

Chemical and Biological Control of Radish Leave Miner Insect *Scaptomyza flava* Fallen (Diptera:Drosophilidae) under the Conditions of Basrah Province

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Abstract

This study was conducted at the district of Shatt Al-Arab in Basra province in order to evaluate several pesticides and *Beauveria bassiana* fungi against radish leaves miner and to estimate the severity of the injury and damage to this insect. Results showed that the highest population density was in March 2016 (1.78 larva/leaf), where the highest infection rate was (76%), and the injury severity showed a significant differences among study intervals, where the injury locations recorded a significant differences among them represented by surpass of outer leaves on other locations (1.8%). All examined pesticides showed high affectivity in controlling the pest with a simple significant difference killing percentage performed by Super Saqr followed by Dragon and Sakhuy (92%, 85.6%, 82.2%) respectively where the lowest killing percentage was performed by biocontrol agent (54.4 %) after 72 h of treatment.

Keywords: *Scaptomyza flava*, Radish leaf miner, Pesticides, Basrah, Iraq.

Introduction:

Radish is a winter herbaceous plant of the Cruciferae – Brassicaceae family. The edible parts of this vegetable are its roots and fresh leaves. In general radish root contains carbohydrate, sugars, dietary fibers, protein and even some fat and fluoride, I addition, it contains various water-soluble vitamins (B1, B2, B3, B5, B6, B9 and C) (Khattak, 2011). The production of the radish per hectare in the world is 10.2 - 11.3 tons per year and the area cultivated in Iraq is estimated at 9113 dunams. And the production capacity is 2833 kg (Khushk and Hisbani,2003). Radish is infected with many insect pests that belong to different order, the most important one is *Scaptomyza flava* from the family Drosophilidae and the order Diptera. This insect also infects many plants from Cruciferae, including cabbage, cauliflower and turnip. The harmful phase of this insect is the larval stage, where it feeds on the chlorophyll found in between the two layers of the leaf epidermis causing transparent white spots on the top surface of the leaf leading to significant economic damage to the plant (Seraj,1994). The first record of *Scaptomyza flava* was in New Zealand in 1964, *Scaptomyza flava* is a polyphagous insect, infesting more than 10 plant families, it was found on the Leguminosae, including peas, it was also found on parsley, cabbage, broccoli and

cauliflower (Martin, 2004; Maca, 1972). In Iraq, it was registered in 2015 in Basrah province on radish plant. The chemical pesticides played an important role in the control of leaf miner insects, in America, field and laboratory tests were conducted to assess the toxicity of the insecticide Abamectin to control the leaf miner insect *Liriomyza trifolii* in adults and the larvae phases on cabbage (Trumble, 1985). The negative effects of these chemicals pesticides represented by environmental pollution and the remaining impact, made the researchers try to use alternative means for these pesticides such as the use of biological control against insect for example the use of fungus *Beauveria bassiana* and the bacteria *Bacillus thuringiensis* (Van lenteren, 2006). This study was conducted due to the economic importance of this insect especially when it is being new to the Iraqi environment and to determine its spread as much as possible, especially at Basrah province and to provide sufficient information on the extent of damages in the plants caused by this insect.

Materials and methods

This research was conducted at Houta, Shatt Al-Arab, Basrah province during the agricultural season 2015/2016 of the four months, December, January, February and March (the growth period of radish plant). Four random sites were chosen in fields of radish (*Raphanus sativus*) cultivation with homogeneous plant density and service operations.

Laboratory Study:

For the purpose of preparing radish seedlings and a laboratory colony for *Scaptomyza flava*. Seeds of radish in a range four of them were planted in a 12 cm diameter and 12 cm height plastic pot containing a mixture of 1 mixed soil:1 bet moss after dusting the soil with fungicide redomyl granulated with 5 g of a commercial pesticide per 1 kg soil.

When the seedlings reached the stage of the four leaves, they were placed in a wooden cage with (1*1*1m) in dimensions and covered all its sides by gauze cloth except its base which was made of wood. The cage was placed in a laboratory in a good lighting location.

Then the infected seedlings of radish with various stages of the *Scaptomyza flava* were collected from Shatt Al-Arab fields in February 2016 and taken to the laboratory. The seedlings were placed in 250 ml glass containers in a liquid nutrient solution with a concentration of 3 ml / liter of water to feed the seedling, keeping it juicy, and then placing the containers in the previously described wooden cage until the insects were fully developed and the adults were left to mate and reproduce inside the cage.

The colony was continuously maintained by replacing the most damaged seedlings with freshly planted seedlings instead. *Scaptomyza flava* was diagnosed with special taxonomic keys, and the samples were also sent to the Museum of Natural History of the College of Science of Baghdad University to confirm the species of *Scaptomyza flava* by Dr. Hanaa Hani AL-Saffar, the insect was added as a new record to the insect collection in Iraq.

