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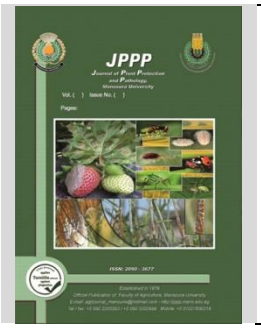
Efficacy of New Isolates of Entomopathogenic Fungus, *Metarhizium anisopliae* (Metsch.), from Sinai Peninsula against Yellow Mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) under Laboratory Conditions.

Altahawi, A. G.[1*]; A. A. Ibrahim[1]; F. M. Eid [1]; S. A. Ahmed[2] and H. M. Mahfouz[2]

[1] Plant Prot. Res. Inst., Agric. Res. Center, Dokki, Giza, Egypt.
[2] Fac. of Agric. Environmental Sci., Arish University.



ABSTRACT

Entomopathogenic fungi were isolated from soil samples collected from North and South Sinai governorates, by the use of Galleria bait method (GBT). The isolates were identified using the conventional methods. The virulence of nine entomopathogenic fungi *Metarhizium anisopliae* isolates (M1, M2, M3, M4, M5, M6, M7, M8 and M9) were tested against *Tenebrio molitre* L. Results proved that (M1) was more effective against larvae compared with all other isolates. the highest concentration of 1×10^8 spores/g revealed 71,56,47,31,37,52,41,46 and 64% mortality percentage for M1, M2, M3, M4, M5, M6 M7, M8, and M9, respectively at tenth day after treatment.

Keywords: Entomopathogenic Fungus, *Metarhizium anisopliae*, Yellow Mealworm, *Tenebrio molitor*, Sinai Peninsula.

INTRODUCTION

Biological control agents such as entomopathogenic fungi can be used as a component of integrated pest management. Entomopathogenic fungi can be isolated from the soil (Korosi *et al*., 2019). Under natural conditions, fungal pathogens are frequent and often cause natural mortalities to the insect populations. Many fungal species such *as Metarhizium anisopliae, Lecanicillium lecanii, Isaria fumosoroseus and Beauveria bassiana* are used as biocontrol agents for controlling various insect pests including termites, black vine weevil, whiteflies, aphids, corn borers, colons and other insects (Ravensberg, 2011).

MATERIALS AND METHODS

Soil samples were randomly collected from nature and cultivated locations. From 0 to 10 cm depth, samples were collected by the use of sterile auger in clean sterile bags and brought back to the laboratory. Each sample were mixed to make homogeneity, coarse debris were removed (Ali-Shtayeh *et al*., 2002). Sample drying or exposure to high temperatures during the mixing process was avoided. *Galleria mellonella* L., greater wax moth was reared in Bio-Insecticides Production Unit, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. Ten larvae of *G. mellonella* were placed into 250 ml plastic container filled with soil. The containers were shaken and incubated at (27±1°C). The larvae were examined daily until 14 days. The surface of dead larvae was sterilized by alcohol (70%), then rinsed with sterile distilled water and the larvae were placed in Petri dishes on moistened filter paper to germination of fungal spores on the cuticle of the insect. The Petri dishes were covered with parafilm to maintain suitable relative humidity and incubated in darkness at 70 ± 5 % relative humidity (R.H.) and 27 ±1°C. Larvae were observed until mycelium appeared. Infected larvae placed into plates of Czapeck's Dox's agar medium. When the isolates showed the macroscopic and microscopic morphological characteristics, the isolates were sent for identification at Fungi Identification Unit, Plant Pathology Research Institute. The obtained isolates were preserved by freeze drying at Bio-insecticides Production Unit then kept under -80 till it needed.

