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Effect of Secondary Metabolite Crude of *Metarhizum anisopliea* Fungus on the Second Larval Stage of the Housefly *Musca domestica*

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Abstract:

The house flies *Musca domestica* (Diptera:musidae) are the primary carrier of many pathogens such as cholera, typhoid, anthrax, and others. The use of chemical pesticides as a basic method of control leads to many problems at the environmental and health level. The use of safe alternatives to chemical pesticides has become an urgent necessity. The research aims to find biological alternatives that are environment-friendly and non-pathogenic to humans in controlling house flies through the possibility of extracting and diagnosing some secondary metabolites produced by the fungus Metarhizium anisopliae and testing their effects on the second larval stage of house flies using different treatment methods that include direct spraying of the larvae, treating the food environment, and the dipping method. Secondary metabolites and toxins of *Metarhizium anisopliae* were extracted in liquid media PDB using a mixture of organic solvents such as ethyl acetate and methanol. The secondary metabolites were identified by gas chromatography-mass spectrometry (GC-MS). The results showed the identification of 10 chemical compounds, including phenol, 2,4-bis(1,1-dimethylethyl ($C_{14}H_{22}O$)., Diethyl Phthalate ($C_{12}H_{14}O_4$), Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$), Phthalic acid, butyl undecyl ester ($C_{23}H_{36}O_4$) , 9,12-Octadecanoic (Z,Z)-, methyl ester ($C_{19}H_{34}O_2$), 9-Octadecanoic acid, methyl ester, ($C_{19}H_{36}O_2$), 9,12,15-Octadecanoic acid,methyl ester,(Z,Z,Z) ($C_{19}H_{32}O_2$), Octadecanoic acid,methyl ester($C_{19}H_{38}O_2$), Oleic Acid ($C_{24}H_{38}O_4$), 9-Octadecanoic acid (Z)-,2-hydroxyl (hydroxymethyl) ethyl ester($C_{21}H_{40}O_4$) ,and Di-n-octyl phthalate ($C_{24}H_{38}O_4$). The results showed that the crude extract of the fungus cause the best mortality rate in the second instar larvae at concentrations of 3 and 5% after 72 hours of treatment when the mortality rates ranged between 60-100%. The mortality rates were directly proportional to the increase in concentration and time with a significant difference. The results also showed that the treatment of the food media was the most effective in affecting the larvae of flies, recording mortality rates that reached 100%, with a significant difference with direct spraying and dipping methods. These results reveal the significant efficacy of the tested secondary metabolite crude of m.anesopalae against Musca domestica which could be used as an ecofriendly alternative for insect control.

Keywords: Diptera, Fungi secondary metabolism, Fungi extract, Insect Control, Musca *domestica.*

Introduction:

Housefly (*Musca domestica*) is the most common fly on the planet. More than 100 infectious diseases have been linked to houseflies in humans and animals [1]. Pathogens which are transferred by *M. domestica* may cause cholera, anthrax, food poisoning, typhoid, diarrhea and shigellosis [2]. House fly is considered as a wide separate and a dangerous insect due to its characteristics which are facultative diapauses characteristics such as broad distribution, and high reproductive capacity leading to the adaptation of various agroecological habitats and it has become a primary pest, through its vomits or excreta and legs, it acts as a mechanical vector for disease transmission through contaminated water and food [3]

