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Bioassay and Metabolic Disturbances of some Entomopathogenic Fungi against *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

In the current study, four entomopathogenic fungi(EPF) were isolated and identified as *Aspergillus niger*, *Penicillium* sp.s, *Cunninghamella echinulate* and *Penicillium* sp. for testing their virulence against *Culex pipiens* that represent a major vector for several pathogens. Based on the obtained data, these EPF exhibited a lethality effect against stages of *C. pipiens* after treating the newly 3rd instar larvae. However, the effect was reflected in the hinder of pupation and adult emergence. The entomopathogenic fungus *A. niger* exhibited the lowest LC_{50}, 5.20E+13 (Spores/ml). EPF revealed a somewhat decrease in both % of pupation and adult emergence. Dramatically, EPF declined all metabolic substances; total carbohydrate, total lipid, and total protein in the treated insects. EPF in the current study proved its virulence against *C. pipiens*. However, it decreased the insect body metabolites.

INTRODUCTION

Mosquitoes are one of most life-threatening insects, as they are major vectors causing several diseases **(Ward & Benelli, 2017)**. Mosquitoes are insects belonging to the order Diptera / family Culicidae, which comprise more than 3500 species **(Foster & Walker, 2019)**. Most species are hematophagous since blood meal is necessary for egg production in the stage of adult female **(Clements, 1992)**. Such blood meals can also result in pathogen transmission to humans and other animals **(Gao *et al.*, 2020)**. The most important mosquito-borne diseases are transmitted by three mosquito genera (*Anopheles*, *Aedes*, and *Culex*).

C. pipiens is the most common species of mosquitoes in Egypt **(Kenawy *et al.*, 1996)**; *C.* species are major vectors of filarial nematodes and the West Nile virus.

Mosquito's control is a vital public health practice throughout the world **(Soni & Prakash, 2011)**. The chemical control of vector insects, despite being effective,

represents health, environmental and climatic hazard. Therefore, the increasing interest in alternative non-chemical strategies over the last decades is not surprising.

The uses of biological control agents such as entomopathogenic fungi for mosquito control are promising to control mosquito populations **(Ferron *et al*., 1991)**.

It was assumed that, over 100,000 species of fungi of which about 750 have been identified to be pathogenic to insects **(Moore-Landecker, 1996; Bidochka *et al.*, 2000)**. The EPF, being low toxic to nontarget organisms and its activity as larvicides, proves to be a promising approach for the biological control of insect pests **(Freed *et al*., 2012)**. However, entomopathogenic microorganisms have the advantage to be generally specific without affecting other natural enemies as compared to most of chemical insecticides **(Perinotto *et al*., 2012)**.

Several studies have been given promising results to control *C. Pipiens* with EPF as *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilicanus*, *Aspergillus* sp., *Candida* sp. and *Penicillium* sp. **(Rashed *et al*., 2014; Abdou *et al*., 2017; Hamama *et al*., 2022)**.

The current study was conducted to investigate the virulence of some EPF against *C. pipiens* and evaluate the metabolite contents in insect to shed some light on the fungal isolates' mode of action.

MATERIALS AND METHODS

1- Rearing of mosquitoes

C. pipiens larvae were brought from the Institute of Medical Entomology in Dokki, Giza, and cultivated in the laboratory at Al-Azhar University's Faculty of Science at 27±2C°, 70-80 % relative humidity, and a 14 L: 10D photoperiod. Adult mosquitoes were housed in cages (40 x 40 x 40cm) and given a 10% glucose solution, while blood was obtained from a pigeon host. The egg rafts were collected and stored in plastic containers with water for hatching. Larvae were raised in plastic trays measuring 25 x 30 x 15cm and given fish food **(Gerberg, 1970; Kasap & Demirhan, 1992)**.

2- Isolation and Identification of EPF:

4 fungal isolates were tested against the early 3rd instar larvae (Table 1).

Table 1 Isolation and identification of the 4 fungal isolates in the current study.

