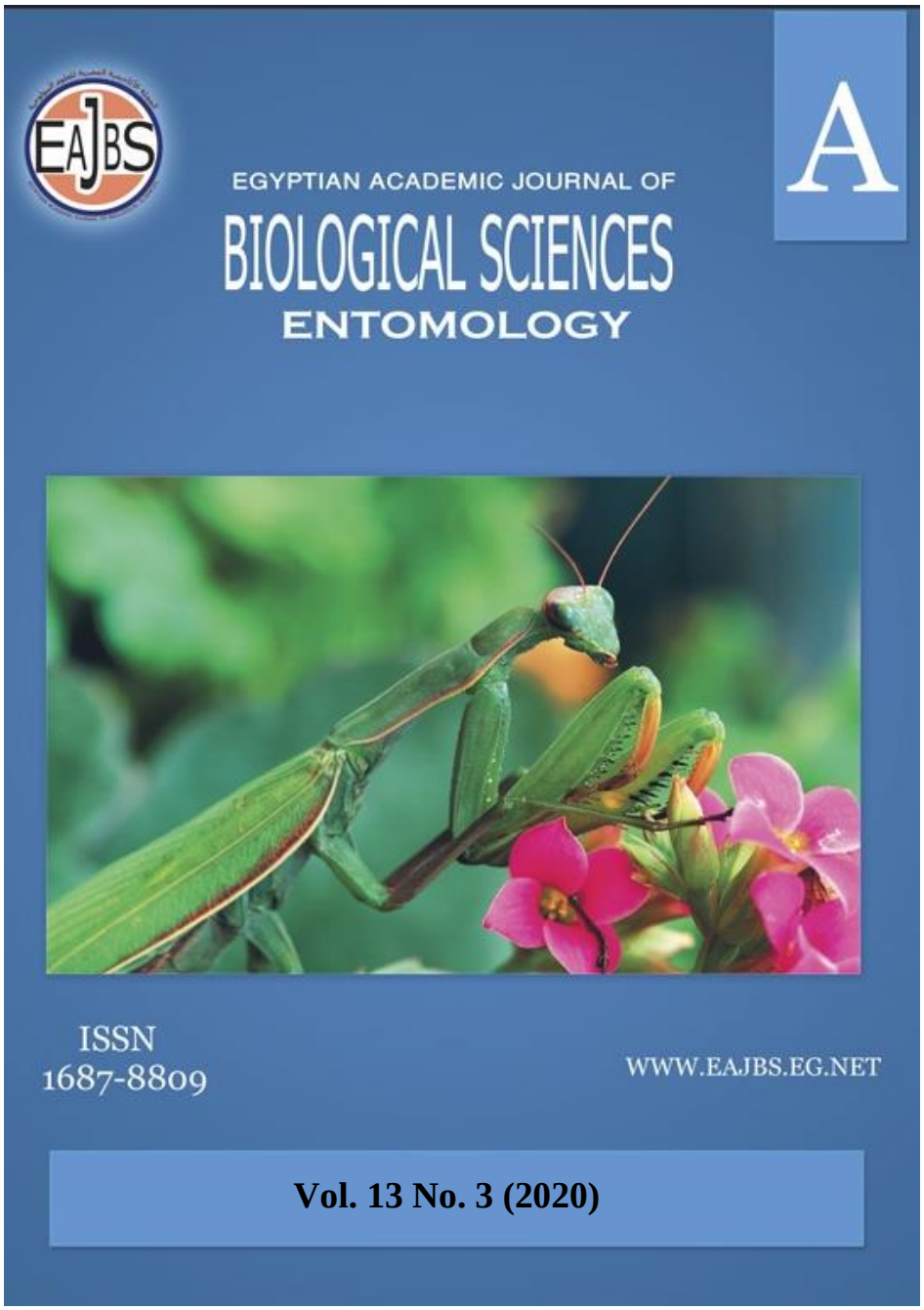

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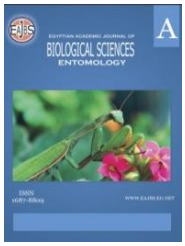

Isolation, Molecular Identification and Host Range Evaluation of *Metarhizium anisopliae* Egyptian Isolate

Yasmein E. Ahmed[1], Suzan A. Ibrahim[1], and Manal F. Abdelall[2]*

1- Cotton Leaf Worm Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), 7 Nady El-Sayied Street, Dokki, Giza, Egypt.
2- Microbial Molecular Biology Department, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), 9 Gamaa Street, 12619, Giza, Egypt.
Email: dryasmeinsayed@gmail.com - drsuzanelrefaey@yahoo.com- manalfm@gmail.com

