

Impact of Some Fungi Species as Biocontrol Agent Against the Root-knot Nematode, *Meloidogyne Incognita*

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Abstract: A laboratory experiment was conducted to evaluate the ability of some fungi species, isolated from certain newly reclaimed areas in Egypt, as bioagent against the root-knot nematode, *M. incognita* infecting some vegetable crops. Generally, the results pointed to the presence of distinctive larvicidal and ovipocidal impact of previous isolated fungi. A proportional relation was found between mortality percentage and tested fungal culture filtrate (FCF) concentrations at different exposure times. CFs of *Fusarium* sp.₂ and *Fusarium* sp.₃ and *Mucor* sp. were the most effective fungi. *Trichoderma viride* was the most effective species followed by *Aspergillus niger* and *Rhizoctonia* sp. in parasitizing nematode eggs. Such species could cease egg embryogenesis or deplete egg content; in addition they could sporulate nematode juveniles. *In vivo* study indicated that *Fusarium* sp.₁, *Fusarium* sp.₄, *Fusarium* sp.₅, *Rhizoctonia* sp. and *Trichoderma viride* were the most effectiveness fungi species against *M. incognita*, while *Aspergillus tamarii* and *Trichothecium roseum* had no or little effect. Accordingly, these isolated fungi could be safety and friendly environment alternatives used in controlling *M. incognita* infected economic vegetable crops.

Key words: Biocontrol agent, parasitic fungi, culture filtrate, *Meloidogyne* spp.

INTRODUCTION

During the last years *Meloidogyne incognita* has become an intense problem that occurred worldwide especially in warm areas. The over used of the conventional nematicides for several years was detrimental to the environment and human health which have increased the need for new safety methods of managing plant parasitic nematodes. Consequently, during the recent years, there has been a growing recognition of the role of some natural enemies to combat plant parasitic nematodes. One interesting and significant aspect involves association of fungi with certain nematodes. It is established that many fungi are known to produce nematocidal or nematostatic compounds.

The effect of fungal filtrates on nematode juveniles and mortality of *Meloidogyne javanica* was studied *in vitro* and *in vivo* by Agha and Montasser (1992). Such filtrates suppressed both galls formation and rate of reproduction. They added that the highest value of *M. javanica* eggs parasitism was recorded by *Cylindrocarpon destructans* and *Acremonium gluacus*. A study was done by Zareen *et al.*, (2001) on the culture filtrate (CF) of *Aspergillus* spp. They demonstrated the biocontrol potential of seven species of the previous genus against *Meloidogyne javanica*. The maximum reduction of gall formation and egg-masses production was by CF of *A. niger*, *A. funigatus* and *A. terreus*. On the other hand, the effect of culture filtrates (CF) of some fungi were investigated by Sidiqqi *et al.*(2001). They studied the effect of culture filtrates of *Trichoderma* spp. on *Meloidogyne javanica* associated with okra and mungbeen. They found a significantly reduction of egg hatching in addition to the killing of second stage juveniles. Olivares-Bernabeu and Lopéz-Llorca (2002) studied the biological factors related with the development and performance of *Chlamydosporium*, *Verticillium lecanii* and *Paecilomyces lilacinus* as biocontrol agents for *Meloidogyne javanica* in laboratory tests. Pathogenicity of egg infection was 70-100% and the severity of the penetrating hyphae/egg was 35-40%. In 2003, Mukhtar and Pervaz evaluated the culture filtrate of *Verticillium* and *Clamydosporium* against *Meloidogyne javanica*. Proportional relations were detected between filtrate concentration, duration of exposure and mortality of larvae. The maximum mortality was observed in 100% concentration and after 72 hours. Larval emergence

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was, however, inversely proportional to filtrate concentration. Significant lower hatching was observed in 100% concentration. Moreover, Khan *et al.* (2004) could proof that, hatching of *M. javanica* eggs were reduced as a result of *Paecilomyces lilacinus* protease and chitinase enzymes, either individually or in combination. They demonstrated the major changes in the egg shell structure as; lipid layer was destroyed, the chitin layer hydrolyzed and the vitelline layer had lost integrity.