The common insecticides pyrethroids, spinosad, pyrethrins, dichlorvos, imidacloprid and cyantraniliprole, are used in house fly control [4]. Due to the dangers and hazards of chemical pesticides accumulating in the environment and being transmit into food, which kill untargeted insects, the world has resorted to using alternative pesticides, such as physical and biological control. The biological repression of insect pests such as viruses, fungus, bacteria, protozoa, and nematodes is known as microbial control [5]. The aqueous and alcoholic extracts of plant were tested on the insect which achieve high mortality rates [6] . Even the use of alternative chemicals such as citric acid and bicarbonate have effects on mosquitoes larvae but their safety on the environment is uncertain [7]. Microbial biocontrol agents (MBCAs) have the ability to eliminate pest populations while show no risk to human health or the environment, as a substitute for chemical pesticides, this is a viable option [8] . This Entomopathogenic fungus (EPF) is thought to be eco friendly and capable of producing toxic compounds that can kill target insects like cyclopeptida destruxin A, B, C, D, and desmethyldestruxin B. The fungus *Metarhizium anisopliae* and *Beauveria bassiana* are two fungi that are known to be entomopathogenic [9]. The secondary metabolites of *B. bassiana and Metarhizium anisopliae* as (n-Hexadecanoic acid; 9, 12-Octadecadienoic acid, methyl ester, (E, E)-; Hexadecanoic acid, methyl ester; 7,10-Octadecadienoic acid, methyl ester and trans-13-Octadecenoic acid; Tetradecanoic acid, 12-methyl-, methyl ester),have an insecticidal activity and antifeeding properties against insect as well as they caused larval growth inhibition [10] . The development of biochemical weaponry like as enzymes, poisons, and other metabolites compounds aid in host infection and invasion. Chitinase, proteinase, and lipase are enzymes that are directly involved in the destruction of the host cuticle, which is the initial and most important barrier to EPF infection,destruxins of *Metarhizium*, beauvericins of *Beauveria*, hirsutellides of *Hirsutella*, isarolides of *Isaria*, cordyols of *Cordyceps*, vertihemipterins of *Verticillium*, and other secondary metabolites disable and accelerate the EPF infection process by directly and indirectly disabling the insect hosts' defense mechanisms, Secondary metabolites range in chemical complexity from simple non-peptide pigments like oosporine to very complex piperazine derivatives [6, 11]. Topical application of crude extracts did not result in death, but combined treatments with fungal suspensions of *M. anisopliae* and their extracts resulted in higher mortality rates than solo *M. anisopliae* and extract treatments [12]. Some entomopathogens' secondary metabolites have been shown to be beneficial in IPM programs against a variety of insect pests [10]. The most common secondary metabolites are produced by *M. anisopliae* during fermentation and, by far, the most thoroughly studied entomopathogenic fungus poisons recognized as key virulence factors hastening the mortality of infected insects. There are 38 Dxs or Dx analogs, which are chemically grouped into five groups named A through E [13]. The insecticidal activities of destroxins against Diptera, leading to the suggestion that destruxins could be utilized as pesticides, these toxins weaken the host's immune system, harm the muscular system, and damage the malpighian tubules, altering excretion and causing eating and movement problems [14]. As a result, the destruxins' function of limiting host mobility would also weaken this behavioral defense mechanism. Indeed, isolates of *Metarhizium* that produce more destruxins are more virulent [15].

The purpose of this research is to identify specific toxin and secondary metabolites of the entomopathogenic fungi *Metarhizium anisopliae* in order to improve its insecticidal action, reduce housefly infestation in the environment, and reduce the disease caused by houseflies .

Materials and Methods:

Chemicals

Materials used were methanol ,ethyl acetate (Thomas Baker, India) as a solvent and as co-surfactant, tween 80 (Thomas Baker, India) as surfactant, and deionized water.

Musca domestica collection and rearing

Musca domestica adults were collected from a farm in Baghdad in Iraq government by using arial net. The adults were placed in wooden cage with a dimension of 30×20×20 cm^3 covered with tulle and one lateral side modified the tulle to enter the hand for supplement food and clean the cage. The adults of housefly were sent to the Natural History Museum of Iraq for identification, they were diagnosed as *Musca domestica*. The experiments were done in the laboratory of the Agricultural Research/ Ministry of Science and Technology in Al Twaitha ⁄ Baghdad ⁄ Iraq.

In the laboratory, plastic containers of 500 ml were filled with cotton webbed with 1:1 sugar and distilled water to the nutrition of adults for obtaining eggs placed under the larval nutrition , while larval grown in plastic container 20 ml capacity containing (float fish diet) that consists of crude protein 28.0 % Min. , crude fat 4.0 % Min. and crude fiber 4.0 % Max in addition to amino acids, vitamins , minerals , after crushed and ,where taking 200 gm of (fish diet) mixed with 10 gm of dry yeast , 5gm of citric acid

and dissolved in 100 ml of distilled water , the nutrient was used to feed larvae and adult taking care of change it all three days [16] . The cage was placed in the rearing wooden cages with a dimension of 30×20×20 cm^3. The rearing cages were placed in the incubator under controlled conditions 27± 2 º C, 65± 5 RH and 10: 14 D/L photoperiod. This feeding is important for *Musca domestica* to get the energy needed for flight and other life activities.

