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Evaluation of *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Arthrobotrys dactyloide* as Biocontrol Agents for *Meloidogyne incognita* under Green House Condition

[1]Abd El-Raheem R. EL-Shanshoury, [2]Samir A. El-Sayed, [1]Yehia A.G. Mahmoud and [2]Doaa M. Khalefa
[1]Department of Botany, Faculty of Science, Tanta University, Tanta, Egypt
[2]Microbial Activity Research Unit, Agriculture Research Center,
Soil and Water and Environmental Research Institute, Giza, Egypt

Abstract: In this study, evaluation of the nematophagous fungi *Pochonia chlamydosporia, Paecilomyces lilacinus* and *Arthrobotrys dactyloide* as biological control agents for *Meloidogyne incognita* was investigated under greenhouse conditions. Experiments confirmed the effectiveness of these predatory and parasitic fungi that actively reduced the number of infective larvae of *M. incognita*. The killing effect of these fungi is similar to the synthetic chemical nematicide Furadan and significantly better than the commercial preparation of bioagent Nameless®. The fungi under consideration have the potentiality to reduce population density of *M. incognita* along the growing season of faba bean plant to 95.4 to 98.9%. These nematophagous fungi enhanced shoot and root growth of Faba bean.

Key words: *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Arthrobotrys dactyloide*, *Meloidogyne incognita*, nematodes

INTRODUCTION

Increase of toxic substances in human food, water and environment has urged a search for alternative methods for controlling pests and plant root pathogen. One possibility for controlling soil-borne pathogens without causing environmental threats is by utilizing microorganisms to overcome the use of potentially harmful pesticides[1]. This has created the possibility to use soil microorganisms to reduce population density of plant-parasitic nematodes and nematophagous fungi for nematode biological control. Nematophagous fungi are common soil inhabitants, infecting living nematodes through different strategies[2]. Plant-parasitic nematodes generally attack plant roots, therefore the ability of nematophagous fungi to colonize roots should be a great advantage. It was found that pea, barley and white mustard rhizospheres harbour higher densities of nematophagous fungi than the root-free soil[3]. The nematophagous fungi *Pochonia chlamydosporia* (*Verticillium chlamydosporium*)[4,5], *Arthrobotrys* sp.[6,7]. and *Paecilomyces lilacinus*[8] have been tested as bioagents for controlling parasitic nematodes in some experiments. Various aspects of biological control of nematodes using fungi have been reviewed[9,10]. The infection mechanisms of nematophagous fungi were reviewed in some detail by Dackman *et al.*[11].

The aim of the current study was to evaluate the efficacy of *Pochonia chlamydosporia, Paecilomyces lilacinus* and *Arthrobotrys dactyloide* for controlling *Meloidogyne incognita* infecting faba bean (Giza, 40), in comparison with commercial bioagent (Nameless) and the synthetic chemical nematicide, Furadan (Carbofuran).

MATERIALS AND METHODS

Microorganisms used: Fungal strains belonging to *Pochonia chlamydosporia* (*Verticillium chlamydosporium*) and *Arthrobotrys dactyloides* were provided from Depto. de Ciencias Ambientales y Recursos Naturales, Universidad de Alicante, Spain, *Paecilomyces lilacinus* AUMC (NO. 612-5) was provided from Department of Botany, Faculty of Science, Assiut Univerity, Egypt. The tested fungi were maintained on potato dextrose agar medium (PDA) at 4°C.

Green house assay for biocontrol of *M. incognita*: Pot experiment was conducted under green house condition to explore effectiveness of *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and

Corresponding Author: Yehia A.G. Mahmoud, Department of Botany, Faculty of Science, Tanta University, Tanta, Egypt

Table 1: Physical and chemical properties of experimental soil

Property	value
Mechanical analysis	
Sand (%)	89.92
Silt (%)	2.20
Clay (%)	7.88
Texture grade	Sandy
Saturation of water in soil (SP)	25.0
pH	7.5
Electrical conductive E C (S cm^{-1})	0.38
Soluble cations (meq L^{-1})	
Ca^{++}	0.90
Mg^{++}	0.55
Na^{+}	0.95
K^{+}	0.60
Soluble anions (meq L^{-1})	
Co_3^{--}	1.35
HCO_3^{-}	0.65
Cl^{-}	1.00
So_4^{-}	0.24
Organic matter (%)	0.36

Arthrobotrys dactyloides to reduce population density of *Meloidogyne incognita*. Seeds of Faba been (Giza, 40) were sown in 30 cm pots containing 10 kg of sandy loam soil (1:1). Chemical and physical properties of used soil are shown in Table 1. Five seeds were sown in each pot, *Rhizobium leguminosanum* bv. Vaceae (Strain ICARD 441) was used to inoculate faba bean seeds before planting using seed coating technique. Then thinned to two plants/pot, just 10 days after germination. Pots were divided into 25 groups, each contained five replicates.