Mourad (2005) studied the efficiency of some fungal filtrates applied as soil drench in controlling *M. incognita* infecting sunflower plants. Fungal filtrate of *Verticillium* spp. significantly reduced number of galls, egg-masses. The role of microflora on eggs and female of *Meloidogyne* spp. collected from plant roots and infested soils in China was clarified by Sun *et al.* (2006). The predominant fungal species were: *Paecilomyces lilacinus*, *Fusarium* spp., *Pochonia chlamydosporium*, *Penicillium* spp., *Aspergillus* spp. and *Acremonium*. The isolated fungi could reduce egg hatch rates to less than 10% contrasted to the control of 65.8%, besides; fungi could kill all hatched juveniles. Kiewnik and Sikora (2006) studied the role of *Paecilomyces lilacinus* as biocontrol agent against *M. incognita*. They found that, this fungus can decrease root galling by 66%, number of eggmasses by 74% and the final nematode population in the roots by 71% compared to the inoculated treatment (control). Dababat and Sikora (2007) investigated the activity of *Fusarium oxysporum* strain 162 (Fo162) against *M. incognita*. The fungus inhibited juvenile penetration, galls formation and egg-masses production within tomato plants. The dual inoculation of the fungus was significantly different when compared with single treatment. The chitinase activity was also increased in culture filtrate of *Trichoderma harzianum*, this increment express the ability of this fungus to infect *M. javanica* eggs (Sahebani and Hadavi, 2008). In 2009, Trifonova *et al.* isolated from infected *M. incognita* females in southern Bulgaria three fungi: *Fusarium oxysporum*, *Verticillium chlamydosporium* and *Gliocladium roseum*. These fungi destroyed from 7.6% to 23.5% of the eggs, but the egg parasitism by fungi was 8.7%.

This study aimed to evaluate the biocontrol potential of some fungi isolated from certain newly reclaimed areas in Egypt against *Meloidogyne incognita*, under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Laboratory and greenhouse experiments were carried out to determine the effectiveness of some fungal species as biocontrol agents against *Meloidogyne incognita*.

Sampling collection:

Fifty five soil and root samples were randomly collected from the rhizosphere of some vegetable plants infected by *Meloidogyne* spp., i.e., egg-plant, tomato and okra growing in certain newly reclaimed area [El-Nasr Lake, Bangar El-Sokkar, El-Tahreer rod, EL-Khatatba, Ain Tony (Siwa) and Sohag]. Samples were subjected to sequence processes for fungi isolation, purification, culturing and identification.

Preparation of Medium:

Potato Dextrose Agar (PDA) medium was used for soil fungi isolation and purification to obtain a pure culture that serves for further identification. The medium was prepared by boiling 200g potato small pieces, in sufficient amount of water for 1h.; then filtration was thoroughly done. Agar (20g) and Dextrose (20g) were added to the previous extraction and it was adjusted to a volume of 1 liter. Two capsules of the antibiotic Streptophenicol 250mg (chloramphenicol 125 mg and streptomycin 125 mg) were added to the medium before sterilizing. Streptomycin and chloramphenicol are the most commonly antibiotics that can be used according to their wide spectrum; besides they are very stable in solution either in acidic medium or during heating. The medium was heated to 70 °C with stirring (Davet and Rouxel, 2000), then, it was sterilized by autoclaving at 121 °C for 21 minutes.

Isolation of Fungi from Soil:

Isolation of fungi from infected plants rhizosphere was done by using dilution plate method. The principles of a such method consist of keeping soil suspension in sterile water, then incorporating different dilutions of this suspension in the isolation medium (Rapilly, 1968). The dilution is prepared by weight 10g soil then adding distilled sterile water for adjusting the volume to 100 ml and agitating the suspension for 30 minutes, this gives a dilution of 10^{-1} . Successive extractions of 10 ml from this suspension with 90 ml distilled sterile water will give dilutions of 10^{-2} , 10^{-3} up to 10^{-6} . The Petri dishes are incubated at 27 °C and observed after 3 to 7 days.