Fungal isolate	Isolation	Identification
Aspergillus niger	Isolated from soil sample obtained from Qaroun Lake, Al-Faiyum Governorate, Egypt	Morphologically according to Pitt, 1973; Singh and Webster, 1973; Hennebert and Subramanian, 1974; Dunn *et al*., 1982
***Penicillium* sp. s**	Isolated from soil sample obtained from Qaroun Lake, Al-Faiyum Governorate, Egypt	Morphologically according to Pitt, 1973; Singh and Webster, 1973; Hennebert and Subramanian, 1974; Dunn *et al*., 1982
***Penicillium* sp. w.**	Isolated from water sample obtained from Qaroun Lake, Al-Faiyum Governorate, Egypt	Morphologically according to Pitt, 1973; Singh and Webster, 1973; Hennebert and Subramanian, 1974; Dunn *et al*., 1982
Cunninghamella echinulate	Identified genetically in previous study	Morphologically and genetically in previous study (Zahran *et al*., 2019)

2.1- **Entomopathogenic fungi (EPF)**

Entomopathogenic fungi used in the present study were *A. niger*, *Penicillium* sp. s, *C. echinulate* and *Penicillium* sp. w. The first three fungi were isolated from soil, whereas the 4th was isolated from the water sample.

3 - **Larvicidal bioassay**

To study the toxicity of the entomopathogenic fungi against the early third instar larvae of *C. pipiens*, seven concentrations of fungi (10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{24} and 10^{36} (spores/ml)) were prepared. Each test concentration (100ml) was poured in a flask of 150ml and tested against 10 early third instar larvae of *C. pipiens*, with tap water serving as control. Three replicates were used.

3.1- Studied Criteria

- Mortality rate was daily recorded and calculated as percentages.
- Pupation rate was expressed as percent of the successfully developed pupae.
- Emergence rate was expressed as percent of the successfully emerged adults.

- General mortality percentages were corrected against those of the control by Abbott's formula **(Abbott, 1925)**.
- LC50 and LC_{75}calculation: corrected percentages of total were plotted versus the corresponding concentrations on logarithmic probability paper to obtain the corresponding log-concentration probit lines. The median lethal concentration (LC50 and LC_{75}) of treated insects was determined from the established regression lines **(Finney, 1971)**.

4- Determination of the main body metabolites

This work was carried out to determine the possible biochemical changes in the main metabolites in the late 3rd instar larvae, the late 4th instar larvae and the late pupal stage of *C. pipiens* as responses to LC50 and LC_{75} of the used EPF after the treatment of the early 3rd instar larvae of *C. pipiens*. Three replicates were used.

4.1 Homogenate preparation

The treated and control insects were weighed and homogenized in saline solution (10 larvae/5 ml saline solution) using a fine electric homogenizer, tissue grinder for 3min. Homogenates were centrifuged at 4000r.p.m. for 15min. under 2°C in a refrigerated centrifuge. The supernatant was used directly or stored at -20°C until the use for biochemical work.

4.2. Total protein content

Quantitative determination of the total protein content was conducted according to the method of **Weichselbaum (1946)** using the kit of diamond diagnostics. Total protein was measured at wave length 546nm by spectrophotometer.

4.3. Total carbohydrate content

Quantitative determination of the total carbohydrate content was conducted using the anthrone reagent according to **Singh and Sinha (1977)**. Total carbohydrate content was measured by the spectrophotometer at a wave length of 620nm.

4.4. Total lipid content

Quantitative determination of the total lipid content was conducted according to the vanillin assay procedure **(Van Handel, 1985**) using a kit of biodiagnostics. Total lipid content was measured spectrophotometrically at a wave length of 525nm.

4.5. Statistical analysis of data

All obtained data were statically analysed by Student's t-distribution using GraphPad Instat3 to test the significance of the difference between means ± SD.

RESULTS

In the current study, four fungal isolates were identified and tested as entomopathogenic fungi against the early 3rd instar larvae of *C. pipiens* (Fig. 1).

