Pathogenesis of *Paecilomyces lilacinus* against the immature stages of *Musca domestica L.*

Abbas H. Burhan , Assist. Prof. Dr. Mohammed R. Annon
Biology of Department , Collage of Science , University of AL-Qadisiya ,Diwaniyah . Iraq .
Corresponding Author : Abbas H. Burhan abaasali306@gmail.com

Key words : *Musca domestica* , *Paecilomyces lilacinus* , HPLC , Paecilotoxin

Abstract

The current research aimed to investigate the effect of suspensions and filtrates(secondary products) of the fungus *Paecilomyces lilacinus* in the control of eggs and larvae of house fly *Musca domestica* where the mortality percentage of eggs amounted to 96.66% at the concentration of ($1*10^9$) spore / ml after 24 hours of treatment, while the lowest rate of 53.33% at the concentration ($1*10^5$) spore / ml in the same time period , The fungal suspension affected the three larval stages with the highest mortality rate (90.00.93.33.96.66) respectively at the concentration of $1 * 10^9$ spore/ ml after 72 hours, whereas the lowest rate reached (20,23.33.33.33%) respectively in the same time. The products of secondary metabolites were detected by HPLC technique at different incubation periods showed highly toxic to the larvae. The concentration 100% with a 28 day incubation period caused high mortality rate (90.00 , 93.33, 96.66) % for the three larval stages respectively and the mortality rate reached (53.33 , 56.66 , 66.66)% respectively for concentration 25% , at the same incubation period and the same of time . This confirmed that this fungus has a very promising future in the control of medical insects.

Introduction

Housefly *M.domestica* (Diptera: Muscidae) is a global insect responsible for causing disturbance, irritability, and food damage, and is an important vector of disease in both people and animals. Correlations between homes and pathogens can lead to outbreaks of diseases such as typhoid, cholera, tuberculosis, dysentery, infantile diarrhea and anthrax.(Lecuona et al.,2005; Förster et al.,2009). Household habits such as walking and feeding on garbage and waste make them highly proliferative agents of pathogenic pathogens to human and animal groups (Ugbogu et al.,2002). , It is important to control *M. domestica* to improve the health of people, livestock and poultry.Traditional pesticides are used primarily to control M. domestica in the short term(Cao XM et al.,2005; Malik et al.,2007), but the indiscriminate use of pesticides has led to serious problems including both pesticide resistance and the residual effects of chemicals used in pesticides. 7. Insect resistance Houses are now a global problem - and are increasing (Scott et al.,2000) At the moment, homes resist almost all traditional insecticide groups, including organic phosphates, organic chlorine, carbamite and pyrethroids. (Azzam ,2002). Problems related to resistance and impacts and high chemical costs have opened the door to other alternatives, such as pathogenic fungi to insects, which have the ability to control this pest insect. (Geden ,2012) Compared to synthetic insecticides, pathogenic fungi have low toxicity to mammals. In addition, their natural spread in the household population provides great potential for managing the household population (Khan et al.,2012) .A large number

of cases of house control have been reported through rapid killings and high infection rates of fungi including *Beauveria bassiana* (Vals). , *Metarhizium anisopliae* (Metsch.) Sorok (Kaufman et al.,2005) and and *Paecilomyces lilacinus* (Mwamburi et al., 2010).
Research efforts are still needed to explore any local isolates of pathogenic fungi that work effectively in local environments and thus can compete with traditional insecticides.
According to the importance of the home as a medical and veterinary pest, the present study is designed to verify the effectiveness of *Paecilomyces lilacinus* on domestic groups consisting of both eggs and larvae.

Materials and methods of work:

I: Isolation of *P.lilacinus***:**

P.lilacinus was isolated from Cadaver of house flies and was grown on the PDA medium. It was diagnosed by taking a small portion of the fungal growth through a sterile vector from the fungus farm and placed on a sterile filter paper pasted into a petri dish cover. The spores then placed the slide cover and studied the colony's characteristics under the light microscope and the fungus was diagnosed using the taxonomic keys (Ellis, 1971; Burgess *et al*., 1988) .

II: Preparation of filtrates(secondary metabolites) and suspensions of *P.lilacinus***:**

The media PDA was free agar and distributed in 250 ml bottle of 150 ml of bottle and the center was sprayed with 0.5 cm diameter tablets of corks from the five-day *P.lilacinus* colony . The filter was incubated at a temperature of 23 ̊m for 10,14,21,28 days and then filtered using the Whatman No.1 filter with Buechner and with the help of the air discharge device, the filter was re-filtered with 0.22µ and 25%, 50% and 75% 100% by distillation with distilled water, and the fungal suspension concentrations, ($1*10^5$, $1*10^6$,$1*10^7$, $1*10^8$, $1*10^9$) were obtained based on the equation (Lacey, 1997) .

III :Biological effect of *P.lilacinus* on the eggs and larval stages:

In this aspect, the effect of the fungus in the egg and the larval stage I, II and III was tested.

A-Effect of *P.lilacinus* on the egg**:**

Prepare 24-hour eggs from the permanent farm female in the lab. and place 100 eggs in each petri dish and spray the eggs with 5 ml of each concentration of fungus suspension with a hand-brush of approximately 15 cm. The comparison treatment was sprinkled with distilled water. Repeat the experiment three times and the same treatment comparison and calculated the proportion of hatching eggs after 24 hours.

