Bio-control of *Dacus ciliatus* Loew, 1862 (Diptera: Tephritidae) on squash crop in two different locations of Sulaymaniyah, by using *Lecanicillium lecanii.*

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Abstract

The current study was conducted to evaluate the efficiency of entomopathogenic fungus *Lecanicillium lecanii* (1×10^6) spore per gram to control *Dacus ciliatus* on summer squash *cucurbita pepo* in two different stations (Tanjaro and Tainal) in Sulaymaniyah, Kurdistan Region-Iraq, of two greenhouses during July to November, 2021, Set up a yellow sticky trap on both sides from each greenhouse to determine the time of the appearance of the adult insect, Fungi of the genus *L. lecanii* are major insect pathogens, which was used with three different concentrations (2.5, 5 and 7.5) g/L, with sterile distilled water as a control treatment, The results of sstatistical analysisshowed that the pathogen *L. lecanii* is significant effect with high concentration (7.5) g/L. in both stations to mortality a pest or reduce its field losses, These findings indicate that *L. lecanii*, is safe for the environment and humans and non-target organisms, as well as for the production of a high quality crop.Therefore, beneficial to farmers when applying an integrated program for control of cucurbit fruit fly pests.

Keywords: Cucurbit Fruit Fly, Squash Crop, *Lecanicillium lecanii*.

Introduction

Tephritidae (Diptera) is the most economically significant family in the world. Tephritidae has over 4800 species in around 500 genera across the world (23and 6). Around 250 species are economically important and widely distributed in temperate in the world especially (1). Fruit flies (Diptera: Tephritidae) are invasive pests of horticulture crops all over the world (31). The direct damage caused by females that oviposit inside the host fruit and larvae eating on the fruit flesh is of economic importance (5). Fruit flies (Diptera: Tephritidae) are one of the most common pests in the world. Also, milkweed and colocynth are also other hosts for this pest in Iran (4,24,3,**2**and 17). In Saudi Arabia, *Dacus ciliatus* Loew is a significant cucurbit pest and it is distributed in Atlantic Islands, Africa, and oriental Asia (16). *Dacus ciliatus* (Loew) (Diptera: Tephritidae), the Ethiopian fruit fly (EFF), is a Cucurbitaceae oligophagous pest (e.g., cucumber, zucchini, and melons) (29). *Dacus ciliatus* is a serious pest of Cucurbitaceae including *Cucumis metuliferus, cucurbita pepo, Cucumis sativus, Cucurbita maxima, Cucumis melo, Citrullus lanatus, Citrullus colocynthis, Coccinia grandis, Momordica balsamina, Momordica charantia, Lagenaria siceraria, Lagenaria aegyptiaca, Trichosanthes cucumerina, Luffa acutangula, Sechium edule,Benincasa sp.*(27). Cucurbit crops (Cucurbitaceae), has been produced for fruits with high nutritional value and commercial worth, Fruits with great value and economical significance. They are vulnerable to a number of insect pests, including the cucurbit fruit fly, *D. ciliatus*, which is one of the most dangerous (36 and 10).

Chemical control is one of the methods of controlling the fly, organophosphate and carbamate are pesticides used to control the insect(21). The insecticides deltamethrin, dimethoate, trichlorofon, spinosad, acetamiprid and Malathion, kill insects through contact and ingestion (19). Use this method to control *D. ciliatus*, also effect and kill other insects, since there is no specific chemical attractant known for this fly, its mean that kill beneficial insect such as parasite and predator (25). Chemical pesticides harm-full to humans, animals, fish and beneficial insects, cause water and air pollution, also cause cancer diseases, according to the world health organization (WHO), about 1000,000 human being are affected by acute poisoning by contact with pesticide (13) .

Previous research has concentrated on discovering un-chemical compounds such as entomopathogenic fungi (EPFs) that can be used as pesticide alternatives to synthetic pesticides (28). Insect pathogen microorganisms are an option to be employed as biocontrol agents (22). In addition to the cost-efficacy, the microbes are harmless to the environment (17). According to the application method, the viability and spreading capacity of the pathogen determines the range of reproductive in the host (30 and 30). Within the area-wide integrated pest management strategy, there is entomopathogen for the management of economically important fruit flies (20 and 35). Entomopathogenic fungi (EPFs) are important biocontrol agents because of their high host specificity, capacity, and pathogenicity to control a wide range of insect pests (14 and 8). Fungal agents are

one of the most promising types of biological pest control agents. (9). the use of fungi in an insect pest control contributes to reducing the use of chemical pesticides that pollute the environment (7). As an insect pathogen, *Lecanicillium lecanii* is an effective biological control agent for a variety of plant diseases, insect pests, and plant-parasitic nematodes (12 and 37).