Bioassay by conidiospores:

Entomopathogenic fungi conidiospores (aerobic spores produced asexually by a fungus) were harvested by scrapping it from surface of agar plates. Heamocytometer (Neubauer improved HBG, Germany) was used to count the spores (Lozano-Tovar *et al*., 2013). Four concentrations of each isolate were prepared: 1×105, 1×106, 1×107, and 1×108 spores/g, also four replicates of each concentration were prepared. Twenty five larvae were placed in 250 ml plastic container and smeared with thin layer of the very concentration. Mortality was assessed daily. The mortality percentage corrected by Abbott's formula (1925). LC50, LC90 and slope values were calculated according to (Finney, 1971), using "Ldp line" software by Bakr (2000).

Rearing Of The yellow wheat mealworm *Tenebrio molitor* L.:

Mealworms were reared in laboratory at room temperature on sterilized bran , crushed maize and sliced potato as food and source of water to nourish larvae, in plastic containers 15 x 50 cm according to (Ahmed *et al* 2001).

RESULTS AND DISCUSSION

The study was aimed to be an attempt to found new natural micro-organisms as new trends of pesticides, these compounds also have advantage of biodegradation, economic affordability, environmental safety and easy handling also it can be termed green pesticides which less risk to human, non-target organisms and environment than traditional pesticides.

About 370 Soil samples were collected from the nature and cultivated fields from North Sinai and South Sinai governorates. Galleria bait method (GBM) (Zimmermann

* **Corresponding author.**
E-mail address: amado.goda@gmail.com
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1986) was used to reveal the presence of entomopathogenic agents, soil samples collected from nature (185 soil sample) did not revealed any pathogenic isolates. On the other hand, data given in Table (1) showed that, occurrence of Metarhizium anisopliae isolates in locations of North and South Sinai governorates, from cultivated soils. The pathogens were found in 9 soil samples out of 185 soil sample representing 16.65%.

The study revealed the presence of nine isolates of entomopathogenic fungus Metarhizium anisopliae; these nine isolates were coded as (M1, M2, M3, M4, M5, M6, M7, M8 and M9).

The present results have revealed that entomopathogenic fungus Metarhizium anisopliae is inhabitant commonly in the soil. The obtained results are in agreement with the previous studies of (Nada, 1999; Ali-Shtayeh *et al*., 2002; Keller *et al*., 2003; Sayed and Abolmaaty 2013; Sahar and Moharram, 2014; Hussein; 2015, and Cabrera-Mora *et al*., 2019).

Table 1. Soil sampling and number of *Metarhizium anisopliae* isolates in from North Sinai and South Sinai.

Regions	North Sinai		Regions	South Sinai	
	Soil Samples	No. of Isolates		Soil Samples	No. of Isolates
Al Arish	25	1	Ras Sedr	25	1
Al Tolol	10	0	Tor	10	1
Bear Al-Abd	25	2	Alwadi	15	1
Al Sadat	10	0	Abo Swera	15	0
Al Najah	10	0	Wadi Feran	10	1
Romanah	15	1			
Gelbanah	15	1			

Bioassay of entomopathogenic fungi *Metarhizium anisopliae* isolates:

The present work aims to study the efficiency of nine isolates (M1, M2, M3, M4, M5, M6, M7, M8 and M9) of the entomopathogenic fungi M. anisopliae, were isolated from North and South Sinai governorates.

The entomopathogenic fungus, Metarhizium anisopliae, was formulated as powder and assessed through applying different conidiospore concentrations of local isolates against third larval instars 3rd yellow mealworm Tenebrio molitor L. (Coleoptera: Tenebrionidae) under laboratory conditions.

Virulence of entomopathogenic isolates against *T.molitre*:

The susceptibility of T. molitre larvae to entomopathogenic fungi M. anisopliae isolates were tested. Percentage mortality values after exposing larvae to series of concentrations of 1×105, 1×106, 1×107, and 1×108 spores/g were shown for ten days after treatment in Table (2). The Lowest concentration of 1×105 spores/g revealed 40, 24, 17, 15, 19, 21, 15, 23 and 22% for M1, M2, M3, M4, M5, M6 M7, M8, and M9, respectively at ten days after treatment. While, the highest concentration of 1×108 spores/g revealed 71, 56, 47, 31, 37, 52, 41, 46 and 64 % for M1, M2, M3, M4, M5, M6 M7, M8, and M9, respectively, when mortality percentage of the perished larvae was assessed after the same successive days, respectively. The mortality percentage gradually increased along with spores concentrations and time Fig (1), (2) and (3).