B -Effect of *P.lilacinus* in larval stages**:**

To determine the biological effect of the fungus in the three larval stages, the first stage larvae were taken within 24 hours after hatching sufficient number of eggs. The larvae of the second and third phases were prepared from sufficient numbers of the previous phase. They were placed individually in glass tubes and containers on sufficient food Sterile larvae were monitored daily until dissociation. Ten larvae from each of the three phases were placed in a sterile 250 ml glass container and treated with bioaccumulation reagents with the said fungal suspension and control treatment in the manner indicated (2-3).

The experiment was repeated three times for each concentration and treatment of control, and the losses were calculated daily for three days. The effect of the fungicide was examined at different concentrations. The percentage of mortality was corrected according to the equation (Abbot, 1925).

IV: Testing the ability of *P.lilacinus* to produce toxins and some secondary metabolites and different incubation periods with HPLC technologies

A - Detection of HPLC toxins production:

The analysis was carried out in the laboratories of the department of Environment and Water of the Ministry of Science and Technology, where the toxins produced by *P.lilacinus*, such as Paecilotoxin, were detected using the Skyam HPLC technique and using a mobile phase (DW: 5% Formic acid: methanol (20: 5: 75) and the C18-ODS separation column (25 cm and 4.6mm) using the Fluorescence detection detector and the mobile phase. (Flow rate = 1ml / min). After the analysis of the standard material was obtained by taking 0.1 g of the standard toxin and dissolving 250 ml in a 250 ml volume vial where the initial concentration was 40 µg / ml or 40 ppm, ml of the initial concentration and complete the volume to 10 ml in a 10 ml volume vial. 100 µl of the last concentration was taken and injected into the HPLC (Tolgyesi*etal*., 2015). Based on (Akiema*etal*. 1999), The concentration of toxins in the fungus samples was calculated.

B - Extraction of amino acids by HPLC:

Take 10 g of the sample and add 15 ml of 6-unit hydrochloric acid and add a solution of 20: 20 phosphate and methanol, put in a sealed vial. After the decomposition is completed, dry under low pressure and then put in the dryer for 24 hours to remove the acid by adding Sodium hydroxide 6 molar is standard and then diluted to 200 ml. It is recommended to use the rotary evaporator to 1 ml.

1- Derivation**:**

Take 10 µl of the standard solution or sample and mix with 10 µl of phenyl isothiocyanat (PTIC) for 1 minute and add 50 µlof sodium acetate at a concentration of 0.1 mM PH = 7 and mix the solution thoroughly for a minute and then take 40 µL In the device.

2 - Determination of amino acids**:**

The analysis was carried out in the Environment and Water Laboratories / Ministry of Science and Technology using the Korean amino acid analyzer (Amino Acid Analyzer) origin, the vehicles were estimated based on the method provided by
(Scriver, 2001).

V: Statistical analysis**:**

The data were analyzed according to the design of the randomized complete analysis (RCD) and the least significant difference was used in diagnosing the statistical differences between the coefficients.

Results and discussion

First: Biological testing of *P.lilacinus* suspension in eggs and larval larvae of *M.domestica***:**

A - eggs

Table (1) shows the effect of different concentrations of fungal suspension in domestic fly eggs with a mean loss rate of $1x10^5$spore / ml of 53.33%. The mortality rate gradually increased to 60%, 73.33 and 86% in the $1*10^6$, $1*10^7$ and $1*10^8$. The highest mortality rate was 96.66% at $1*10^9$spore / ml. There was a significant difference between all concentrations. The higher the concentration, the higher the percentage of mortality (Fiedler and Sosnowska, 2007). Many researchers have speculated that there is no hatching For the eggs when dealing with the fungus mentioned that the fungal plays an important role in facilitating the process of penetrating the

fungus egg easily and thus able to enter into the egg and break the embryos because of the metabolic materials produced by fungi such as toxins and unsaturated fatty acids or cause mutations on the eggs and thus eggs unable from Maturation and ovals (Jatala, 1986; Bonants*et al*., 1995; Khan, 2006).

P.lilacinus parasites on nematode eggs in general, with low nematode communities, such as banana nematodes, *R. similison* banana, *Heterodera*spp. and *H. schachtii* and *H. glycines* are significantly treated when eggs are treated with the fungus (Davide and Zorilla, 1985). *P.lilacinus* reduced egg hatching rates by 63% when tested for its biological efficacy against *Meloidogynehapla* (Bonants*et al*., 1995). Olivares-Bernabeu and López-Llorca (2002) isolated the fungus from nematode eggs and *H.avenae* and tested the effect of laboratory on the rate of hatching eggs, as the percentage of the infected eggs 70-100%. (Pau *etal*., 2012) found that nematode eggs treated with the suspensions of three different strains PLA, PLB, and PLM of *P.lilacinus* decreased significantly between 88-89% compared to control factor after seven days of treatment and interpreted fungus Mentioned by several researchers as (Khan, *etal*.,2006) penetrating the fungal filaments of the mentioned fungus to the cuticle layer using an electron microscope.