The aim of this work was to evaluate the potential of EPFs, *Llecanii* to control *D. ciliatus*, in squash crops, to reduce the use of chemical pesticides that pollute the environment, and use the pathogen that is safe for humans.

Materials and Methods:

The study was conducted at two field sites near Sulaimani, the first site is located in the Tanjro district is located in the southeast of the city of Sulaimani, 24 km away from it, it consists of one greenhouse with an area of (468) m^2 (width 9 m and length 52 m). The second site is located in the Tinal area, west of Sulaimani, approximately 48 km from the city center, and in the same area is the first site. After plowing and preparing the land from July, From the date, 29/7/2021planted squash seeds directly in both stations from the U-turn variety, In the form of a plot, and each plot consists of 4 seedlings, a distance between one seedling and another 80 cm, and a distance between one plot and another 120 cm, watered in a drip method. This experiment was carried out during the autumn agricultural season of 2021, The results were analyzed using a randomized complete block design (RCBD) and means were compared by Duncan's multiple range test ($P \leq 0.05$), using statistical software ready (XL-STAT.21.2.59614 (Addinsoft2019). The powder fungus *Lecanicillium lecanii*, obtained from the plant protection office – Baghdad was taken by Professor Dr. Hussam Aldin Abdulla Muhamad, the number of spores fungus is (1×10^6) spores and this production is from June2021.The pathogen was weighed with the scale of the sensor type (OHAUS) into three weights (2.5, 5and 7.5) g/L. fig (1),for the purpose of spraying three times, spray with a 2-liter hand-held sprinkler for the plants in the field. Fig1 (B), Set up a yellow sticky trap with a size of (14×20) cm, and glue on both sides each greenhouse to determine the time of the appearance of the adult insect (30).Fig (1,A+B).

Fig 1 (A): Pathogen weights by means of a sensitive scale of the type of (OHAUS).

Fig 1 (B): yellow sticky trap to determine the time of the

appearance of the adult insect.

The first treatment after seed germination and from stage 5-6 leaves from date 16/8/2021 after showing the flowers with fungus *L. lecanii* and with three different concentrations (2.5, 5, and 7.5) g/L of water. According to the recommendations, with block control for each treatment and spray with water only, The second treatment, three weeks after the first spray, from the date 7/9/2021 with the same fungus and the same concentrations as the first treatment with a control spray with water. The third treatment four weeks after the second spray on the date 5/10/2021 also with the same concentrations and the same fungus as the first and second treatment with a control spray with water, Fig (2).

Fig (2): spray squash plants with different concentrations and different Entomopathogenic fungi.

The first collecting data are from the date 11/9/2021 to 10/11/2021 weekly in both stations, Collecting data in terms of the number of fruits, total weight/ grams of fruit in each block, the number of infested fruits, total weight/grams of infested fruit, the number of holes, percentage of fruits infested, the severity of the injury for pest to all the different concentrations and the control treatment, percentage of infested was calculated using the following formula(24and 38).

$$\text{percentage fruit infestation} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

$$\text{The severity of the injury} = \frac{\text{area of injury fruits}}{\text{fruit area}} \times 100$$

Results and Discussion

Mycoses of the fungi with three concentrations sprayed on a plant from both greenhouses and after collection and analysis, the results showed that the pathogen *Lecanicillium lecanii* with high dose is more effective to control *D. ciliatus* in the greenhouse, table (1), showed Different concentrations of *L. lecanii* on *D. ciliatus*, the number of the hole in the fruit, number and weight infestation percentage of squash crop at Tanjaro location in 2021. The non-significant result of the number of fruit infest (1.333) eggs/fruit in the concentration of 2.5g/L on 03/10/2021 and the significant result (0.000) eggs/fruit in the concentration of 7.5g.L^{-1} on (26/09/2021); however, there is a difference between the concentrations and the control, the significant concentration is 7.5g/L and the smallest result of fruit infest compared to the controltreatment, thisresult of the percentage of fruit infests, it showed that the non-significant result is (50.000)fruit/hole in concentration (2.5) g/L on 11/10/2021, the same result as to control treatment (50.000) fruit/hole on 11/10/2021 and 10/11/2021, and smallest result (1.528) fruit/hole in concentration (7.5) g.L^{-1}in 26/10/2021,The result showed that the control and the concentration of 2.5g.L^{-1} are the same and nan-significant,