Crude extraction of toxin and secondary metabolism from fungi *M. anisopliae*:

Mass culturing of *Metarhizium.anisopliae* :

The fungi *Metarhizium anisopliae* was taken from Agriculutral Research Dpartment\ Ministry of Agriculture. The fungus was grown on PDA plates at 25±5°C for 3-5 days, to optain pure culture, 5 g/ml of chloramphenicol was added to Potato Dextrose Agr (PDA) after sterilized as antibacterial agent to prevent growth bacteria [17].

Production of fungal toxic proteins and secondary metabolits in Potato Dextrose Broth (PDB) medium:

In order to produce the secondary metabolic compounds of fungus *Metarhizum anisopliae* , 1cm piece of fungus was taken from three-day old PDA grown cultures by borer and cultured in 500 mL of PDB medium which consist of 200 ml potato water and water 800 mL , 20 g sugar, and 500g amoxylin in conical flasks and were incubated at 25°C and humedity 80% in an incubatore with shaking in different time for a period of 10- 12 days. [18, 19, 20]

Classical solvent extraction method:

The majority of isolation procedures still utilize simple extraction procedures with organic solvents of different polarity, water and their mixtures. The method includes maceration. Following incubation, the fungus biomass was separated from the culturing medium with gauze (soft) cloth in order to use the broth portion, which contained toxin, secondary metabolism, and spores. The extraction of crude secondary metabolites was cmpleted with Whatman filter paper No.1 separated the light-yellow color component of the fungal mat (organic phase). , 500 ml broth, 750 ml solvent (1:1 ethyl acetate and methanol) which were mixed with the fungal broth component for cold extraction with shaking for 2-3 days at 28 ºC. The solvent was separated from the water by separating funel, and the solvent was filtered using a micropore filter 0.2 Mm to clear it. A vacuum evaporator at 80 °C was used to separate the combined solvent from concentrated extracts. Deionized water and tween 80 were used to dissolve the crud extract [21] .

Determine toxines and secondary metabolits in crude extract of *Metarhizum anisopliae* :

To determine toxines and secondary metabolits in crude extract of *Metarhizum anisopliae* used technique (Gas Chromtography –Mass Spectrophotometery) (GC-MS) .A Clarus 680 30 m × 0.25 mm ID × 250 μm silica column was used for GC analysis of the chemical constituents. This column was packed with elite-5MS 5% biphenyl 95% dimethylpolysiloxane. The chemical constituents were separated by using He at a constant flow of 1 mL/min as a carrier gas. The crude extracts (1 μL) were injected to the GC-MS instrument at 260 °C during the column running time. The temperature ramp was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and finally 300 °C, where it was held for 6 min. The mass detector conditions were 240 °C; ion source temperature at 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 s and scan interval of 0.1 s. The fragments from 40 to 600 Da were collected) [22].

Bioassay of crud extract of *M. anisopliae* against the 2nd larval stage of *Musca domestica:*

Experiment was conducted to bioassay of crud extract of *M. anisopliae* against 2nd larval stage of *Musca domestica* (diagnosis by head capsule) by dissolving the methanolic crude extract in de ionized water and tween 80 to separate and dissolving the compound . The bioassay has three testing concentrations 1, 3, and 5% and each concentration had three replicates of 30 larvae in each concentration (10 larvae to each replicate).

Direct spray technique:

90 of 2nd larval stage from culture divided to 3 replicates to each concentration(10 larvae to each replicate) ,spray it with the concentration 1 , 3,and 5% attended previously each separately ,then put the treated larvae on non treated medium cages that put in an incubator at 25±5 º C , 70% RH . Larval mortality rate was recorded after 24, 48, and 72h.

