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Isolation And Diagnosis Of Fungi Associated With The Larvae Of The Mosquitoes *Culex Quinquefasciatus* And Its Biological Control

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***Abstract*—A laboratory experiment was conducted in the laboratories of the University of Basrah / College of Agriculture to isolate and diagnose the fungi accompanying the larvae of the Culex quinquefasciatus. Isolated fungi (Aspergillus candids ,Aspergillus niger ,Aspergillus terreus, Penicillium expancum, Cladosporium cladosponoieds, and Penicillium sp) of Culex quinquefasciatus mosquitoes and at different densities and and showed The results of isolation and diagnosis of fungi associated with mosquitoes C. quinquefasciatus, Aspergillus candids were recorded and the highest incidence of was 80% with of Aspergillus niger, which recorded an emergence rate of 47.33% while other fungal species Aspergillus terreus, Penicillium expancum, Cladosporium cladosponoieds, and Penicillium sp have an appearance ratio of 33.33, 26.00, 20.00 and 16.00%, respectively As for the fungal fungi isolated, the percentage of killing was increased by increasing the length of time and the type of fungus and the age of larvae, where the superiority of A.niger, where the rate of killing is 100 and 90% for the second and fourth respectively in 72 hours and the record of P.expancum the lowest killing rate amounted to 40.00 and 56.76% for the second and fourth respectively in 72 hours.**

***Keywords*—C. quinquefasciatus, accompanying fungus, biological control**

I. INTRODUCTION

Culex quinquefasciatus mosquitoes are known to transmit pathogens that kill humans and animals and spread in tropical and subtropical regions of the world (Jasinskiene et al.,1998), This type transmits pathogens such as nematodes, which cause lymphatic filariasis and viruses Including the Zika fever virus known as Zika fever (Fernández et al.,2016), It also transmits worms(Wuchereria bancrofti) that infect millions of people around the world and cause what is known as the elephant's disease (Cirimotich et al.,2011), Due to the medical importance of all kinds of mosquitoes, scientists have been interested in combating them for hundreds of years And used various chemical pesticides, but these pesticides were harmful to humans and their environment, On the other hand, these targeted fungi have acquired the ability to adapt to and develop immunity against pesticides (Ibarra and Castero,2008), Therefore, there is a need for the development of non-toxic and safe alternatives to

humans and animals by other biological species of bacteria, fungi, The pathogenic fungi of insects have been among the important factors for their spread and wide presence in nature and are characterized by their high specialization in dealing with specific lesions , several species entomopathogenic fungi like *Beauveria bassaina , Isaria fumosorosea and Metarhizium anisapliea* have been developed commercially to control aphids speciaes *etc*.. ,of (Shiff,2002 ; Mohammed et al. , 2018).

Aspergillus sp. is a fungus associated with insects and belongs to the Ascomycota Division (Kamalulddin,2018), And tested its effect on mosquito larvae *Culex. Quinquefasciatus* (Govindarajan et al.2005) And the fungus Penicillium sp, which belongs to the Division of fungus cyst also has many important enzymes that are whose factors affecting the insects and tested its impact on the larvae of mosquitoes Cx. quinquefasciatus (Khalaf et al.2004).

Objective

Given the good importance of mosquitoes *Cx. Quinquefasciatus* and the investigation of new ways of biological control, Previous available researches have contributed to the isolation of very few pathogenic fungi, The aim of this study was to isolate some fungi associated with mosquitoes and its diagnosis and evaluation of its ability to resist mosquito larvae *Cx. Quinquefasciatus.*

II.MATERIALS AND METHODS

Biological control using fungi

Isolation and diagnosis of fungi associated with *C. quinquefasciatus*

A number of dead ***C. quinquefasciatus*** mosquitoes were brought in and signs of infection were observed where the sodium hypochlorite was sterilized with 10% concentration for 2 minutes Then washed twice with distilled distilled water and then passed on the filter paper sterilizer to remove the remaining water and then transferred the insects to a set of plastic Petri dishes in the diameter of 9 cm container on 20 ml medium Potato Dextrose Agar of medium (APD). It was distributed at a rate of five insects per dish and then incubated the dishes in the incubator at a temperature of 2 + 25 ° C and for 3 - 4 days after isolating the developing fungi and then scrubbed again and incubated on slant circles. (Domsch et al.,1980; Simmons et al.,2000; Watanabe, T. and Shiyomi et al.,1975). Then the percentage of the appearance and frequency of isolated fungi was calculated according to the equations mentioned(Krebs and Davies,2009).

$$\text{Percentage of Fungi appearance} = \frac{\text{Number of times the fungus appeared in the samples}}{\text{Total number of samples}} * 100$$

$$\text{Percentage of fungus frequency} = \frac{\text{Number of fungus isolates}}{\text{Total number of fungus}} * 100$$

Preparation of a bacterial suspension of fungi isolated from mosquito insect

Sterilized glass flasks of 500 ml were used, each of which contained 250 ml of the medium of the liquid potato extract(Potato Dextrose broth) And Then rinse each flask by taking a 0.5 cm drop tablet from the isolated fungal colony at kept for 5 days incubating the fertilized vials in the incubator at 25 ° C for seven days Taking into account the daily stress of the distribution of fungal growth during the incubation period and then put the components of the containers containing the liquid medium and fungi colonies in Blender electric mixer made in Taiwan for five minutes, Adjust The concentration of spores used on 610 x 1 pg / ml for each fungal species present with in the aid of the Haemocytometer count (Lacey,1997).

