LARVICIDAL EFFECT OF EXTRACELLULAR SECONDARY METABOLITES OF DIFFERENT FUNGI AGAINST THE MOSQUITO, *CULEX MOLESTUS FORSKAL*

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ABSTRACT

In this study, the efficacy of eight fungi isolate against four instarlarvae of mosquito vector *CulexmolestusForska* were tested. Fungi isolated and identified were *Aspergillus niger*, *A.flavus*, *A.candidus*, *Penicillium sp.*, *Candida sp.*, *Alternaria alternaria*, *Fusariumsolani* and *Trichophytonviride*. The larvae were treated with different periods ranged from 48 to 96 hours. The results showed the presence of a significant effect of the fungal filtrates *A.niger* and *Penicillum sp.* on the mortality of larvae as the percentage of mortality reached 74.85% and 64.44%, respectively compared to the rest of the fungi. The concentration of 100% surpassed the remaining examined concentrations yielding the highest percentage of mortality 96.66% and 100% for the fungus *A.niger* and 68.33% and 100% for the fungus *Aspergillus*, respectively within 48 and 96 hours after treatment.

Keywords: Larvicidal, filtrates, *Aspergillus niger*


INTRODUCTION

Mosquitoes are a group of 3530 species, which are within 43 genus and include the family Culicidae 3601 species and subspecies¹, one of its most essential races registered in Iraq *Uronotaenia, Theobaldiae Aedes, Culex Culex*. Most species of the genus are spread from the far north of Iraq to the south especially in the Mosul area, where they are located in tropical, humid and dry areas. The aquatic environment is the environment of mosquitoes in the non-adult stage, where they reside in open ponds with rich material content organic and stagnant water³. Alkarawipointed out the presence of Mosquitoes in heavy sewage which has a high percentage of nitrogen and zero oxygen⁴.

The *Culex* genus contains several types, and that type *Cx.molestusFroskal* is the most prevalent within the human environment⁵. *CulexmolestusFroskal* one of the unique types of mosquitoes due to its ability of the male tomate in small places without the need to fly in swarms (Stenogamy) and the ability of adults females to lay the first bunch of eggs without needing a blood meal (autogeny)⁶. All characters whose expression differs widely across the complex, have clear consequences for distribution and abundance.

Due to the medical importance of mosquitoes, scientists have been interested in fighting them for hundreds of years and have used various chemical pesticides. However, these pesticides cause damage to humans and the environment from one side, and on the other hand, the target insect gained the ability to adapt quickly with toxic substances and to start in developing immunity against it⁷. Therefore the need to develop a non-toxic and safe
method represents one of the methods of biological control by other biological types of insects, worms, bacteria, fungi or others. The promised fungi pathogenic to insects is an essential factor because of its widespread in nature as well as being inexpensive and highly specialized to face specific pests.

The present study determines the efficacy of extracellular secondary metabolites of eight fungal culture filtrates against Cx. Mosquitolarvae under laboratory conditions.

**MATERIAL AND METHODS**

**Sample collection**

Eight fungal species namely *Aspergillus niger*, *A. flavus*, *A. candidus*, *Penicillium sp.*, *Candida sp.*, *Alternaria alternaria*, *Fusarium solani* and *Trichophyton viride* were isolated in our previous study. Isolates were grown on potato dextrose agar. The dishes were preserved in the refrigerator until use.

**Preparation of fungal filtrates**

A broth potato dextrose agar was prepared according to the standard doctrine. The agar was distributed in 250 ml beakers, and the medium was inoculated with discs with a diameter of 0.5 cm of the isolated fungal cultures at the age of 7 days, each alone, at a rate of three discs for each beaker. The beakers were incubated at 27°C and shaken every three days to distribute the fungal growth. After two weeks filtering the inoculations using the Whatman No.1 filter paper using a Buchner cone with the assistance of a vacuum pump, the filtration was repeated utilizing the fine filter containing a fine filter paper with 0.22 millimicrons boreholes and the following concentrations were prepared: 25%, 50%, 75%, and 100%.