Hostaffer *et al*., (2016) tested the effect of the fungus mentioned in the egg hatching of the nematode worms where the hatching rate decreased to 68.43% compared to 56.43% and 52.25% for *Trichodermaharizianum* and *T. virens* respectively. The results of the laboratory experiments showed the ability of the fungus under investigation to inhibit hatching of parasitic nematode eggs on the tomato *Meloidogynejavanica* by 94%, especially when using the concentration of 3000 pg / ml 48 hours after laying eggs in the petri dishes inside the incubator (AL-Jarmany, 2016) .

. Al-Yasiri (2014) noted that the house fly egg mortality rate was 18.66% for the concentration of $2 * 10^3$spore/ ml. It increased to 36.66% for the concentration of $2 * 10^6$spore / ml when treated with pods belonging to the fungus *Metarrhizium anisopliae*.

B- larval stages

Table 1 shows the percentage of larvae mortality after treatment with pre-prepared suspensions of the fungus. The concentration of $10^5 \times 1$ spors / ml was the lowest (33.33, 23.33 and 20) % for the larval first, second and third larvae, while the highest percentage was larval (90.00, 93.33% and 96.66)% respectively after exposure to $1*10^9$spore/ ml respectively after 72 hours of treatment. A positive correlation between concentration and mortality was found to be statistically significant and the results were statistically significant. Oka *et al* (2010) and Park*et al*. (2004) that the fungus mentioned penetrates the layer of cuticle with the help of enzymatic and accompanied by the secretion of a number of substances Such as acetic acid and toxins known as Paecilotoxins or leucinotoxins, which cause the cessation of chemical reactions in the cell by inhibiting ATP synthesis in mitochondria and phosphorylation processes causing larvae to die.

The results of the present study are similar to the effect of *Paecilomyces fumosoroseus* in the fourth larval stage of the *Trialeurodes vaporariorum* ,where the larval stage mortality was 84-100% after 7 days of Avery *et al*., (2004), while the third and fourth larvae reduced the same fly by 60% When treated with *P.lilacinus*(Sonowska and Fiedler, 2006). Marti *et al*., (2006)

recorded the first time in the world the carrier of *Triatoma infestans* Klug (Hemiptera: Reduviidae) was infected with the fungus and was a great success in eliminating the insect with a 100% mortality rate for fifth larvae instar in Argentina.

Lopez et al. (2014) pointed out that *P.lilacinus* superior to *B.bassiana* fungi in the elimination of larvae of the second stage of a cotton insect with a loss of 60% compared to control treatment.

The results of the current study coincided with that of Abdul-Raheem *et al* (2016) that *P. fumosoroseus* fungi affected the development and growth of larvae of the third stage of housefly and the percentage of loss was 54%. Yasiri (2014) noted that the fungus *M. anisopliae* caused larvae larvae The first of the house flies with a mortality of 56.66% at the concentration of 2 ×103 spore / ml.

Table (1) Effect of different concentrations of *P. lilacinus* suspensions in the percentage of larval mortality of *M.domestica*

stage	Con.	Percentage of eggs and larval larvae after 1 hour 24 h	48 h	72 h
Eggs	$1*10^5$	53.33	-	-
	$1*10^6$	60	-	-
	$1*10^7$	73.33	-	-
	$1*10^8$	80	-	-
	$1*10^9$	96.66	-	-
control		0	0	0
Larval instars	$1*10^5$	20	26.66	33.33
First	$1*10^6$	26.66	40	46.66
	$1*10^7$	40	53.33	56.66
	$1*10^8$	66.66	83.33	86.66
	$1*10^9$	90	93.33	96.66
control		0	0	0
Second	$1*10^5$	16.66	20	23.33
	$1*10^6$	23.33	33.33	36.66
	$1*10^7$	36.66	50	53.33
	$1*10^8$	60	73.33	76.66
	$1*10^9$	86.66	90	93.33
control		0	0	0
Third	$1*10^5$	13.33	16.66	20
	$1*10^6$	20	30	33.33
	$1*10^7$	30	46.66	50.00
	$1*10^8$	53.33	70	73.33
	$1*10^9$	80	86.66	90.00
Control		0	0	0

LSD between phases = 2.89, LSD for time = 2.89, and LSD between concentrations = 3.73 LSD, for inter-factor correlation = 11.20

II. Investigation of secondary metabolites of *P.lilacinus*: Paecilotoxin and amino acids using high performance liquid chromatography (HPLC)

Table 2 and Fig. 2 and 1 show the emergence of Paecilotoxin and amino acids separated by Retention time (Rt) and the sequence of their forms with peaks and times of standard sample retention. The time of detention for metabolites was 16.02 - 5.38 min). The fungus *P.lilacinus* produced the poison known as Paecilotoxin and amino acids throughout its incubation period on the liquid medium (PDB) and at different concentrations. The fungus produced the highest concentration after 28 days of incubation, which was 1.84 µg / (10 days) and this corresponds to Wattanalai et al., (2014) , where he explained that the increase in concentrations The metabolic

material is increased by increasing the incubation period, the nature of the used culture medium and the fermentation conditions.

Paecilotoxin poisons are a combination of amino acids with straight peptide chains associated with unsaturated fatty acids that have played a major role in combating many insects and nematodes (Khan et al., 2003).