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ABSTRACT

Metarhizium anisopliae is the most popular entomopathogenic fungi used successfully in microbial control. Isolation, identification, and estimation of *M. anisopliae* Egyptian isolate host range has been done in this study. Our isolate was identified molecularly by amplification of Internal transcribed spacer (ITS) region using polymerase chain reaction technique (PCR). The sequence of ITS was compared with other *M. anisopliae* isolates published in gene bank and our isolate showed 98% homology with most published *M. anisopliae* isolates so submitted in gene bank database as a new Egyptian isolate with accession no MT102079.The pathogenicity was evaluated by applying five spore suspension concentrations against four insect pests, *Spodoptera littoralis* (2nd and 4th instar larvae), *Galleria mellonella* (3rd and 5th instar larvae), *Tetranychus urticae* (nymph) and *Rhynchophorus ferrugineus* (3rd and 5th instar larvae), and our results revealed that *M. anisopliae* was highly effective against all tested insects. The highest concentration used (10^9) caused a high mortality rate after 10 days post-treatment reached 96% for *T. urticae* nymph which appears to be highly sensitive insect tested in this study followed by *S, littoralis* 2nd and 4th instar larvae which showed 96 and 90% mortality rate respectively. High pathogenicity of the tested isolate also noticed in *G. mellonella* in which the highest concentration used (10^9) induced 93 and 86% for 3rd and 5th instar larvae respectively, while the same instars of *R. ferrugineus* were moderately affected and 73 and 66% mortality rate was achieved by the same concentration after 10 days post-treatment.

INTRODUCTION

Fungi are known as the most common parasite of insects, and thus important insect population regulators. In nature, there are over 1,000 species of the phyla Entomophthoromycota, Blastocladiomycota, Microsporidia, Basidiomycota, and Ascomycota, which are believed to invade and destroy insects (Vega *et al*., 2012). The phylum Entomophthoromycota is freshly developed from the previous phylum Zygomycota and contains mostly insect pathogenic species (Boomsma *et al*., 2014). Entomopathogenic

fungal experience can potentiate cost-effectiveness mycoinsecticides applications for in-field pest control. *Metarhizium anisopliae* is a suspected one of representative fungus organisms that have been identified as successful eco-friendly mycoinsecticides. *M. anisopliae* can grow naturally in soils all over the world and causes disease in different insects by grows on cuticles of the insect host and acting as a parasitoid. A subtilisin-like serine protease that initiates protein degradation appears to be the main enzyme involved in this growth. A subtilisin-like serine protease is the main protein that was secreted during invasion into the host cuticle (Illana *et al*., 2013). This protease activity is then accompanied by the action of exopeptidases including carboxypeptidases, which activate the host's individual amino acids for nutritional use. While fungi usually contain serine carboxypeptidase activity, *M. anisopliae* has discovered a novel carboxypeptidase by Stleger *et al.* (1994).

Insect pests are the main competitors to humans for agriculture resources (Oerke and Dehne, 2004), they are responsible for 50% of all losses in crop production (Metcalf, 1996). The examples of the most destructive insects are *Spodoptera littoralis, Galleria mellonella*, *Tetranychus urticae*, and *Rhynchophorus ferrugineus. S. littoralis* is the Egyptian cotton leafworm and the major cotton insect pest in the Middle East, especially in Egypt. It is a polyphagous therefore they feed on a wide range of economically important crops. It causes a huge loss in yield by reducing the nutrients and making plants susceptible to bacterial and fungal attacks. Because of the excessive use of chemicals, both pests developed resistance to almost all known classes of insecticides (EPPO, 1997 and Brambila *et al*, 2010).

Although the small size of *T. urticae*, it is capable of causing very rapid and severe damage, due to their great reproductive capability. Severe infestation of *T. urticae* damage plant leaves and both quality and yield of the crops decline (Hussey & Parr, 1963). *R. ferrugineus* is the most destructive insect pest of palms all over the world. *R. ferrugineus* larvae start their feeding within the palms apical growing point creating great tunnels lead to extensive injury to palm tissues and general weakness of the palm trunk structure. The area with establishing *R. ferrugineus* reduces vitality in infested date palms resulting in tree death and commercial date production is affected (Wattanapongsiri, 1966). Also, *G. mellonella* is a pest of honeybee products, its larva has destructive feeding habits in which they feed on pollen, honey, wax, honeybee skin. Damage occurs as the larvae create tunnels through the hexagonal cell walls and over the comb surface and these tunnels make holes through which honey leaks. A great infestation of honey bee colonies by *G. mellonella* larvae leads to colony loss, absconding, and reduction in the number of the migratory bee swarms. *G. mellonella* adults and larvae also are potential vectors of different pathogens (Sehnal *et al*., 1986).

The chemical insecticides are commonly used to protect the agriculture products from insect pests by make suppression of their populations, but the extensive use of these insecticides cause serious problems (Golshan *et al*., 2013) include, environmental pollution and negative effects to human health and beneficial organisms plus insect's resistance which developed to these insecticides (Ffrench-Constant *et al*., 2007). As a result of these problems, there is an urgent requirement to develop an alternative form of chemical insecticides (Wilson and Tisdall, 2001). The most effective alternative form is microbial bio-control agents (Nicholson, 2007). The microbial biocontrol agents (MBCAs) refer to the use of pathogenic microorganisms like viruses, bacteria, fungi, and nematodes as regulators to insect pest populations. MBCAs have a complicated mode of action that makes it difficult for pests to develop resistance against them, plus they have no hazard effects on human health and environment, so they applied throughout the world with great success and advantages. Entomopathogenic fungi are promising agents and the most important among all MBCAs because they are numerous pathogenic strains with broad host range, comparatively

inexpensive, convenient to use, safe to apply, and safe to human, beneficial organisms and environment (Hussain *et al*. 2014).