Evaluation of the insect population density and the percentage of infection and the impact of environmental conditions

Three insect samples were taken from each field monthly by collecting 10 random plants samples from each field. Three leaves were selected from each plant (external, central and internal), to calculate the population density of the *Scaptomyza flava*. The percentage of infection was determined on the basis of the number of healthy and the infected plants with the insect out of 50 radish samples chosen randomly from each field. A thermometer and hygrometer were used to record relative temperatures and humidity during the study.

Damage percentage:

The infested leaves were collected and placed down a transparent paper that is divided into square centimeters, the total leaf area and the area of the damaged part were calculated (white spot as a result of the biting and feeding between the leaf epidermis). The damage percentage was calculated according to the following equation (Shaban and Al-Malah, 1993) equation 1 :

$$\text{Damage percentage} = \frac{\text{damaged part area (white spot volume)}}{\text{total leaf area}} \times 100, \quad 1$$

Chemical and biological control:

Pesticides of different chemical groups were selected to study their effectiveness in killing the larvae with the recommended concentration (Table 1).

Beauveria bassiana was also used in killing the larvae, samples of it were obtained from the plant protection laboratory and then preparing the fungus suspension by adding a 0.5 cm diameter from the one week aged colony that growing on the sterile culture media to 9.5 ml sterile distilled water for five minutes to separate the spores from its sporophore and 1×10^8 pg/ml concentration was prepared depending on the Haemocytometer (Mehdi, 2002). Healthy radish leaves were collected, taking in regard the equal leaves area and placed them in glass bottles of 8*14 cm in capacity, put on each leaf 10 larvae in the fourth stage and wet cotton was placed at the base of the bottle to prevent drying plant leaf.

Three replicates of larvae for each treatment were treated with the above-mentioned pesticides in the recommended concentration of the company with 0.5 ml spray solution/repeat by micro syringe. On the other hand, the leaves were sprayed with suspension of the fungus at a concentration of 1×10^8 pg/ml. The control treatment was sprayed with distilled water, blocking the vial mouth with a cloth of gauze to prevent insect escape in addition to ventilation, the bottles were incubated at temperature 2+28, relative humidity 60% and light duration 6-10 /day (Lacey, 1997).

The number of dead larvae was recorded after one hour of spray, and the percentage of mortality was calculated and corrected according to equation 2:

$$\text{Corrected percentage of mortality} = \frac{\text{the ratio of death in treatment} - \text{the ratio of death in control}}{100 - \text{the ratio of death in control}} \times 100, \quad 2$$

The experiment was carried out according to the random design as multi-factorial experiments after the angular conversion of the percentage. The averages were compared according to the least significant difference at $P > 5\%$ (Al-Rawi and Khalaf Allah, 2000).

Results and Discussion:**Population density and infection ratio of radish leaves miner:**

The results show in Table (2), the variation in the population density of the *Scaptomyza flava* on the radish plant during the different periods of the study. The highest population density was 1.78 larvae/leaf and ratio of infection was 76% that recorded during the month of March with significant differences with other months at temperature 26°C and humidity 51.25%, while the population density and the rate of infection during the month of December was the lowest, 1.11 larvae/leaf and 10% respectively, at a temperature of 13.35°C and humidity 65.6%. That might be ascribed to the increase in temperatures and humidity during March and the decrease in them during December, Al-Zubaidi, (1992) mentioned that the increase in temperature at certain limits leads to high metabolism, which in turn leads to a rapid growth and increase of proliferative activity and thus increase the number; and for this reason also a strong positive correlation was found between population density and temperature degrees, reached 0.940358 as in Figure 1.A, which shows a positive relationship between temperature and population

density during the study period. The present results did not agree with Al-Zubaidi, (1992), who pointed out that the larvae need a wet environment to the degree of saturation in consequence to their presence within closed tunnels and if the tunnels rupture, the humidity will decline even a small extent will lead to its destruction and therefore found a strong negative correlation between the relative humidity and density Population was 0.88274, as shown in Figure 1.B, which indicates an inverse relationship between the humidity and the population density of the insect

As illustrated in Table (2), the location of the leaf can affect the population density in which the external leaves showed the highest density 1.67 larvae/ leaf that might be ascribe to the leaf composition and its surface area and the abundance of chlorophyll content. The preference insect factors for the plant, include the external structure of the plant represented by the thickness of the leaves and the presence or absence of capillaries on the leaves and the surface area and their color and other factors of the leaf (Mohamad, 1980).