Table 2. Mortality % of *Tenebrio molitor* L. treated with different concentrations of *Metarhizium anisopliae* Conidiospores.

Line Name	Concentration/g	Mortality % in 10 days post-treatment									
		1	2	3	4	5	6	7	8	9	10
M1	$1x10^8$	0	0	0	42	49	55	61	64	70	71
	$1x10^7$	0	0	0	31	34	37	45	47	54	58
	$1x10^6$	0	0	0	29	33	36	39	43	47	49
	$1x10^5$	0	0	0	19	23	28	31	35	38	40
M2	$1x10^8$	0	0	0	30	33	37	43	47	52	56
	$1x10^7$	0	0	0	18	23	27	33	37	43	46
	$1x10^6$	0	0	0	16	18	22	26	30	33	36
	$1x10^5$	0	0	0	6	9	14	16	20	22	24
M3	$1x10^8$	0	0	0	20	24	29	34	38	43	47
	$1x10^7$	0	0	0	6	12	18	20	22	29	34
	$1x10^6$	0	0	0	3	11	14	17	19	22	26
	$1x10^5$	0	0	0	1	4	9	12	14	17	17
M4	$1x10^8$	0	0	0	9	13	18	22	24	27	31
	$1x10^7$	0	0	0	2	9	13	15	18	20	25
	$1x10^6$	0	0	0	0	4	8	13	15	17	20
	$1x10^5$	0	0	0	0	4	4	9	12	15	15
M5	$1x10^8$	0	0	0	13	17	21	25	29	34	37
	$1x10^7$	0	0	0	9	14	16	20	23	26	31
	$1x10^6$	0	0	0	4	9	13	15	18	21	26
	$1x10^5$	0	0	0	1	6	9	12	15	17	19
M6	$1x10^8$	0	0	0	28	34	40	43	47	50	52
	$1x10^7$	0	0	0	20	25	30	33	37	41	46
	$1x10^6$	0	0	0	16	18	20	24	25	28	30
	$1x10^5$	0	0	0	7	9	12	16	18	20	21
M7	$1x10^8$	0	0	0	15	18	22	25	29	35	41
	$1x10^7$	0	0	0	14	16	19	21	23	27	28
	$1x10^6$	0	0	0	5	7	12	14	16	18	20
	$1x10^5$	0	0	0	2	6	9	11	13	15	15
M8	$1x10^8$	0	0	0	27	28	28	31	35	39	46
	$1x10^7$	0	0	0	15	18	22	26	30	34	38
	$1x10^6$	0	0	0	11	13	18	21	22	26	29
	$1x10^5$	0	0	0	9	11	13	17	19	21	23
M9	$1x10^8$	0	0	0	33	38	44	48	53	59	64
	$1x10^7$	0	0	0	24	27	30	35	37	41	45
	$1x10^6$	0	0	0	14	18	20	24	25	28	31
	$1x10^5$	0	0	0	8	10	14	16	18	20	22

Control treatment have no dead individuals during experiment.

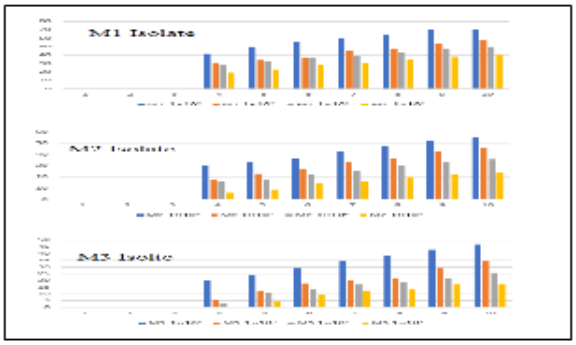

Fig. 1. Mortality % of *Tenebrio molitor* L. treated with different concentrations of *Metarhizium anisopliae* (M1, M2 and M3) isolates.