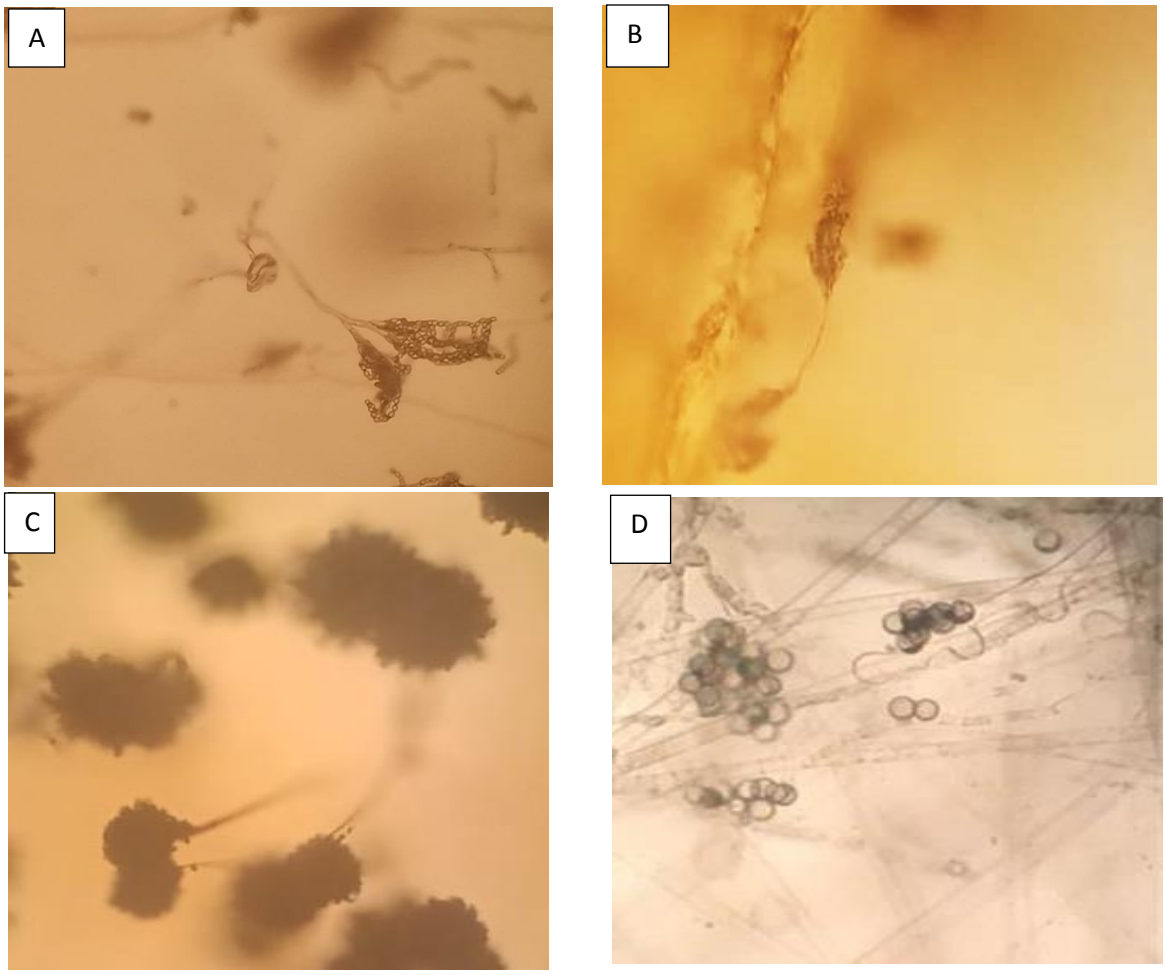

Fig. 1. Photos of preliminary identification of 4 fungal isolates. Where, **A:** *Penicillium* sp. w; **B:** *Penicillium* sp. s; **C:** *A. niger*, and **D:** *C. echinulate.*

1. Lethality effect of EPF

Penicillium sp. w induced different mortalities in the stages of *C. pipiens* after the treatment of the newly 3rd instar larvae (Table 2). Slight mortality was recorded in the treated 3rd instar larvae by 20 and 10% at concentration level 10^{36} and 10^{24} (Spore/ml) vs. 0.0 % of control larvae. The highest concentration caused a lethal effect in 50, 80 and 100% for total larval mortality, pupal mortality and adult mortality, respectively, compared to 0.0, 10.0 and 0.0 % of the control insects. General mortality increased progressively with concentrations. The lethal effect of *Penicillium* sp.w was reflected on the reduction of pupation and adult emergence.

Penicillium sp. s exhibited the highest mortality against the 3rd instar larvae of *C. pipiens* with 100 and 90 % at the two highest concentration levels, 10^{36} and 10^{24}, (Spores/ml) vs 0.0 % of control larvae (Table 3). Pupation % decreased to 10 and 70 % at

concentration levels of 10^{24} and 10^{12} (Spores/ml) vs 100.0 % of control pupae. Complete adult mortality was recorded at the concentration level of 10^{24} (Spores/ml). General mortality increased with the increase concentration level to 30, 40, 30, 30, 50, 100 and 100% vs 10 % of control insects.