Indirect technique (treated rearing medium):

5 g of medium was used to rear *Musca domestica* put in container 50 ml , sprayed medium and container put un treated 2nd larval stage . As 3 replicates of each concentration 1, 3, and 5%, 30 larvae in each concentration (10 larvae to each replicate), the experiment was put in an incubator at 25±5ºC, humidity 70%. The mortality larvae was isolated and reported daily for 27 h.

Dipping technique:

In dipping method , 90 of 2nd larval stage was divided into 3 replicates of each concentration 1, 3,and 5%) dipping 30 larvae with 1ml of each concentration for 1 minute each separately ,then transfer to non treated medium cage with smooth brush (10 larvae to each replicate). Then, the cages were put in an incubator at 25±5ºC humidity 70%. The isolated and reported larval mortality rate was recorded after 72 hours.

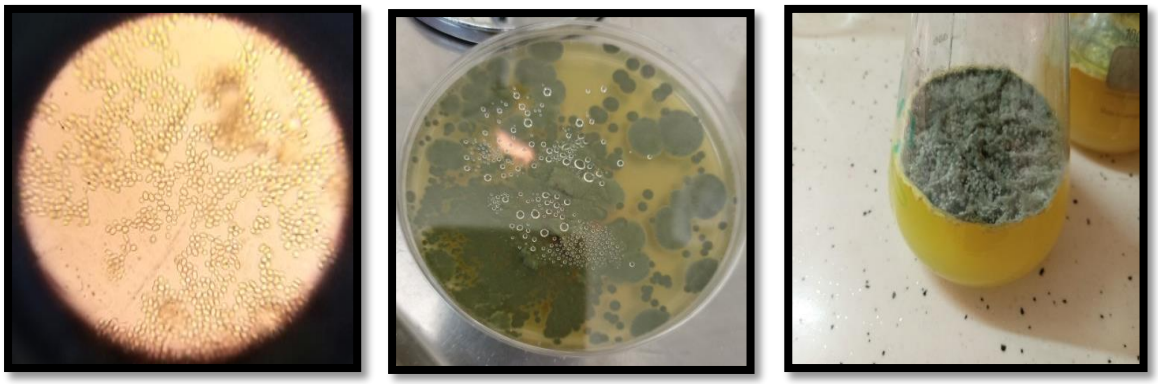
Statistical analysis:

The experiments were carried out according to factorial experiments using a completely randomized design (CRD), and the differences between the means of the treatments were tested according to the value of the least significant difference at the probability level of 0.05. The results were analyzed by the statistical program Genstat 10. The lethal and sub-lethal concentrations were determined by a probit analysis software program [23].

Results:

Growth *M. anisopliae*

Fungi ***M.*** *anisopliae* characterized with radial growth and green cray color and seriales of elongated sporeses, Fig 1 (a,b,c).



A B C

Figure 1. (a) serial boards spores 40X . (b) radical growth of *Metarhizium anisopliae* in PDA (c) growth fungus in PDB .

crude extract of *Metarhizum anisopliae* :

Crude extract of *Metarhizum anisopliae* obained was yallow –riddish and gellatenouse in nature texture with bad smell , figure 2 .

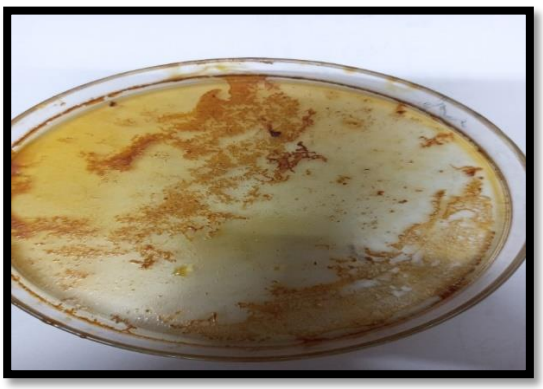


Figure 2. Gelatinouse nature of crud extract of *M. anisopliae* after drying.