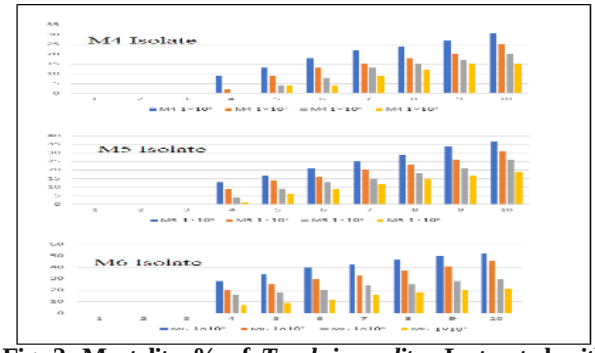

Fig. 2. Mortality % of *Tenebrio molitor* L. treated with different concentrations of *Metarhizium anisopliae* (M4, M5 and M6) isolates

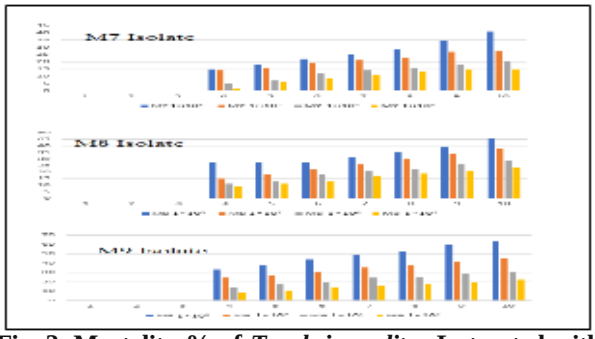

Fig. 3. Mortality % of *Tenebrio molitor* L. treated with different concentrations of *Metarhizium anisopliae* (M7, M8 and M9) isolates

Fig (4) image A illustrated petrified Larvae of *Tenebrio moliter* infected by *Metarhizium anisopliae* .Image B show sporulation of *Metarhizium anisopliae* on *Tenebrio moliter* cadaver after incubation in humid container.

As shown in Table (3) & Fig(5) mortality means were 10.3, 7.45, 5.875, 3.6, 4.4, 7.35, 4.625, 5.85 and 8.475 for M1, M2, M3, M4, M5, M6 M7, M8, and M9 , respectively.

Results in Table (3) Fig(6 & 7) showed The LC_{50} value of M1 was $2.63x10^{9}$ spores/g (slope 0.181) ,while it revealed greater LC_{50} for M2, M3, M4, M5, M6, M7 M8 and M9 were ($2.3\ x10^{10}$, $1.65x10^{11}$, $2.54\ x10^{14}$, $5.86x10^{14}$, $1.29x10^{10}$, $2.61x10^{12}$, $2.95\ x10^{12}$ and $3.14\ x10^{9}$) spores/g, respectively.

Fig. 4. Image A show petrified Larvae of *Tenebrio moliter* infected by *Metarhizium anisopliae*. Image B show sporulation of *Metarhizium anisopliae* on *Tenebrio moliter* cadaver after incubation in humid container.

Table 3. Toxicity of the tested *Metarhizium anisopliae* isolates against larvae of *Tenebrio molitor* L. calculated on tenth day post-treatment.