MATERIALS AND METHODS

The Microorganism:

M. anisopliae was isolated from dead adult red palm weevil showing signs and symptoms of fungal infection (sluggish and inactive adults, firm and fanatic cadavers, and sometimes fungal mycelium growth enveloping the body or emerging from body segments) collected from infected date palms from El-kassasin district, Ismailia governorate, Egypt.

The red weevil cadavers were surface sterilized by dipping for one minute in 70% ethanol, then rinsing twice by distilled serialized water, and put individually on petri dishes contain Czapek Dox media supplemented with streptomycin (0.04g/L) to allow the growth of their content of fungi. The petri dishes were incubated for 72 hours at 27^{o}C. After the incubation period, the fungal growth will appear on the media plates and small part of the fungal mycelium was taken by sterilized inoculation loop and transferred to newly prepared Czapek Dox media contain the same concentration of streptomycin to prevent the bacterial growth, and the inoculation cycles were repeated several times till clear and visible growth of fungi appeared.

Identification of Fungal Isolate: -

I-Light microscope identification:

Firstly, fungal culture slide was prepared by taking a small part of fungal mycelium from the edge of the colony, spread by a sterilized loop on glass slide contain one drop of sterilized water and covered with coverslip then visualized under a light microscope. The diameter of the hyphae, conidiophore branching, arrangement, and shape of conidia was observed to make preliminary identification.

II-Molecular Identification:

Genomic DNA from a fresh culture of fungal mycelium was extracted as described by Sambrook *et al*. (1989). The mycelium sample was frozen in liquid nitrogen and crushed thoroughly using a sterile mortar and pestle. Then 500 µl CTAB buffer {100mM Tris-HCl, pH 8.0, 1.4M NaCl, 20mM EDTA, 2% w/v hexadecyl-trimethyl-ammonium bromide (CTAB)} and 5 µl of 2-mercaptoethanol (0.1 %) were added and mixed thoroughly. The homogenate sample was incubated at 60°C for 30 minutes. Homogenate sample was centrifuged for 5 minutes at 14,000 xg. The supernatant was transferred to a new tube. An equal volume of chloroform: isoamyl alcohol (24:1) was added. The sample was centrifuged for 1 min at 14,000 xg to separate the phases. The upper aqueous phase was transferred to a new tube. DNA was precipitated by adding 0.7 volume cold isopropanol and was incubated at -20°C for 15 minutes. The sample was centrifuged at 14,000 xg for 10 minutes. The DNA pellet was washed with 500 µl ice-cold 70% ethanol. Dried DNA was dissolved in 50 µl nuclease-free water. DNA quantity and integrity were checked on an agarose gel.

1. **ITS Region Amplification of The Fungal Isolate:**

The fungal isolate was identified using ITS region (ITS1 and 2 and the 5.8S gene) sequencing as molecular tools. Amplification of the ITS region from the extracted DNA of fungal isolate was performed using polymerase chain reaction (PCR), oligonucleotide primers pair, (forward (TW81): GTTTCCGTAGGTGAACCTGC and reverse (AB21): ATATGCTTAAGTTCAGCGGGT) were synthesized by Vivantis Co. (Selangor Darul Ehsan, Malaysia), (Curran *et al*., 1994).

Every reaction will contain 14.5 µL of deionized sterile water, 2.5 µL of buffer 10X, 1 µL of MgCl2 (50 mM), 0.5 µL of dNTP´s (20mM), 2 µL of each primer (10 pM), 0.5 µL of Taq polymerase (5U/mL) and 2 µL of sample DNA. PCR amplification was carried out under the

following conditions: initial denaturation 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 minute, annealing 65 ˚C for 1 minute and elongation at 72 °C for 1 minute and a final extension at 72°C for 7 min. The amplified product was separated by electrophoresis on 1.0 % agarose gel was stain with ethidium bromide (0.5 µg/ml) and was visualized on UV gel documentation system (BioRad, USA). PCR product was purified from unincorporated PCR primers and dNTPs using QIAquick PCR Purification Kit (Qiagen), following the manufacturer`s instructions. The purified PCR product of ITS gene was subjected to sequence analysis using the Big TriDye sequencing kit (ABI Applied Biosystems) by the facility of LGC, Germany. A homology search was performed for DNA sequence for ITS region obtained from the sequencer using the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) database, USA (http://www.ncbi.nlm.nih.gov). The obtained sequences were compared to ITS sequences in the National Center for Biotechnology Information (NCBI) GeneBank database using the BLASTN algorithm (Altschul *et al*., 1990), and closely related sequences were downloaded. The sequence and closely related sequences were manually imported into and aligned in the Molecular Evolutionary Genetics Analysis software, ver. 7.0 (Kumar *et al*., 2016), with the Clustal W tool. DNA sequence obtained in this study was submitted to the NCBI database (accession No. MT102079)

Pathogenicity Tests:

I-Conidial Suspension Preparation: -

The tested isolate was propagated on Czapek Dox agar medium for preparation of the conidial suspension. The inoculated plates were incubated for 15 days at 27ºC and 50–60% RH. After incubation period sterile spatula was used to harvest the conidia from the surface of the fungal culture by scraping them in distilled serialized water contain 0.02% Tween-80. The concentration of the stock solution was estimated by using Neubauer hemocytometer (Alves and Moraes, 1998). Five concentrations were prepared from the stock solution by serial dilution to be used in pathogenicity experiments.