**Collection of mosquito samples**

Egg rafts were collected from one of the massive sewage discharge locations whose dimensions were (2.5X2.5)m and which is exposed and surrounded by sugar cane plants (*Typha sp.*), on March 2018 in the district of Ramadan, Kerbela Governorate.

The rafts were placed in strict laboratory conditions in order to hatch and were breed until reaching the fourth-instar larval stage where samples were then taken for purposes of identification using the diagnostic key. To confirm the identification process, a biological phenomenon was utilized, namely, the ability of this type of females to lay its first raft of eggs without needing a blood meal.

**Mosquitos breeding**

The colonies were filtered into three generations, where each of the egg rafts were transferred into a 100 ml plastic container. After the eggs hatch and transform into larvae, they are nourished with bread crumbs. When they transform into pupae, they are transferred into breeding cages with dimensions of (30 X 30 X 30) cm, which are enclosed with steel mesh on the four sides and with Tulle cloth on the fifth, in addition to a wooden base. The adults were supplied with a 10% concentration of sugary solution rather than blood. This method of breeding was utilised to attain adult and the fourth-instar larval to be used in identification and carrying out the needed experiments.

**Study the effect of the fungal filtrates against the fourth – larval instar of the Cx. Molestus**
A total of 40 larvae of the fourth larval stage were taken for each concentration. Bread crumbs were added as nutrition. The four containers were then covered with a piece of tulle and placed in the incubator at 27 °C. The mortality percentage was calculated after 48 and 96 hours, and values were corrected according to the Orell and Schenilderequation.

Corrected mortality percent =

\[
\frac{(\text{mortality in treatment} \% - \text{mortality in the control} \%)}{(100 - \text{percent of mortality in the control})} \times 100
\]

Statistical analysis
All results were analyzed according to the complete randomized factorial design, and the significance among averages was calculated using the least significant difference at a probability level of 0.05.

RESULTS AND DISCUSSION
The effect of the isolated fungi filtrates on the mortality percentage of the fourth larval stage of the *Cx. molestus* Froskal were noted, and results were presented in table 1. The results show the presence of a significant difference in the corrected mortality for the fourth-instar larvae of the *Cx. Molestus*. The two fungi *Penicillium sp.* and *A. niger* recorded the highest mortality rates reaching up to 74.85 % and 64.44 %, respectively. While the two fungi *Candida sp.* and *T. viride* recorded the lowest mortality rates of 30.86 % and 37.56 %, respectively in comparison to control.

<table>
<thead>
<tr>
<th>Concentration of filtrates</th>
<th>Larval mortality after</th>
<th>The average concentration of the filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtrates of fungi</td>
<td>48 hour</td>
<td>96 hour</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>50.00</td>
<td>50.00</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>10.00</td>
<td>33.33</td>
</tr>
<tr>
<td><em>A. candidus</em></td>
<td>14.99</td>
<td>20.55</td>
</tr>
<tr>
<td><em>Penicillium sp.</em></td>
<td>20.00</td>
<td>33.33</td>
</tr>
<tr>
<td><em>Candida sp.</em></td>
<td>12.22</td>
<td>23.33</td>
</tr>
<tr>
<td><em>A. alternaria</em></td>
<td>24.99</td>
<td>36.12</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>42.77</td>
<td>48.33</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>23.57</td>
<td>36.66</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Average time intervals</td>
<td>22.06</td>
<td>31.29</td>
</tr>
<tr>
<td>L.S.D. 0.05</td>
<td>For filtrates = 0.6442</td>
<td>For concentrations = 0.4471</td>
</tr>
</tbody>
</table>
The results also demonstrated the presence of a significant effect of the concentrations of the different fungal filtrates on the mortality percentage of fourth-instar larvae as the concentration 100 % superseded the rest of the concentrations in larval mortality.