Isolation of Fungi from Root Tissues:

After washing root tissues, they were disinfected, cut into small pieces (2-3 mm) and placed on the medium surface by forceps or well embedded in the medium and incubated for 4-5 days at 20-25°C. The colonies which appeared were transplanted to PDA medium for purification.

Isolation of Fungi from Egg-masses Surface:

Egg-mass surface was disinfected with 0.5 % sodium hypochlorite, washed in distilled sterile water and placed in Chitin Agar medium to determine the fungi chitinolytic activity. Medium was composed of Agar, 20.0; Chitin, 4.0; K₂HPO₄, 0.7; MgSO₄·7H₂O, 0.5; KH₂PO₄, 0.3; FeSO₄·7H₂O, 0.01; MnCl₂·4H₂O, 0.001; ZnSO₄·7H₂O, 0.001, in grams per liter of distilled deionizer water adjusting pH to 8 ± 0.2 at 25 °C (Atlas, 1995). The medium was autoclaved for 15 min at psi pressure 121°C then pour into sterile Petri dishes. Fungal colonies that will grown from an egg-mass were transferred to PDA medium for establishing pure culture (Nitao *et al.* 1999). Fungi were purified by using hyphal-tip method. Few spores from the conidiophores tips of sporulating fungus were collected and transferred to a nutrient medium. After fungi purification, they were preserved in tubes on common media such as PDA.

Yeast Extracts Sucrose Broth Media:

Yeast – sucrose broth media was used for the cultivation of fungi to obtain their secondary metabolites. It was prepared by dissolving the solid ingredients; yeast (20 g) and sucrose (150 g) in 1 liter of cold distilled water then heated at 60 - 70 °C with stirring. The broth media were dispensed into flasks and sterilized by autoclaving at 121 °C for 15 minutes. The flasks were inoculated and incubated for 21 days at 28 °C (Paterson and Bridge, 1994). At the end of incubation period, the cultures were passed through Whatman filter paper N.1 to remove the mycelial mats. Filtrates thus obtained were designated as 100% concentration and were amended to pH 7.

Effect of Culture Filtrates on *M. Incognita* Juveniles:

The previous fungal filtrates were tested, *in vitro* experiment, against *incognita* juveniles, (J₂) obtained from pure stock culture propagated in a greenhouse on eggplant. Three dilutions from each fungal metabolite were prepared, i.e., 1/10, 1/25 and 1/50. Treatments of each dilution were replicated three times, in addition to those of control treatment (distilled water and medium only). Ten milliliters of each diluted metabolite were poured into sterile Petri dishes. Fifty freshly hatched *M. incognita* (J₂) in 0.8 ml of distilled water were added to each treatment, and all Petri dishes kept at room temperature (25 ± 3 °C). After 24, 48 and 72 h data were recorded. Dead (unmoved) *M. incognita* juveniles in each Petri dish were counted under a stereomicroscope to calculate mortality percentage of the nematode. Immobile *M. incognita* J₂ were prodded with a needle to check for a response. After 72 h, *M. incognita* juveniles were collected from each treatment and kept for 24 h in distilled water to ensure nematode immobility.

Fungal parasitism of *M. incognita* eggs:

Four egg-masses were touched to the surface of each sporulating fungal cultures grew on PDA. Inoculated egg-masses were placed on 1.5% water agar in Petri dishes and incubated at 25°C. After seven days, egg-masses were squashed in water between a microscope slide and cover slip and were then viewed with a compound microscope. Eggs were considered parasitized when hyphae were observed within the eggs (Gaspard *et al.*, 1990).

In vivo Study:

A greenhouse study was done to assert the nematicidal effect of the previous fungal filtrates against *M. incognita*. One month old, eggplant seedlings were transplanted individually into clay pots (15cm.diameter) filled with sterilized soil mixture of 2 sand: 1 clay (v/v).The fungi treatments besides control were replicated three times and arranged in complete randomized block design in a greenhouse bench at 35±5. The pots were watered and fertilized when needed. One week after transplanting, each seedling received 1000 J₂ of *M. incognita* that were add to soil with 50 ml of different fungal metabolite in holes around the root system. Thirty five days after inoculation, the plants were uprooted, washed and stained in lactophenol acid fuchsin. Number of galls, eggmasses and eggs/eggmass were counted per plant. Nematode final population and rate of reproduction were then calculated.