Determining toxines and secondary metabolits in crude extract of *Metarhizum anisopliae* :

GCmass technique (Gas Chromtography –Mass Spectrophotometer) Analysis:

GasChromatography -Mass Spectrometry results obtained from *M.anisopliae* indicated the presence of several major compounds (secondary metabolism) , as shown in Table 1 and Figure 3 . The highest compounds were(Phenol, 2,4-bis(1,1-dimethylethyl ($C_{14}H_{22}O$), Diethyl Phthalate ($C_{12}H_{14}O_4$), Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$), Phthalic acid, butyl undecyl ester ($C_{23}H_{36}O_4$), 9,12-Octadecanoic (Z,Z)-, methyl ester ($C_{19}H_{34}O_2$), 9-Octadecanoic acid, methyl ester , (E) ($C_{19}H_{36}O_2$), 9,12,15-Octadecanoic acid,methyl ester,(Z,Z,Z) ($C_{19}H_{32}O_2$), Octadecanoic acid,methyl ester($C_{19}H_{38}O_2$), Oleic Acid ($C_{24}H_{38}O_4$), 9-Octadecanoic acid (Z)-,2-hydroxyl (hydroxymethyl) ethyl ester($C_{21}H_{40}O_4$) ,and Di-n-octyl phthalate ($C_{24}H_{38}O_4$) . Di-n-octyl phthalate ($C_{24}H_{38}O_4$) and 9-Octadecanoic acid (Z)-, 2hydroxyl (hydroxymethyl)ethyl ester ($C_{21}H_{40}O_4$) were the highest with the highest RT/min. This agrees with [24, 25].

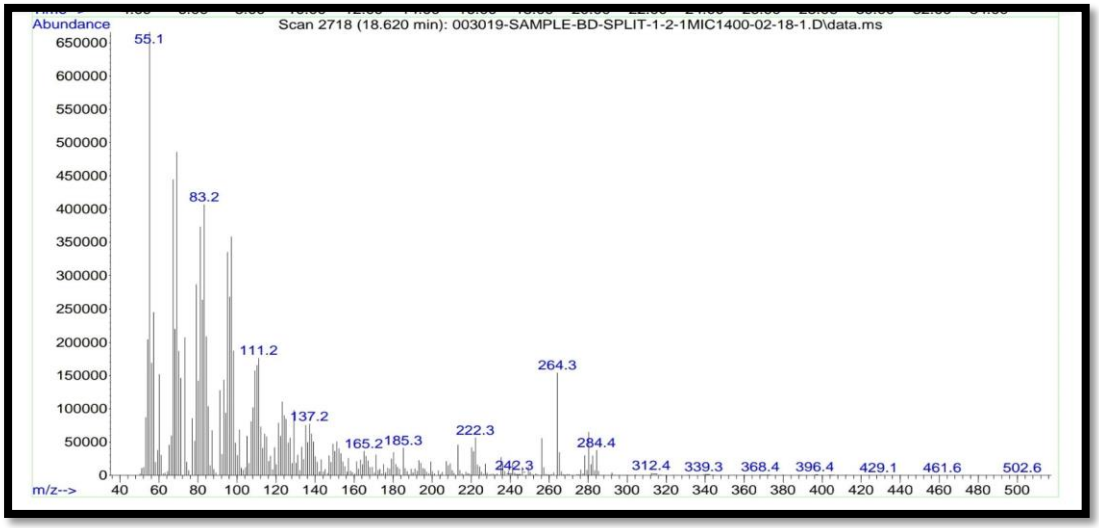


Figure 3. GC-MS analysis of methanolic extract.

Table 1. Major bioactive compounds identified in methanol extracts of *M. nisolpiae* using Gas chromatography –Mass Spectrometry analysis.

Compound	(R.T./min)	Molecular formula	Prob %	Area %
Phenol, 2,4-bis(1,1-dimethylethyl	11.724	$C_{14}H_{22}O$	54.7	0.932
Diethyl Phthalate	12.740	$C_{12}H_{14}O_4$	51.6	1.418
Hexadecanoic acid, methyl ester	15.712	$C_{17}H_{34}O_2$	42.6	1.748
Phthalic acid, butyl undecyl ester	16.205	$C_{23}H_{36}O_4$	11.0	4.81
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.866	$C_{19}H_{34}O_2$	12.6	10.505
9-Octadecenoic acid, methyl ester, (E)-	17.924	$C_{19}H_{36}O_2$	6.03	26.763
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.056	$C_{19}H_{32}O_2$	33.9	9.818
Octadecanoic acid, methyl ester	18.13	$C_{19}H_{38}O_2$	30.9	4.37
Oleic Acid	18.706	$C_{24}H_{38}O_4$	17.6	32.226
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.795	$C_{21}H_{40}O_4$	13.1	5.005
Di-n-octyl phthalate	22.900	$C_{24}H_{38}O_4$	18.4	2.406

