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Isolation of fungi associated with *Meloidogyne incognita* and their effect on its eggs hatching in the Green Mountain region of Libya

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Abstract

Aim of study: The aim of the study was to isolate the fungi associated with root-knot nematodes and to determine their effect on its eggs hatching in the Green Mountain region of Libya.

Material and methods: Soil samples infected with root-knot nematode were collected from the some farms of the Green Mountain region in Libya. Sample collection was carried out during May 2007 within ten different areas. Two fields planted with tomato plants were chosen from the each area. Different types of culture media were used to isolate the fungus species. Fifteen fungi were isolated from the rhizosphere of infected plants. Then, the effect of some isolated and identified fungi on the rate of hatching of nematode eggs was tested.

Main results: Isolation results indicate that some of the isolated fungi were considered as parasites on the nematodes. In the study area, *Arthrobotrys* sp. had the highest percentage of occurrence (70%) and the least one was *Penicillium* sp. (12.25%). Three inoculum levels of 0.75, 1.5, 2×106 fungal propagules were tested and the highest hatching inhibition (90.5%) was recorded in *Paecilomyces lilacinus* and the lowest inhibition level (58.4%) was observed in *Vertecillium chlamydosporium*.

Research highlights: As a result of this study, more successful outcomes can be obtained during the biological control of root-knot nematodes.

Keywords: Root-knot Nematode, Fungi isolation, Egg hatching inhibition, Green Mountain, Libya.

Libya'nın Green Mountain bölgesinde kök-ur nematodu (*Meloidogyne incognita*) ile bağlantılı mantarların izolasyonu ve yumurtaların açılımı üzerine etkisi

Özet

Çalışmanın amacı: Bu çalışmada Libya'nın Green Mountain bölgesinde kök-ur nemetodu ile bağlantılı mantarların izole edilmesi ve bu mantarların kök-ur nemetodu yumurta açılımları üzerine etkisinin araştırılması amaçlanmaktadır.

Materyal ve yöntem: Bu çalışmada Libya'nın Green Mountain bölgesindeki bazı tarım alanlarından kök-ur nematodu enfekteli toprak örnekleri alınmıştır. Araziden örnek alımları Mayıs 2007'de 10 farklı alanda yapılmıştır. Her bir alandan domates ekili iki tarla seçilerek örneklenmiştir. Mantar türlerini izole etmek için farklı besi ortamları kullanılmış olup enfekte olmuş bitki rizosferlerinden 15 mantar türü izole edilmiştir. Daha sonra izole edilip teşhis edilen bazı mantar türlerinin nematod yumurtaları açılım oranları üzerine etkisi test edilmiştir.

Sonuçlar: Izolasyon sonuçları izole edilen bazı mantar türlerinin nematodlar üzerinde parazitik özellikte olduğunu göstermiştir. Çalışma alanında *Arthrobotrys* sp. varlığının en yüksek yüzdeye (%70) sahip olduğu, en az görülen türlerin ise *Penicillium* sp. (%12.25) olduğu tespit edilmiştir. Araştırmada mantar üreme yapılarının 3 inokulum seviyesi (0.75, 1.5 ve 2×106) test edilmiştir. Sonuç olarak, en yüksek yumurta açılımı engellenme (%90.5) *Paecilomyces lilacinus*'da ve en düşük (%58.4) ise *Vertecillium chlamydosporium* uygulamasında görüldü.

Araştırma vurguları: Bu çalışmanın sonucu olarak, kök-ur nematodu ile biyolojik mücadele kapsamında daha başarılı sonuçların alınabileceği düşünülmektedir.

Anahtar Kelimeler: Kök-ur nematodu, Mantar izolasyonu, Yumurta açılımı engeli, Green Mountain, Libya.

Introduction

Nematodes are small worms that can only be seen by a microscope. These worms live in different environments such as soil and water (Alhazmi, 1992). The general shape of the plant nematode is usually a cylindrical tube with two pointed ends and the average of its length is about 0.2-1.2 mm and width is 15-35µm (Al-Assas, 2003).

Nematodes may cause direct or indirect damages on host plants. The direct damage is to attack and feed on the different tissues of the plant causing plant weakness or death, whereas indirect damage is the transmission of various pathogens as well as breaking plant resistance against those pathogens (Agrios, 1985). There are many examples showing the seriousness of parasitic nematodes on the plant, for example, nematodes have caused big losses in potato crops in Britain, rice in Southeast Asia, cotton in Egypt, coffee in Brazil, corn in America, which are important crops in those countries (Ahmed, 1998). Although, it is difficult to obtain accurate estimates of the losses caused by plant nematodes, but researchers indicated that the estimated loss in the annual growth rate of major crops in the developed countries is 8.8% and in developing countries is 14.6% (Al-Assas, 2003). Root- knot nematode is considered one of the three most important types of nematodes that cause economic damage to ornamental plants and crops (Moens et al., 2009).