Study of the suspended effect of fungus spores in the second and fourth larval stages of mosquitoes in Laboratory

Khalaf et al,.(2004) followed the study of the effect of suspensions of fungi in mosquito larvae, Where 20 larvae of the second phase were placed in 250 mL polypropylene plastic containers containing 40 ml of water after purification And then add 10 ml of the bacterial suspense containing a concentration of 610 x 1 pg / mL of bacterial suspense for each fungus of isolated fungi and by three replicates per treatment, The treatment of the control was added to the breeding water only. All the treated pots were covered with pieces of tulle and tied with a rubber band. The pots were then transferred to the incubator at 25 ± 2 ° C, 70% RH and 8-9 hours light period and the number of dead larvae By observing them daily. And then calculated the percentage of death after one day and three days of treatment with fungus, the same steps were carried out on the fourth phase larvae,After the experiment was completed, the fungi were isolated from the treated larvae, which showed the pathogenicity to prove the isolated fungal diseases and therefore their use in the biological resistance.

III.RESULTS AND DISCUSSION

Biological control using fungi

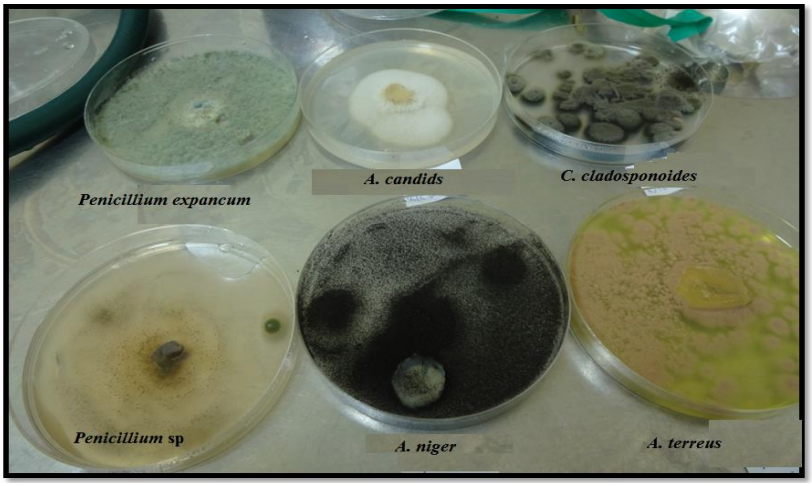
Isolation and diagnosis of fungi associated with mosquitoes *C. quinquefasciatus*

The results of Table (1) showed the diagnosis of six different types of fungi isolated from mosquitoes *C. quinquefasciatus* (Picture 1) *Aspergillus candids* recorded the highest incidence rate of 80% and frequency ratio of 6.80%, *Aspergillus niger*, which recorded an emergence rate of 47.33% and a frequency of 4.26%, while other fungal species, *Aspergillus terreus, Penicillium expancum, Cladosporium cladosponoieds,* and *Penicillium sp* showed the proportion of 33.33, 26.00, 20.00 and 16.00% respectively and frequency ratio of 2.12, 2.88, 2.70 and 2.35%, respectively, And that these results were consistent with several studies indicated the isolation of many fungi from mosquito larvae *C. quinquefasciatus* , Where (Khalaf et al.2004)isolated *Aspergillius niger, Aspergillius flavus, Fusarium, Penicillium sp,* and *Mucor hiemalis* were isolated from mosquitoes *C. Quinquefasciatus*, and (Coast and Oliveria,(1998)

isolated the *Penicillium sp* from mosquitoes carrying tropical diseases in Brazil.

Table 1. Isolated fungi and the percentage of appearance and frequency on mosquito larvae

Isolated fungi	Percentage	
	Appearance	Frequency
Aspergillus niger	**47.33**	**4.26**
Aspergillus candids	**80.00**	**6.80**
***Penicillium* sp**	**16.00**	**2.35**
Aspergillus terreus	**33.33**	**2.12**
Cladosporium cladosponoides	**20.00**	**2.70**
Penicillium expancum	**26.00**	**2.88**



Picture 1. Fungi isolated from mosquito larvae *C. quinquefasciatus*

Studying the effect of the fungal suspense in the second and fourth stages of mosquitoes in the laboratory.

