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Abstract. The biological efficacy of of Metarrhizium anisopliae and Entomophothra muscae in the biological control of the first and fourth larval phases and adult male and female mosquitoes was studied. Culex quinquefasciatus Say Mosquito Breeding Cx. quinquefasciatus Say was conducted in the laboratory at a temperature of 25 ± 2 ° C, relative humidity 65 ± 5 of light a period of 12 hours / day the adult phase. He gave the fungus M. anisopliae at concentration of /1 x 106 spore lml had the highest mortality ratio of 78.19% for the first larval age after 48 hours, while the suspension for E.muscae at concentration 1 x 106 sporel mlgave the highest mortality rate of 69.63% for the fourth larval age after 48 hours. M. anisopliae at concentration / 1 x 106 spore 1 ml highest mortality ratio 84.83% for the first larval age after 48 hours while the suspended for mushroom E. muscae at concentration 1 x 106 spore / ml gave the highest mortality rate 67.63% for the fourth larval age after 48 hours . The highest killing rate was 97.66% for males and 99.91% for females after 72 hours when using the concentration of 1 × 610 spore / ml of the fungus M. anisopliae, while the killing rate was 99.54% for males and 98.43% for females after 72 hours when concentrated /1 x 106 spore l ml of for E.muscae fungus.

1. Introduction

There are about 35 species of mosquitoes in nature, including about 3700 [1]. Culex quiuquefasiatus is one of the most common species in the central and southern regions of Iraq [2]. Confirms [3] that the misleading sites of plants and weeds are more attractive to female mosquitoes. Eggs from exposed sites, mosquitoes Cx. quiuquefasiatus, one of the most common mosquito species in the world, is a biological vector for many pathogens of humans and animals, including the transmission of filariasis parasite [4]. Meningitis virus as well as the Rift Valley fever virus [5]. Insect pathogenic fungi are one of the most common and most distinctive pathogens of insect diseases fungi produce spores that stick to the insect's body and then germinate when the conditions are right. Therefore, send a tube that produces enzymes that release enzymes at the point of contact with the cucicle of the insect, analyzing the proteins, chitin and lipids involved in the synthesis of cuticle. M. anisopliae and E. muscae are fungi associated with insects, and are considered insect pathogenic fungi due to their widespread presence in nature. [6] Bio-control using some fungi is currently one of the best ways to control mosquitoes. Through the use of spores containing Cx quiuquefasiatus [7].

This research aims to isolate M. anisopliae and E. muscae fungi associated with C. quinquefasciatus Say mosquitoes and compare the biological efficiency of M. anisopliae and E. muscae in their biological control larvae of Cx. quinquefasciatus.

2. Materials and Methods

2.1. Preparation of Permanent Farm for Mosquitoes Cx. quinquefasciatus Say

Multiple ponds were selected at sites in Salahuddin province where C. Quinquefasciatus in these ponds because they are rich in organic materials, were collected from different sites for each pond during the period from March 2016 to September 2016 and then transferred to the laboratory and emptied in plastic ponds, and the larval food of ground mice, consisting of corn, wheat and protein was added by 1: 1: 1 by 2 g per basin [8]. Insect breeding was carried out in the laboratory at laboratory at a temperature of 25 ± 2 ° C, relative humidity 65 ± 5 of light a period of 12 hours / day.

2.2. Fungi Isolation

Cx. quinquefasciatus Say larvae were sterilized by ethyl alcohol 70% For one minute, sodium hypochlorite was used for 30 seconds for sterilization and placed on filter paper for disposal of sterile residue. Four replicates were prepared from Potate dextrose agar medium, and five larvae were placed in each repeater. [9] By sterile forceps, the dishes were incubated in a Syrian-made Jard incubator at a temperature of 25 ± 2 C for seven days, a 0.4 cm tablet was taken from the edge of the growing colony around the larvae and placed in a petri dish containing 20 ml of the above food medium and used for this purpose a sterile needle Incubate at 28 ± 2 ° C for seven days.