II-Tested insects:

The pathogenicity of *M. anisopliae* isolate was estimated on four insects species which belonging to four family: Noctuidae, Pyralidae, Tetranychidae, and Curculionidae **(Table 1).** *S. littoralis* and *G. mellonella* (Lepidoptera: Noctuidae and Pyralidae) that were reared in Insect Pathogen Unit, Plant Protection Research Institute-Agriculture Research Center. *T. urticae* (Tetranychidae: Trombidiformes) which were collected from cucumber agriculture greenhouse follow Agriculture Research Center. *R. ferrugineus* (Curculionidae: Coleoptera) were collected from infected date palms from Elkassasin district, Ismailia governorate, Egypt.

Table 1: Details of the insects bioassayed with the tested fungal isolate (*M. anisolpae*) to check its host specificity.

Species	Common Name	Order	Family	Stage
Spodoptera littoralis	Cotton Leaf Worm	Lepidoptera	Noctuidae	Larvae
Galleria mellonella	Greater wax moth	Lepidoptera	Pyralidae	Larvae
Tetranychus urticae	Red spider mite	Trombidiformes	Tetranychidae	Nymph
Rhynchophorus ferrugineus	Red Palm Weevil	Coleoptera	Curculionidae	Larvae

III-Efficacy Tests:

For *S. littoralis*, *G. mellonella* and *R. ferrugineus*:

Virulence of the tested fungal isolate was evaluated by testing five concentrations (10^5, 10^6, 10^7, 10^8 and 10^9) adjusted from spore mother suspension against 2nd and 4thinstar larvae of *S. littoralis* and 3rd and 5th instar larvae of *G. mellonella* and *R. ferrugineus*. Thirty

larvae were dipped for 20 seconds in spore suspension, then left to dry on filter paper, and transferred to plastic bottles contain artificial diet developed by Shoery and Hale (1965) for S. *littoralis*, artificial diet developed by Birah *et al* (2008) for *G. mellonella* and sugarcane setts for *R. ferrugineus*, with three replicates for each treatment. Plastic bottle with thirty larvae dipped for 20 seconds in distilled sterilized water contain 0.02% tween 80 also replicated three time serves as control treatment (Baskar *et al*. 2012).

For *T. urticae*:

The bioassay experiment was performed in a rearing unit consisting of a piece of sponge (1 cm height) covered with a moist cotton pad. A sufficient amount of water was added to the dish to moisten the sponge fully. *T. urticae* nymph sited on cucumber leaf disc were placed on the cotton pad and sprayed with each concentration by fine perfume sprayer at a distance of 20 cm from the rearing unite. 30 nymphs were used in one replication and replicated three times, with three replicates for control nymph treated with serialized distilled water contain 0.02% tween 80.

Statistical Analysis:

The mortality rate for each treated pest was recorded continuously for 10 days, Concentrations of the treated fungal isolate and the mortality data were computed to be analyzed and to determine the fifty percent lethal concentration (LC_{50}) by using Ldp line software (Bakr, 2000).

RESULTS AND DISCUSSION

Identification of Fungal Isolate:

Initial morphological identification using light microscope of the fungal isolate showed the cylindrical shape of conidia arranged in parallel chains on branching, densely interwined conidiophores. The isolate was then identified by molecular sequences of the ITS region. To obtain the coding sequence of ITS (Internal Transcribed Spacer 1, 2, and 5.8S subunit), DNA was extracted from purified fungal isolate (Fig. 1). Polymerase chain reaction (PCR) amplification was performed with forward universal primers (TW81) and reverse universal primers (AB21). The amplified bands at ~750 bp as shown in (Fig. 2). The amplified fragment was sequenced and homology searches in the database. The amplified DNA sequence of the ITS region (Internal Transcribed Spacer 1, 2, and 5.8S subunit) of the purified isolate was submitted to NCBI Genbank under the Accession number MT102079.

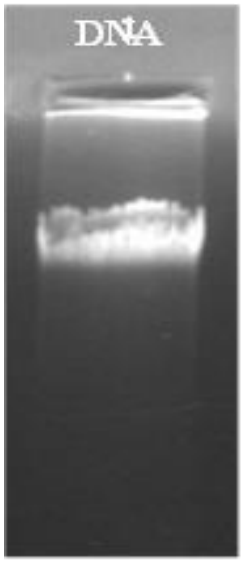

Fig. 1: Agarose gel electrophoresis of genomic DNA extracted from fungal mycelium sample

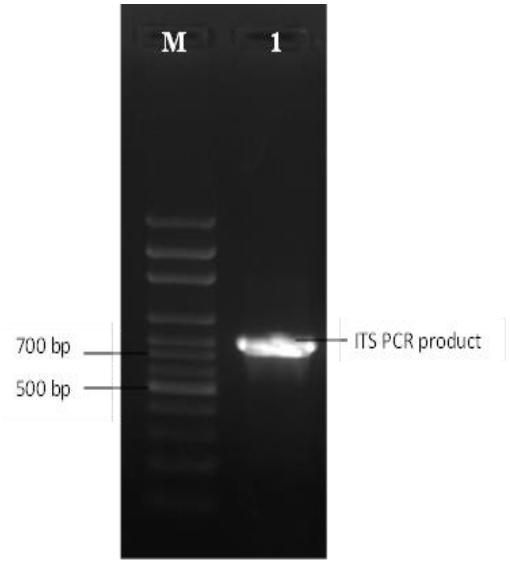

Fig. 2: ITS PCR products of fungal isolates on 1% (w/v) agarose gel.

