

Laboratory Evaluation of the Entomopathogenic Fungi *Penicillium Marneffeii* and *Verticillium lecanii* Against *Culex Pipeins Molestus*

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Abstract

Background: The biological efficacy of *Penicillium marneffeii*, *Verticillium lecanii* in the biological control of the second and third larval phases and adult male and female mosquitoes was studied. *Culex pipeins molestus*. **Aim of the study:** This research aimed to isolate *P. marneffeii* and *V. lecanii* associated with *C. pipeins* mosquitoes and compare the biological efficiency of *P. marneffeii* and *V. lecanii* in their biological control second and third instar and adult for the *Cx. pipeins*. **Materials and methods:** study was conducted for a period of 3 months from March 2020 to May 2020. **Results:** killing increased with increasing concentration due to an increase in the number of developing spores. The results of the laboratory tests are shown *V. lecanii* fungi. at concentration 2×10^7 spore / ml highest mortality ratio 89.63% for the second larval age after 72 hours, the killing rate was 81.59% for males and 92.67% for females after 72 hours when using 2×10^7 spore / ml. **Conclusion:** Entomopathogenic Fungi *P. marneffeii* and *V. lecanii* can use secondary metabolites in the manufacture of a fungicide for the purpose of controlling *Cx. pipeins* mosquito.

Keywords: *Penicillium marneffeii*, *Verticillium lecanii*, *Culex pipeins*

Introduction

Agricultural pest control using fungal pathogens formed during last five-fifth years fertile ground for scientific research, it has been defined more than 700 species of pest pathogenic¹. The pest pathogen is undoubtedly an important biological enemy. In general, the pathogenic forms reach the host and then spread and penetrate his body, depending on their stock food. Whereas, these spores grow within the host in succession². The form of monocytes or multicellular cells (hence, bud spores Protoplast), taking advantage of the food sources of the overworked host². The pathogen develops and dies, while the fungus results in a number. The infection secures the spread of the fungus. A large source of spores. Pathogen, or when the fungus resorts to produce a resistant fungal structure that allows for prolongation. How long it is without host³.

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 cuticle of the insect, analyzing the proteins, chitin and lipids involved in the synthesis of cuticle. *P. marneffeii* and *V. lecanii* are fungi associated with insects, and are considered insect pathogenic fungi due to their widespread presence in nature⁴. There are about 35 species of mosquitoes in nature. *Culex pipeins* is one of the most common species in the central and southern regions of Iraq⁵. Misleading sites of plants and weeds are more attractive to female mosquitoes⁶. Eggs from exposed sites, mosquitoes *Cx. pipeins*, 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^{6,7}. Bio-control using some fungi is currently one of the best ways to control mosquitoes. Through the use of spores containing *Cx. pipeins*⁸.

Aims and Objective

This research aimed to isolate *P. marneffeii* and

V.lacani associated with *C. pipeins* mosquitoes and compare the biological efficiency of *P. marneffi* and *V. lecenii* in their biological control second and third instar and adult for the *Cx.pipeins*.

Materials and Methods

Fungi Isolation

Cx. pipeins 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 Potato dextrose agar medium, and five larvae were placed in each repeater. By sterile forceps, the dishes were incubated in a Syrian-made Jar 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. Multiple ponds were selected at sites in Salheldin province where *C. pipeins* in these ponds because they are rich in organic materials, were collected from different sites for each pond during the march to May 2020 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 basin⁹.

Insect breeding was carried out in the laboratory at a temperature of 25 ± 2 ° relative humidity 65 ± 5 light a period of 12 hours / day. *P. Marneffi* and *V. lecenii* isolated from *Cx.pipeins* larvae were diagnosed by fungal growth and caterpillar color by taking a small portion of the fungal growth and placing it on a glass slide with a drop of Icto phenol cotton blue and then cover the slide on the slide and examined under a microscope under 40x magnification. .

Preparing commenter fungi

Glass flakes 250 ml were used for the preparation of the suspension for *P. maneffi* and *V. lecenii* 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 fugi using a modified erythrocyte slice count to calculate the number of Haemocytometer spores¹⁰. Following the concentration.

2×10^5 spore / ml , 2×10^6 spore / ml , 2×10^7 spore / ml .

Bioassay in larval stages of *Cx.pipeins* molestues

Four containers/ type of fungus were used, each containing 20 larvae per instars II and III and each concentrstion of *P. marneffi* and *V. lecenii* tested by using 50 ml distilled water was used to control . The larvae were test 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

28 ± 2 °C and a light period of 72 hours, the mortality rate was calculated after 48 hours and 72 houres.