Data were statistically analyzed by using the Fisher's Least Significant Differences (L.S.D.) according to Gomez and Gomez, (1984). Treatment means were compared by the Duncan's Multiple Range Test at 5% level of probability.

RESULTS AND DISCUSSION

Occurrence of Some Fungi Genera in Certain Newly Reclaimed Areas:

Fungi genera recovered from seven newly reclaimed localities are listed in table (1). Data in general, indicated that members of six fungi genera were found in association with some vegetable crops (okra, eggplant and tomato plants) infected with *Meloidogyne* spp. These genera, were *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Trichoderma* and *Trichothecium*.

Nasr-Lake was the richest area in isolated fungi, whereas 3 species of *Aspergillus* were recovered from soil and roots, i.e., *A. niger*, *A. terreus*, *Aspergillus* sp. and *A. tamarii* besides *A. parasiticus* that was associated with roots. Also, *Penicillium expansum* and *P. italicum* were isolated from soil and roots, while *Mucor* sp. was only associated with roots. In contrary, Banger El-Sokkar and Sohag were the poorest areas where *P. expansum* and *Trichocethium roseum* were only isolated from plant roots. While, of El-Kassara soil samples, three species of *Fusarium* were isolated. In addition, *Rhizoctonia* sp. was isolated from egg-masses surface.

Trichoderma viride was isolated from soil, plant roots samples and egg-masses surface samples of El-Tahreer rod, but two species of *Fusarium* occurred only in soil and roots samples. From Ain-Tony (Siwa), *Aspergillus tamari* was isolated from soil and roots samples. *A. parasiticus* was detected within roots while, *Penicillium expansum* was isolated from soil. On the other hand, nil fungi were isolated from El-Khatatba area (Figure,1). Therefore, it is apparent that fungi genera associated with vegetable plant roots infected with *Meloidogyne* spp. and their egg-masses, showed diversity with prominent occurrence of six fungi genera.

In vitro Experiments:

Chitinolytic Activity:

The previous isolated fungi were tested for their chitinolytic activity on the basis of colony diameter (CD). As shown in table (2) highly chitinolytic activity was obtained by *Trichoderma viride* where its CD measured 7 cm. Also, CD of *Fusarium* species ranged between 6.1 - 4.1 cm. However, *Aspergillus* species colony diameter were diminished gradually whereas, *A. niger* and *A. tamarii* exhibited moderate chinolytic activity (5.1 cm.), as it was decrease to 4.2 cm. with *A. parasiticus*. Other *Aspergillus* spp. and *A. terreus* recorded 3.9 and 3.4 cm., respectively. *Rizoctonia* sp, expressed chinolytic activity (CD) as 5.2 cm Continuous decrement in colony diameter was obtained by both *Mucor* sp. and *Penicillium italicum* which gave 3.8 cm. Moderate chitinolytic activation occurred with *Trichothecium roseum* (3.4 cm). The lowest activation (2.9cm.) was recorded by *Penicillium expansum*.

Accordingly, the ability of fungus to decompose chitin in media could be used as an indicator to asses its parasitism potentiality.

Impact of Culture Filtrates Against Juveniles of *M. Incognita*:

Fungal culture filtrates (CF) of the previous isolated fungi showed significant effect on *M. incognita* juveniles within different treatments. Data presented in table (3) indicate in general that all treatments caused juveniles mortality. Proportional relation were noticed between CF concentration, exposure time and juveniles mortality percentage. Namely, when CF concentration increased the effect on juveniles was more pronounced as exposure time elapsed. Thus, S/10 concentrations was the most effective treatment at 12 h exposure time for all isolated fungi genera. Accordingly, nematicidal potential of *Fusarium* sp.₂ and *Fusarium* sp.₃, in killing *M. incognita* juveniles with all concentrations and at different exposure time was greatly cosidered *Fusarium* sp.₄ had only the same potential effect with S/10 conc. at 72 h. *Fusarium* sp.₁ and *Fusarium*. sp.₅ had, however, moderate effect. *Mucor* sp. had- to same extent- similar effects to those of *Fusarium* sp.₂ and sp.₃.

