREFERENCE OF THE LEAF MINER *LIRIOMYZA* SPP. (DIPTERA: AGROMYZIDAE) ON EGGPLANT (*SOLANUM MELONGENA* L.) AND SQUASH (*CUCURBITA PEPO* L.) CROPS AND ITS RELATION WITH THE SECONDARY COMPOUNDS IN PLANT

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Abstract

A field study was carried out on eggplant (*Solanum melongena* L.) and Squash (*Cucurbita pepo* L.) crops under the conditions of the plastic house in the Plant Protection Department / College of Agricultural Engineering Sciences / University of Baghdad, to determine which is the most preference from the leaf miner *Liriomyza* spp. As well as its relation with the secondary compounds existing in the plant that's well-known for its importance in the defense system of the plant. The results showed a significant difference in the leaf miner *Liriomyza* spp. preference to infection over eggplant (*S. melongena* L.) and Squash (*C. pepo* L.), Squash was the most preference from the insect and the general average of insect population density on Squash throughout the growing season was 39.2 individual / 10 leaves compared with eggplant which was 7.83 individual / 10 leaves. The study results also showed a variation in the leaves content of eggplant and Squash of secondary compounds (Phenols, Flavonoids, Tannins and Alkaloids) and their different concentrations when the infestation with the insect, as it is increase when it increases and decrease when it decreases with as a defensive means. Finally, the study findings showed the presence of Tannins was in very small quantities below the level of sensitivity over the Squash leaves, which is not preferred by insects and it is one of the compounds that known as their toxic effect to insects, while eggplant resistance to the insect was attributed to its high content of Phenols, Tannins, total Alkaloids and Flavonoids compared to Squash.

Key words: Host Plant preference, Leaf miner *Liriomyza* spp., eggplant,Squash, Secondary compounds, Tannins.

Introduction

Eggplant (*Solanum melongena* L.) and Squash (*Cucurbita pepo* L.) have considered one of most important vegetable crops in Iraq, the nutritional importance of those two crops were because both vegetables contain Carbohydrate, volatile oils and a few quantities of A, B, B2 vitamins, Iron salt, Calcium and Phosphorus, in addition to its medicinal benefits in treating some diseases. Moreover, Eggplant (*S. melongena* L.) is classified as one of the top ten types of vegetables for therapeutic uses (Danny *et al*., 2000; Al- Rikabi and Abdul -Jabbar, 1981; Vinson *et al*., 1998; Kwon *et al*., 2008).

Eggplant (*S. melongena* L.) and Squash (*C.pepo* L.) crops attacked from a number of insects and non-insect pests, where the leaf miner belonged to the genus of *Liriomyza* (Agromyzidae: Diptera) is one of the most dangerous pests that attack a number of plants belonging to 31 plant families, including vegetable crops that has an economic importance such as: (Cucumbers, Tomatoes, Potatoes, Eggplants and ornamental plants) caused a destroying of the leaf area through feeding their larvae between the two surfaces of the leaf (Spencer, 1973). The leaf miner *L.sativae* (a wide host range) on many crops, including the Solanaceae such as Tomato, Leguminosae such as Beans, *Vicia faba* and Gourd family Cucurbitaceae such as *Cucurbita pepo*, *Cucumis melo*., Water melon, *Cucumis sativus* (Abul-Nasr and Assem, 1961 and Spencer, 1990).

A leaf miner preference that belonged to the genus *Liriomyza* for the different varieties of plants back to the presence of Trichomes, which its distribution and density varies among the leaves and are important factors in the election of the host by adults (Fagoonee and Toory, 1984, Knodel *et al*., 1985). Many researchers have

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indicated that Trichomes produce secondary metabolic compounds, including glycoalkolodis, Phenols and Flavonoids compounds, which are a defensive compound against many insects (Goffreda *et al*., 1990, Elliger *et al*., 1981). Finally, in order to determine the effective crop in their inhabitant's lives of the leaf miner *Liriomyza* spp, this study was aimed to food preference of this insect and its relation with the secondary compounds responsible for resistance in the plant.