Damage ratio caused by radish leaves miner insect:

The results showed no significant differences during the study periods in the percentage of damage on radish leaves in January, February and March table 3. The higher percentage of damage was 32.7% in February while the least damage to leaves was 14.8% in December which differ significantly with February due to the favorable environmental conditions that serve the insect, which increases the percentage of damages to plant leaves and this is consistent with Seraj, (1994).

Which indicated that the damage caused by insect larvae is only the tunnels within the tissues of the plant resulting from the nutrition and behavior of egg laying by female adult under favorable conditions that increase the numerical density of the insect.

While the effect of the location of the leaf can be noticed by the percentage of damage, which was 50.1% in the external leaves of the radish plant that significantly exceeded the record of other leaves locations. While the lowest rate of damage recorded on internal leaves was 7.9%. The interaction between the periods of study and the location of the leaf played a role in the percentage of damage, in which the highest damage rate was 67.9% during March on the external leaves, while the lowest rate of damage was 6.5% during December on the internal leaves

This may be due to the conditions surrounding the plant. The increase and decrease of the temperature has a role in increasing or decreasing the activity of insect damage to the plant as well as the location of the plant leaves if exposed to direct sunlight or its presence in the shade has a role in insect activity, The exposure to the sun increase the insect activity more than if it was in the shade where the area affected in the leaves exposed to the sun is greater than those in the shade in addition to the number of larvae in the leaves exposed to the sun more abundant than the shade (MacGarvin *et al.*, 1986).

Chemical and biological control:

All the used pesticides were highly effective against the insect of the radish leaves, with significant differences between them (Table 4). Super Saqr pesticide was most effective in comparison to the rest of the pesticides followed by the Dragon pesticide, the pesticide Sakhuy while the least effective one was *B. Bassiana*. The percentage of mortality resulted by these pesticides was 92%, 85.6%, 82.2% and 54.4% Respectively, after 72 hours of treatment. The high effectiveness of Super Saqr pesticide can be attributed to the systemic status of the plant tissue with sufficient concentration to kill a large number of *Scaptomyza flava*.

Balikai and Yelshetty, (2001) proved the higher activity of the Dimethoate 30 EC in insects' control and increase in the production of seeds compared to treatment with other pesticides in the fight against aphids.

As for the pesticide Dragon its effectiveness is due to the common effect of the materials Thiamethoxam and Lambda dacyhalothrin where the first one affects significantly in nicotinic acetylcholine receptors which located in the membranes of the nerve fibers of the central and peripheral nervous system of the insect. These pesticides have a continuous transmission of nerve impulses, which causes commotion, paralysis and death of the insect (Horowitz *et al.*,1998). With regard to pesticide Lamb dacyhalothrin which belongs to the synthetic pyrethroids which is known for its effect on the transmission of the nervous system orders by interfering with the channels of sodium channels and it also affect the membrane effort in the region of the nervous axons (Liu and Casida,1993), thus the pesticide Dragon target all the aspects of insect life from its nerve centers , digestive system as well as contact toxicity. The Sakhuy is a highly contact toxic pyrothroide pesticide which penetrates the body of the insect and affects the sensory receptors in the nervous system causing a shock to the insect and death within hours, it gave excellent results and rapid control.

While the *B. bassiana* effectiveness might be due to its ability to penetrate the body of the insect through the body wall , the parts of the mouth , the respiratory openings and areas between the rings or through the anus opening by the secretion of the enzyme protease to degrade the complex proteins that give the hardness of the insect cuticle to simple proteins , as well as its ability to secrete the enzyme chitinase, which works on the degradation of chitin . After the entry of *B. bassiana* into the insect body the fungus begins to produce the toxin Beauvercin that causes the death of the insect.

Table.1. Pesticides are used in current study

Commercial pesticide	Effective substance	Chemical group	Mode of action	Concentration
Dragon	Lambda-cyhalothrin Thiamethoxam	Synthetic pyrethroids Neonicotinoid	Systematic & Contact	0.5m/L
Sakhy	Alphacypermethrin	pyrethroids	Contact	1m/L
Super saqr	Dimethoate	Organophosphate	Systematic	1m/L