Also, *Penicellium expansum* and *P. italicum* caused 100 % mortality only with S/10 at all exposure times; however, other concentration levels caused variable % mortality. Likely, CFs of *Aspergillus* species had variable effect. Evidently, effects of *A. parasiticus* CFs was similar to those of other genera with. However, *Aspergillus* sp. exhibited a highly effect with S/10 at all exposure times, while *A. niger*, *A. terreus* and *A. tamari* varied in their mortality effects. Similarity results were obtained by *Trichoderma viride* and *Fusarium*. sp.₅. CF of *Rhizoctonia* sp. had gradual effect killing as its concentration level was increased. CF of *Trichothecium roesum* had the least effect amongst those of other fungi.

The obtained results results are in accordance with those of Anke and Sterner (1997) and Chitwood (2002). Variability in nematicidal potential of CFs of the tested fungi against *M. incognita* could be attributed to nature of their secondary metabolites which may contain many nematicidal compounds. Furthermore, these metabolites may vary among isolates and/or species. Recently, Zhang *et al.* (2007) reviewed and summarized all natural products isolated from fungi that have nematicidal effect. They isolated some substances such as terpenoids, macrolide compounds, penicillic acid, nafenredin, patulin, enniatin compound and sterols.

Table 1: Occurrence of some fungi species isolated from certain newly reclaimed areas cultivated with eggplant tomato and okra plants.

Localities	Bangger	El-Nasr lake	El-Tahreer Rod	El-Kassara	El-Khatat ba	Siwa (Ain Tony)	Sohag
Fungi isolated from egg masses surface of root-knot nematodes							
<i>Rhizoctonia sp.</i>	-	-	+	-	-	-	-
<i>Trichoderma viride</i>	-	-	-	+	-	-	-
Fungi isolated from vegetable plant roots infected with root-knot nematodes.							
<i>Trichothecium roseum</i>	-	-	-	-	-	-	+
<i>Trichoderma viride</i>	-	-	-	+	-	-	-
<i>Penicillium expansum</i>	+	-	-	-	-	-	-
<i>Penicillium italicum</i>	-	+	-	-	-	-	-
<i>Mucor sp.</i>	-	+	-	-	-	-	-
<i>Fusarium sp.5</i>	-	-	-	+	-	-	-
<i>Fusarium sp.4</i>	-	-	-	-	-	-	-
<i>Fusarium sp.2</i>	-	-	-	-	-	-	-
<i>Fusarium sp.1</i>	-	-	-	-	-	-	-
<i>Aspergillus tamarii</i>	-	-	-	-	-	+	-
<i>Aspergillus parasiticus</i>	-	+	-	-	-	+	-
<i>Aspergillus sp.</i>	-	+	-	-	-	-	-
<i>Aspergillus terreus</i>	-	+	-	-	-	-	-
<i>Aspergillus niger</i>	-	+	-	-	-	-	-
Fungi isolated from soil							
<i>Trichoderma viride</i>	-	-	-	+	-	-	-
<i>Penicillium italicum</i>	-	+	-	-	-	-	-
<i>Penicillium expansum</i>	-	-	-	-	-	+	-
<i>Fusarium sp.4</i>	-	-	+	-	-	-	-
<i>Fusarium sp.3</i>	-	-	+	-	-	-	-
<i>Fusarium sp.2</i>	-	-	+	-	-	-	-
<i>Fusarium sp.1</i>	-	-	-	+	-	-	-
<i>Aspergillus tamarii</i>	-	+	-	-	-	+	-
<i>Aspergillus sp.</i>	-	+	-	-	-	-	-
<i>Aspergillus terreus</i>	-	+	-	-	-	-	-
<i>Aspergillus niger</i>	-	+	-	-	-	-	-

Table 2: Chitinolytic activity of isolated fungi.