The two highest concentration levels (10^{36} and 10^{24} Spores/ml) of *A. niger* induced mortality against the 3rd instar larvae of *C. pipiens* with 70 and 60%, respectively, compared to 0.0% of the control larvae (Table 4). Pupation and adult emergence % decreased with the increase in the concentration levels. Complete adult mortality was registered at the two highest concentration levels, whereas the same concentration levels caused 50 and 33.3 % of pupal mortality vs 0.0 and 10.0% mortality of control for both adults and pupae, respectively. Overall, general mortality was increased with the increase of the concentration levels to 30, 50, 50, 40, 50, 100, 100 at 10^{36}, 10^{24}, 10^{12} 10^{11}, 10^{10}, 10^{9}, 10^{8} (Spores/ml), respectively, vs 10 % of control insects.

C. echinulate showed slight mortality against 3rd instar larvae of *C. pipiens* with 30 % at the two highest concentration levels. Some mortalities were recorded in other stages in no certain trend (Table 5). The highest concentration level (10^{36}, Spores/ml) caused complete adult mortality. The fungus retarded the % of both pupation and adult emergence. Remarkable progressive mortality for general mortality was recorded.

According to the values of LC_{50} for general mortality, the fungus *A. niger* was more effective than the other tested fungi after treatment of the newly 3rd instar larvae of *C. pipiens* (Table 6). In ascending orders, LC_{50} was recorded by 5.20E+13, 9.30E+15, 8.70E+16 and 2.9E+17 (spores/ml) for *A. niger*, *Penicillium* sp. s, *C. echinulate* and *Penicillium* sp. w.

Table 2 Lethality activity of the fungal isolate, *Penicillium* sp. w after treatment the newly 3rd instar larvae of *C. pipiens.*

Conc. (spores/ml)	Larval instar mortality (%)			Pupation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)	General mortality (%)	Corrected Mortality (%)
	3rd	4th	Total Larval mortality (%)						
10^{36}	20.0	37.5	50.0	50.0	80.0	20.0	100.0	100.0	100.0
10^{24}	10.0	22.2	30.0	70.0	42.9	57.1	50.0	80.0	77.7
10^{12}	0.00	50	50.0	50.0	20.0	80.0	0.00	60.0	55.5
10^{11}	0.00	40	40.0	60.0	33.33	66.7	0.00	60.0	55.5
10^{10}	0.00	20	20.0	80.0	0.00	100	12.5	30.0	22.22
10^{9}	0.00	0.00	0.00	100.0	0.00	100	10.0	10.0	0.00
10^{8}	0.00	10.0	10.0	90.0	0.00	100	0.00	10.0	0.00
Control	0.00	0.00	0.00	100.0	10.0	90	0.00	10.0	---

Table 3 Larvicidal activity of the fungal isolate, *Penicillium* sp. s after treatment the newly 3rd instar larvae of *C. pipiens.*

Conc. (spores/ml)	Larval instar mortality (%)			Pupation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)	General mortality (%)	Corrected Mortality (%)
	3rd	4th	Total Larval mortality (%)						
10^{36}	100.0	0.00	100.0	---	---	---	---	100.0	100
10^{24}	90.0	0.00	90.0	10.0	0.00	100.0	100.0	100.0	100
10^{12}	0.00	30.0	30.0	70.0	14.3	85.7	16.7	50.0	44.4
10^{11}	0.00	0.00	0.00	100.0	10	90.0	22.2	30.0	22.2
10^{10}	0.00	10.0	10.0	90.0	0.00	100.0	22.22	30.0	22.2
10^{9}	0.00	10.0	10.0	90.0	22.2	77.8	14.3	40.0	33.3
10^{8}	0.00	10.0	10.0	90.0	22.2	77.8	0.00	30.0	22.2
Control	0.00	0.00	0.00	100.0	10	90.0	0.00	10.0	---

Table 4 Larvicidal activity of the fungal isolate, *A. niger* after treatment the newly 3rd instar larvae of *C. pipiens.*