2.3. Diagnosis and description of the fungus M. anisopliae and E. muscae

M. anisopliae and E. muscae isolated from Cx. quinquefasciatus Say larvae were diagnosed by fungal growth and caterpillar colour by taking a small portion of the fungal growth and placing it on a glass slide with a drop of methylene blue dye and then put the lid of the slide and examined under a microscope under 40x magnification.

2.4. Preperaing Commenter Fungi

Glass flakes 100 ml were used for the preparation of the suspension for M. anisopliae and E. muscae by taking a 0.5 cm diameter tablet from the fungal colony growing on P.D.A. The tablet was placed in 9.5 ml sterile distilled water in the jug above and shaken for five minutes to remove the spores from its spore mounts. Culture was incubated at 25 ° C for 7 days, and for the purpose of distributing the fungal growth was shaken daily, a piece of paper was used for filtration and get commenter fungi.

Using a modified erythrocyte slice count to calculate the number of Haemocytometer spores [9]. In order to estimate the number of spores per unit volume and for the purpose of obtaining the average number of spores per square, place 1 ml of the base suspension on the slab and put the lid on the slide and then calculate the number of spores in each of the four large squares in the corners of the slide at a magnification of 40X According to the equation [10].

(1) $$\text{Number of spores} = \frac{N}{8} \times 6^{10} \times 10$$

N = Number of existing
8 = The sum of spores
6^{10} = Commonly dilution correction
10 = The correction coefficient volume
Concentrations were then prepared
1x102 spore / ml, 1 x 104 spore / ml, 1 x 106 spore / ml M. anisopliae
1x102 spore / ml, 1 x 104 spore / ml, 1 x 106 spore / ml for E. muscae

2.5. Bioassay in Larval Stages of Cx. quinquefasciatus Say

Four plastic containers were used, each containing 20 larvae per phase I and IV and each concentration of M. anisopliae and E. muscae tested. The larvae were left in the plastic pots for 2 minutes.The larvae were then transported by a soft brush to glass jars of 200 ml each distilled water container and the larval food at 10 mg / ml and placed in the incubator at a temperature of 2±.28 ° C and a light period of 12 hours, after which the mortality rate was calculated after 12,.24, 36, 48 hours and corrected using an equation [11]. The mortality rate was calculated and corrected using an equation.

2.6. Bioassay in Adult Mosquitoes. Cx. quinquefasciatus Say

Numbers of pupae were placed individually in 20 mL tubes and cotton was used for the purpose of closing the nozzle of the tubing and waited to become adults. 10 adult males and females were each distributed separately in a 1-liter wide-mouthed pot with three replicates per concentration. In addition to the control factor, each repeater was sprayed by a fungus at a distance of 5 cm, while the control factor was sprayed with water and placed in the incubator at a temperature of 2±.28 ° C and a light period of 12 hours, after which the mortality rate was calculated after 12,.24, 36, 48, 72 hours and corrected using an equation [11].

$$\text{Precentage of mortality} = \frac{\text{number in comparison} - \text{number in treatment}}{100 - \text{number in comparison}} \times 100$$

2.7. Statistical Analysis

The results were analysed based on multiple comparisons between the coefficient rates of the experiment using the complete random design (CRD). The results were analyzed using Duncan Multiple Range Test to find the differences between the coefficients according to the significant differences between them and at the level of significance specified for the test ($P < 0.05$).