Fungi	Colony diameter (cm)	Fungi	Colony diameter (cm)	Fungi	Colony diameter (cm)
<i>Aspergillus niger</i>	5.1	<i>Fusarium sp.₁</i>	4.1	<i>Mucor sp.</i>	3.8
<i>Aspergillus terreus</i>	3.4	<i>Fusarium sp.₂</i>	5.8	<i>Penicillium expansum</i>	2.9
<i>Aspergillus sp.</i>	3.9	<i>Fusarium sp.₃</i>	5.4	<i>Penicillium italicum</i>	3.8
<i>Aspergillus tamarii</i>	4.2	<i>Fusarium sp.₄</i>	6.1	<i>Rhizoctonia sp.</i>	5.2
<i>Aspergillus parasiticus</i>	5.1	<i>Fusarium sp.₅</i>	5.8	<i>Trichothecium roesum</i>	3.4
				<i>Trichoderma viride</i>	7.0

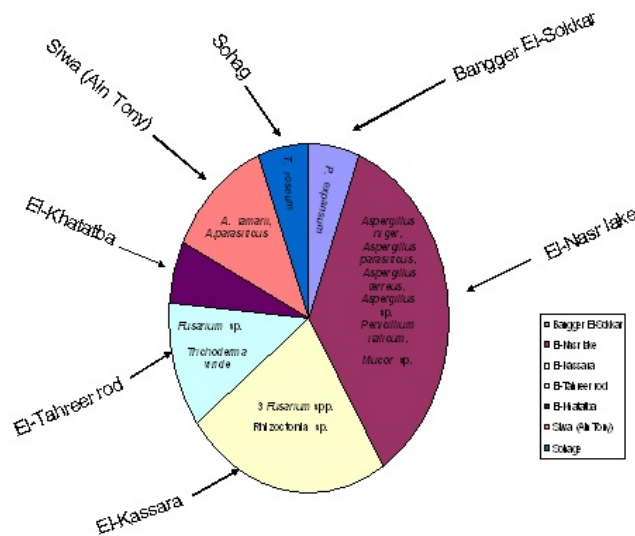


Fig. 1: Distribution of some fungi genera isolated from certain newly reclaimed areas.

Table 3: Effect of certain fungal metabolites concentrations on mortality percentage of *Meloidogyne incognita* juveniles under different exposure times .

Fungi	Average of % nematode juveniles mortality								
	24 h			48 h			72 h		
	Fungal filtrate concentration								
	S/10	S/25	S/50	S/10	S/25	S/50	S/10	S/25	S/50
<i>Aspergillus niger</i>	55 de	15 fgh	12 efg	70 cd	45 c	31 d	78 be	69 d	62 cd
<i>Aspergillus terreus</i>	17 g	15 fgh	12 efg	49 e	41 d	33 d	71 c	69 d	61 cd
<i>Aspergillus sp.</i>	96 a	9 gh	6 fg	100 a	67 be	58 c	100 a	79 be	73 bc
<i>Aspergillus parasiticus</i>	16 g	11 gh	8 efg	59 de	59 bc	23 b	85 abc	77 bcd	64 cd
<i>Aspergillus tamaritii</i>	73 b	65 b	65 b	81 dc	74 b	72 c	91 ab	83 b	80 b
<i>Fusarium sp.1</i>	52 e	35 cde	27 c	71 a	64 bc	53 c	84 abc	73 cd	69 bed
<i>Fusarium sp.2</i>	100 a	100 a	99 a	100 a	100 a	100 a	100 a	100 a	100 a
<i>Fusarium sp.3</i>	100 a	100 a	91 a	100 a	100 a	95 a	100 a	100 a	95 a
<i>Fusarium sp.4</i>	59 e	21 efg	18 cde	85 b	65 bc	56 c	100 a	87 b	76 bc
<i>Fusarium sp.5</i>	65 c	45 c	24 cd	68 cd	63 bc	55 c	77 abc	70 d	64 cd
<i>Mucor sp.</i>	100 a	89 a	74 b	100 a	100 a	97 a	100 a	100 a	100 a
<i>Penicillium expansum</i>	100 a	28 def	0 g	100 a	60 bc	49 c	100 a	79 bc	67 bed
<i>Penicillium italicum</i>	100 a	37 cd	11 efg	100 a	71 bc	30 d	100 a	85 b	73 bc
<i>Rhizoctonia sp.</i>	59 e	21 efg	17 def	65 d	63 bc	47 c	75 bc	69 d	62 cd
<i>Trichothecium roseum</i>	15 h	9 h	5 g	32 f	19 e	16 e	55 d	21 e	19 e
<i>Trichoderma viride</i>	62 cd	38 cd	17 de	69 cd	39 d	23 d	79 bc	70 cd	57 d
Control	0 h	0 h	0 g	0 g	0 e	0 e	0 e	0 e	0 e