Conc. (spores/ml)	Larval instar mortality (%)			Pupation (%)	Pupal mortality (%)	Adult emergency (%)	Adult mortality (%)	General mortality (%)	Corrected Mortality (%)
	3rd	4th	Total Larval mortality (%)						
10^{36}	70.0	33.3	80.0	20.0	50.0	50.0	100	100.0	100.0
10^{24}	60.0	25.0	70.0	30.0	33.3	66.7	100	100.0	100.0
10^{12}	0.00	30.0	30.0	70.0	14.3	85.7	16.66	50.0	44.44
10^{11}	0.00	10.0	10.0	90.0	11.1	88.9	25.0	40.0	33.33
10^{10}	0.00	20.0	20.0	80.0	12.5	87.5	28.6	50.0	44.4
10^{9}	0.00	10.0	10.0	90.0	11.1	88.9	37.5	50.0	44.4
10^{8}	0.00	10.0	10.0	90.0	11.11	88.9	12.5	30.0	22.2
Control	0.00	0.00	0.00	100.0	10.0	90.0	0.00	10.0	---

Table 5 Larvicidal activity of the fungal isolate, *C. echinulata* after treatment the newly 3rd instar larvae of *C. pipiens.*

Conc. (spores/ml)	Larval instar mortality (%)			Pupation (%)	Pupal mortality (%)	Adult emergency (%)	Adult mortality (%)	General mortality (%)	Corrected Mortality (%)
	3rd	4th	Total larval mortality (%)						
10^{36}	30.0	42.9	60.0	40.0	25.0	75.0	100.0	100.0	100.0
10^{24}	30.0	28.6	50.0	50.0	40.0	60.0	66.7	90.0	88.88
10^{12}	0.00	30.0	30.0	70.0	28.6	71.4	20.0	60.0	55.5
10^{11}	0.00	20.0	20.0	80.0	12.5	87.5	14.3	40.0	33.33
10^{10}	0.00	0.00	0.00	100.0	20.0	80.0	12.5	30.0	22.2
10^{9}	0.00	10.0	10.0	90.0	11.1	88.9	12.5	30.0	22.2
10^{8}	0.00	0.00	0.00	100.0	0.00	100.0	10.0	10.0	0.00
Control	0.00	0.00	0.00	100.0	10.0	90.0	0.00	10.0	---

Table 6 LC_{50} and LC_{75} values of the fungal isolates after treatment the newly 3rd instar larvae of *C. pipiens* for general mortality.

EPF	LC_{50} (Spores/ml)	LC_{75} (Spores/ml)
***Penicillium* sp. w**	2.9E+17	2.10E+25
***Penicillium* sp. s**	9.30E+15	7.50E+23
A. niger	5.20E+13	5.40E+22
C. echinulata	8.70E+16	4.20E+24

2. Effect of EPF on insect body metabolites

Total carbohydrate content was decreased drastically in all insect ages after treatment the newly 3rd instar larvae of *C. pipiens* by LC_{50} and LC_{75} of all EPF (**Table 7**). More than 50% reduction of total carbohydrate content was recorded in the late pupal stage by LC_{75} of both EPF *Penicillium* sp. w and *C. echinulate*, 0.79±0.13 and 0.75± 0.07 (mg/dl), respectively vs 1.90±0.01 of control.

Total lipid content was decreased in all insect ages of *C. pipiens* after treatment of newly 3rd instar larvae with EPF without exception (**Table 8**). Remarkable decrement of total lipid was recorded by more than 50% in late 4th instar larvae at LC_{50} of *A. niger* (change%: -59.5) and in late pupal stage at LC_{75} of *Penicillium* sp. w, LC_{75} and LC_{50} of *Penicillium* sp. s and at LC_{50} of *A. niger* (change %: -53.5, -57.1, -56.6 and -63.7, respectively).

All EPF was extremely decreased total protein content in all ages of of *C. pipiens* after treatment of newly 3rd instar larvae without exception. *A. niger* exhibited the highest effect in prevent the insect to maintain normal protein (**Table 9**).

Table (7): Total carbohydrate content (mg/dl) in the body homogenate of *C. pipiens* as influenced by treatment of the newly 3rd instar larvae with LC_{50} and LC_{75} of EPF.

