EFFECT OF SINGLE AND MIXTURE OF ANTAGONISTIC FUNGI ON THE CONTROL OF ROOT-KNOT NEMATODE,MELOEDOGYNE INCOGNITA

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Abstract

Three antagonisitc fungi reduced the percentage of egg hatching and larvae activity compared to control treatment under laboratory conditions . *The antagonistic fungi were Paecilomyces lilacinus, Trichoderma harzianum* and *Epicoccum* sp. Significant reduction was observed in the viability of larvae of root - knot nematode when two different antagonsitic fungi were conbined as compared to treatments whith single biocontrol agents. These three different fungi were used under green-house and field conditions. Although there was a clear and significant difference in the effect of different antagonistic fungi under green-house conditions there was no differrence between *Paecilomyces* and *Epicoccum* under field conditions. Significant reduction in disease incidence or root-knot. index were observed where the rate of application of antagomisits was increased from one gram / pot to two grams / pot . On the other hand, no significant difference was observed between two grams / pot and three grams / pot for *Paecilomyces* and *Trichoderma. Paecilomyces lilacinmus* proved to be a good biocontrol agane in reducing the disease incidence or suppressing root - knot index. It also retarded egg hatching and reduced the viability of larvae, if it was used alone or with other antagonistic fungi in green - house and field conditions as compared to the control .

INTRODUCTION

Biological control is gaining increasing significence throughout the world due to the high costs of chemical pesticides and hazards involved. Shahzad & Ghaffar (1987) found that *Paecilomyces lilacinus* (Thom) Samson, a parasite of *Meloidogyne* eggs (Jatala 1985), effectively restricted root - knot disease on mung beans , okra and gram. Use of P. *lilacinus* showed better results than those obtained by Furadan, a nematicide commonly used . Similarly, *Pasteuria penetrans* (Thorne) Sayre & Starr a parasite of root - knot nematode (Mankaau 1980 , Sayre 1980) showed control of these pathogens similar to that by nematicides (Stirling 1984). The effects of *Paecilomyces lilacinus* and *Pasteuria penetrans* showed similar results in supprssing root-knot nematodes as compared to the control . Using these pathogens together, suppression in RKI was significant (P = 0.05) as compared to treatments with single biocontrol agents . Use of microorgnisms in the biological control of root kmot nematodes holds promise, and the combined use of more than one biocontrol agent can be more beneficial. Abd-El-Moity *et. al.* (1985) found that *Trichoderma harzianum* (isolate No. 25) reduced the root-knot *M. javanica* and also increased the fresh weight of treated plants as compared with control . The biocontrol agent of *Peacilomyces lilacinus* reduced the root-knot nematode, *M. incognita* population in the soil by 66.05 - 77.3 % on okra, while the nematicide treatment of isazofos (Miral lOG.) gave on 86 . 8 % reduction , and significant yield increases compared with the control (David & Zorilla 1986). Some isolates of *Trichoderma harzianum* and *T. koningi* were found antagonistic and produced metabolites toxic to *Pythium debaryanum.* The results indicated that the effectiveness of the biocontrol agents was no statistically significant from chemicals or soil pasteurization where oospores were used as inoculum . However , the biocontrol agents were significantly better when the mycelium was used for inoculation (Abdel-Rahim and Abu-Surrieh 1990).

Stephan *et al.,* (1990) found that all tested nematicides , solar heating and the fungus *Pacillomyces lilacinus* reduced the number of root-knot nematode , *M. javanica* on cucumber and egg plant . The fungus application showed least satisfactofy effect with no significant differences in yield compared with the control. Saifullag and Saeed (1988) showed that *P. lilacinus* can effectively control the golden cyst nematode in the soil in Pakistan on potatoes. Coating tubers with the fungus was more effective than fungal application to the soil . Hewlett *et al* . 1988 found that the population densities of *P. lilacinus* in the inoculated plots did not control *M. javanica* and no assitive or synergistic activity was observed in any of the fungicide - nematicide combinations on tobacco. The low number of fungal propagules detected on root segments and at soil levels below 15 cm indicate that *P. lilacinus* was unable to conlonize the rhizosphere of tobacco roots and did not move down through the soil with the roots. Galls and egg-masses collected from tobacco roots below 15 cm level, therefore were not exposed to a high population of the fungus.