(9)Amino acids in phenylalanine, Valine, Methionine, Methylphenylalanine, Aspartic acid, Glutamate, Leucine, Tyrosine and Serine. Acid the first six amino acids had the highest concentration at incubation period for 28 days with (12.31, 5.59, 12.15, 2.83, 3.81 and 2.70 microg / mL respectively, while the remaining three amino acids had the highest concentration at incubation Mushrooms (21) days with (12.28, 30.56 and 5.53) microgram / ml respectively.

HPLC results showed that *P. lilacinus* growing on the plant medium (Karanja cake medium) could produce Paecilotoxin between a time interval of 3.75-7.5 minutes (Sharma et al., 2016). This is similar to the current research results when the time of retention of this toxin was recorded at 5.38 minutes Mori *et al* (1981) noted that *P. lilacinus* had a leucinostatin antibody and an amino acid pyiperidinecrboxylic acid 4-methyl-6 (2-oxobutyl), 2 which is an antibacterial to a Gram positive bacteria isolated by HPLC.

Frisvad et al., (1990) noted the diagnosis of oxytoxin from *Penicillium* fungus using high-performance liquid technology. Al-Ghanmi (2016) reported that *A.niger* extracts oxalic acid, Naphtha-r-pyrun, Malformin A, C and OCHRATOXIN by using high-performance liquid technology and has been tested for mosquito control of *Culex quinquefaciatus. B. australiensis* produces a prehelminthosporal (PHL) toxin with a concentration of 21.69 µg / ml using high performance liquid technology. While the concentration of the toxin Zeralonine produced by the fungus *F. oxysporium* was the highest among the other compounds where the concentration was (45.06) microgram / followed by poison Fusaric acid concentration ((21.55 microgram / ml, while the B2 has the highest concentration of 7.85 Microgram / ml when estimating the toxicity of these fungi against *Culex Quinquefaciatus* mosquitoes.

Table (2) Concentrations of the secondary metabolites of *P.lilacinus* with their retention times compared to HPLC standard samples

N0.	Name	Rt. (st.)	Rt. (sample)	Sample con. ml/µg for incubation period			
				10 day	14 day	21 day	28 day
1	**Paecilotoxin**	5.18	5.38	0.23	1.65	1.64	1.84
2	**Phenylalanine**	10.87	10.88	6.72	10. 64	11.81	12.31
3	**Valine**	12.19	12.19	1.39	3.03	4.13	5.59
4	**Methionine**	12.56	12.57	2.29	5.67	6.77	12.15
5	**Leucine**	13.18	13.18	9.92	7.93	12.28	7.21
6	**Tyrosine**	13.42	13.43	0.40	8.70	30.56	8.22
7	**Methylphenylalanin e**	13.87	13.87	0.20	1.04	2.15	2.83
8	**Aspartic acid**	14.56	14.56	0.57	2.78	0.86	3.81
9	**Glutamate**	14.92	14.93	1.44	1.59	1.22	2.70
10	**Serine**	16.02	16.02	4.77	5.19	5.53	5. 20