There are several ways to control root-knot nematodes including usage of chemicals, applications of natural ways and soil solarization. Several experiments in this regard were conducted, for example; Dabaj (2003) had got good results using transparent and black plastics to control root- knot nematodes in the soil as compared to the use of methyl bromide. Due to the high cost of chemical control in plant nematodes and on the other hand the danger of chemicals to humans and the environment, researches are recently directed towards the possibility of reducing plant parasitic nematode populations in the soil by using microorganisms such as bacteria and fungi. The use of fungi for controlling plant parasitic nematode has been increased in the recent years. There are number of recent reports of attempts to identify fungi with activity against plant parasitic nematode (Alhazmi, 1992). The research and studies have clarified the nature of fungi parasitism on plant parasitic nematodes. Some of the commercial formulations have been produced from certain fungi such as; *Trichoderma* spp., *Arthrobotrys* spp. (Stirling & West, 1991). The aim of the study was to isolate the fungi associated with root-knot nematodes, without considering the difference in the presence of nematodes in the study area.

Materials and Methods

Sample collection

Sample collection was carried out during May 2007 within ten areas of the Green Mountain region in Libya. These areas are as under Sousse, Al- Hamama, Al-Henniye, Al-Mansoura, Cyrene, Al-Wasyta, Al-Byda, Omar Mukhtar, Al-Faydia, Massa. Two root-knot nematode infested fields planted with tomato plants were chosen from the each area.

Ten soil samples were collected on a zig-zag pattern over the field. The total soil samples were 100, each of them weighing about 500 g were collected from the rhizosphere of 5 plants from each sample site (Verdejo-Lucas et al., 2002). Samples were placed in nylon bags mentioning the necessary data and then transferred to the laboratory to isolate the fungus associated with root-knot nematodes (Al-Assas, 2003).

Fungi isolation

Different types of culture media were used to isolate as many number of fungus species as possible from the study area, (Al-Bony, 1990).

1. Potato Dextrose Agar (PDA)
2. Corn Meal Agar (CMA)
3. Malt Extract Agar (MEA)
4. Sabouroud Maltose Agar (SMA)
5. Liquid Potato Dextrose (PD)
6. Water Agar (WA)

The above mentioned nutrient media were prepared by dissolving the recommended weight of each type in 1 liter distilled water and then sterilized in autoclave for 20 minutes, then poured in Petri dishes having 10 cm diameter. About 1 gram of each soil samples were scattered in a Petri dish prepared in the previous step. Five Petri dishes were allocated for each type of nutrient media, Dishes were left in incubator at a temperature of 25 C° and they were checked daily during the period of 2-3 weeks to isolate the developing fungus in the form of pure isolates (Saadabi & Al-Amein, 2001).

Fungi identification

A semi-permanent slide of all pure fungal isolates obtained previously had been prepared in the fungus laboratory, Faculty of Agriculture - Omar AL-Mukhtar University, Al-Byda-Libya. Then isolated fungi were identified by using a compound microscope. For identification, morphological shape, fungal spores, mycelium, color of fungus, fungal traps if applicable and taxonomic keys were used (Barnett & Hunter, 1972; Toussoun & Nelson, 1976).

Processing of root-knot nematode eggs

Meloidogyne incognita (Kofoid & White) Chitwood had been propagated on pepper plants (California wender) planted in sterile soils within pots inside a greenhouse, Department of Plant Protection, Omar Al - Mukhtar University. Plants had been well taken care of to get eggs of *M. incognita* for subsequent tests.

Effect of some isolated fungi on M. incognita egg hatching

Five fungi species isolated from soil and nematodes in the study region were selected to study their effect on *M. incognita* egg hatching ratio. Those fungi were *Arthrobotrys* sp., *Trichoderma* sp., *Paecilomyces lilacinus* (Thom.), *Acremonium strictum* (W.Gams) *and Vertecillium chlamydosporium* (Godard). Fungi were cultured on PDA media in 10cm diameter Petri dishes at 25 C °. Propagules suspensions were prepared by blending (10 days old) 0.5 cm diameter disk in sterilized distilled water for 20 seconds. Three inoculums levels of 0.75, 1.5**,** and 2×10^6 fungus propagules (1 ml suspensions) were separately mixed into 5g of sterilized soil placed in 10cm containers and incubated for a week. *M. incognita* egg masses were collected from infected pepper plants (California wender) which were processed in the previous step and the mean number of eggs in each mass was counted which was found to be 550 eggs. Three egg masses were incorporated in each container that contained soil and fungus propagules suspensions. The experiment was conducted with three replicates. Sterilized water was used for control treatment. After one week, nematodes had been extracted from the soil in the containers by using Baermann trays method and by using hemocytometer, the mean number of hatching larvae were counted (Al-Assas, 2003).