MATERIALS AND METHODS

Three different antagonistic fungi and one species of *Meloidogyne* were used in these studies . These were *Paecilomyces lilacinus* , *Trichoderma harzianum, Epicoccum* sp. and one root - knot nematode , *Meloidogyne incognita.*

Propagation of antagonists :-

The antagonistic fungi were grown on liquid gliotocxin fermentation medium for 7 days and under complete darkness . Cultures were then blended and numbers of propagules were adjusted to 3 X 10 ^{6} propagules / ml . Granules of the antagonists were prepared according to the method developed by Abd. El - Moity (1986) . Eggs of *M. Meloidogyne* were collected from tomato crops in the field. By means of morphometric studies (perineal patterns) the nematode was identified as *M. incognita* . This nematode was cultured on tomato plants cv. Pritchard in the greenhouse. The nematode eggs extracted from tomato roots were washed with 0.5 % sodium hypochlorite.

Laboratory experiments :-

Five ml of antagonistic fungi or five ml of nematode inoculum (500 eggs / 1 ml .) were placed in small vials (5- ml. in capicity) and were incubated at 25^{o} C . Samples of these mixturess were examined under the microscope every 24 h for one week and the percentage of hatchimg and viability of larvae were recorded.

Green-house experiments : -

1- Evaluation of different antagonistic fungi:

Pots , 20 cm in diamter , filled with light clay soil were used . Fifty ml. of nematode suspension (about 100 eggs per 1 ml .) were added to each pot. Five tomato transplants, var. Pritcherd, were transplanted in each pot. Antagonistic fungi were added at time of planting at the rate of 1 gram granules/pot. Pots were then kept in air-conditioned greenhouse at 20 - 25^{o} C. After two months , plants were

Table 1. Host Plants of *S. amygdali* in Egypt.

No.	English name	Scientific name	Locality.	Author	Year
1	Peach	*Prunus persica*	---	Willcocks	1924
2	Apricot	*Pr. arminiaca*	---	"	"
3	Plum	*Pr. Triflora*	---	"	"
4	Almond	*Pr. amygdalus*	---	"	"
5	Apple	*Pyrus Malus*	---	"	"
6	Pear	*Py. Communis*	---	"	"
7	Quince	*Py. Cydonia*	Damietta	Present survey	1980
8	Nectarin	*Pr. persica nectarina*	Giza	" "	1981
9	Pecan	*Carya illinoesis*	Qalubia	" "	"
10	Loquat	*Eriobotrya japonica*	Alexandria	" "	1985
11	Olive	*Olea europea*	Fayoum	" "	1986
12	Pomegranate	*Punica granatum*	Matrouh	" "	"
13	Annonas	*Annona squamosa*	Alexandria	" "	"
14	Sweet orange	*Citrus sinensis*	Suez	" "	"
15	Carob	*Ceratonia siliqua*	Alexandria	" "	1987
16	Jujube	*Zizyphus vulgaris*	Suez	" "	1989
17	Persimmon	*Diospyrus Kaki*	Behera	" "	1990

Table 2. Percentages of *S. amygdali* infestation in peach orchards allover the governorates of Egypt.