P. chlamydosporia, *P. lilacinus* and *A. dactyloides* were grown at 25°C for 7 days in yeast peptone glucose broth, which contained the following: yeast-extract 3 g L^{-1}, peptone 10 g L^{-1} and glucose 20 g L^{-1}[12] on a rotary shaker (170 rpm). At the end of incubation period, the resultant growth was used for soil inoculation. These fungi were individually incorporated into the soil at three doses (30 mL pots $^{-1}$) (1 mL=5.64x 10^4 cfu mL^{-1}), the doses were added one time or divided equally two or three times. The doses of the fungi were added at planting (nematodes-post infection) or after 15 days of planning (nematode pre-infection). Other two groups of pots, contained Nameless® (commercial preparation of nematodes biocontrol agent produced by Soils, Water and Environment Res. Inst. Agric. Microbiol. Res., Dept., Giza, Egypt) or Nematiciedes furdan. They were added on dose rate of 30 mL pot $^{-1}$ and 0.005 g pot^{-1}, respectively. Pots were arranged in a Complete Randomized Block Design, watered and received the normal agricultural practices.

Pot infested with nematode received juvenile larvae of second stage of *M. incognita* at dose rate of 20 mL pot^{-1} (100 larvae ml L^{-1}), after 15 days of planting, (post-infection)or at planting (pre-infection). The treated plants were compared with non-infested (healthy plant) and infected (control).

After two months of planting, the plants in each pot were uprooted and the roots were gently separated from soil, washed with flow water and dried by pressing lightly between blotting paper. A modified Root Gall Index (RGI) and Egg Mass Index (EMI) were calculated according to Taylor and Sasser[13] and averages of galls and *Rhizobium* nodules were counted. Nematode larvae population density after harvest (Pf) were extracted from soil and counted using Oosten brink's elutritor technique. The collected larvae were miscropically counted. Reduction of nematode population density in soil samples was also calculated according to Tilton formula, as follows:

Tilton formula =

$$\left[1-\frac{\text{Population denstiy in the treated pot after application}}{\text{Population denstiy in the treated pot before application}}\times\frac{\text{Population denstiy of the control pot before application}}{\text{Population denstiy of the control pot after application}}\right]\times 100$$

The growth response of faba bean (roots and shoot fresh and dry weight) was also recorded. Data were subjected to statistical analysis and means were compared using the Least Significant Difference (LSD at p = 0.01 and 0.05), according to Gomez and Gomez[14].

RESULTS

Data presented in Table 2 clearly revealed that *Verticillium chlamydosporum* (V10) has the potentiality to reduce population density of *Meloidogyne incognita* to great extend along the growing season of faba bean plants, either with post or pre-infection. High reduction percentage in the number of the juveniles ranged from 97.1 to 98.9% during the growing season was recorder in comparison with (control). Data also revealed that the infectivity of the nematode was tremendously. Thus, number of eggs mass per root system, were significantly (p<0.01, 0.05) decreased from 116 (control) to 2 with *V. chlamydosporum* (V10) inoculated plants, resulting reduction of 98.2% either post- or pre-infection. Data also, revealed superiority of *V. chlamydosporum* (V10)

Table 2: Evaluation of *Verticillium chlamydosporum* for controlling *Meloidogyne incognita** infected faba bean plant, under greenhouse conditions