Results and Discussion

Fungi isolation

As shown in Table 1, fifteen fungal isolates were found to be associated with *Meloidogyne* nematodes in study region. The most frequently occurring fungus was *Arthrobotrys* sp, having the most percentage of occurrence (70%) and the least one (12.25) was *Penicillium* sp. The proportion of fungi presence differed in the study areas and this may be due to the difference in fertility of the soil in different regions which was in correspondence with the studies of Saddaby and Al-Amein (2001) and also corresponded with a previous study by Bin–Saud and Dongly (2003).

Table 1. Fungi occurrence in study region

Fungi	% of Occurrence
Arthrobotrys sp.	70
Trichoderma sp.	53.6
Paecilomyces lilacinus	66.2
Vertecillium chlamydosporium	48.7
Acremonium strictum	39.9
Aspergillus sp.	41.4
Dactylaria sp.	45.5
Harposporium sp.	29.8
Mucor sp.	28
Pythium sp.	24
Cephalosporium sp.	44
Fusarium sp.	61
Rhizopus sp.	63.3
Alternaria tenius	21.4
Penicillium sp.	12.25

Table 2. Distribution of fungi in the study areas

İsolated fungi/study areas	Massa	Cyrene	Al-Henniye	Al-Hamama	Omar Mukhtar	Al-Faydia	Al-Wasyta	Sousse	Al-Mansoura	Al-Byda
Arthrobotrys sp.	+	+	+	-	-	-	+	+	+	+
Trichoderma sp.	-	-	-	-	-	+	+	-	+	-
Paecilomyces lilacinus	+	-	-	-	-	+	-	+	-	-
Vertecillium chlamydosporium	-	-	+	+	+	+	-	-	-	+
Acremonium strictum	-	-	-	+	+	-	-	+	-	-
Aspergillus sp.	-	-	-	+	+	-	+	+	-	-
Dactylaria sp.	+	-	+	-	-	+	-	+	-	-
Harposporium sp.	-	-	+	+	-	+	-	-	-	-
Mucor sp.	-	-	-	-	+	-	-	-	-	+
Pythium sp.	-	+	-	-	-	+	-	-	-	-
Cephalosporium sp.	-	-	+	-	-	-	-	+	+	-
Fusarium sp.	+	+	+	-	-	-	+	+	+	+
Rhizopus sp.	-	+	-	+	-	-	-	-	-	-
Alternaria tenius	-	-	-	+	+	-	-	-	+	+
Penicillium sp.	-	+	+	-	-	-	+	-	-	-

(+)Means the presence of fungus in the area, (-)Means fungus does not exist in the area

Effect of some isolated fungi on M. incognita egg hatching

As shown in Table 3, results indicated that *M. incognita* egg hatching was inhibited by propagules suspensions of the fungi under study which varied significantly between treatments. Percentage of egg hatching was calculated and the highest hatching inhibition (90.5%) was recorded in *Paecilomyces* and the least inhibition level (58.4%) was observed in *Vertecillium* treatment. Some of these fungi were effective to certain degree, inhibiting the egg hatching process of *M. incognita*. The differences in response of nematodes to the tested fungi may be due to the differences in the nature of toxic metabolites produced by fungus. So, it may be due to the reason that some of our isolated fungi are capable of producing toxic substances in the presence of suitable substrate in the rhizosphere of plants**,** and the presence of high population of these fungi in the rhizosphere where nematode population is also high may help in reducing the plants` damage that is caused by nematodes by the suppression of their population. Some studies have indicated the importance of availability of organic matter in the soil, which helps in increasing the number of beneficial fungi (Zhen et al., 2014). On the other hand, it increases the growth of plants, which improves the resistance of plants against pests (Main and Rodriguez, 1982; Main et al., 1982).

Table 3. Effect of Some Isolated Fungi on *M. incognita* egg hatching

Fungus	Concentration (ml)	Mean	% of Hatching	% of Inhibition
Arthrobotrys sp.	0.75	10.0	33.2	66.8
	1.5	6.0	26.9	73.1
	2	7.0	29.1	70.9
Trichoderma sp.	0.75	11.0	27.3	72.7
	1.5	12.0	18.9	81.1
	2	8.5	30.1	69.9
Paecilomyces lilacinus	0.75	3.5	20.4	79.6
	1.5	3.0	17.2	90.5
	2	0.8	9.5	82.8
Acremonium strictum	0.75	9.0	28.8	71.2
	1.5	4.3	19.1	80.9
	2	9.0	26.8	73.2
Vertecillium chlamydosporium	0.75	14.0	41.6	58.4
	1.5	11.0	38.4	61.6
	2	11.0	44.5	55.5
Aspergillus niger	0.75	13.0	41.8	58.2
	1.5	14.0	42.7	57.3
	2	14.0	44.1	55.9
Control		33.0	100.0	00.0
LSD		14.7	16.6	

Conclusion

Various biological agents can be used to control root-knot nematodes, including beneficial fungi that attack nematodes at different stages, such as eggs, larvae, and adults. Where it can reduce the density of nematodes significantly, if the appropriate conditions are available and reduces the use of chemicals.

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