Governorate	% Infestation		Governorate	% Infestation	
	Average	Range		Average	Range
Matrouh	16.2	0 - 32	Giza	31.4	12 - 49
Alexandria	28.6	15 - 43	Fayoum	4.4	0 - 8
Kafr El-Sheikh	9.6	0 - 17	Beni-Seuf	6.8	0 - 19
Damietta	10.4	0 - 18	Minia	3.2	0 - 6
Behera	37.0	19 - 87	Assiut	5.0	0 - 7
Gharbia	42.2	18 - 64	Sohag	14.6	6 - 23
Dakahlia	56.6	12 - 81	Quena	18.6	0 - 35
Skarkia	20.8	0 - 66	Aswan	6.8	0 - 10
Ismailia	25.2	0 - 72	North Sinai	25.4	0 - 46
Menofia	35.4	8 - 99	South Sinai	7.4	0 - 12
Qalubia	29.6	13 - 46			

General average : 21.58 %
General range : 0 - 99%

Table 1. In vitro effect of the antagonistic fungi on egg hatching and activity of larvae one week after inoculation with *Meloidogyne incognita.*

Treatments	% of non hatrchin eggs	% of heaching eggs		% of active juveniles
		active	in active	
Paecilomyces lilacinus	41.6	26.7	31.7	45.7
Trichoderma harzianum	22.2	20.0	57.8	25.7
Epicocum sp.	37.0	35.2	27.8	55.9
Paecilomyces lilacinus + *Trichoderma harzianum*	23.5	49.4	27.1	64.6
Paecilomyces lilacinus + *Epicocum* sp.	41.6	6.3	52.1	10.7
Trichoderma harzianum + *Epicocum* sp	36.7	4.3	59.0	6.3
Paecilomyces lilacinus + *Trichoderma harzianum* + *Epicocum* sp.	26.0	29.7	44.3	40.0
Control	9.8	76.5	13.7	84.8

In Tabe (2) single or combined antagonistic fungi reduced the root-knot disease as compared to control . Using these agents together (*Trichoderm + Paecilomyces*) supperssion in RKI was greater as compared to treatmernts where the biocontrol agents *Trichoderma* or *Epicocum* were used alone. Using *Trichoderma + Epicocum* or *Paecilomyces + Epicocum* showed similar results in suppressing root - knot index lower than that nesulting from *Trichoderma or Paecilomyces* alone. These data showed that *Epicocum* adversely inerfered with the antagonistic effect of *Paeecilomyces* or *Trichoderma* when used togethr. Clear antagonism appeared with *Paecilomyces* alone or combined with Trichoderma . On the other hand , *Epicocum* sp. was the least antagonistic fungus against *M. incognita.*

Table 2. Effect of different antagonistic fungi on the root-knot nematode , *Meloidogyne incognita* under green-house conditions.

Treatments	Root - knort Index (RKI)	% incidence
Paecilomyces lilacinus	0.9	17.3
Trichoderma harzianum	1.4	27.2
Epicocum sp.	2.1	39.1
Paecilomyces lilacinus + *Trichoderma harzianum*	1.1	20.5
Paecilomyces lilacinus + *Epicocum* sp.	1.6	30.1
Trichoderma harzianum + *Epicocum* sp.	1.6	31.2
Control 1	3.0	58.8
Control II	2.7	50.6

The different activites of the biocontrol fungi appeared in reducing the root - knot disease and root-knot index compared with the control. There was no difference between the rate of two or three grams of *Paecilomyces or Trichoderma* pot while no difference was observed between one, two or three grams of *Paecilomyces* or *Trichoderma*, pot while no difference was observed between one, two or three grams / pot for *Epicocum* sp Table 3 . Therfore, the rate of 2 g/pot was considered an appropnate biocontrol dose. Increasing the rate (from 2 gr. to 3 gr ./ pot) does not increase the suppression of RKI on tomato roots by any appreciabe amount . Croshier *et al* . (1985) in Chile obtained an increase in the percentage of *Meloidogyne javanica* eggs parasitized by increasing the rate of *Paecilomyces lilacinus* (from O.O to 1.1gr / pot) on tomato roots . Sifullah and Saeed (1988) , in Pakistan, concluded that *P. lilacinus* can effectively control the golden cysl nematode, (*Globodera rostochiensis*) in the soil by using two rates of either 7 or 14 g of infected wheat grains per pot.