Bioassay of *M. anisopliae* extract in the 2 [end] larval stage of housefly:

The results in Table 2 show the effect of concentration 1,3,and 5% with 3 techniques as, in direct spray technique in the concentration 1% were 0.0 , 0.0 , and 6.67 % mortality respectively after 24, 48 , and 72 hours of treatment . On the other hand, in the concentration 3% the mortality rate was 23.3 , 40.0 , 60.0 % respectively after 24, 48 ,72 hours .In the concentration 5% mortality rate was 63.3 ,73.33 , 73.33 % respectively after 24, 48 , and 72 hours of treatment . Statistical analysis reported a significant difference in mortality percentage with increasing time in rate 2.22 , 41.11 , 70 respectively , the concentration 3 % was the best to obtain higher rate mortality at LSD 0.05 .As the results in Table 2 record, the lowest mortality rates were 0.0 , 23.3 , 63.3 in the concentration 1, 3, and 5% after 24 hour of treatment , and the highest mortality rates were 6.67 ,60, 73.33 % in concentration 5% after 72 h , at LSD 0.05 showed a significant difference between concentration and the important interaction between treatment factors by increasing larval mortality with increasing time of exposure to extract and concentration .

In rearing medium with cage treatment technique, the results showed the effect of concentration 1, 3, and 5 % in period 24, 48, 72 hours of treatment. The concentration 1% percentage mortality was 36.67,36.67, 36.67 % respectively in 24,48,72 hours , while the concentration 3% showed percentage of mortality 66.7 , 83.33, 93.33 % respectively after 72 hours of treatment. The concentration 5% gave 0.0, 6.7, 100 % percentage of mortality respectively after 72 hours of treatment.

Theirwas a significant difference at LSD 0.05. The results showed the interaction effect between treatment factors had significant effect on the increasing mortality rate with significant difference, as well.

In dipping technique , the results showed the mortality percentage in concentration 1% was 10, 10,

10 % respectively after 72 hours, while in concentration 3 , and 5% the rate mortality was 0.0, 0.0. 100 % after 72 hours of treatment. Statistical analysis showed the significant effect of the treatment factors interaction. The result in rearing medium only technique showed the difference between application methods as high effect was with sprayed both rearing cage and larvae with nutrient medium in statistical analysis at LSD 0.05. As statistical analysis of factors experiment show that the concentration 3% was the best.

Results in Table 3 showed sub lethal and median lethal concentration under different approach to control population of housefly, treated rearing cage more effect with LC50 was 0.26 , which maybe returned to increase the exposure to the toxic by treating rearing cage with nutrient media contain larvae , while lower LC50 was in dipping technique as 0.04. This agrees with the results in Table 2.

Table 2. Toxicity of *M. anisopliae* derived secondary metabolites on 2ed larvae of *Musca domestica* under different application methods.

Application methods	Conc.%	% corrected mortality / Time			Mean
		24 h	48 h	72 h	
direct spray technique	1	0.0	0.0	6.67	3.33
	3	23.3	40.	60.0	41.11
	5	63.3	73.33	73.33	70
	Control	0	0	0	0
	Mean	28.9	36.37	46.7	28.33
rearing medium treated technique	1	36.67	36.67	36.67	36.67
	3	66.7	83.33	93.33	81.11
	5	0.0	6.7	100	35.6
	Control	0	0	0	0
	Mean	34.44	42.22	76.67	51.11
Dipping	1	10.0	10.0	10.0	10.0
	3	0.0	0.0	100	33.33
	5	0.0	0.0	100	33.33
	Control	0	0	0	0
	Mean	3.3	3.3	70.00	25.53
	Total mean	16.67	20.83	48.33	
	Conc.mean	16.3	51.85	46.3	
LSD 0.05 Appl.md = 3.55 conc.= 4.09	Time = 3.54	Apl.md×	conc ×time	=12.28	

Table 3. Lethal and Sub lethal dose of toxicity of *M. anisopliae* derived secondary metabolites on 2 nd larvae of *Musca domestica* under different application methods.