The highest mortality rate was detected at 100 % upon the treatment with the fungal filtrates of Penicillium sp., A. flavus and A. niger after 96 hours in comparison with the mortality rates recorded in the lowest concentrations which decrease whenever the concentration decreases. This result is consistent with a previous study, which indicates that the rate of mortality increases as the interval of time prolongs and as the concentration of the filtrate increases. The interval of the treatment had a significant effect as well on the effectiveness of the fungal filtrate, as it recorded the highest rate of mortality after 96 hours following the treatment, and it reached 79.44 % in comparison to the exposure interval after 48 hours following the treatment which yielded the least mortality rate at 22.06 %.

As regards the overlap between the concentrations and the time intervals, the same table demonstrates that the highest mortality rate occurred after 96 hours from the time of treatment in a concentration of 100 % and reached 100 % with the treatment with the fungal filtrates Penicillium sp., A. flavus and A. niger, respectively whereas the least mortality rates of larvae occurred when treatment was by the filtrates of the same fungi at concentrations of 50 %, 78.33 % and 44.99 %, respectively after the same interval of exposure. Meanwhile, the mortality rate was nonexistent in case of the control treatment, which overall confirms the presence of a positive correlation between the concentration and mortality rate.

The effect against insects may be due to fungi ability to produce toxic substances that affect the insectile pests. Increasing mortality rates as the concentration increased might be due to the increasing numbers of the growing spore when they attack the host and the weakening the insect’s immune system as the immune system can only defend the body at low concentrations, yet at high concentrations, its efficiency could decrease. Several studies have also shown that the fungi pathogenic to insects can penetrate the larval spiracles which lead to the occlusion or closure of the larynxes or windpipes causing asphyxiation of the larvae and in turn their mortality.

Larval mortality can occur without the fungus storming the body cavity but just due to launching active biological substances which help in the disease development, such as the digestion of the basal membrane cells. These substances are the fungal poisons secreted by the fungus which interferes, not only with the development of the intact insect but with the growth and metamorphosis as well, because it acts through speedily draining the body nutrients and thus the larval mortality. The effect of the toxins could be through affecting the circulatory system as it could impede the physiology of circulation. On the other hand, these toxins could work by increasing the membrane permeability to specific ions which leads to the transfer of unusual ions and thus disruption of the intact cell function and the organelles such as the mitochondria leading to the larval mortality. The current results are inconsistent with the findings of Scholeteet al., which demonstrated that the fungus Leptolegnia caudate produces mortality rates of 100 % against the larvae of A. culicifacies. A study was done about the effectiveness of the fungus Aphanomyces against the larvae of the Cx. quinquefaciatus showed that the raw filtrate of the fungus causes a mortality rate reaching 80 % for the larval instars after seven days of the treatment. Fayadet al. also stated that the raw fungal filtrate of the fungus B. bassiana was effective in the control of the whitefly insect, as it achieved a high mortality rate reaching 54.2 % and 43.69 % in the pupae and the adult. Alamara (2009) showed that the concentration 100 % for the fungal filtrate of the fungus B. bassiana
was effective in killing the larvae of *Trogodermagranarium* as the mortality rate using this concentration was 42.10% with a significant superiority to the rest of the concentrations and that the mortality rate decreases with the decrease of the concentration. Dewan and his group found that the filtrates of the two fungi *A. terrus* and *A. sydowii* produced the highest mortality rates for the third instar larvae of the housefly, as it reached 70.17% and 64.91%, respectively. The study by Mahdi et al. also showed that the presence of a significant effect for the concentrations of the fungal filtrate of *B. bassiana* on the mortality of the fourth-instar larvae of *Cx. pipienspipiens* and that the concentration 100% surpassed the rest of the dilutions in producing the highest mortality rates as the highest mortality rate was 50% and 100% during 2 and 4 days, respectively. A study by AlJamily documented the highest mortality rates in the four larval instar of the fungus *Cx. quinquefaciatus* by utilizing the raw fungal filtrate recorded of the fungus *A. parasiticus* 100% where it reached 81.66%, 70.33%, 63.33% and 58.33%, respectively after the elapsing of 72 hours.

**CONCLUSION**

There is asignificant effect of the fungal filtrates of *A. niger* and *Penicillum sp.* on the mortality of the mosquito larvae.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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