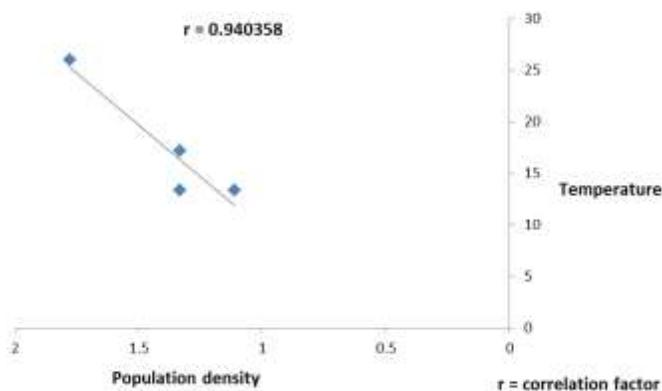


Figure. 1. A

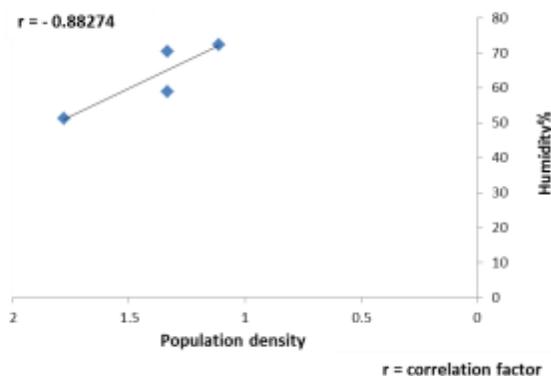


Figure 1. B

Figure. 1,A. Correlation coefficient between temperature and population density of insect.

1,B. Correlation coefficient between humidity and population density of insect.

Table 2. Population density and infection ratio of radish leaves miner

Study periods	Larvae density (larva/leaf)			Population density (larva/leaf)	Infection ratio%
	outer	meso	inner		
December	1.33	1	1	1.11	10
January	1.67	1.33	1	1.33	20
February	1.67	1.33	1	1.33	50
March	2	1.67	1.67	1.78	76
Average of leaf location effect	1.67	1.33	1.16	1.38	39
L.S.D 0.05	Period=0.43	Leaf location=0.37	Interaction=0.74		

Table 3. Damage ratio caused by insect feeding during study periods

Study periods	Damage ratio % Leaf location			Average %
	Outer	meso	inner	
December	20	18	6.5	14.8
January	52.4	27.1	10	29.8
February	60.4	30.5	7.3	32.7
March	67.9	21.8	8	32.5
Effect of leaf location average	50.1	24.3	7.9	27.45
L.S.D 0.05	Periods=8.35	leaf location=9.98	interaction=16.88	

Table.4. Effect of used pesticides and *B. bassiana* on radish leaf miner insect

Percentage of radish leaf miner mortality				
Pesticide	after			Average of pesticides effect
	24hours	48hours	72hours	
Dragon	70	90	96	85.6
Sakhuy	56.7	93.3	96.7	82.2
Super saqr	90	92	94	92
B. bassiana	40	56.7	66.7	54.4
Time average	66.7	85	90	80.6
L.S.D for pesticide = 16.59				
L.S.D for time = 14.37				
L.S.D for pesticide and time interaction = 28.74				

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المكافحة الكيميائية والبيولوجية لحشرة حفار أوراق الفجل
(*Scaptomyza flava* Fallen Diptera: Drosophilidae) ضمن ظروف
محافظة البصرة

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الملخص

أجريت هذه الدراسة في قضاء شط العرب بمحافظة البصرة بهدف تقييم كفاءة عدة مبيدات من مجاميع كيميائية مختلفة والفطر *Beauveria bassiana* ضد حفار أوراق الفجل *Scaptomyza flava* Fallen، خلال الموسم الزراعي 2015/2016، كما تم تقدير شدة الإصابة والضرر لهذه الحشرة. أظهرت النتائج أن الكثافة العددية لهذه الحشرة كانت أقصاها في شهر آذار 1.78 يرقة/ورقة، وسجلت أعلى نسبة إصابة في الشهر ذاته إذ بلغت % 67، أما نسبة الضرر فلم تكن هناك فروق معنوية بين فترات الدراسة بل تساوى ضرر الحشرة خلال هذه الفترات، في حين سجل موقع الإصابة فروق معنوية فيما بينها، حيث تفوقت الأوراق الخارجية بنسبة الضرر عن بقية المواقع حيث بلغت % 1.8؛ أما نتائج اختبار المبيدات فقد أثبتت النتائج أن جميع المبيدات المستعملة فعالة بدرجة عالية ضد هذه الحشرة مع وجود فروق معنوية بسيطة بينها إذ سجل المبيد Super Saqr أعلى نسبة قتل، تلاه مبيد Dragon ثم Sakhuy بنسب بلغت 92، و85، و82% على التوالي، في حين سجل الفطر الاحيائي أقل نسبة قتل بلغت % 54.4 بعد 72 ساعة من المكافحة.

الكلمات المفتاحية: *Scaptomyza falava* Fallen، حفار أوراق الفجل، مبيدات حشرية، البصرة، العراق.