Treatments	Bio agent Add (mL)	Final No. of *M. incognita* pot^{-1}	Reduction (%)	Egg mass No. on root pot^{-1}	Reduction (%)	*Rhizobium* Nodules/root	Fresh Shoot weight (g) pot^{-1}	Fresh Root weight (g) pot^{-1}	Dry Shoot weight (g) pot^{-1}	Dry Root weight (g) pot^{-1}
Control (infected plant)		7020	0	116	0	22	36.6	5.8	8.2	1.75
V. chlamydosporum (only)	30 mL ** (one dose)	0	0	0	0	79	85.2	13.7	10.2	2.1
Healthy plant (non infected)		0	0	0	0	74	58.8	9.3	19.9	2.1
Pre-infection of *Meloidogyne incognita*										
V.chlamydospo-rum	30 mL (one dose)	140	98	3	97.4	100	84.6	11.2	11.3	
V.chlamydospo-rum	30 mL (two doses)	140	98	3	97.4	91	99.2	18.2	13.2	3.2
V.chlamydospo-rum	30 mL (three doses)	140	98	2	98.2	92	102.0	20.1	14.1	4.1
Post-infection of *Meloidogyne incognita*										
V.chlamydospo-rum	30 mL (one dose)	72	98.9	2	98.2	91	83.1	11.6	11.1	3.8
V.chlamydospo-rum	30 mL (two doses)	200	97.1	3	96.5	94	94.0	17.1	12.1	
V.chlamydospo-rum	30 mL (three doses)	200	97.1	3	97.5	94	91.2	16.1	9.1	2.5
LSD 0.05				12.84		36.2	21.33	3.47	1.6	1.142
0.01				17.26		48.70	28.69	4.67	2.18	1.536

*Initial number of *M. incognita* was 2000 larvae pot^{-1}, **1 mL=5.64x 10^4 cfu mL^{-1}

Table 3: Evaluation of *Paecilomyces lilacinum* (P20) for controlling *Meloidogyne incognita** infected faba bean plant, under greenhouse conditions

Treatments	Bio agent Add (mL)	Final No. of *M. incognita* pot^{-1}	Reduction (%)	Egg mass No. on root pot^{-1}	Reduction (%)	*Rhizobium* Nodules/root	fresh Shoot weight (g) pot^{-1}	fresh Root weight (g)pot^{-1}	Dry Shoot weight (g) pot^{-1}	Dry Root weight (g) pot^{-1}
Control (infected plant)		7020	0	116	0	22	36.6	5.8	8.2	1.75
P. lilacinus (only)	30 mL ** (one dose)	0	0	0	0	88	84.57	11.2	11.3	3.1
Healthy plant (non infected)		0	0	0	0	74	58.8	9.3	9.9	2.1
Pre-infection of *Meloidogyne incognita*										
P. lilacinus	30 mL (one dose)	180	97.4	2	98.4	100	92.1	13.2	11.1	3.1
P. lilacinus	30 mL (two doses)	320	95.4	3	97.4	82	93.6	14.1	14.1	4.2
P. lilacinus	30 mL (three doses)	320	95.4	3	97.4	83	101.2	17.5	13.3	4.1
Post-infection of *Meloidogyne incognita*										
P. lilacinus	30 mL (one dose)	72	98.9	3	97.4	91	93	15.8	12.1	2.2
P. lilacinus	30 mL (two doses)	200	97.1	4	96.5	98	83.2	12.1	9.1	1.95
P. lilacinus	30 mL (three doses)	200	97.1	2	98.2	93	88.6	14.3	11.1	3.1
LSD 0.05				5.44		2.14	2.83	1.0	0.9	0.16
0.01				7.32		2.87	2.81	1.3	1.25	0.22

*Initial number of *M. incognita* was 2000 larvae pot^{-1}, **1 mL=5.64x 10^4 cfu mL^{-1}

to enhance the growth of faba bean plant, as well as *Rhizobium* nodulation. Therefore, plant shoot and roots dry weight were increased from control 8.20 and 1.75 to 14.1 and 4.1 g pot^{-1}, respectively when *V. chlamydosporum* (V10) was added to the soil at three doses. The corresponding figures for *Rhizobium* nodules number from control 22 to 94 and 92 nodule $root^{-1}$ system, respectively.

The results recorded in Table 3 indicated that *Paecilomyces lilacinus* (P20) succeeded to reduce the population density of *M. incognita* along growing season of faba bean. Reduction in the number of juveniles ranged from 95.4 to 97.4% and from 97.1 to 98.9% in comparison with control either with pre- or post infection, respectively. The numbers of egg masses per root system were significantly ($p<0.01$, 0.05) decreased from 116

Table 4: Evaluation of *Arthrobotrys dactyloides* (A25) *for controlling Meloidogyne incognita** infected faba bean plant, under greenhouse conditions