the concentration of 7.5g.L^{-1}is significant. About weight of fruit infest showed a non-significant result (160.000) weight/total in concentration 2.5g.L^{-1} on 19/10/2021, and the significant result (31.044) weight/total in concentration 7.5g.L^{-1} on 26/10/202, however the result in first three weeks (0.000) weight/total but can't rely on them because the plant was at the beginning of production.

*Number with the same letters in each column is not different significantly by Duncan's Multiple Range Test (P≤0.05.

Table (1): Different *Lecanicillium lecanii* concentrations on *D. ciliatus*, number of holes, number, Weight, and infestation percentage of squash crop at Tanjaro location during 2021.

day	concentration gram/litter	Total of fruit	Number of fruit infest	Number of hole	%infest of fruit	Total/weight	weight of fruit infest
11/09/2021	2.5	3.333 ab*	0.000 b	0.000 c	0.000 b	502.000 ab	0.000 b
	5	3.667 a	0.000 b	0.000 c	0.000 b	638.667 a	0.000 b
	7.5	3.333 ab	0.000 b	0.000 c	0.000 b	449.667 bc	0.000 b
	0	3.333 ab	0.000 b	0.000 c	0.000 b	453.000 bc	0.000 b
19/09/2021	2.5	2.333 a-e	0.000 b	0.000 c	0.000 b	345.667 b-g	0.000 b
	5	3.000 abc	0.000 b	0.000 c	0.000 b	405.000 b-e	0.000 b
	7.5	2.667 a-d	0.000 b	0.000 c	0.000 b	397.333 b-f	0.000 b
	0	2.667 a-d	0.000 b	0.000 c	0.000 b	382.333 b-f	0.000 b
26/09/2021	2.5	2.667 a-d	0.667 ab	0.667 abc	22.222 ab	343.667 b-g	77.000 ab
	5	3.000 abc	0.333 ab	0.333 bc	11.111 b	377.000 b-f	43.333 ab
	7.5	3.000 abc	0.000 b	0.000 c	0.000 b	355.333 b-g	0.000 b
	0	2.667 a-d	0.667 ab	1.000 abc	27.778 ab	352.000 b-g	86.000 ab
03/10/2021	2.5	2.667 a-d	1.333 a	1.333 abc	38.889 ab	349.333 b-g	158.000 ab
	5	3.333 ab	0.667 ab	0.667 abc	22.222 ab	388.667 b-f	77.000 ab
	7.5	3.000 abc	0.333 ab	0.333 bc	11.111 b	366.667 b-f	41.333 ab