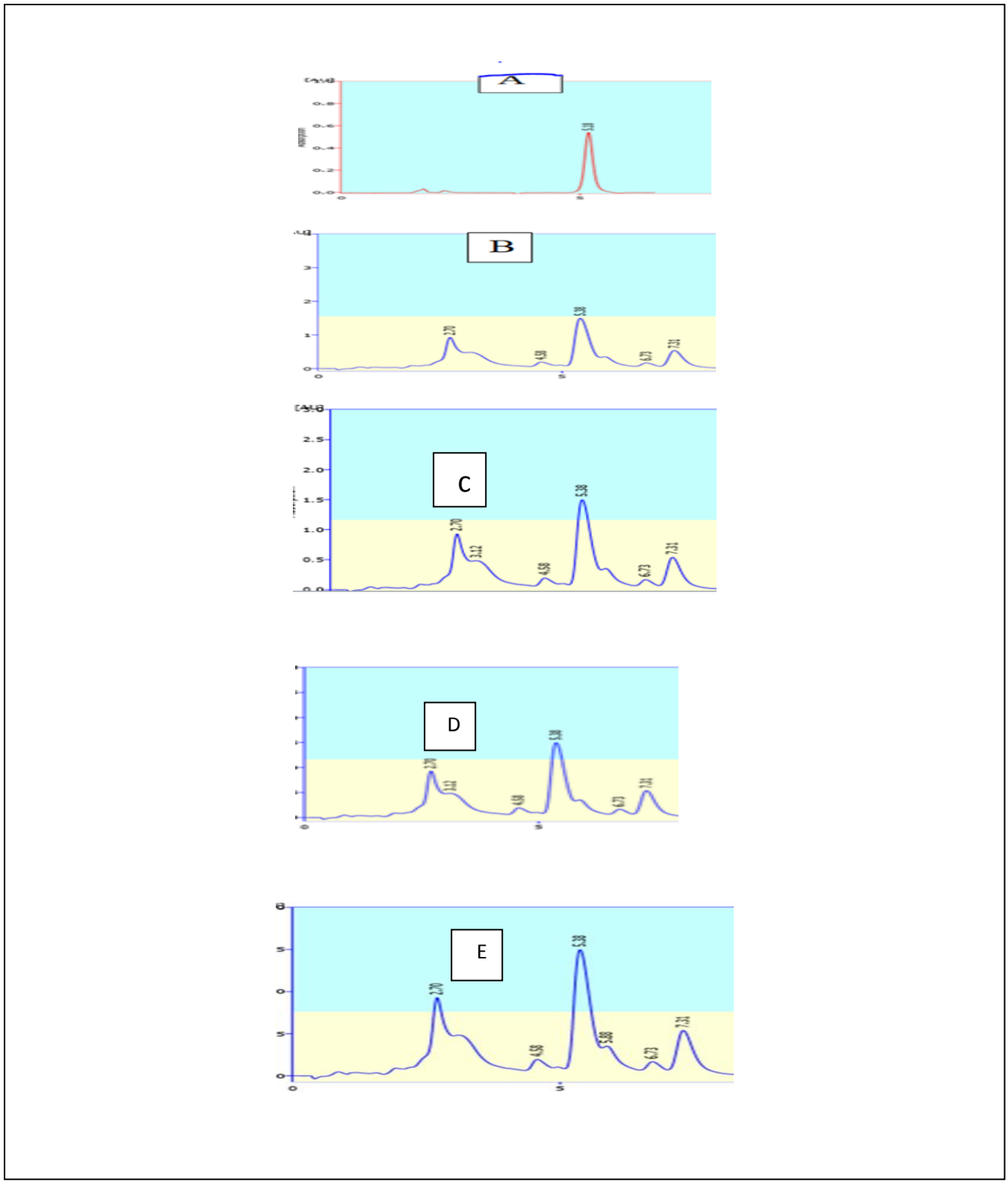

Figure (1) High performance liquid chromatography of Paecilotoxin for *P. lilacinus*(A, B, C, D, E) samples for incubation periods (10,14, 21 and 28) days respectivel .

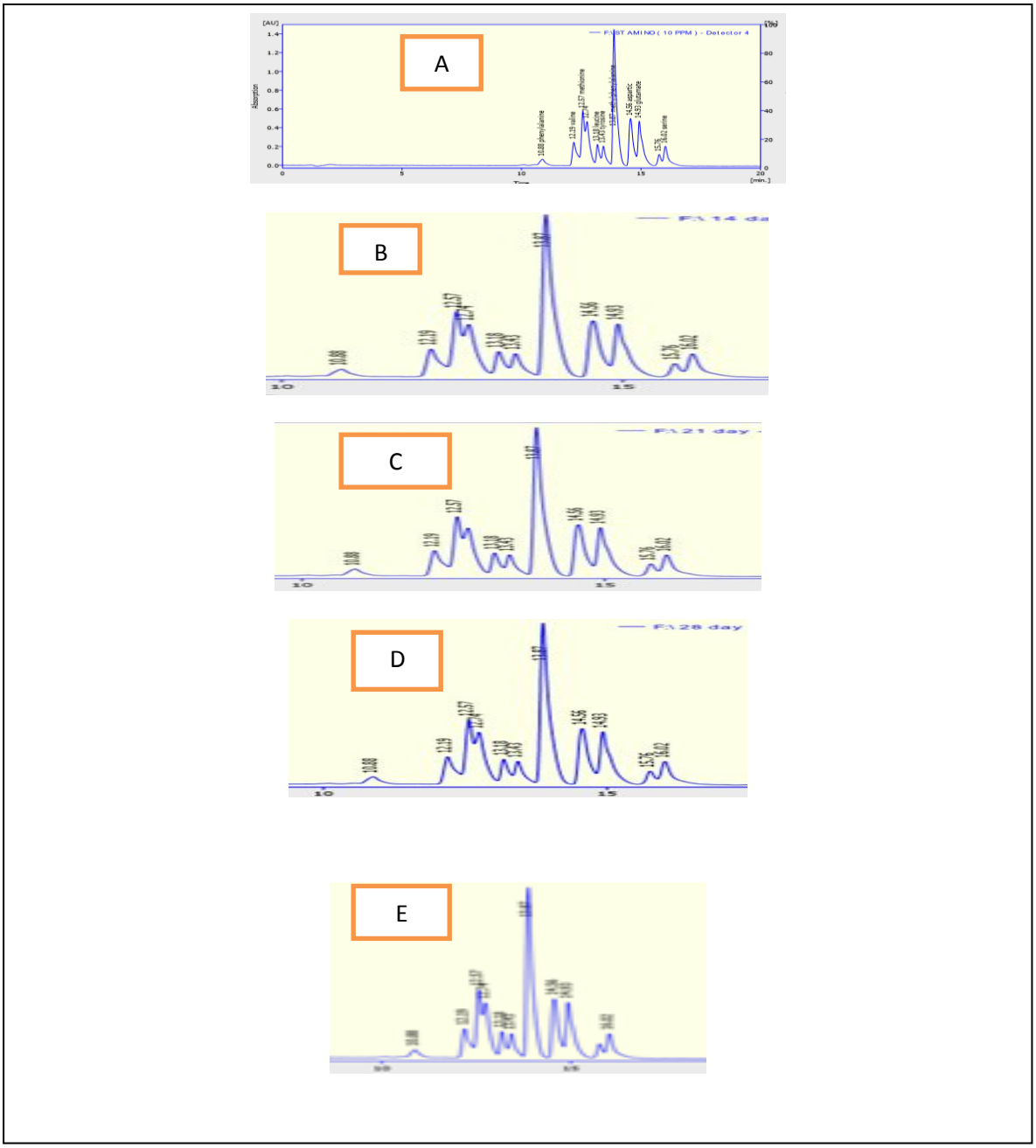

Figure (2) Data of the high performance liquid chromatography of the amino acids of *P. lilacinus* (A) standard and (B,C,D,E) **samples for incubation periods (10,14, 21 and 28) days respectivel .**