Materials and Methods

Eggplant (*S. melongena*) and Squash (*C. pepo*) crops planting

This experiment was conducted in a plastic house belonged to the Plant Protection Department / College of Agricultural Engineering Sciences / University of Baghdad in the season 2018-2019, the plastic house area of (237.5) m^2 *and* with a dimension (25m length, 9.5m width, 3.5m height). The soil was sterilized by solarization method and prepared for planting by conducting all necessary agricultural operations and accordance with the approved recommendations. After preparing the soil well, the plastic house was divided for the purpose of planting it with eggplant seedlings and Squash, where the experiment was designed according to a Randomized Complete Block Design (RCBD) with three replicates, each replicate contains two experimental units. Two types of plant, Eggplant (*S. melongena* L.) and Squash (*C. pepo* L.) were planted on 15/9/2018 to know its sensitivity and preference by this insect with an average of one cultivar per crop and these cultivars are Iraqi wonder F1) for Eggplant crop and cultivar (Ismalia F1) for Squash. A weekly sampling program has been set, which started from the first planting of seedling for these two crops and the appearance of real leaves on 3/10/2019 to 9/5/2019, where 10 plant leaves were selected randomly from each experimental unit and crop, therefore the sampling size becomes 30 plant leaves. These leaves were placed in polyethylene bags numbered according to the crop and replicate and taken to the laboratory to calculate these leaves contain eggs, larvae, pupae *and* extract its average per one leaf as follows:

• Egg: Eggs numbers that placed in the feeding punctures on the leaf epidermis and implanted, were calculated with the ovipositor.

• Larvae: The leaves were tested under the light microscope, where the initial ages that small in size were calculated under the microscope, while the visual inspection eye for the large size larval ages.

• Pupae: The numbers of pupae that located on the top surface of the leaves are calculated after each leaf was placed separately in plastic boxes of different sizes, depending on the leaf size. Each of which was placed with filter paper moisturized with water to keep the leaves from drying, then put it in the incubator at a temperature of 25 ± 2 and relative humidity 65-70%. These boxes are monitored to calculate the number of emerging adults until the leaves are completely dry.

Determination of leaves content of secondary metabolic compounds (Phenols, Flavonoids, Tannins, Alkaloids) on Eggplant and Squash:

For the purpose of identifying some important secondary metabolites in Eggplant and Squash crops and their relation to leaf miner *Liriomyza* spp, chemical and qualitatively analyzes has been carried out, including:

• Phenols: The method that used by (Khadijeh *et al*., 2014) was implemented to detect the total phenolic compounds using the Folin- ciocalteu, which is prepared firstly and then the standard matter (Gallic acid) was prepared. A weight of sample was taken, then a 500 µl of Folin- ciocalteu and 1.5 ml of sodium carbonate with concentration of 20% were added to it and mixed with the addition of 10 ml of distilled water. The reaction left for two hours, then its absorption was recorded along a wavelength of 765 nm. A standard curve of Gallic acid was prepared by using the Calibration curve and is measured in (mg) based on dry weight before starting the above procedure.

• Total Flavonoids: A one gm of the sample was taken and 50 ml of Ethanol, 0.3 ml of Sodium Nitrite $NaNO_2$ at a concentration of 5% and 0.3 ml of trioctyl Ammonium chloride $AICI_3$ were added to it *and* left for 6 minutes, then a 2 ml of Sodium hydroxide NaOH was added and left for 15 minutes *and* read over a wavelength of 510 nm, a standard curve of rutin matter was prepared using a calibration curve and measured in units (mg) based on dry weight (Shahid and Shoib, 2015) before the above procedure.

• Total Tannin: A one gm of the model was taken, then a distilled water added to it and placed in a water bath, then iron chloride was added, the dark green color appearance indicates the presence of the Tannin, Finally, a 2 ml of Sodium Chloride and 5 ml of gelatin was added to and 1 ml of the extract, then the titration process was carried and measured along a wavelength of 450 nm (Mekhaldi *et al*., 2014).