	0	3.333 ab	1.333 a	1.667 abc	41.667 ab	390.667 b-f	209.667 a
	2.5	1.667 c-g	1.000 ab	2.333 ab	50.000 ab	238.000 d-j	134.667 ab
11/10/2021	5	2.000 b-f	0.667 ab	0.667 abc	27.778 ab	276.667 c-i	103.667 ab
	7.5	2.000 b-f	0.333 ab	0.667 abc	16.667 b	287.000 c-i	53.333 ab
	0	1.667 c-g	1.000 ab	2.000 abc	50.000 ab	218.667 e-j	122.000 ab
	2.5	3.667 a	1.000 ab	1.333 abc	25.000 ab	508.667 ab	160.000 ab
19/10/2021	5	3.000 abc	0.667 ab	1.667 abc	19.444 b	455.333 bc	113.667 ab
	7.5	2.667 a-d	0.333 ab	0.333 bc	11.111 b	427.333 bcd	59.000 ab
	0	3.000 abc	1.333 a	2.667 a	38.889 ab	432.667 bcd	194.000 a
	2.5	1.667 c-g	0.333 ab	0.667 abc	38.889 ab	282.333 c-i	57.667 ab
26/10/2021	5	2.000 b-f	0.667 ab	1.333 abc	16.667 b	300.000 c-i	104.667 ab
	7.5	0.989 fg	0.222 b	0.094 c	1.528 b	158.800 hij	31.044 b
	0	2.000 b-f	1.000 ab	1.667 abc	44.444 ab	300.667 c-i	152.000 ab
	2.5	1.333 d-g	0.667 ab	2.333 ab	50.000 ab	218.333 e-j	103.333 ab
03/11/2021	5	1.333 d-g	0.333 ab	0.333 bc	16.667 b	206.333 e-j	52.333 ab
	7.5	2.000 b-f	0.333 ab	0.333 bc	11.111 b	311.333 b-h	46.333 ab
	0	1.333 d-g	1.000 ab	2.000 abc	83.333 a	201.667 f-j	143.000 ab
	2.5	0.667 fg	0.333 ab	0.667 abc	50.000 ab	108.667 ij	53.000 ab
10/11/2021	5	0.333 g	0.333 ab	0.667 abc	33.333 ab	57.000 j	49.000 ab
	7.5	1.000 efg	0.333 ab	0.333 abc	16.667 b	159.333 g-j	22.238 b
	0	1.000 efg	0.667 ab	1.000 abc1	57.73 b	149.000 hij	105.333 ab

Table (2): Different *Lecanicillium lecanii* concentrations on *D. ciliatus*, number of the hole, number, weight, and infestation percentage of squash crop at Tainal location during 2021.

day	concentration gram/litter	Total of fruit	Number of fruit infest	Number of holes	%infest of fruit	Total/ weight	weight of fruit infest
11/09/ 2021	2.5	3.667 a*	0.000 c	0.000 d	0.000 c	474.333 abc	0.000 c
	5	3.667 a	0.000 c	0.000 d	0.000 c	495.667 ab	0.000 c
	7.5	3.000 ab	0.000 c	0.000 d	0.000 c	472.333 abc	0.000 c
	0	3.333 a	0.000 c	0.000 d	0.000 c	444.667 abc	0.000 c
19/09/ 2021	2.5	3.333 a	0.000 c	0.000 d	0.000 c	499.667 ab	0.000 c
	5	3.000 ab	0.000 c	0.000 d	0.000 c	467.667 abc	0.000 c
	7.5	3.333 a	0.000 c	0.000 d	0.000 c	518.000 a	0.000 c
	0	3.000 ab	0.000 c	0.000 d	0.000 c	471.333 abc	0.000 c
26/09/ 2021	2.5	3.000 ab	0.333 bc	0.667 cd	11.111 bc	366.000 a-e	43.333 bc
	5	3.000 ab	0.333 bc	0.333 cd	8.333 bc	371.667 a-e	39.667 bc
	7.5	3.333 a	0.000 c	0.000 d	0.000 c	396.333 a-d	0.000 c
	0	3.000 ab	1.000 bc	1.333 bcd	27.778 abc	379.667 a-e	119.667 bc
03/10/ 2021	2.5	3.333 a	0.667 bc	1.333 bcd	19.444 abc	404.667 a-d	116.000 bc
	5	2.667 abc	0.333 bc	0.333 cd	11.111 bc	355.333 a-e	40.667 bc
	7.5	3.000 ab	0.333 bc	0.333 cd	8.333 bc	361.333 a-e	39.333 bc
	0	3.333 a	1.000 bc	1.333 bcd	27.778 abc	394.000 a-d	106.333 bc
11/10/ 2021	2.5	2.333 a-d	0.667 bc	1.000 cd	27.778 abc	324.333 a-f	107.333 bc
	5	2.000 a-e	0.667 bc	1.000 cd	27.778 abc	259.333 c-g	93.667 bc
	7.5	3.000 ab	0.333 bc	0.667 cd	8.333 bc	369.333 a-e	41.000 bc
	0	1.000 de	0.667 bc	1.000 cd	50.000 ab	151.000 fg	97.333 bc
19/10/ 2021	2.5	2.333 a-d	0.667 bc	1.667 a-d	27.778 abc	373.000 a-e	95.667 bc