Third: Bioassay of *P. lilacinus* filtrates(secondary metabolites) on the housefly fly larvae *M.domestica*

Table (4-5) shows the effect of different concentrations of the fungus secondary metabolites produced from different incubation periods of 10, 14, 21 and 28 days in the larval larvae of the domestic fly insect. The larvae of the first stage were the most affected compared to the second and third stage larvae. The mortality rate was 20% for the first larval stage with a 10-day incubation period. The mortality rate increased to 30.00, 56.66 and 66.66% during the period of 14, 21 and 28 days respectively for the same stage. The mortality rate was 16.66, 33.33, 46.66

and 56% during the same incubation periods of the second larval stage, while the third larvae mortality rate was 13.33, 30.33, 43.33 and 53.33% respectively. (100.3%), while the concentration was 100% with a significant difference of 63.33, 6.66, 86.66, 96.33%, 53.33, 76.66, 83.33, 93.33% and 46.66 and 56.66 respectively. And 76.66% and 90.00%) for the three larvae and incubation periods mentioned above. The increase in mortality was observed by increasing the concentration and the time interval. This is confirmed by the results of the statistical analysis through the moral differences between the treatments. On the secretion of a large number of metabolic substances and various types of mycotoxin fungal toxins and increased concentration lead to the accumulation of toxic substances in the body cells of the insect, which causes an explosion of these cells, which increases the rate of loss rates (Wattanalaietal., 2004). The cause of the larval death was explained by the containment of leucino toxin and other metabolites such as oleic acid, linoleic acid and linolenic acid, which have high toxicity to larvae and arthropods (Park *etal*., 2004 ;Beven *et al*. 1998) notes that the biological efficacy of the metabolic material of *P. lilacinus* is due to its effect on the ionic system in the pores of the bipolar layer of the plasma membrane of the cells or its effect on nerve receptors.

Table(3): Effect of *P. lilacinus* of filtrates on larval stages of housefly with different incubation periods

Period of lap		Percentage of eggs and larval larvae after 1 hour			
		10 day	14 day	21 day	28 day
The phase	Con. %				
First	25	20.00	30.00	56.66	66.66
	50	26.66	36.33	63.33	83.33
	75	43.33	53.33	80.00	93.33
	100	63.33	76.66	86.66	96.33
control		0	0	0	0
Second	25	16.66	26.33	46.66	56.66
	50	20.00	33.33	53.33	80.00
	75	26.66	43.66	76.66	86.66
	100	53.33	66.66	83.33	93.33
control		0	0	0	0
Third	25	13.33	23.33	43.33	53.33
	50	16.66	30.00	50.00	76.66
	75	23.33	40.00	73.33	83.33
	100	46.66	56.66	76.66	90.00
control		0	0	0	0

LSD between phases = 2.47, LSD for time = 2.85, and LSD between concentrations =2.85 LSD, for inter-factor correlation = 9.89

The results of this study were similar to those of Rowbach *et al*., (1986) when larvae of the first stage were exposed to insects such as *Nilaparvata lugens* (Homoptera: Delphacidae) to *P. lilacinus* and *Metarhizium anisopliae* (63) The study indicated that this fungus has a significant role in the control of third and fourth stage larvae of mosquitoes, particularly *Aedes aegypti* mosquitoes, with a mortality rate ranging from 84-88% (Agarwala et al., 1999). Jubouri (2008) recorded the highest mortality of its record *A. niger* has reached 90% after four days of treatment and this is consistent with the findings of the current study.

Bioassay results showed that *P lilacinus*, which was developed on the Karanja cake medium, significantly affected the larvae of *Meloidogyne incognita* larvae, with a 100%

mortality rate, while Czapeck-Dox had a loss of 78.28% and the effect of the fungal filtrates was more toxic Incubation period and the toxic effect of the given fungus to nematodes and insects varies according to the medium used (Sharma et al., 2014) (Nithao et al., 2002; Mukhtar*et al*. 2013and 73)% at 100% and 75% respectively against larvae of the first stage of domestic fly (Yasiri, 2014). To find out the effect of fungus *P.lilacinus* on nematodes causing the complexity of the root crop in tomato *Meloidogy nejavanica* have high toxicity against the second larval stage, as the mortality amounted to 57 percent after 72 hours of treatment AL-Ajramy, 2016)

Al-Khalidi, 2018, reported that the toxic effect of 100% *P.lilacinus* filtrate caused mortality 57.90% of the larvae of *Tylenchulus semipenetrans.*

References

Abdel-Raheem, A. M. and Eldafrawy B. M. (2016). Economic Entomology& Agricultural Zoology Dept. , Faculty of Agriculture, Menoufia University, Menoufia, Egypt.