Line Name	LC_{50}(spores/g) Lower limit-Upper limit	Index	Slope	LC_{90}(spores/g) Lower limit-Upper limit	Mean±SE
M 1	$2.63x10^{9}$ $7.9x10^{8} – 1.65x10^{10}$	100	0.181	$3.15x10^{16}$ $6.34x10^{14} – 1.61x10^{17}$	10.3±2.35a
M 2	$2.3\ x10^{10}$ $5.7x10^{9}$ - $1.68x10^{11}$	11.438	0.22	$1.51x10^{16}$ $4.88x10^{14} – 2.19x10^{18}$	7.45±0.85bc
M 3	$1.65x10^{11}$ $3x10^{10}$ - $1.98x10^{12}$	1.597	0.236	$4.38x10^{16}$ $1.11x10^{15} – 9.73x10^{18}$	5.875±0.70cd
M 4	$2.54\ x10^{14}$ $4.3x10^{12}$ - $4.17x10^{17}$	0.001	0.168	$1.06x10^{22}$ $3.47x10^{18} – 2.46x10^{28}$	3.6±0.48d
M 5	$5.86x10^{14}$ $5.7x10^{12}$ - $4.72x10^{18}$	0.0004	0.139	$9.86\ x10^{23}$ $5.53x10^{19} – 2x10^{32}$	4.4±0.54d
M 6	$1.29x10^{10}$ $3.8x10^{9}$ - $6.9x10^{10}$	20.441	0.242	$2.58x10^{15}$ $1.36x10^{14} – 1.61x10^{17}$	7.35±0.86cb
M 7	$2.61x10^{12}$ $2.1x10^{11} – 1.41x10^{14}$	0.101	0.2	$6.69x10^{18}$ $3.50x10^{16} – 2.94x10^{22}$	4.625±0.59d
M 8	$2.95\ x10^{12}$ $1.8\ x10^{11} – 3.18\ x10^{14}$	0.089	0.164	$1.91x10^{20}$ $3.07x10^{17} – 9.87x10^{24}$	5.85±0.67cd
M 9	$3.14\ x10^{9}$ $1.3\ x10^{9} – 1.02\ x10^{10}$	83.969	0.278	$1.28x10^{14}$ $1.4x10^{14} – 2.48x10^{15}$	8.475±0.99ba

a, b, c Means within the same row having different superscripts significantly different at level (P ≤ .05)
Index compared with M1

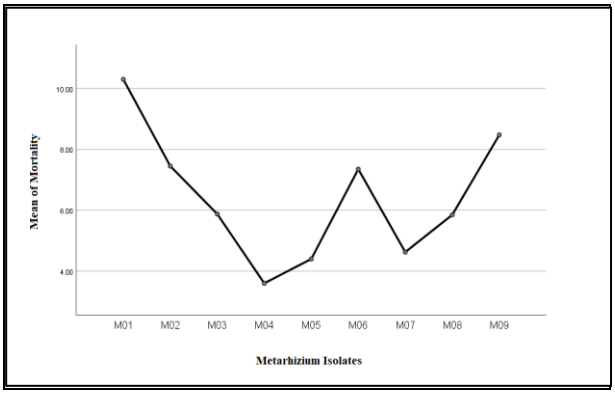

Fig. 5. Mean of mortality of *Tenebrio molitor* L. treated with different concentrations of *Metarhizium anisopliae* conidiospores.

The obtained results showed that isolate M1 caused highest mortality in shortest time, LT_{50} value was 6.28 days. While, for the other isolates M2, M3, M4, M5, M6, M7 M8 and M9 LT_{50} values were 8.26, 10.02, 15.77, 12.93, 8.41, 12.36, 10.41, 7.4days, respectively as shown in Table (4) & Fig(8). Results as shown in Table (3 & 4), proved that M1 was the most effective isolate against *Tenebrio molitor* L. One way ANOVA statistical analysis revealed that there was significant effect ($F_{8,351}$=7.42, $P \leq .05$) between the nine isolates M1, M2, M3, M4, M5, M6 M7, M8, and M9 on larvae of *Tenebrio molitor* L .