Phylogenetic Analysis:

The phylogenetic relationship was derived from comparisons of the ITS gene sequences and a dendrogram was constructed with other *Metarhizium* species (Fig. 3).

The result of BLASTn program showed that our *Metarhizium* (MT102079) isolate has 98% homology with most published isolates such as *M. anisopliae* (EU307890), *M. anisopliae* (AB027383), *M. anisopliae* (EU307923). The phylogenatic tree showed that the fungal isolate in this study was closely related to all other database isolates although it was treated as outgroup as showed in (Fig. 3). This finding in the present study was not compatible with Chandra Teja & Rahman (2016) who found that all isolates were closer to each other.

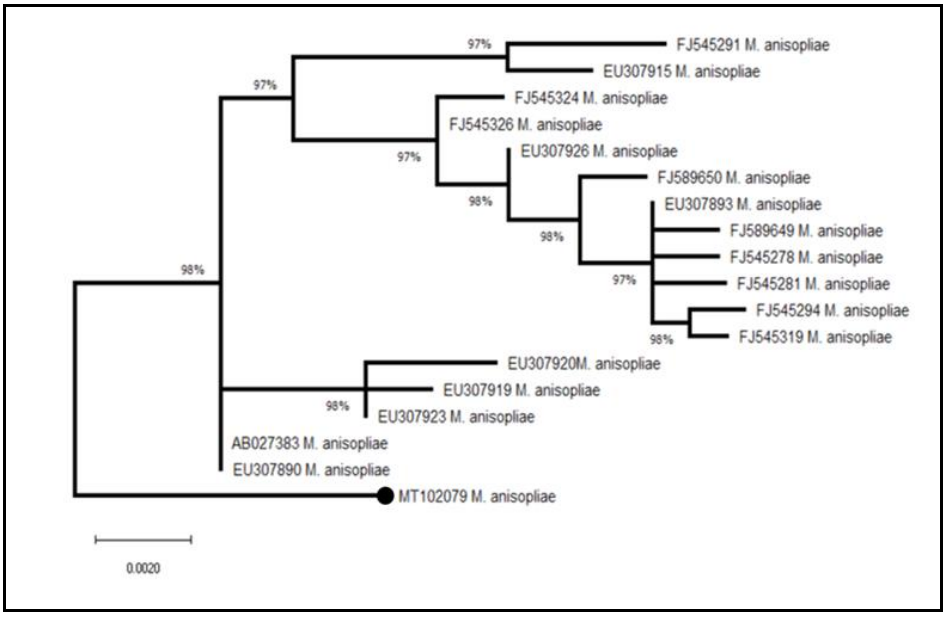

Fig. 3: A phylogenetic tree of *M. anisopliae* isolate (MT102079) of study (shown in black circle) with other species of *Metarhizium* based on the nucleotide of ITS sequences constructed by neighbor-joining method. Evolutionary analyses were conducted in MEGA 7. alignment of the sequences was done with CLUSTALW, bootstrap values (in percent) are calculated from 1000 resamplings. The scale bar shows the genetic distance. The number presented next to each node shows the percentage bootstrap value of 1000 replicates.

Pathogenicity Tests:

The tested fungal isolate was found pathogenic to all tested insect species. The results data showed that the mortality percentage increased by increasing the fungal spore concentrations Shairra (2007) and that the younger larval instar was more susceptible to the tested fungus than the older larval one Purwar and Sachan (2005).

High *pathogenicity* was observed in *T. urticae* nymph (Table 5) in which the mortality percentage ranged from 73 to 96 % for the five tested concentrations after 10 days post-treatment. *S. littoralis* was more susceptible to the tested fungal concentrations following T. urticae with highly sensitive 2nd instar larvae showed mortality percentage ranged from 70 to 96 % and more tolerant 4th instar larvae with mortality percentage ranged from 56 to 90 % for the same concentrations after the same days mentioned above (Table 2). High mortality percentage (93 and 86 %) also recorded for the highest tested concentration 10^9 spore/ml used for 3rd and 5th instar larvae of *G. mellonella* respectively (Table 4). The median impact of the tested isolate was observed in 3rd and 5th instar larvae of *R. ferrugineus* with mortality percentage ranged from 23 to 73 % and 13 to 66 % respectively (Table 3). A linear

relationship between tested fungal concentrations and mortality percentages (Fig. 4, 5, 6, and 7) was made by Ldp line software program for calculation of LC_{50} values after 10 days post-treatment. The lower LC_{50} was 7.7 x 10^2 spore/ml for *T. urticae* followed by 1.6 x 10^3 and 2.8 x 10^4 for 2nd and 4th instar larvae of *S. littoralis*. The calculated LC_{50} for 3rd and 5th instar larvae of *G. mellonella* and *R. ferrugineus* was 5.7 x 10^3 and 4.9 x 10^4, 8.5 x $10^{6,}$ and 5.9 x 10^7 respectively.