Agarwala,S; Sagar ,S; Shailen ,S. ; Sehgal (1999) . Published in The Journal of communicable diseases 31(3):193-6..

Akiyma, H. and Chen D.Y. (1999). Simle HPLC determination of aflatoxin B1.B2.G1.G2. in nut and corn , J. of food Hygienic scocity of Japan. 37 (4): 195-201 .

Al-Ajrami, H. H. M.(2016). Evaluation the effect of *Paecilomyces lilacinus* as a biocontrol agent of Meloidogyne javanica on tomato in gaza strip . Msc. Thesis . Faculty of science . The Islamic University-Gaza .

Avery , B. ; Monique , J. f. ; Simmonds, S. J.(2004). Effect of different photoperiods on the growth, infectivity and colonization of Trinidadian strains of *Paecilomyces fumosoroseus* on the greenhouse white fly, *Trialeurodes vaporariorum*, using a glass slide bioassay. Journal of Insect Science, Issue 1, 1, 38, https://doi.org/10.1093/jis/4.1.38

Azzam S, Hussein E.(2002). Toxicities of several insecticides to the house fly Musca domestica from different regions in Jordon. Sarhad J Agric.;18:69–75.

Beven L, Duval D, Rebuffat S, Riddell F., Bodo, B. (1998). Membrane permeabilisation and antimycoplasmic activity of the 18-residue peptaibols, trichorzins PA. Biochim Biophys Acta 1372:78–90**.**

Bonants, P. J., Fitters, P. F., Thijs, H., den Belder, E., Waalwijk, C., & Henfling, J. W. D. (1995). A basic serine protease from *Paecilomyces lilacinus* with biological activity against Meloidogyne hapla eggs. Microbiology, 141(4), 775-784.

Burges, H.D. (2007). Techniques for Testing Microbials for Control of Arthropod Pests in Greenhouses', in Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and other Invertebrate Pests, eds. L.A. Lacey and H.K.

Cao XM, Song FL, Zhao TY, Dong YD, Sun CX, Lu BL. (2006). Survey of Deltamethrin resistance in houseflies (Musca domestica) from urban garbage dumps in Northern China. Environ Entomol.;35:1–9.

David , R. G. Zorilla R.A. and (1985). Evaluation of a fungus Paclomyces lilacinus (Thom.) Samson for the biological control of the potato cyst nematode , Globodera rostachiensis Woll , as compared with some nematicides . Phil. Agric. 66 : 397- 407.

Ellis, M.B.; (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, UK.

Kaya (2nd ed.), Dordrecht, The Netherlands: Springer, pp. 37.

Fiedler , Z and Sosnowska,D.(2007). Nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson is also a biological agent for control of greenhouse insects and mite pests. Bio Control 52:547–558 .

Förster M, Klimpel S, Sievert K.(2009). The house fly (Musca domestica) as a potential vector of metazoan parasites caught in a pig-pen in Germany. Vet Parasitol.;160:163–167.

Frisvad JC, Thrane U, Samson RA, Pitt J (2006) Important mycotoxins and the fungi which produce them. Adv Exp Med Biol 571:3–31

Geden CJ.(2012). Status of biopesticides for control of house flies. J Biopest.;5:1–11.

Ghanmi, Amira Abdul Hadi Rahman. 2016). Comparison of the efficiency of some fungi and their secondary metabolites in the control of Culex quinquefasciatus mosquito. Master Thesis - Faculty of Science - University of Qadisiyah

Hostaffer, B.D; Oliveira, S. F. A; Souza, M. F.F; Persici, B.M; Pötter. L; Silveira, A; Antoniolli, Z.I and Brayer, P. D.I. (2017). Effect of Paecilomyces lilacinus, Trichoderma harzianum and Trichoderma virens fungal extracts on the hatchability of Ancylostoma eggs. Rev Iberoam Micol. ,34(1):28-31.

Jatala,P. (1985). Biological control of nematodeisn.:Sasser, J. N. and Carter C. C. (Eds). An advanced treatise on Meloidogyne. Biology and Control. Raleigh, Department of Plant Pathology. North Carolina State University &USAID : 302308.

Jumaili, Sarah Nair Abdul Amir (2017). Evaluation of the efficacy of some fungi associated with some Culex quinquefasciatus in its fight against life. Master Thesis . Faculty of Science - University of Qadisiyah.

Kaufman PE, Reasor C, Rutz DA, Ketzis JK, Arends JJ.(2005). Evaluation of Beauveria bassiana applications against adult house fly, Musca domestica, in commercial caged-layer poultry facilities in New York State. Biol Control.;33:360–367.

Khan, A.; Williams, K. L. and Nevalainen, H. K. M. (2004). Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of Meloidogyne javanica juveniles. Biol. control., 31: 346-352.

Khan S, Guo LH, Maimaiti Y, Mijit M, Qiu DW.(2012) Entomopathogenic fungi as microbial biocontrol agent. Mol Plant Breed.;3:63–79.