The obtained results came in harmony with Oreste *et al.*(2012) who tested the pathogenicity of four *M. anisopliae* isolates against *T. molitor* larvae in laboratory assays presented Mortality were 100, 89, 82 and %99, respectively, and LT_{50} were 4.1, 6.6, 7.4, 11.5 and 11.5 days respectively.

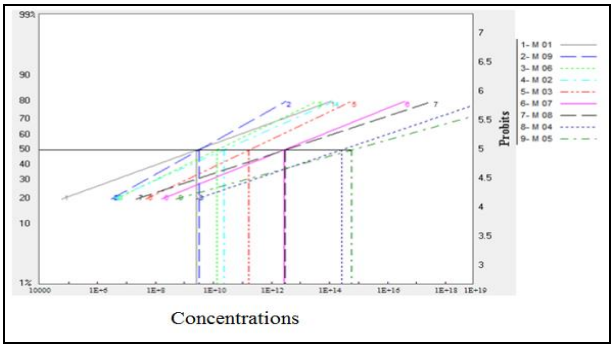

Fig. 6. LC50 regression lines of entomopathogenic fungus isolates (for M1, M2, M3, M4, M5, M6 M7, M8 and M9) against larvae of Tenebrio molitor L.

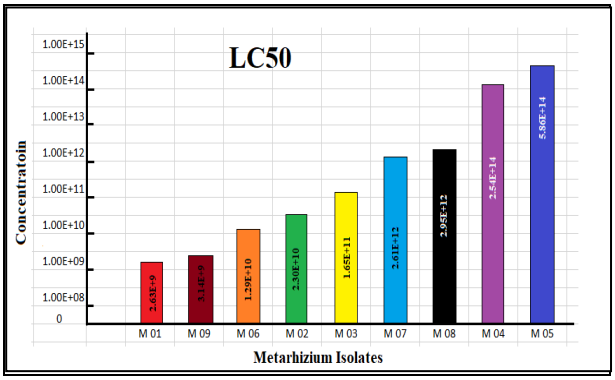

Fig. 7. Comparative LC_{50} of (M1, M2, M3, M4, M5, M6 M7, M8 and M9 against larvae of *Tenebrio molitor* L.

Table 4. LT50 of entomopathogenic fungus isolates (for M1, M2, M3, M4, M5, M6 M7, M8, and M9), against larvae of *Tenebrio molitor* L.

Line Name	LT_{50}(Days) Lower limit-Upper limit	Index	Slope	LT_{90} (Days)
M 01	6.28 5.1 – 9.01	100	4.17	12.75
M 02	8.26 7.08 – 11.66	76.04	3.38	19.78
M 03	10.02 8.76 - 14.33	62.69	3.06	26.34
M 04	15.77 13.52 - 25.33	39.85	2.46	52.36
M 05	12.93 11.29 – 19.41	48.61	2.70	38.61
M 06	8.41 7.22 - 11.93	74.75	3.32	20.48
M 07	12.36 10.82 – 18.48	50.86	2.75	36.1
M 08	10.41 9.13 – 16.12	60.35	2.88	29.1
M 09	7.4 6.24 – 10.3	84.95	3.68	16.5

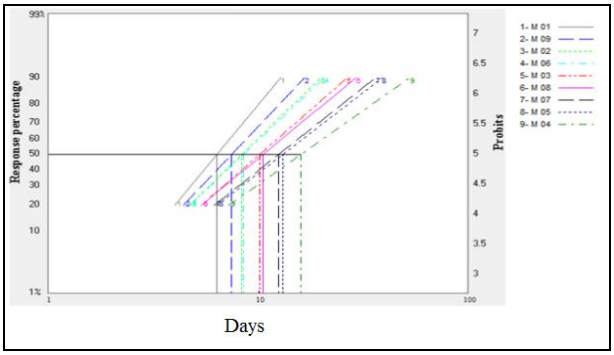

Fig. 8. LT50 regression lines of (M1, M2, M3, M4, M5, M6 M7, M8 and M9), 1× 108 spores/g concentration against larvae of Tenebrio molitor L.