Table 2: Efficacy of *M. anisopliae* spore suspension against 2nd and 4th instar larvae of *S. littoralis* after 5, 7- and 10-days post treatment.

Concentration (Spore/ ml)	Mortality percentage (%)					
	2nd instar larvae			4thinstar larvae		
	5 days	7 days	10 days	5 days	7 days	10 days
10^5	20	39	70	13	40	56
10^6	23	50	76	20	50	66
10^7	36	60	83	29	59	73
10^8	46	70	90	39	69	83
10^9	53	78	96	50	77	90
LC_{50}	3.3 x 10^8	9.8 x 10^5	1.6 x 10^3	8.8 x 10^8	9.8 x 10^5	2.8 x 10^4
Lower limit of LC_{50}	4.2 x 10^7	2.5 x 10^4	0.0013	1.1 x 10^8	1.3 x 10^4	0.67 x10^1
Upper limit of LC_{50}	1.1 x 10^{11}	5.8 x 10^6	5.6 x 10^4	2.8 x 10^{11}	6.7 x 10^6	3.5 x 10^5

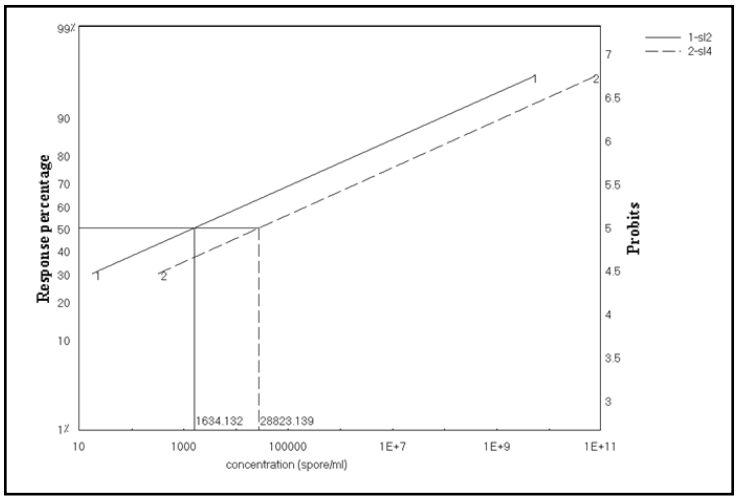

Fig. 4: Linear relationship between Mortality percentage and five concentrations *M. anisopliae* spore suspension treated against 2nd and 4th instar larvae of *S. littoralis* after 10 days post treatment**.**

Table 3: Efficacy of *M. anisopliae* spore suspension against 3rd and 5th instar larvae of *R. ferrugineus* after 5, 7- and 10-days post treatment.

Concentration (Spore/ ml)	Mortality percentage (%)					
	3rd instar larvae			5th instar larvae		
	5 days	7 days	10 days	5 days	7 days	10 days
10^5	6	16	23	3	10	13
10^6	13	25	43	3	20	33
10^7	23	40	56	13	26	40
10^8	30	53	60	20	40	50
10^9	40	63	73	26	50	66
LC_{50}	5.4 x 10^9	7.2 x 10^7	8.5 x 10^6	5 x 10^{10}	8.3 x 10^8	5.9 x 10^7
Lower limit of LC_{50}	4.5 x 10^8	1.6 x 10^7	1.3 x 10^6	2.2 x 10^9	1.2 x 10^8	1.4 x 10^7
Upper limit of LC_{50}	1.3 x 10^{13}	7.7 x 10^8	3.8 x 10^7	1 x 10^{16}	9.4 x 10^{10}	5.3 x 10^8

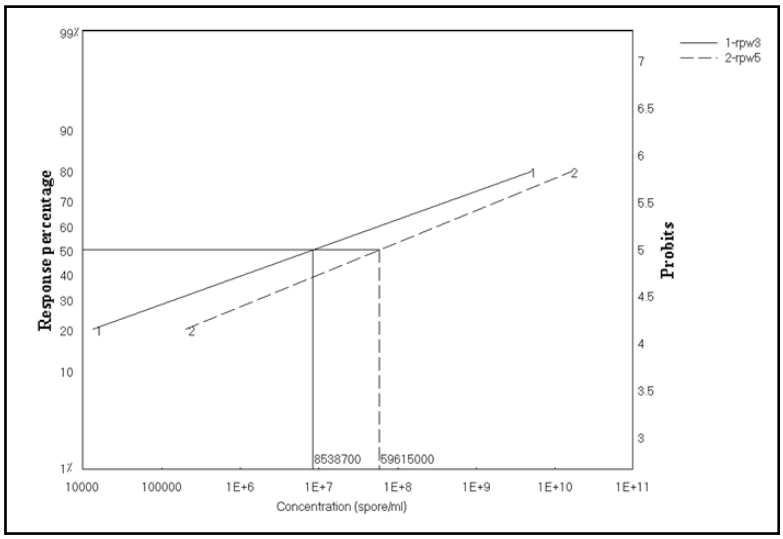

Fig. 5: Linear relationship between Mortality percentage and five concentrations *M. anisopliae* spore suspension treated against 3rd and 5th instar larvae of *R. ferrugineus* after 10 days post treatment.