Bioassay in adult mosquitoes. *Cx.pipeins* molestues

Numbers of pupae were placed individually in 20 mL tubes and cotton was used for the purpose of closing the nozzle of the tube 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 $2 \pm .28$ ° C and a light period of 12 hours, after which the mortality rate was calculated after 48, 72 hours.

Statistical Analysis

The results were analyzed 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 coefficients according to the significant differences between them and at the level of significance specified for the test ($P < 0.05$). .

Results and Discussion

The results of the laboratory tests are shown in the table(1) .The fungus *P. marneffi* at concentration $2 \times$

6⁷ spore /ml gave highest mortality ratio 83.92% for the second larval age after 72 hours while the fungus *P. marneffeii* . at concentration 2 × 6⁷ spore /ml highest mortality ratio 68.83% for the third larval age after 72 hours of treatment the rate was 0% 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 II and III where as the proportion of killing increased with increasing concentration due to an

increase in the number of developing spores as well as the impact of the immune system, which can not defend the body at high concentrations of the fungus ¹¹.

The statistical analysis shows that there are significant differences in the killing rate for the phases at the level of 0.05 and the killing rates decrease with increasing larval age and when exposing the second and third larval phases. The reason is that the immune system of the second larval instar is incomplete and the insect body will be thin.

Table (1) shows larval stages of *Cx. pipiens* molestues affected by various concentrations.

Type of fungi	Phases	Concentration Spore / ml	Percentage of mortality		Average concentration effect	Average tretment effect
			24 h	72 h		
<i>. P marneffeii</i>	Second	2x10 ⁵	34.52	73.41	53.83 C	62.92 A
		2 x 10 ⁶	43.74	78.68	61.21 B	
		2 x 10 ⁷	63.58	83.92	73.74 A	
	Third	2x10 ⁵	23.89	56.83	40.36 D	47.75 B
		2 x 10 ⁶	28.65	62.69	45.67 D	
		2 x 10 ⁷	45.62	68.83	57.22 C	
		Control	0	0	0 E	
	Second	Average time effect	47.28 b	78.66 a		
	Third		32.72 b	62.78 a		

* Vertically similar letters and horizontally similar small letters mean no significant differences in Dunkin’s multiple-range test.

Bioassay of fungi suspension in adult mosquitoes.
Cx. pipiens molestus

The results of the laboratory tests are shown in the table(2) . *P. marneffei* . Against mosquito adults. with the highest homicide *Cx. pipiens molestus* rate was 79.17% for males and 89.42% for females after 72 hours

when using 2×10^7 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 in the *P. marneffei* destruction of adult males and female mosquitoes *Cx. pipiens molestus*.

Type of fungi	Adult	Concentration Spore / ml	Percentage of adult mortality		Average concentration on effect	Average treatment effect
			24 h	72 h		
<i>P. marneffei</i>	Male	2x10 ⁵	45.94	67.41	56.67 D	65.07 B
		2 x 10 ⁶	56.95	73.83	65.39 C	
		2 x 10 ⁷	67.72	79.17	73.17 B	
	Female	2x10 ⁵	53.72	65.96	59.84 D	70.81 A
		2 x 10 ⁶	68.21	84.36	76.28 B	
		2 x 10 ⁷	78.18	89.42	A 83.88	
		Control	0	0	0	
	Male	Average time effect	62.50 b	78.71a		
	Female		66.70 b	79.91 b		

* Vertically similar letters and horizontally similar small letters mean no significant differences in Dunkin’s multiple-range test.

The results of the laboratory tests are shown in the table(3) *V.lecanii* fungi. at concentration 2×6^7 spore / ml highest mortality ratio 89.63% for the second larval age after 72 hours while giving to the fungus *V. lecanii* at concentration 2×6^7 spore/ml highest mortality ratio 72.23% for the third larval age after 72 hours of treatment while the rate was 0% 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 mortality rate for larval phases II and III, whereas 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 can defend the body at high concentrations of the fungus Its efficiency¹³. The statistical analysis shows that there are significant differences in the killing rate for the phases at the level of 0.05 and the killing rates decrease with increasing larval age and when exposing the second and third instar.

The reason is that the immune system of the second larval phases is incomplete and the insect body will be thin which is porous by the fungi spores. The findings were consistent with¹⁴. 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 This laboratory study converged with the study¹⁵. Which proved that the biological control using the fungus *Aspergillus niger* at the concentration of 1×10^4 spore /ml for the larvae of different phases of mosquitoes.