3. Results and Discussions

3.1. Biological testing M. anisopliae in larval stages of Cx. quinquefasciatus Say.

He gave to fungi. M. anisopliae at concentration 1 × 610 spore / ml highest mortality ratio 78.19% for the first larval age after 48 hours while giving to the fungus. M. anisopliae at concentration 1 × 610 spore / ml highest mortality ratio 69.63% for the fourth larval age after 48 hours of treatment while the rate was 1% in the control coefficient, and the relationship between concentration and rate was direct and that this relationship was clear between the duration of exposure and rate For larval phases I and IV, where the proportion of killing increased with increasing concentration and exposure period in killing rates increased concentration is due to an increase in the number of developing spores as well as the impact of the immune system, which cannot defend the body at high concentrations of the fungus Its efficiency [12]. The statistical analysis shows that there are significant differences in the killing rate for the four phases at the level of 0.05 and the killing rates decrease with increasing larval age and when exposing the first and fourth larval phases. The reason is that the immune system of the first larval phases is incomplete and the insect body will be thin which is porous by the fungi spores.

Table 1. Shows larval stages of Cx. quinquefasciatus Say affected by various concentrations of the fungus.

Type of fungi	Phase	Concentration	Percentage of adult mortality			Effect rate of fungi
			12 h	24 h	48 h	
M. anisopliae	First	$1x10^2$	44.32	51.27	61.38	52.32 B
		$1 x 10^4$	48.12	55.48	67.43	57.01 B
		$1 x 10^6$	56.17	69.57	78.19	68.03 A
		Control	1	1	1	
		Average	49.68 b	59.16 a	52.00 b	
	Fourth	$1x10^2$	35.14	41.13	54.66	43.8 B

	1 x 10^4	40.51	42.59	64.71	49.27 B
	1 x 10^6	49.53	51.56	69.36	56.90 A
	Control	2	2	2	
	Average	31.19 a	34.30 a	31.19 a	

*Averages and horizontally attached to the same letter do not differ significantly according to Duncan multi-range test.

3.2. Bioassay of Fungal Suspension in Adult Mosquitoes. Cx. quinquefasciatus Say

Table 2 shows the effect of different concentrations of the fungus trapped. In M. anisopliae. Against mosquito adults. Cx. quinquefasciatus Say with the highest homicide rate was 97.66% for males and 99.91% for females after 72 hours when using 1 ×106 spore The mechanism of action of the fungus against male and female adults is the penetration of the thin areas of cuticle after adult spraying with the fungus, the fungus grows and the problem of filamentous growth is multiplied between the tissues of the body of the insect then send the fungus carriers outward and then the death of the insect [12].

Table 2. Effect of different concentrations of fungi suspensions. M. anisopliae in the destruction of adult males and female mosquitoes. C. quinquefasciatus Say

Type of fungi	Adult	Concentration	Percentage of adult mortality			
			12 h	24 h	48 h	72 h
M. anisopliae	Male	1x10^2	76.65	82.06	86.67 B	89.72
		1 x 10^4	74.95	81.83	89.51 B	94.91
		1 x 10^6	78.43	94.43	95.69 A	97.66
		Control	3	3	3	3
		Average	62.50 b	65.3 b	78.71a	71.32 a
	Female	1x10^2	65.50	75.69	65.96 B	88.75
		1 x 10^4	69.21	75.71	69.36 B	96.87
		1 x 10^6	77.73	82.81	77.85 A	99.91
		Control	2	2	2	2
		Average	53.21 b	59.05 b	65.04.86 b	95.05 a

*Averages and horizontally attached to the same letter do not differ significantly according to Duncan multi-range test.