Table 4: Efficacy of *M. anisopliae* spore suspension against 3rd and 5th instar larvae of *G. mellonella* after 5, 7- and 10-days post treatment.

Concentration (Spore/ ml)	Mortality percentage (%)					
	3rd instar larvae			5thinstar larvae		
	5 days	7 days	10 days	5 days	7 days	10 days
10^5	22	36	63	16	26	50
10^6	30	46	73	23	36	63
10^7	40	56	83	32	45	70
10^8	49	66	86	43	53	76
10^9	60	83	93	53	64	86
LC_{50}	1.0×10^8	1.8×10^6	5.7×10^3	4.5×10^8	3.3×10^7	4.9×10^4
Lower limit of LC_{50}	1.4×10^7	1.5×10^5	0.0556	6.0×10^7	4.0×10^6	6.1089
Upper limit of LC_{50}	7.4×10^9	9.0×10^6	1.1×10^5	1.0×10^{11}	1.2×10^9	7.6×10^5

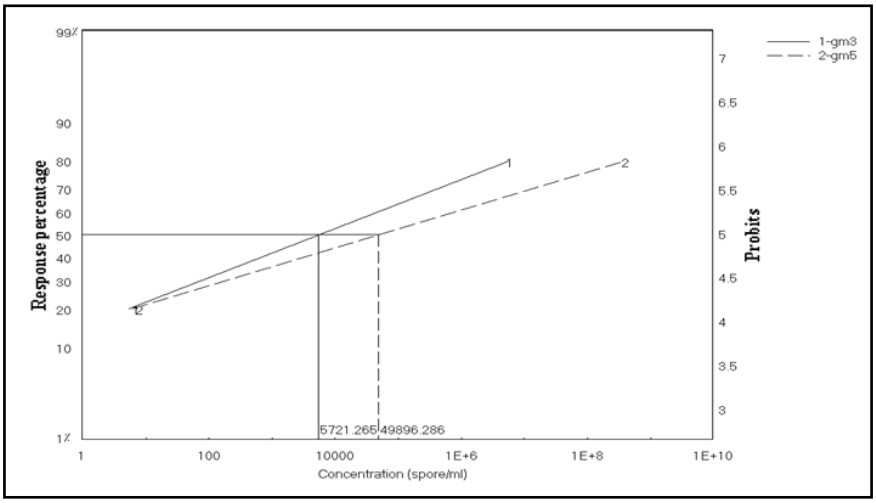

Fig. 6: Linear relationship between Mortality percentage and five concentrations *M. anisopliae* spore suspension treated against 3rd and 5th instar larvae of *G. mellonella* after 10 days post treatment**.**

Table 5: Efficacy of *M. anisopliae* spore suspension against *T. urticae* nymph after 5, 7- and 10-days post treatment.

Concentration (Spore/ ml)	Mortality percentage (%)		
	5 days	7 days	10 days
10^5	30	45	73
10^6	45	60	83
10^7	55	75	90
10^8	64	86	96
10^9	76	96	96
LC_{50}	4.2×10^6	2.3×10^5	7.7×10^2
Lower limit of LC_{50}	1.6×10^5	6.2×10^3	0.0009
Upper limit of LC_{50}	3.8×10^7	1.2×10^6	2.9×10^4

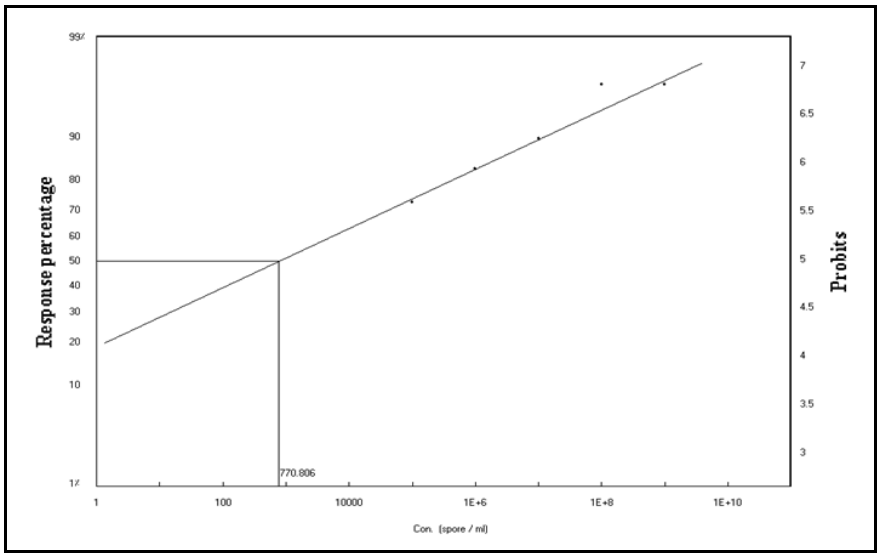

Fig. 7: Linear relationship between Mortality percentage and five concentrations *M. anisopliae* spore suspension treated against *T. urticae* nymph after 10 days post treatment.