Cx.quinquefasciatus gave a killing rate of 87.68%. Murder The results of the study were similar with¹⁶ a 90% killing rate when exposure of *Cx.quinquefasciatus* mosquito larvae to *Metarrhizium anisopliae* spores at 2×10^6 spore / ml. This study is consistent with a study¹⁷ that demonstrated that the use of different concentrations of fungus

Metarrhizium brunneum resulted in 95% killing of the first larval stage of *Cx.pipiens*.

Table (3). Effect of different concentrations of fungi suspension. *E. muscae* in the destruction of mosquitoes. *Cx. pipiens* molestues.

Type of fungi	Phase	Concentration Spore / ml	Percentage of adult mortality		Average concentration effect	Average concentration effect
			24 h	72h		
<i>V. lecanii</i>	Second	2x10 ⁵	49.73	56.52	53.12 D	71.91 A
		2 x 10 ⁶	76.62	79.21	77.91 B	
		2 x 10 ⁷	81.26	89.63	85.44 A	
	Third	2x10 ⁵	38.18	67.53	52.85 D	58.32 B
		2 x 10 ⁶	49.42	55.18	52.33 D	
		2 x 10 ⁷	67.43	72.32	69.87 C	
		Control	0	0	0	
	Second	Average time effect	63.20 b	75.12 a		
Third	51.67 b		65.02 a			

*Vertically similar letters and horizontally similar small letters mean no significant differences in Dunkin’s multiple-range test.

Table (4) shows the effect of different concentrations of the *V. lecanii* in mosquitoes. The killing rate was 81.59% for males and 92.67% for females after 72 hours when using 2×10^7 spore / ml concentration. The mechanism of action of the fungus against male and female adults is the penetration of the thin areas of the cuticle after 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 ¹⁸ Current results show similarity with some previous research, where the use of *V. lecanii* against females *Cx. pipiens* resulted in a 100% killing rate within four days and when the use of *V. lecanii* against mosquito adults *Cx. pipiens* 93% at 2×10^{10} spore / ml concentration after 168 hours while females killed 96% at the same concentration and duration ¹⁹. Males lost 93.33% and females 90% after 168 hours.

Table (4). Effect of different concentrations of *V. lecanii* on the destruction of adult males and mosquitoes destruction of mosquitoes. *Cx. pipiens molestus*.

Type of fungi	Adult	Concentration Spore / ml	Percentage of adult mortality		Average concentration effect	Average concentration effect
			48 h	24 h		
<i>V. lecanii</i>	Male	2x10 ⁵	34.73	68.51	51.62 B	61.29 B
		2 x 10 ⁶	42.32	71.94	57.13 B	
		2 x 10 ⁷	68.67	81.59	75.13 A	
	Female	2x10 ⁵	51.35	73.16	62.25 C	75.58 A
		2 x 10 ⁶	67.56	83.37	75.46 B	
		2 x 10 ⁷	68.31	92.76	89.03 A	
		Control	0	0	0	
	Male	Average time effect	48.57 b	74.01 a		
	Female		62.07 b	71.11 a		

* Vertically similar letters and horizontally similar small letters mean no significant differences in Dunckin's multiple-range test.

The *P.marneffi* and *V.lacanii* fungus infects mosquito larvae naturally in aquatic environment. Use an insect pathogenic fungus to reduce its spread about pesticide within integrated management programs are an important alternative chemicals that, in many areas, cause the disturbance of the vital balance they led to the emergence of secondary pests as major pests as a result of damaging their vital enemies prevalent in these

areas. Especially as many of these pathogenic fungus it is naturally present in many of our arab environments to return pioneering role in limiting the spread of many of the pests throughout our homeland. The study proved that entomopathogenic fungi *P.marneffei* and *V.lecanii* have a significant effect on the life second, third instar and adult of *Cx.pipiens* . It can be introduced into the pest management program using secondary metabolites

in the manufacture of a fungicide for the purpose of controlling *Cx.pipiens* mosquito.

Conclusion

The results of the laboratory tests are shown *V.lecanii* fungi at concentration 2×6^7 spore / ml highest mortality ratio after 72 hours The study proved that the insect entomopathogenic *P. marneffeii* fungi. *V.lecanii* has a significant effect on the life of the larvae of the second and third instar, as well as the adult male and female mosquitoes *Cx.pipiens*, so that a fungicide can be manufactured for the purpose of controlling mosquitoes *Cx.pipiens*.

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Ethical Clearance: Nil

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