Conc. (spores/ml)		Late 3rd instar larvae		Late 4th instar larvae		Late pupal stage	
		Mean±SD	Change %	Mean±SD	Change %	Mean±SD	Change %
Penicillium sp. w	LC_{75}	1.17±0.10 c	-27.8	1.10±0.14 c	-35.7	0.79± 0.13 d	-53.8
	LC_{50}	1.38±0.04 a	-14.8	1.12± 0.08 c	-34.5	1.29±0.11 c	-24.6
Penicillium sp. s	LC_{75}	1.30±0.01 b	-19.8	1.14±0.06 c	-33.3	0.89±0.05 d	-48.0
	LC_{50}	1.49±0.02 a	-8.0	1.44±0.04 a	-15.8	1.27±0.05 c	-25.7
A. niger	LC_{75}	0.94±0.06 d	-42.0	1.06±0.08 d	-38.0	1.23±0.04 d	-28.1
	LC_{50}	1.47±0.03 a	-9.3	1.49±0.12 a	-12.9	1.17±0.10 d	-31.6
C. echinulata	LC_{75}	1.21±0.13 b	-25.3	0.96±0.08 d	-43.9	0.75±0.07 d	-56.1
	LC_{50}	1.12±0.15 c	-30.9	1.26±0.08 b	-26.3	1.12±0.11 d	-34.5
Control		1.62±0.06	---	1.71±0.10	---	1.90±0.01	---

Mean ± SD followed with the letter (a): is not significantly different (P > 0.05), (b): significant (P < 0.05), (c): very significant (P < 0.01), (d): extremely significant (P < 0.001)

Table (8): Total lipid content (mg/dl) in the body homogenate of *C. pipiens* as influenced by treatment of the newly 3rd instar larvae with LC_{50} and LC_{75} of EPF.

Conc. (spores/ml)		Late 3rd instar larvae		Late 4th instar larvae		Late pupal stage	
		Mean±SD	Change%	Mean±SD	Change%	Mean±SD	Change%
Penicillium sp. w	LC_{75}	11.6± 0.8 d	-41.1	13.8± 0.7 c	-34.3	10.5± 0.7 d	-53.5
	LC_{50}	17.7± 0.5 a	-10.2	14.5± 0.3 c	-31.0	13.0± 1.1 d	-42.5
Penicillium sp. s	LC_{75}	10.6± 0.6 d	-46.2	11.9± 1.3 d	-43.3	9.7± 0.4 d	-57.1
	LC_{50}	12.8± 1.1 d	-35.0	11.7± 1.8 d	-44.3	9.8± 1.2 d	-56.6
A. niger	LC_{75}	17.6± 0.8 a	-10.7	16.0± 0.6 b	-23.8	17.7± 0.4 c	-21.7
	LC_{50}	11.7± 0.6 d	-40.6	8.5± 0.7 d	-59.5	8.2± 1.1 d	-63.7
C. echinulata	LC_{75}	15.8± 1.1 b	-19.8	14.2± 1.2 c	-32.4	14.5± 0.1 d	-35.8
	LC_{50}	16.9± 0.7 a	-14.2	18.2± 1.6 a	-13.3	11.8± 1.1 d	-47.8
Control		19.7± 0.8	---	21.0± 0.3	---	22.6± 0.6	---

a, b, c, d: see footnote table 7.

Table (9): Total protein content (mg/dl) in the body homogenate of *C. pipiens* as influenced by treatment of the newly 3rd instar larvae with LC_{50} and LC_{75} of EPF.

Conc. (spores/ml)		Late 3rd instar larvae		Late 4th instar larvae		Late pupal stage	
		Mean±SD	Change%	Mean±SD	Change%	Mean±SD	Change%
***Penicillium* sp. w**	LC_{75}	12.7± 0.7 d	-42.5	13.6± 0.6 d	-47.7	16.7± 0.6 d	-65.4
	LC_{50}	10.3± 1.5 d	-53.4	10.9± 0.5 d	-58.1	13.3± 0.4 d	-72.5
***Penicillium* sp. s**	LC_{75}	12.3± 1.3 d	-44.3	16.4± 1.0 d	-36.9	16.7± 0.4 d	-65.4
	LC_{50}	11.8± 0.4 d	-46.6	16.9± 0.5 d	-35.0	14.7± 0.6 d	-69.6
A. niger	LC_{75}	7.5± 0.3 d	-66.1	12.6± 0.6 d	-51.5	10.7± 0.8 d	-77.8
	LC_{50}	6.1± 0.8 d	-72.4	5.4± 0.5 d	-79.2	10.9± 0.3 d	-77.4
C. echinulata	LC_{75}	11.8± 0.7 d	-46.6	13.3± 0.4 d	-48.8	29.8± 1.2 d	-38.3
	LC_{50}	8.0± 0.6 d	-63.8	9.2± 0.5 d	-64.6	10.9± 1.0 d	-77.4
Control		22.1± 0.6	---	26.0±1.0	---	48.3±2.6	---

d: see footnote table 7.