Means followed by the same letters (s) are not significantly by (p0.05) accordingly to Duncan's multiple - range test.

Fungal Parasitism of *M. Incognita* Eggs:

The tested fungi have variable potent parasitism against *M. incognita* eggs. This is mainly related to the fungi characters and mechanical force which enable fungi to penetrate host cell; besides production of lytic enzymes (Steirling,1991 and Khan *et al.*, 2004). *Trichoderma viride* was the most vigour fungus either for eggs or juveniles followed by *A. parasiticus* and *Rhizoctonia sp.*(Figure, 2).

In Vivo Study:

All tested fungi had variable effect on *M. incognita* infecting eggplant. The ability of these fungi species to reduce gall formation of *M. incognita* can be arranged between 2 galls/ plant due to *Fusarium sp.*₂ and 55 galls/ plant due to *Aspergillus tamaritii*. Negative effect of the other fungi decreasing gall formation could be arranged in descending order as follows: *Fusarium sp.*₂ and *Fusarium sp.*₄> *Rhizoctonia sp.*> *Fusarium sp.*₁> *Aspergillus tamaritii* and *Trichoderma viride*> *Aspergillus sp.*, *Mucor sp.*, and *Penicillium expansum*> *Aspergillus niger*, *Aspergillus terreus* and *Fusarium sp.*₃> *Penicillium italicum*> *Trichothecium roseum* (Table, 4).

Also, the tested fungi significantly succeeded in reducing eggmass production. Plants treated with *Fusarium sp.*₄, *Fusarium.sp.*₁, *Fusarium.sp.*₂, *Fusarium.sp.*₁, *Rhizoctonia sp.*, *Aspergillus parasiticus*, *Aspergillus sp.*, *Trichoderma viride* and *Mucor sp.* had, relatively the least counts of eggmasses ranging between 1 to 6 eggmasses/ plant. While *Penicillium expansum*, *Aspergillus niger* and *A. terreus* did reduce it to 8-13 eggmasses. *Fusarium sp.*₂ reduced eggmass production to 18 egg-masses. However, an augmentation in eggmass count occurred, in treatment of *Penicillium italicum* (23 eggmasses), while those of *Trichothecium roseum* and *Aspergillus parasiticus* recorded 39 and 51, respectively. On the other hand, control treatment recorded 148 eggmasses/plant.

Fecundity (number of eggs /eggmass) of *M. incognita* was significantly restricted as a result to application of fungal culture filtrates. The highest fecundity value was noted with *Aspergillus niger* (393 eggs/egg-mass). Then the fecundity was diminished gradually in descending pattern to record the number of eggs/egg-mass as follows: *Trichothecium roseum* (300), *Aspergillus parasiticus* (275), while *Fusarium species* achieved 288, 279 and 261 for *Fusarium sp.*₂, *F.sp.*₁ and *F. sp.*₅, respectively. Members of *Penicillium* recorded 247 and 226 eggs/eggmass for *P. expansum* and *P. italicum*, respectively. *Trichoderma viride* and *Rhizoctonia sp.* CFs filtrate minimized *M. incognita* fecundity to 176 and 139 eggs/eggmass, respectively. CF of *Fusarium sp.*₄ overtopped those of all other fungi species in reducing egg production *M. incognita* (56 eggs/eggmass). Moreover, a such filtrate was the most successful in reducing all nematode criteria when compared with those of the other fungi species. Therefore, this fungus must be considered as a potent bioagent for combating and restricting *M. incognita*.