The results of Table (2) showed that the treatment of *A. niger* and *A.candids* showed high mortality rates *of C. quinquefasciatus* in laboratory if the mortality rate was 85.00 and 74.17% respectively followed by the treatment of other fungi *Penicillium sp , A.terreus, C.cladosponoides* and *Penicillium expancum*, which had a mean larval mortality rate of 65.83, 58.33, 52.50 and 50.00%, respectively, which was significantly higher than the control ratio of 0.00%. The period of time exceeded 72 hours in the effect on the larvae of the second phase of mosquito *C. quinquefasciatus* significant difference over the period of time 24 hours if the rate of larval mortality rate 69.52 and 40.71 each, respectively.Table (2) shows the results of the effect of the fungal suspension on the mortality rate of the fourth stage larvae of mosquitoes *C. quinquefasciatus* in the laboratory. The table showed that the treatment of A. *niger* and A.*candids* yielded a high mortality rate of

71.68 and 65.00% respectively Followed by the treatment of other fungi *Penicillium* sp, A.*terreus*, C.*ladosponoides* and *Penicillium expancum*, which had an average larval mortality rate of 57.50, 49.17, 40.11 and 42.50%, respectively, it which exceeded the high morbidity differences from the control treatment of 0.00%. Followed by the treatment of other fungi *Penicillium sp*, *A.terreus*, *C.ladosponoides* and *Penicillium expancum*, which had an average larval mortality rate of 57.50, 49.17, 40.11 and 42.50%, respectively, which exceeded the high morbidity differences from the control treatment of 0.00%. The period of time was 72 hours, affecting the fourth stage larvae of mosquitoes *C. quinquefasciatus* significantly longer than 24 hours if the larvae mortality rate was 59.57 and 33.57% respectively. The high mortality rate of larvae in fungi is due to the inability of larvae to feed as a result of the adhesion of fungus spores to the mouthparts, which are performed by mouth brushes, resulting in lack of food and starvation (Silva et al.2004). These findings were consistent with the findings of (Khalaf et al.2004) when the highest mortality rate for *C. cinquefasciatus* was found when larvae were treated with the bacteria *Apergillus sp*, due to the ability of these species to secrete Kaite enzymes in the insect body, The toxic secretions produced by some species (Doherty et al.,1977).The results in tables (2, 3) show that the fourth stage larvae mortality is lower than that of mosquitoes. This is due to the fact that the late stages have decreased feeding rate and the skin becomes your name in preparation for entering the pupa stage, thus reducing the chances of entry or Break the spores of fungi to their bodies, This result is consistent with (Bukhari et al.,(2010) when mosquito larvae *A. gambiae* and *A. astepensi* were treated with *Buvaria bassiana*. The larvae of the fourth stage larvae of both species were lower than that of the larvae of the first and second larvae. This is due to the thickness of the cuticle in the larvae of the second phase which is less than in the larvae of the fourth stage, and the first phases continue to grow into two other stages until they reach the role of the pupa or may have some kind of chemical or mechanical defenses against fungal infection and increase the death by increasing the period of time may be due to the fact that fungal cones need a longer period so that the fungal threads can analyze the wall of the body of the insect and the entry of fungus so the rates of killing and destruction of larvae increases after the passage aganist several days of treatment (Lyz,1998).As stated by (Amin,(2007), prolonging the exposure of the insect to the fungus germs leads to an increase in the rate of insect killing. This may be due to the ability of fungi to produce quantities of enzymes that have the ability to analyze the body wall of the insect as this stage is essential in causing the injury and draining the contents And the increase in the number of fungal spores increases the chances of germination and injury and thus the inability of the insect to repel the fungus attack and thus increase the chances of disease and killing, and the length of time working to allow sufficient time for the spores of mushrooms germination and events of pathogenicity(**Hussain,2018)**.

Table 2. Effect of different fungus suspension on the percentage of second larvae in the Lab.

Fungus suspension	Percentage of second stage larvae deaths		Mean fungi
	24hours	72Hours	
A. niger	70.00	100	85.00
A.candids	51.67	96.67	74.17
Penicillium sp	50.00	81.67	65.83
A.terreus	48.33	68.33	58.33
C. cladosponoides	40.00	65.00	52.50
P. expancum	25.00	75.00	50.00
Control	0.00	0.00	00.00
time period Means	40.71	69.52	
R.L.S.D $_{0.01}$ fungus suspension =24.88 R.L.S.D $_{0.01}$ Time period = 18.74 R.L.S.D $_{0.01}$ Interaction = N.S			

Table 3. Effect of different fungus suspension on the percentage of fourth stage larvae of mosquitoes in the laboratory

Fungus suspension	Percentage of fourth stage larvae deaths		Mean fungi
	24 hours	72 Hours	
A. niger	53.34	90.00	71.67
A.candids	50.00	80.00	65.00
Penicillium sp	48.33	66.67	57.50
A.terreus	38.34	60.00	49.17
C. cladosponoides	16.66	63.66	40.11
P. expancum	28.33	56.67	42.50
Control	0.00	0.00	00.00
time period Means	235.00	417.00	
R.L.S.D $_{0.01}$ fungus suspension =12.422 R.L.S.D $_{0.01}$ Time period = 12.116 R.L.S.D $_{0.01}$ Interaction = N.S			

IV.Conclusions

The isolation of the fungus from the mosquito larvae and the use of the broccoli suspension had a clear effect against the larvae of this mosquito, The percentage of killings increased by increasing the length of time by different species of fungus and larval age, The second larval stage was more influential than the fourth larval stage.

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