Methods of application	slope	LC25	LC50	LC90	Regression Equation	χ^2
direct spray	16.66	1.7	3.2	5.59	Y=16.66 x - 3.34	0.654*
rearing medium	15.83	0.26	1.32	3.84	Y=15.83 x + 29.16	0.843*
Dipping	22.5	0.04	2.11	3.89	Y=22.5 x +2.5	0.689*

Discussion:

We noticed from the results that the secondary compounds identified by GC mass9, 12-Octadecadienoic acid, methyl ester, (E, E)-Nematicide & Insectifuge compound ، Hexadecanoic acid, methyl ester Pesticide [26], act as pesticide and repellent [27].

Treated larvae with 1, 3, and 5 % concentration of *M. anisopliae* required 72 hours to reach the mortality rate of 100% of methanolic extract crud (secondary metabolites) of fungus *M. anisopliae*, while it reported their significant difference between concentrations ,and the concentration 5% gave the highest mortality after 72 hours. Infected larva manifested numerous symptoms of unregulated movement, anti-feeding, change color from wax to brown then black and no development in growth larvae and not reaching the pupa stage. 1%, 3% low concentration have fatal attracting effect while high concentration 5% has a repellent and anti-nutritive effect. The differences between application methods have high effect, sprayed both rearing cage and larvae with treated medium, which gave more chance to exposure the larvae to the second compound. The result agrees with (Ortiz , 2010). Testing the crude extract offers an alternative approach and is recommended when assessing the risks of metabolites for registration purposes.

The toxicological risk evaluation of metabolites produced by the insect pathogenic fungus Metarhizium anisopliae was spurred by growing sensitivity to secondary metabolites from fungal biological control agents (BCAs), Individual metabolites (destruxins A, B, and E) as well as the whole crude extract from liquid cultures were used to compare the two ways of assessing individual metabolites (destruxins A, B, and E) or the complete crude extract from liquid cultures. The crude extract contains synergistic and antagonistic actions. The findings show that identifying and assessing each unique metabolite produced by a BCA is not only time and money consuming, but also does not provide a complete picture. When analyzing the risks of metabolites for registration reasons, testing the crude extract is an alternate approach that is advised [28]. Several entomopathogen-secreted secondary metabolites have been reported to exhibit anti feeding and insecticidal effects [29, 30].

Also, it might be due to the presence of an endogenous lipid layer in *M. anisopliae*. Above all, ecdysis is a physiological mechanism that eliminates increasing infections as well as the old cuticle, boosting the chances of the host surviving [31]. Immune modulator secretion and protease repressor secretion are two primary methods by which an invading pathogen overcomes host immune defenses, *M. anisopliae* produced a collagen-like immune evasion protein that served as an anti-adhesive protective covering, masking antigenic cell wall-glucans and prevented haemocytes from recognizing the hyphal bodies [32] .They're cyclopeptides and cyclodepsipeptides that are physiologically active and cause direct cytotoxicity [33]. Increasing sensitivity towards secondary metabolites from fungal biological control agents (BCAs) has prompted the toxicological risk assessment of metabolites produced by the insect pathogenic fungus *Metarhizium anisopliae*. Main destruxins A, B and E. Evaluation of the cytotoxic activity of these different compounds suggested that a wide range of metabolites with synergistic effects are present in the crude extract , These metabolites also play a role in antimicrobial peptide synthesis and phagocytosis resistance. Destruxins from *Metarhizium*, on the other hand, are implicated in the creation of oxidative stress, which causes many of the host antioxidant enzymes to be destroyed [34]. However, one drawback to using fungi to manage arthropods is that they may kill their hosts too slowly. These toxins weaken the host's immune system, harm the muscular system, and damage the Malpighian tubules, altering excretion and causing eating and movement problems [14]. .As a result, the destruxins' function of limiting host mobility would also weaken this behavioral defense mechanism. Indeed, isolates of *Metarhizium* that produce more destruxins are more virulent [15].