Treatments	Bio agent Add (mL)	Final No. of *M. incognita* pot^{-1}	Reduction (%)	Egg mass No. on root pot^{-1}	Reduction (%)	*Rhizobium* Nodules/root	Fresh Shoot weight (g) pot^{-1}	Fresh Root weight (g) pot^{-1}	Dry Shoot weight (g) pot^{-1}	Dry Root weight (g) pot^{-1}
Control (infected plant)		7020	0	116	0	22	36.6	5.8	8.2	1.75
A.dactyloides (only)	30 mL** (one dose)	0	0	0	0	78	86.22	14.1	10.1	3.4
Healthy plant (non infected)		0	0	0	0	74	58.8	9.3	9.9	2.1
Pre-infection of *Meloidogyne incognita*										
A. dactyloides (only)	30 mL (one dose)	80	98.8	2	98.2	80	87.2	12.1	12.9	4.4
A. dactyloides (only)	30 mL (two doses)	180	97.4	3	97.4	92	86.1	14.2	11.1	2.9
A.dactyloides (only)	30 mL (three doses)	120	98.2	3	97.4	81	89.5	15.1	12.1	2.3
Post-infection of *Meloidogyne incognita*										
A. dactyloides (only)	30 mL (one dose)	140	98	3	97.4	93	93.9	20.2	15.1	3.5
A.dactyloides (only)	30 mL (two doses)	120	98.2	3	97.4	98	89	18.1	12.1	2.9
A. dactyloides (only)	30 mL (three doses)	160	97.7	4	96.5	80	85	14.3	16.1	3.6
LSD 0.05				3.12		1.51	0.17	0.43	2.17	0.28
0.01				4.20		2.04	0.23	0.58	2.9	0.387

*Initial number of *M. incognita* was 2000 larvae pot^{-1}, **1 mL=5.64x 10^4 cfu mL^{-1}

Table 5: Comparative study between Nameless, the nematicide Furadan and selected fungi *Arthrobotrys dactyloidesa* (A25), *Paecilomyces lilacinus* (P20) and *Verticillium chlamydosporum* (V10) for controlling *Meloidogyne incognita** infecting faba bean plants, under greenhouse c

Treatment	Bio agent Add (mL)	Final No. of *M. incognita* pot^{-1}	Reduction (%)	Egg mass No. on root pot^{-1}	Reduction (%)	*Rhizobium* Nodules/root	Fresh Shoot weight (g) pot^{-1}	Fresh Root weight (g) pot^{-1}	Dry Shoot weight (g) pot^{-1}	Dry Root weight (g) pot^{-1}
Control (infected plant)		7020	0	116	0	22	36.6	5.8	8.2	1.75
Healthy plant (non infected)		0	0	0	0	74	58.8	9.3	9.9	2.1
Nameles®	0.005 g pot^{-1}	480	68	13	88.7	75	60.40	10.2	8.1	2.1
Nematicids (Furadan)	30 mL pot^{-1}	280	96.0	5	95.6	50	45	7.8	6.93	1.41
Pre-infection of *Meloidogyne incognita*										
V. chlamysosp -orum	30 mL** (three doses)	140	98	2	98.2	92	102	20.1	14.1	4.1
P.yc es lilacinu	30 mL (one dose)	180	97.4	2	98.2	100	92.1	13.2	11.1	3.1
A.dactyloides	30 mL (one dose)	80	98.8	2	98.2	80	87.2	12.1	12.9	4.4
Post-infection of *Meloidogyne incognita*										
V. chlamysosp -orum	30 mL (one dose)	72	98.9	2	98.2	91	83.1	11.6	11.1	3.8
P. lilacinu	30 mL (one dose)	72	98.9	3	97.4	91	93	15.8	12.1	2.2
A. dactyloides	30 mL (two doses)	120	98.2	3	97.4	98	89	18.1	12.1	2.9

Ondition *Initial number of *M. incognita* was 2000 larvae pot^{-1}, **1 mL=5.64x 10^4 cfu mL^{-1}

control to 2 in *P. lilacinus* inoculated plants; resulting reduction 98.2%. Application of the fungus showed significant increase in plant growth parameters ($p<0.05$, 0.01) as well as *Rhizobium* nodules over nematode control.