Also results were agree with Pajar (2013) where Four *M. anisopliae* were used against *T. molitor* , larval mortality ranged between 81.25% and 100% in 7 days . As well , Results were in harmony with Mora *et al.* (2016) who evaluated the pathogenicity of four *M. anisopliae* strains against *Tenebrio molitor*, with three concentrations (1×10^7 , 1×10^8 and 1×10^9 conidia.ml^{-1}). The Ma58MI *M. anisopliae* strain presented 82% mortality in *T. molitor*, LC_{50} = 1.00×10^6 conidia.ml^{-1} and LT_{50} = 4.05 days, calculated on eighth day post-treatment.

Also, results came in same trend with Karaborklu *et al.* (2019) who tested virulence of Forty-five *M. anisopliae* isolates against *Tenebrio molitor* larvae. Insecticidal activity fluctuated between 20% and 100%. Also, results were in line with Also, Waweru (2019) conducted bioassay of *M. anisopliae* using $1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ conidia/ml against *T. molitor*, under laboratory conditions. The LC_{50} was 9.4 x 10^4 conidia/ml calculated after eight days post-treatment. Lethal time for $1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ conidia/ml was 10.5, 8.1, 6 and 4.8 days, respectively. On the other hand, result were not in agreement with Michalaki *et al.* (2006) studied the virulence of *M.*

anisopliae against larvae of The confused flour beetle *Tenebrio confusum* was moderate, with mortality levels in all cases lower than 55% after 7 days of exposure even at 8 x 10^{10} conidia kg^{-1} wheat or flour .

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فاعلية عزلات جديدة لفطر *Metarhizium anisopliae* الممرض للحشرات معزولة من شبه جزيرة سيناء ضد دودة جريش الذرة تحت الظروف المعملية

أحمد جوده الطحاوي [١]، أحمد عدلي محمد [١]، فوزي محمد حسن [١]، سيد علي أحمد [٢] و حاتم محمد محفوظ [٢]

[١]معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - الجيزة

[٢] كلية العلوم الزراعية البيئية - جامعة العريش - شمال سيناء

فى هذه الدراسة جمعت عينات التربة من مختلف المناطق الزراعية والطبيعية في شمال وجنوب سيناء واستخدمت طريقة مصيدة دودة الشمع الموصي بها في عزل الفطريات الممرضة للحشرات.وقد أظهرت النتائج وجود تسع عزلات لفطر *Metarhizium anisopliae* الممرض للحشرات و تم تكويدها بالرموز(M1 و M2 و M3 و M4 و M5 و M6 و M7و M8 و M9) . وتم دراسة قدرة عزلات الفطر *Metarhizium anisopliae* علي امراض الحشرات . تم عمل عدوي لحشرة دودة جريش الذرة علي صورة بودر بأربعة تركيزات 5×10^{1}و 6×10^{1} و 7×10^{1} و 8×10^{1} جراثيم/جرام ووجد ان جميع عزلات الفطرممرضة للحشرة و كانت اعلي نسبة موت بعد اليوم العاشر للمعاملة عند اعلي تركيز 8×10^{1} هي 71٪ للعزلة M1 وأقل نسبة موت 31٪ للعزلة M4 .وقد كانت قيمة LC_{50} للعزلة M1 $2.63x10^{9}$ جرثومة/جرام ، بينما كانت قيم LC_{50} أكبر لباقي العزلات .كما أظهرت النتائج ان العزلة M1 سببت أعلي نسبة موت في أقصر وقت ، حيث بلغت قيمة LT_{50} 6.28 يوم بينما كانت قيم LT_{50} أكبر لباقي العزلات . وأوضحت الدراسة انه هناك فروق معنوية بين العزلات ضد دودة جريش الذرة.