Conclusion:

It is believed that methanolic crude extract (secondary metabolites and toxin) of fungus *Metarhizium* anisopliae has a natural pesticide activity. It can be concluded that methanolic crude extract (secondary metabolites and toxin) of fungus *Metarhizium* anisopliae can be used as a safe and effective alternative in the control of vector-borne diseases caused by Mucidae larvae.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' contributions statement:

H. I. AL. and S. A. K. conceived of the presented idea and supervised the findings of this work. While, A. A. A. Hammed did all the experiments and verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

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تاثير مركبات الايض الثانوي الخام للفطر *Metarhizum anisopliea* في الطور اليرقي الثاني لحشرة الذبابة المنزلية *Musca domestica*

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الخلاصة :

يعد الذباب المنزلي *Musca domestica* الناقل الاولي للعديد من المسببات المرضية مثل الكوليرا والتيفوئيد والجمرة الخبيثة وغيرها. ان استخدام المبيدات الكيميائية كطريقة اساسية في المكافحة يؤدي الى العديد من المشاكل على المستوى البيئ والصحي. ان استخدام بدائل امنة عن المبيدات الكيميائية اصبحت ضرورة ملحة. يهدف البحث الى ايجاد بدائل حيوية صديقة للبيئة وغير ممرضة للانسان في السيطرة على حشرة الذباب المنزلي عن طريق امكانية استخلاص وتشخيص بعض مركبات الايض الثانوي المنتجة من قبل الفطر *Metarhizium anisopliae* فضلا عن اختبار تاثيرها ضد الطور اليرقي الثاني للذباب المنزلي باستعمال طرق معاملة مختلفة شملت الرش المباشر لليرقات ومعاملة البيئة الغذائية وطريقة الغمر. تم استخلاص المركبات الايضية الثانوية والسموم للفطر *M. anisopliae* بالوسط السائل PDB باستخدام مزيج من المذيبات العضوية مثل خلات الاثيل والميثانول. شخصت المركبات الايضية الثانوية بواسطة جهاز الكروماتوغرافي الغازي – مطياف الكتلة (GC-MS) . أ ظهرت النتائج تشخيص 10 مركبات كيمياوية شملت Phenol, 2,4-bis (1,1-dimethylethyl (C14H22O), Diethyl Phthalate ($C_{12}H_{14}O_4$), Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$), Phthalic acid, butyl undecyl ester ($C_{23}H_{36}O_4$) , 9,12-Octadecanoic (Z,Z)-, methyl ester ($C_{19}H_{34}O_2$) , 9-Octadecanoic acid, methyl ester , (E) ($C_{19}H_{36}O_2$) , 9,12,15-Octadecanoic acid,methyl ester,(Z,Z,Z) ($C_{19}H_{32}O_2$), Octadecanoic acid,methyl ester($C_{19}H_{38}O_2$), Oleic Acid ($C_{24}H_{38}O_4$) , 9-Octadecanoic acid (Z)-, 2-hydroxyl (hydroxymethyl) ethyl ester($C_{21}H_{40}O_4$), Di-n-octyl phthalate ($C_{24}H_{38}O_4$) اظهرت النتائج ان المستخلص الخام للفطر قد حقق افضل نسب القتل في يرقات الطور الثاني عند التركيزين 3 و5 % بعد 72 ساعة من المعاملة اذا تراوحت نسب القتل بين 60-100 % وان نسب القتل تتناسب بشكل طردي مع زيادة التركيز ومرور الزمن وبفارق معنوي . كما بينت النتائج ان معاملة البيئة الغذائية كانت الاكثر فاعلية في التأثير على يرقات الذباب محققة نسب قتل وصلت الى 100 % وبفارق معنوي عن طريقتي الرش المباشر والغمر. تكشف هذه النتائج عن الفعالية المعنوية لخام مركبات الايض الثانوي للفطر M.aneopalae ضد يرقات *Musca domestic*a والذي يمكن استخدامه كبديل صديق للبيئة لمكافحة الحشرات.

الكلمات المفتاحية: رتبة ثنائية الاجنحة، المركبات الايضية الثانوية الفطرية، مستخلص فطري، مكافحة الحشرات، الذباب المنزلي *Musca domestica*