The previous trends were also estimated with *Arthrobotrys dactyloides* (A25) as shown in Table 4, whereas, fungus reduced numbers of the juveniles in nematodes infested soil resulting reduction ranged from 97.4 to 98.8% and from 97.7 to 98% with pre- and post infection, respectively during the growing season. The number of egg masses were also significantly ($p<0.01$, 0.05) decreased. Thus, reduction in egg masses ranged from 97.4 to 98.2% and from 96.5 to 97.4%, were

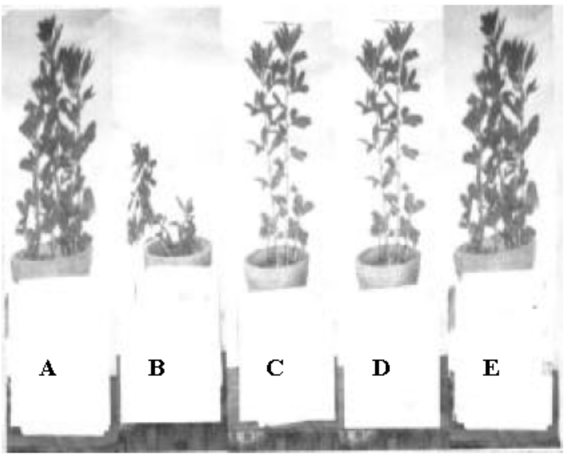

Fig. 1: Biocontol of *Meloidogyne incognita* infecting faba bean (Giza, 40) using *Arthrobotrys dactyloides* (A25), *Peacilomyces lilacimus* (P20) and *Verticilium chlamydosporum* (V10) under greenhouse conditios as follow: (A) Healthy plant, (B) *Meloidogyne incognita* infected plant, (C) *M. incognita* plant and treated by arthrobotrys dactyloides (A25), (D) *M. incognita* plant and treated by *Paecilomyces lilacinus* (P20), (E) *M. incognita* plant and treated by *Verticillium chlamydosporum* (V10)

determined with pre and post infection, respectively, in comparison with control treatment.

Generally, from after mentioned data, it could be confirmed potentialities of *Arthrobotrys dactyloides* (A25), *Paecilomyces lilacinus* (P20) and *Verticillium chlamydosporum* (V10) for controlling *M. incognita* infested soil cultivated with faba bean plants, as well as their potentialities to improve faba bean plant growth (Fig. 1). The selected fungal also revealed pronounced effect on *Rhizobium* to invade roots system. The potentialities of the selected fungi strains to reduce population density of *M. incognita* were almost similar to that of chemically synthetic (Furdan) and better than Nemaless® (Commercial preparation of bio-Nemticide) as shown in Table 5.

DISCUSSION

This study confirmed the suppressive effect of *Arthrobotrys dactyloides*, *Paecilomyces lilacinus* and Pochonia chlamydosporia on the population density of the *Meloidogyne incognita* as well as their potentialities to reduce invasion of juveniles larvae to plant root system of faba bean. Similar results were reported by Al-Hazmi *et al.*[15]. they found that addition of *A. conoides* to *M. incognita* infested soil suppressed juvenile number and root galls development. In this concerns, Duponnois *et al.*[16]. confirmed that different species of *Arthrobotrys nematophagous* fungi and several strains of *A. oligospora* were antagonistic against nematodes of *M. incognita* and *M. mayaaguensis* juvenile's *in vitro*. In another study Persson and Jansson[17] reported that *A. dactyloides*, *A. superba* and *Monacrasporium ellipasasporum* were the most frequently in the tomato rhizospere that was infected by *M. incognita*. The network-forming *A. superba* grew rapidly during the first two weeks after introduction to soil, while the other fungi tested had slower growth rates. Bordallo *et al.*[18] stated that nematophagous fungi colonized endophytically monocotyledon and dicotyledonous plant roots. *A. oligospora* seemed to be more aggressive than *V. chlamydosporium* on barley roots. Both fungi induced cell wall modifications, but did not prevent growth. The response of root cells to colonization by nematophagous fungi may have profound implications in the performance of these organisms as biocontrol agents of plant parasitic nematodes. This idea was confirmed by our study and others. In this connection De Leij and Kerry[19] reported the potential of *V. chlamydosporium* as a biological control agent against *M. arenaria* on tomato plants. Significant population reductions greater than 80% after the first nematode generation were achieved. De Leij *et al.*[20] reported in a microplot experiment on sandy loam *V. chlamydosporium* controlled population of M. hapla on tomato plants by more than 90%.

Some reports confirmed that *P. lilacinus* colonised root tissue in its interaction with *M. incognita*[21,22]. The authors showed that *P. lilacinus* propagules in the soil were correlated to the initial dose applied and decreased progressively through time with increased dosage.

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