• Alkaloids: A 20 gm of the sample was taken and 200 ml of Methanol was added to it and left for 24 hours, then placed in the Soxholet extractor device *and* the output is dried in the rotary evaporator device with a temperature of 45°C until full dry limit (Manjunath *et al*., 2012).

Table 1: Average number of leaf miner *Liriomyza* Spp. in eggplant and Squash crops for the season 2018-2019.

Sampling date	Average number of leaf miner individual's/10 leaves		Average
	Eggplant	Squash	
3/10/2018	12.7	14.6	13.7
10/10/2018	18.9	30.8	24.9
17/10/2018	15.2	45.2	30.2
24/10/2018	17.9	46.3	32.1
31/10/2018	36.8	148.7	92.8
7/11/2018	24.8	111.6	68.2
14/11/2018	15.2	79.9	47.6
21/11/2018	9.1	104.1	56.6
28/11/2018	15.1	116.7	65.9
5/12/2018	14.1	101.6	57.9
12/12/2018	9.5	64.9	37.2
19/12/2018	8.2	24.4	16.3
26/12/2018	8.8	21.5	15.2
2/1/2019	10.1	56.9	33.5
9/1/2019	20.3	24.2	22.3
16/1/2019	7.5	15.7	11.6
23/1/2019	10.5	33.1	21.8
30/1/2019	3.3	46.2	24.8
6/2/2019	13.7	52.9	33.3
13/2/2019	17	57.7	37.4
20/2/2019	15.6	30.7	23.2
27/2/2019	17.8	18.9	18.4
6/3/2019	15.7	13.2	14.5
13/3/2019	19.3	-	9.7
20/3/2019	20.3	-	10.2
27/3/2019	32.8	-	16.4
3/4/2019	36.4	-	18.2
10/4/2019	16.2	-	8.1
17/4/2019	33.1	-	16.6
24/4/2019	47.8	-	23.9
2/5/2019	39.6	-	19.8
9/5/2019	28.7	-	14.4
General average	17.83	39.2	28.5
Values LSD	Plant type: 7.52*; time: 15.63*; interaction: 19.72*		

• Alkaloids detecting: A two ml of the extract was taken with the addition of HCL by calibration of 5%, then two drops of the Dragon reagent were added to detect the formation of form crystals as an indication of the presence of alkaloids.

Statistical analysis

The field experiment was designed according to the Randomized Complete Block Design (R.C.B.D.) *and* the data were analyzed statistically using the variance analysis table. Furthermore, the least significant difference LSD was adopted at a probability of 0.05 for comparison of average results based on the statistical program for this purpose (SAS, 2012).

Results and Discussion

The results of table 1, showed average number of leaf miner *Liriomyza* spp. in the plastic house for the period from 3/10/2018 to 9/5/2019 on the Eggplant (*S. melongena*) and Squash (*C. pepo*) crops, the general average of insect density on Eggplant and Squash along the growing season has reached (17.83, 39.2) individual / 10 leaves, respectively, the infection started on plants after seedling germination at the beginning of October of 2018, where the highest average recorded during the fourth week on Eggplant and Squash by (36.8 and 148.7) individual/10 leaves, respectively. Their numbers gradually declined during January of 2019 was lowest average was recorded during fourth week by (3.3) individual/10 leaves Eggplant, while the lowest average was occurred in the second week of January by (15.7) individual/10 leaves / squash. Furthermore, the pest intensity increased in February, recorded the highest average during the fourth week by (17.8) individual/Eggplant, while the highest average of Squash for this month during the second week amounted to (57.7) individual / 10 leaves. Finally, the infection on Squash disappeared in the beginning of March, as a result of the end of the plant's life and a few individual remained on the Eggplant.

The results of statistical analysis showed that there was a significant difference between Eggplant and Squash, where the Squash was the most infected by pest from Eggplant. However, This was due to the difference of the physical and chemical properties of the two studied plants, which resulting in the attraction or exclusion the pest. The results above showed that the insect of leaf miner had accompanied the two plants under study throughout the growing season from the beginning of the seedling emergence

Table 2: Determination of leaf content of Phenols, Flavonoids, Tannins and Alkaloids.