Khalidi, Asraa Jalil Hussein. (2018). The combined effect of the fungus Paecilomyces lilacinus and the digestion of the plant Jaafari Tagetes erecta in the control of citrus nematodes Tylenchulus semipenetrans in Wasit province.

Lacey , L. A. (1997) . Manual of techniques in Insect pathology (Biological techniques) . Academic press . Sadiego. London . Boston . 408pp.

Lopez ,D.C., Zhu-Salzman1, K., Ek-Ramos2, M.J. , Sword1 ,G. A. (2014). The Entomopathogenic Fungal Endophytes *Purpureocillium* (Formerly *P. lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid

reproduction under Both Greenhouse and field Conditions. PLoS ONE 9(8): journal.pone . 0103891.

Lecuona RE, Turica M, Tarocco F, Crespo DC.(2005). Microbial control of Musca domestica (Diptera: Musciadae) with selected strains of Beauveria bassiana. J Med Entomol.;42:332–336.

Marti1, G.M. ,Claudia, Lastra2,C. L., Pelizza2, S. A. & Garcı´a2, J. J.(2006) Isolation of *Paecilomyces lilacinus* (Thom) Samson (Ascomycota: Hypocreales) from the Chagas disease vector, Triatoma infestans Klug (Hemiptera: Reduviidae) in an endemic area in Argentina. Mycopathologia (2006) 162: 369–372.

Malik A, Singh N, Satya S.(2007). Housefly (Musca domestica): a review of control strategies for a challenging pest. J Environ Sci Health B.;42:453–469.

Miao L., Theresa FN, Qian P.Y .(2006). Effect of culture conditions on mycelia growth, antibacterial activity and metabolite profiles of a marine-derived fungus Arthrinium c.f. saccharycola. Appl Microbiol Cell Physiol 72:1063–1073.

Mori, Y.; Tsuboi, M.; Suzuki, M.(1982). Isolation of luucinostatin A and one of ITS constituents, the new amino acid 4-methyl -6-(2-oxobutel)-2-piperidinecarboxylic acid from *Paecilomyces lilacinuis*. The journal of antibiotics . Vol . xxxv .4

Mukhtar T., Kayani M.Z., Hussain M.A. (2013). Nematocidal activities of Cannabis sativa L. and Zanthoxylum alatum Roxb. against meloidogyne incognita. Ind Crop Prod 42:447–453.

Mwamburi LA , Laing MD. , Miller R. (2011) . Laboratory screening of insecticidal activities of Beauveria bassiana and *Paecilomyces lilacinus* against larval and adult housefly (Musca domestica). African Entomology 2011a; 18:38-46.

Nitao J.K., Meyer S. L.F., Olive J.E., Chitwood D.J .(2002) .Isolation of falvipin, a fungus compound antagonistic to plant parasitic nematodes. Nematology 4:55–63.

Olivares-Bernabeu, C. and López-Llorca, L. V. (2002) . Fungal eggparasites ujhbujof plant-parasitic nematodes from Spanish soils.Rev. lberoam Mycol., 19: 104-110.

Park JO, Hargreaves JR, McConville EJ, Stirling GR, Ghisalberti EL, Sivasithamparam,K. (2004). Production of leucinostatins and nematicidal activity of Australian isolates of Paecilomyces lilacinus (Thom) Samson. Lett Appl Microbiol;38:271–6.

Rowbach, V. ; Ryba, J. ; Hanslova , H. and Weiser J. (1987). The efficiency of beta-exotoxin on *Bacillus thuringiensis* on susceptible and resistant Housefly. In: Proceedings of the International Conference of Medical and Veterinary Dipterology ; 262-265.

Scott JG, Alefantis TG, Kaufman PE, Rutz DA.(2000). Insecticide resistance in house flies from caged-layer poultry facilities. Pest Manage Sci;56:147–153.

Scriver CR, Beaudet AL, Valle D, Sly WS, Childs B, Kinzler KW, Vogelstein

B, eds.(2001). The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York, NY: McGraw-Hill, Inc;;1665-2105.

Sharma1 ,A. , Sharma1 S. ,Aditya Mittal A. , Naik1 S. N.(2016). Evidence for the involvement of nematocidal toxins of Purpureocillium lilacinum 6029 cultured on Karanja deoiled cake liquid medium. World J Microbiol Biotechnol 32:82

Tolgyesi , A . ; Stroka , J. and Zwickel ,T. (2015) . Simultaneous analysis of *Alternaria* toxtins and citrinin in tomato: an optimisd method using liquid chromatography- tandem mass spectrometry . Food Additives and Contaminants: Part A, 32,No. 9, 1512-1522.

Ugbogu OC, Nwachukwu NC, Ogbuagu UN.(2006). Isolation of Salmonella and Shigella species from house flies (Musca domestica L.) in Uturu, Nigeria. Afr J Biotech.;5:1090–1091.

Wattanalai, R.; Wiwat, C.; Boucias, D.G.a Tartar, A. (2004). Chitinase gene of the dimorphic mycopathogen, Nomaraea rileyi. J. Invert. Path. 85: 54-57.

Yasiri, Ali Morteza Kazem. (2014). Effect of some biological control factors in some aspects of life of domestic fly Musca domestica (L.) (Diptera: Muscidae). Master Thesis - Faculty of Science - University of Qadisiyah