	5	3.000 ab	0.333 bc	0.333 cd	8.333 bc	401.000 a-d	43.667 bc
	7.5	3.000 ab	0.333 bc	0.667 cd	11.111 bc	414.667 a-d	47.333 bc
	0	3.333 a	2.000 a	3.333 a	58.333 a	476.333 abc	300.000 a
	2.5	2.000 a-e	0.667 bc	1.667 a-d	33.333 abc	325.333 a-f	108.000 bc
26/10/2021	5	2.000 a-e	0.333 bc	0.333 cd	11.111 bc	290.667 b-g	41.000 bc
	7.5	1.050 cde	0.117 c	0.094 d	2.963 c	154.278 fg	12.133 c
	0	2.333 a-d	1.000 bc	1.333 bcd	38.889 abc	327.333 a-f	137.333 b
	2.5	2.333 a-d	1.000 bc	1.667 a-d	44.444 ab	351.333 a-e	141.000 b
03/11/2021	5	2.000 a-e	0.667 bc	1.667 a-d	27.778 abc	288.000 b-g	105.333 bc
	7.5	1.333 b-e	0.333 bc	0.333 cd	16.667 abc	206.000 d-g	56.667 bc
	0	2.000 a-e	1.333 ab	2.000 abc	55.556 a	298.667 a-g	179.333 b
	2.5	0.667 e	0.333 bc	0.667 cd	50.000 ab	94.000 g	106.667 bc
10/11/2021	5	1.000 de	0.667 bc	1.333 bcd	33.333 abc	165.667 efg	46.333 bc
	7.5	1.050 cde	0.117 c	0.094 d	2.963 c	154.278 fg	12.133 c
	0	1.000 de	0.667 bc	1.000 cd	50.000 ab	151.000 fg	97.333 bc

*Number with the same letters in each column is not different significantly by Duncan's Multiple Range Test (P≤0.05.

In general, the results of the Tanjaro station are similar to the results of the Tinal station, table (2) showed Different concentrations of *L. lecanii* on *D. ciliatus*, the number of the hole on fruit, number, and weight infestation, percentage of squash crop at Tainal location during 2021, which are non-significant result in concentration (2.5)g.L^{-1}, in 03/11/2021 is (1.000) eggs/fruit and significant result (0.117) eggs/fruit from concentration (7.5)g.L^{-1}. On 10/11/2021, its result of the Number of fruit infest, also the results of percentage of fruit infest is non-significant in concentration 2.5g/L. is (50.000) fruit/hole and significant result in concentration (7.5) g.L^{-1} is (2.963) fruit/hole both results in date 10/11/2021, also the same result in 26/10/2021 to concentration (7.5)g.L^{-1}. About weight of fruit infest, the nan-significant result in concentration (2.5) g.L^{-1} is (141.000) weight/total on 03/11/2021, and the significant result is (12.133) weight/total in concentration 7.5g.L^{-1}in two different weeks 26/10 and 10/11/2021.

Both results showed that the *L. lecanii* is

effective to control *D. ciliatus*, it's Agree with Kovac *et al*.(15). Who was noticed that the fungus is effective for the control of different insect pest, the efficacy of various fungal concentrations on *D. ciliatus* indicated an increase in mortality rates as the concentration was increased(9). It agrees with this result that the effectiveness of different concentrations of *L. lecanii* on *D ciliatus* in both tables showed decreasinginfestation rates with an increase in the concentration of the pathogen. Also the results agree with Singh and Kaur (33). Who showed that the *L. lecanii* is an insect pathogen that is used commercially to control greenhouse pest.The results of both station showed the fungus *L. lecanii*, as a pathogen is effective to reduce the pest population in field, this result is agree with Mahmoud(18). These pathogen with high concentrations, are more effective than normal concentrations and reduce the population density to less than half the population, and as a result, the damage is reduced to less than half the damage, it is agree with Goettel *et al*.(11).

Conclusion:

According to the results obtained from this study, the following points could be concluded:

1- *L. lecanii.* is a potential biocontrol agent that can be employed in the greenhouse to combat insect pests, especially family Diptera.

2- *L. lecanii.* is a potential biocontrol agent that can be employed in the greenhouse to combat insect pests, especially family Diptera.

3- We recommend that to meet the current needs of Ethiopian Fruit Fly management, a link has been established between researchers and companies, especially agro-pharmaceutical companies.

4- Attention to the use of bio-pesticides instead of chemical pesticides to reduce harm to humans and the environment as well as microorganisms.

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