Plant type	Sampling date	Phenols mg Gallic /gm	Flavonoids mg Rutin / gm	Total Alkaloids %	Total Tannins %
Eggplant	1/11/2018	53.2	29.5	20.6	3.5
	1/12/2018	33.6	20.4	13.1	2.4
	1/2/2019	43.6	25.8	16.9	3.1
Squash	1/11/2018	29.8	16.4	2.9	0.5
	1/12/2018	19.7	11.9	1.3	0.1
	1/2/2019	22.6	14.2	2.1	0.3

until the end of the season at the beginning of May, with its numbers varying according to the type of host plants. The pest preference for the Squash plant can be attributed to the leaf area capacity and the amount of chlorophyll in the leaves, where the highest leaf area and amount of chlorophyll were found in the Squash, while the lowest leaf area and chlorophyll was in the Eggplant.

These results are consistent with (Facknath, 2005) findings, where the *L. trifolii* leaf miner preferences leaves that have a large surface area in egg-laying and feeding by adults. Therefore, the leaves of the Squash were superiority to an adult's infection because they were large in size compared to the Eggplant leaf. Many researchers have pointed out that leaf miner *L. trifolii* preferred feeding and laying eggs on plant leaves with high nitrogen content. In other words, whenever the amount of chlorophyll in the vegetable juice increased resulted to increase infected by the leaf miner (Minkenberg and Fredrix, 1989; Minkenberg and Ottenhem, 1990).

Moreover, the plant content measurement of Phenols, Flavonoids, Tannins and Alkaloids was described in table 2, showing that there were a difference in the secondary compounds content for Eggplant and Squash leaves.

The highest concentration of Phenols (estimated based on Gallic acid), Flavonoids, total Alkaloids and total Tannins were in the Eggplant leaves during the period of (1/11/2018) were amounted to (53.2 mg Gallic / gm, 29.5 mg Rutin / 20.6, 3.5 gm), respectively, compared with the lowest concentration of Squash that recorded (29.8 mg Gallic / gm, 16.4 mg Rutin / gm, 2.9, 0.5), respectively. The concentration of these materials has also decreased from the previous concentrations during the period (1/12/2018) on Eggplant by (33.6 mg Gallic / g, 20.4 Rutin / g, 13.1, 2.4) respectively, while on Squash were (19.7 mg Gallic / g, 11.9 mg / Rutin, 1.3, 0.1), respectively.

Finally, these Concentrations increased in Eggplant during the period (1/2/2019) and reached to (43.6 mg Gallic / g, 25.8 mg Rutin / g, 16.9, 3.1) respectively, while on Squash reached to (22.6 mg Gallic / g, 14.2 mg Rutin / gm, 2.1, 0.3) respectively.

The study findings showed the presence of Tannins was in very small quantities below the level of sensitivity over Squash leaves, which is not preferred by insects and it is one of the compounds that known as their toxic effect on insects.

The results showed that the Phenols concentration was high at the beginning of the infection on Eggplant and Squash and gradually decreased as plant life progressed and infection. This indicates its importance in the plant defense system, as it increases with infection increased and decreases when infection decreased with as a defensive means *and* this is consistent with (Montoya *et al*., 2006) findings Flavonoids, Terpenoids, Alkaloids and Phenols are the important secondary compounds that are related to insects, where the fat soluble Flavonoids in the leaves produce phenolic resins that prevent insect feeding and these Flavonoids can also combine with proteins to form the Tannins, which the insects do not preferred (Gould and Lister, 2006).

(Feeny, 1970) pointed out that Tannins in plant leaves have a wide range of activity and have also proved effective against fungi, viruses, insects and mammals. The resistance of Eggplant plants to insect may be due to its high content of Phenols, Tannins, total Alkaloids and Flavonoids compared with Squash.

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