Table 3. Effect of different rates of antagonistic fungi on root-knot nematode , *Meloidogyne* incognita under green - house conditions.

Treatments	Rate of application g/ 20 cm pot	RKI	% disease incidence
Paecilomyces lilacinus	1	1.6	30
	2	1.2	23
	3	1.3	23
Trichoderma harzianum	1	1.8	35
	2	1.4	28
	3	1.6	29
Epicocum sp.	1	2.3	39
	2	2.0	38
	3	1.9	37
Control 1 (with pathogen)	-	3.2	62
Control II (with organic matter)	-	2.9	58

Under field condition , data indicated that biocontrol agents reduced the percentage of nematode disease and root-knot index of *M. incongnita* as compared to control (Table 4) . These data conformed with Stephan *et al* (1990) which indicated that all tested nematicides, solar heating and the fungus *Paecilomyces lilacinus* reduced the number of root-knot nematode, *Meloidogyne javanica* on cucumber and eggplant. Althohgh no differences between the treatmetns in suppressing the RKI , were obsserved, however, satisfactory control was obtained. In general, the biocontrol agent, *Paecilomyces lilacinus* gave good results in retarding egg hatching, suppressing the RKI , and reducing the nematode incidence when used alone and or combined with other antagonistic agents under greenhouse and field conditions as compared with control.

Table 4. Efficacy of *Paecilomyces lilacinus* , *Trichoderma harzianum* and *Epicocum sp.* in the control of the root - knot nematode, M. incognita on tomato under field conditions.

Treatments	Root - knort Index (RKI)	% incidence
Paecilomyces lilacinus	1.6	23.3
Trichoderma harzianum	1.7	30.2
Epicocum sp.	1.6	38.3
Control 1	3.0	69.8
Control II	2.4	55.2

(RKI) : Root - knot index " O = no galls ; I = 1 -2 galls;

2 = 3 - 10 galls ; 3 = 11 - 30 gals ; 4 = 41 - 100 galls and

5 = 100 + galls (Taylor & Seasser ; 1987)

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تأثير بعض الفطريات المضادة بمفردها ومعا على مقاومة نيماتودا تعقد الجذور (ميلود جين انكوجنيتا)

توفيق حافظ عبد المعطى ، فاروق وهبه رياض ، صلاح العراقى
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تم اختيار ثلاث فطريات مضادة وهى

Paecillumyces lilacinus, Trichoderma harzianum , *Epicocum* sp.

حيث قللت نسبة فقس البيض وايضاً حيوية اليرقات لنيماتودا تعقد الجذور تحت ظروف المعمل . وقد لوحظ ان اضافة فطرين مختلفين معاً اعطى نقصاً ايجابياً فى حيوية اليرقات بالمقارنة بإستعمال فطر بمفرده.

وقد استعملت هذه الفطريات الثلاثة تحت ظروف الصوبة والحقل .

وبالرغم من ان هناك اختلافات بين هذه الفطريات تحت ظروف الصوبة ، لوحظ عدم وجود اختلافات واضحة تحت ظروف الحقل .

وكان هناك نقص واضح فى نسبة او معامل التعقد عندما ازيد معدل اللقاح للفطر ١ جم / أص الى ٢ جم / أص. بينما لايوجد اختلاف واضح بين الجرعات ٢ ، ٣ جم / أص للفطر , *Paecilomyces* *Trichoderma*ويعتبر الفطر *Paecilomyces lilacinus* افضل الفطريات المضادة المختبرة فى تقليل نسبة حدوث المرض وخفض معامل التعقد وايضاً يؤثر على تأخير فقس البيض وتقليل نسبة حيوية اليرقات اذا ما استعمل بمفرده او مع فطر آخر تحت ظروف الصوبة أو الحقل اذا ما قورن بالمقارنة .