DISCUSSION

1. Toxicity effect (mortality)

Microbe based control agents offer a substitute to chemical control, as it can be more selective than chemical insecticides (**Abdel-Baky and Abdel-Salam, 2003**). The EPF are unique because fungi can directly infect the host insect by penetrating into the cuticle and do not need to ingest by the insect to cause disease.

In the present study, 4 EPF; *A. niger*, *Penicillium* sp. s, *C. echinulate* and *Penicillium* sp. w, exhibited its lethality activity against *C. pipiens* after treatment the early 3rd instar larvae. The lethal effect was extended in the resulted stages, pupae and adult from treatment. The calculated LC_{50} exhibited that *A. niger* was more effective followed by *Penicillium* sp. s, *C. echinulate* and *Penicillium* sp. w.

Several previous studies have been proved that EPF was promising biocontrol agents against *C. pipiens* (**Pedrini *et al.*, 2007; Hamid *et al.*, 2013**). *M. anisopliae* showed maximum larval mortality of *C. pipiens* (**Benserradj and Mihoubi, 2014; Hamama *et al.*, 2022**). However, *M. anisopliae was* more effective than *B. bassiana* against *Culex quinquefasciatus* larvae (**Alves *et al.*, 2002**).

Aspergillus ochraceus, A. kanagawaensis and one strain of *A. sulphureus* were most effective, causing mortality in at least 80% of the larvae of the two mosquito species tested, *Aedes fluviatilis* and *C. quinquefasciatus* (**de Moraes *et al.*, 2001). Rashed *et al.* (2014)** recorded that *A. niger, Penicillium citrinum* followed by *A. ochraceus* were highly

virulent and rapid acting species against *C. pipiens* larvae, they caused 68.4±2.6, 67.4±0.7 and 61.1±3.5% mortality, respectively. **Singh and Prakash (2011)** reported the lethal effects of *A. niger* with LC_{50}, LC_{90}, and LC_{99} values of *C. quinquefasciatus* were 0.76, 3.06, and 4.751 μL/cm2. Moreover, in case of the *Anopheles stephensi* it was observed as 1.43, 3.2, and 3.86. While in case of *Aedes aegypti* it was recorded as 1.43, 2.2, and 4.1 μL/cm2. On the hand, other studies proved the ability of *Aspergillus* sp. metabolites and extracts against the mosquito larvae (**Ragavendran *et al*., 2019; Baskar *et al*., 2020**).

In the current study, EPF exhibited a retard effect for both % if pupation and adult emergence. This data was in harmony with other studies in *C. pipiens* after treatment with EPF (**Hamama *et al*., 2022**). The FPF has been successfully reduceing mosquito vectors population in laboratories and field trials (**Blanford *et al*., 2005; Scholte *et al*., 2005; Mohanty and Prakash, 2009**). The *Aspergillus clavatus* has been found highly pathogenic against larvae of *Ae. aegypti*, *C. quinquefasciatus*, and *Anopheles Gambiae* (**Seye *et al*., 2009**).

Our data corroborated the findings of other studies. that recorded, extending mortalities that appeared in the next stage other than the treated stage as pupal and adult mortalities that result from treatment of larvae of *Spodoptera littoralis* by *Cunninghamella sp*. and *Rhizopus sp*. (**Hamadah *et al*., 2018),** *Leptinotarsa decemlineata* with *B. bassiana* (**Wraight and Ramos, 2005),** *S. littoralis* by the use of biocide products **(Abdel-Rahim, 2011).** Also, some studies recorded pupal mortalities that extended after treatment the larvae of some insects by some EPF as in *Galleria mellonella* **(Shoukry *et al*., 2019)**, *Earias insulana* **(Abd-ElAzeem *et al*., 2019).**

The death of the larvae could be the result of various factors, among of them the high conidia concentration in contact with the midgut epithelium of the larvae. This blockage condition inhibits feeding and larvae die before colonization by the fungus. In this process, larvae were put into dietary stress as conidia are indigestible and hence larvae can't get benefit from ingested food properly (**Lacey *et al*., 1988**). There is a new entry to fungi through the siphon tip during the respiration process. *M. anisopliae* with hydrophobic conidia allowing hyphal growth into the tracheal system causing suffocation to the host and eventually its death (**Mannino *et al*., 2019**).