Table 3 shows the different larval stages of Cx. quinquefasciatus Say and affected by various concentrations of the fungus. E. muscae at concentration 1 × 610 spore / ml highest mortality rate 84.83% for the first larval age after 48 hours and gave the fungus E. muscae at concentration 1 × 610 spore / ml highest rate 67.63% for the fourth larval age after 48 hours of treatment while the killing rate was 2% in control coefficient, the relationship between concentration and killing rate was direct and that this relationship was evident between the duration of exposure and the killing rate for the four different larval phases where the killing rate increased with increasing concentration and exposure period. , the cause of the increase in the proportion Killing by increasing the concentration is due to the increase in the number of developing spores as well as the immune system which cannot defend the body at high concentrations of the fungus. The reason is that the immune system of the first larval phases is incomplete and the insect's body wall is thin and easily penetrated by the fungus spores. The findings were consistent with [13]. Describe the relationship between the concentration of spores and the percentage of killing as a direct relationship, where the greater the concentration rate, the greater the proportion of killing as the results of the study are similar to what he found [1]. This obtained a 90% killing rate when exposing the mosquito larvae Cx.quinquefasciatus to the spores of the Metarrhizium anisopliae at the concentration of 2 × 610 spore / ml lab oratory study converged with the study [14]. Which proved that biological control using the fungus Aspergillus Niger at a concentration of 1 × 410 spore / ml for different larval stages of Cx..quinquefasciatus mosquitoes gave a killing rate of 87.68%, [6] when using three fungal species against the four larval stages of the mosquitoes Cx.quinquefasciatus

that suspended M. anisopliae at a time of 120 hours and at a concentration of 2 × 610 spore / ml recorded the highest mortality rate of 77.7%, and this study is consistent with the study carried out [2] which proved that the use of different concentrations of the fungus M. brunneum led to a 95% killing of the phase First larval mosquitoes for Cx.pipines.

Table 3. Effect of different concentrations of fungi suspension. E. muscae in the destruction of mosquitoes. Cx. Quinquefasciat

Type of fungi	Phase	Concentration	Percentage of adult mortality			Effect rate of fungi
			12 h	24 h	48 h	
E. muscae	First	$1x10^2$	55.51	65.5	78.75	52.32 B
		$1 x 10^4$	66.22	69.58.48	83.467	57.01 B
		$1 x 10^6$	78.56	83.67	84.48	68.03 A
		Control	1	1	1	
		Average	49.68	59.16	52.00	
	Fourth	$1x10^2$	45.30	46.57	61.05	43.8 B
		$1 x 10^4$	44.32	49.33	68.31	49.27 B
		$1 x 10^6$	50.82	55.32	67.63	56.90 A
		Control	2	2	2	
		Average	53.01 a	38.30 b	49.82 b	

*Averages and horizontally attached to the same letter do not differ significantly according to Duncan multi-range test

Table (4) shows the effect of different concentrations of the E. muscae in mosquitoes the killing rate was 99.54% for males and 98.43% for females after 72 hours when using 1 × 610 spore / ml concentrat. The mechanism of action of the fungus against male and female adults is the penetration of the thin areas of the kyocl after the adults spray the fungus, the fungus grows and the problem of filamentous growth between the tissues of the insect's body subsequently increases. The fungus sends conidic carriers outward and then the death of the [12] Current results show similarity with some previous research, where the use of M.ainsopeliae against females Cx. Quinquefasciatus resulted in a 100% killing rate within four days and when the use of M.anisopeliae against mosquito adults Cx. 93% at 2 x 510 spore / ml concentration after 168 hours while females killed 96% at the same concentration and duration [15]. Males lost 93.33% and females 90% after 168 hours.

Table 4. Effect of different concentrations of E. muscae suspensions. In the destruction of adult males and mosquitoes destruction of mosquitoes. Cx. quinquefasciatus Say

Type of fungi	Phase	Concentration	Percentage of adult mortality			
			12 h	24 h	48 h	
E. muscae	Male	$1x10^2$	74.12	76.46	91.26	92.65 B
		$1 x 10^4$	76.16	84.25	93.22	95.23 B
		$1 x 10^6$	80.28	86.73	96.32	99.54 A
		Control	3	3	3	3
		Average	58.39 b	62.36b	70.71 a	72.60a
	Female	$1x10^2$	75.45	81.61	87.16	92.75 B
		$1 x 10^4$	84.21	89.65	87.16	94.27 B
		$1 x 10^6$	89.53	90.85	95.77	98.43 A
		Control	2	2	2	2
		Average	66.02b	66.02b	69.57 a	71.86a

*Averages and horizontally attached to the same letter do not differ significantly according to Duncan multi-range test.

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