In conclusion, it is benefit to use the most effective fungi as bio-agent against *M. incognita* and possibly against other nematode species.

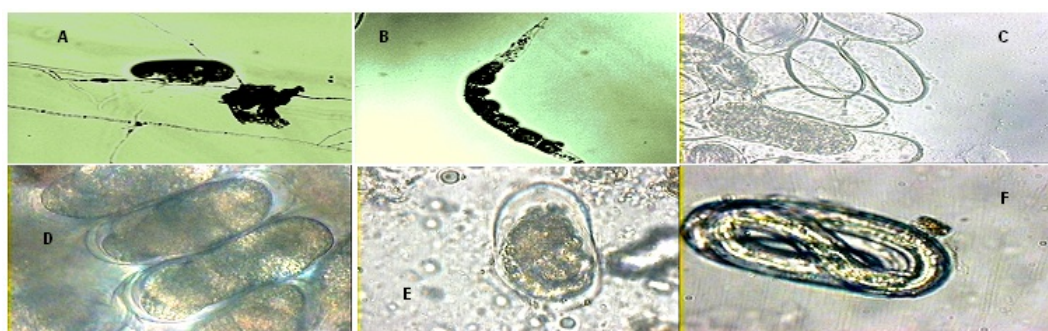


Fig. 2: A) *M. incognita* eggs are destroyed by *T. viride*. B) Large number of *T. viride* zoospores line up inside a *M. incognita* juvenile. C) Empty eggs beside others with stopped embryogenesis as a result of *Rhizoctonia* sp. parasitism. D) Ceasing of embryogenesis as a result of *A. niger* parasitism. E) A damaged egg by *A. parasiticus* F) A well developed juvenile of *M. incognita* inside egg (control treatment).

Table 4: Effect of fungal filtrates on the reproduction of *Meloidogyne incognita* infecting eggplant, *Solanum melongena* L.

Fungi	Nematode criteria/ plant				
	Galls/ root	Egg masses/ root	Eggs/egg mass	Final population	Reproduction rate
<i>Aspergillus niger</i>	15 ef	10 efg	393 a	3930	3.930
<i>Aspergillus terreus</i>	17 de	13 ef	162 de	2754	2.754
<i>Aspergillus</i> sp.	7 fg	5 fg	237 bcd	3318	3.318
<i>Aspergillus tamarii</i>	6 fg	4 fg	275 b	1375	1.375
<i>Aspergillus parasiticus</i>	55 b	51 b	297 b	12177	12.177
<i>Fusarium</i> sp. ₁	5 g	2 g	279 b	837	0.837
<i>Fusarium</i> sp. ₂	3 g	3 g	288 b	1440	1.440
<i>Fusarium</i> sp. ₃	18 de	18 de	257 b	3341	3.341
<i>Fusarium</i> sp. ₄	3 g	1 g	56 f	56	0.056
<i>Fusarium</i> sp. ₅	2 g	2 g	261 b	261	0.261
<i>Mucor</i> sp.	7 fg	6 fg	168 de	1008	1.008
<i>Penicillium expansum</i>	7 fg	8 fg	247 bc	1482	1.482
<i>Penicillium italicum</i>	26 d	23 d	226 bcd	3841	3.842
<i>Rhizoctonia</i> sp.	4 g	3 g	139 e	417	0.417
<i>Trichothecium roseum</i>	43 c	39 c	300 b	8400	8.400
<i>Trichoderma viride</i>	6 fg	5 fg	176 cde	704	0.407
Control	155 a	148 a	408 a	52224	52.224

Means followed by the same letters (s) are not significantly by ($p \leq 0.05$) accordingly to Duncan's multiple - range test.

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