However, the death of *C. pipiens* stages in the current study might be as a result of secondary metabolites or hydrolytic enzyme activity of EPF. The secondary metabolites possess functions in impairing the immune response of the host insect, causing death (**Gillespie and Claydon 1989; Zibaee *et al*., 2011**). Several studies on EPF have been revealed the presence of hydrolytic enzyme activity or cuticle-degrading enzymes; as chitinase, protease and lipase that play the main mechanism of fungal infection to insect host and inducing mortality (**Hamadah *et al*., 2018; Abd-ElAzeem *et al*., 2019**). Nevertheless, **Mitchell and Cali (1994)** suggested that the toxic effect of the EPF is that they destroy the fat bodies; lose their ability to synthesize and capacity to store nutrients.

Otherwise, The *A. niger* is the best producer of extracellular lipase (**Falony *et al.*, 2006**). This can explain why *A. niger* was the most potent EPF in iducing toxicity than other fungi in the current study.

2. Impact of EPF on metabolites availability:

Microbial degradation of insect metabolites as lipid, protein, and chitin by production of enzymes as lipase, protease, and chitinase. It has taken the worldwide attention for insect control and has become the goal of extensive research **(Barra *et al.*, 2015).** In the present study, insect metabolites; total carbohydrate, total lipid and total protein were drastically declined in the treated *C. pipiens*. This data agreed with **Hamama *et al*. (2022)** who recorded different quantitative decrease in total soluble proteins, lipids, and carbohydrate of *C. pipiens* after treatment 3rd instar larvae by *M. anisopliae, B. bassiana*, and *Paecilomyces lilicanus*. *M. anisopliae* was superior to *B. bassiana* upon reducing total protein, carbohydrate, and lipid of the green stink bug, *Nezara viridula* (**Nada, 2015**). Also, **Omar (2022)** recorded reduction of all metabolites in *Pectinophera gossypiella* after treatment with *M. anisopliae* and *B. bassiana.*

Anopheline and culicine mosquitoes when subjected to stress by bio-insecticides, they expressed decreasing carbohydrate, lipid and protein concentrations. This decrease may because of anti-feedent due to blocking of the alimentary canal by the entomopathogen affecting carbohydrate concentration (**Sharma *et al.*, 2011**) and increased metabolism under toxicant stress (**Remia *et al.*, 2008)**. Decline in lipid concentration under stress, may be due to energy production is mainly through lipid catabolism (**Sharma *et al.*, 2011**). The reduction in total protein may be due to that the proteins are among the most important compounds of insects that bind with foreign compounds **(Ahmad and Forgash, 1976**).

Depletion of metabolites by EPF in the current study may exhibit the role of microbial enzymes in hydrolyze cuticle metabolites and insect hemocoel for the nutrient absorption process which is a prerequisite for fungal growth and biomass build-up inside its host and this represent one of induction of mortalities (**Butt *et al.*, 2016; Mondal *et al.*, 2016**).

CONCLUSION

The current study exhibited that the EPF have potential against *C. pipiens* under laboratory conditions. Further studies are needed to shed some light about the possible application of EPF under field conditions and to establish the mode of action against mosquitoes.

ABBREVIATIONS

C. pipiens: *Culex pipiens*; *C. quinquefasciatus*: *Culex quinquefasciatus*; EPF: entomopathogenic fungi; *B. bassiana*: *Beauveria bassiana*; *M. anisopliae*: *Metarhizium anisopliae*; *A. niger*: *Aspergillus niger*; *C. echinulate*: *Cunninghamella echinulate*; *S. littoralis*: *Spodoptera littoralis*; *Ae. Aegypti*: *Aedes aegypti*; *A. ochraceus*: *Aspergillus ochraceus.*

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