Entomopathogenic fungi are considered the most common organism that causing diseases to different insect pests Majeed *et al* (2017) and Mustu *et al*., (2015). They have a characteristic place among insect pathogens (Richard *et al*., 2010) in which they infect insects directly through the exoskeleton, in contrast to other pathogens like viruses and bacteria which penetrate and infect the hosts through midgut after ingestion so there is great interest in the use of entomopathogenic fungi as a potential biological control agent of different insect pests (Hajek & St. Leger, 1994 and Evans, 1999). Some entomopathogenic fungi have a narrow host range while others have wide host range like *M. anisopliae* and *B. bassiana*. Our results proved the pathogenicity of *M. anisopliae* against the four tested insect pest and these agree with Tamai *et al*. (2002) who investigated the effects of 45 isolates of *B. bassiana*, *M. anisopliae*, and *Paecilomyces farinosus* on *T. urticae* and found that *M. anisopliae* was more pathogenic than other fungi used in the study. Bugeme *et al*. (2014) and Ullah and Lim (2015) studied the effect of different concentrations of *M. anisopliae* and *B. bassiana* on *T. urticae* and noticed highest mortality rate, 98% was achieved by *M. anisopliae* isolate so stated that *M. anisopliae* is the highly effective agent that can be used in the control of both *T. urticae* nymphs and adults.

Many authors studied the efficacy of *M. anisopliae* against the same tested insect pests and their findings were in harmony with our results. Souad., *et al* (2014), Godonou., *et al* (2009) and Husinan., *et al* (2014) indicated that the strains of *M. anisopliae* and *B. bassiana* affected the younger instar of *S.littoralis* and confirmed the high pathogenicity of *M. anisopliae* which showed highest mortality percentage than *B. bassiana* against *S. littoralis* larvae. El-Husseini (2019) estimated different *M. anisopliae* concentrations against third and fifth instar larvae of *S. littoralis* and revealed that the highest concentrations induced high mortality percentage reached 100% after seven-day post-treatment.

High virulence of *M. anisopliae* was achieved and viewed in high mortality percentage which reached 100% after seven-day post-treatment of *G. mellonella* larvae in laboratory bioassay (Klingen *et al*., 2002 and AbdelRaheem *et al*,. 2016)

Several workers evaluating the pathogenicity of *M. anisopliae* to various developmental stages of RPW and confirmed that *M. anisopliae* had a great potential effect against *R. ferrugineus* with mortality rate reached 100% at the fifth-day post-treatment. (Gindin, *et al* 2006 and Sun *et al*. 2016)

Conclusion:

The high efficacy and favorable results obtained in this study against the four tested insect pests proved that our *M. anisopliae* Egyptian isolate contain virulence characters so we recommend the use of this fungus in the integrated pest management programmers to control these pests as an effective biological control agent and a promising alternative to chemical insecticides.

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ARABIC SUMMARY

عزل وتعريف جزيئي وتقدير المدي العوائلي لفطر الميتاريزيم انيسوبلي (عزلة مصرية)

ياسمين السيد أحمد[1] ، سوزان عبد الله ابراهيم[1] ومنال فاروق عبد العال[2]

1- قسم بحوث دودة ورق القطن – معهد بحوث وقايه النباتات – مركز البحوث الزراعيه – جيزة – مصر

2-قسم البيولوجيا الجزيئيه الميكروبيه -معهد بحوث الهندسه الوراثيه الزراعيه–مركز البحوث الزراعيه–جيزة – مصر

نظرا لكفاءة فطر الميتاريزم انيسوبلي واستخدامه وتطبيقه بنجاح في برامج المكافحه البيولوجيه المتكامله فقد تم عزله من حشرة سوسه النخيل المصابه وتعريفه جزيئا باستخدام الشفرة الجينيه لمنطقه ITS region وهي المنطقه المعتمدة عالميا للتشخيص الجزيئي المتقدم وقد تم مقارنه تتابع هذه المنطقه بعزلات الميتاريزم انيسوبلي الاخري المسجله في قاعدة بيانات بنك الجينات وقد اظهرت هذه العزله تطابق بنسبه 98% مع معظم العزلات لذلك تم تسجيلها كعزله مصرية جديدة في بنك الجينات برقم MT102079. تم اختبار المدي العوائلي لهذه العزله على اربعه من أخطر الافات الاقتصاديه وهم دودة ورق القطن الكبري – دودة شمع النحل الكبري – سوسه النخيل الحمراء – أكاروس العنكبوت الاحمر.

أظهرت النتائج ان أكثر الحشرات تأثراً بسميه الفطر هي حوريات أكاروس العنكبوت الاحمر حيث بلغت نسبه موت الحوريات المعامله باعلي تركيز للفطر تم استخدامه (10^9) هي 96%، كما أظهرت هذه العزله تأثيرها السام العالي على كلا من عمري دودة ورق القطن الكيري ودودة شمع النحل الكبري بنسب موت 96% للعمر الثاني و90% للعمر الرابع لدودة ورق القطن الكبري و93% للعمر الثالث و86% للعمر الخامس لدودة شمع النحل الكبري. وقد كانت سوسه النخيل الحمراء اقل الحشرات تأثراً بسميه العزله حيث حقق اعلي تركيز للفطر تم تطبيقه على كلا من العمر الثالث والخامس للحشرة 73% و66% على التوالي.