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Biocontrol activity of some bacterial isolates against *Meloidogyne incognita*

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ABSTRACT

The influence of some bacterial isolates of *Mycobacterium phlei*, *Micrococcus* spp., *Escherichia coli*, *Bacillus subtilis*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Sarcina* spp. were evaluated as biocontrol agents against the root-knot nematode, *Meloidogyne incognita* infecting eggplant, (*Solanum melongena*) under greenhouse conditions. The inoculum of each isolate containing $1x10^9$ cells ml^{-1} was added to the soil at the rate of 2.5, 5 and 10 ml per pot. Potential effect of such agents on development and reproduction of *M. incognita* were estimated. Results indicated that most of the tested bacterial isolates significantly reduced numbers of galls, developmental stages, egg masses in roots, and second stage juveniles (J_2) in soil. Consequently, nematode rate of reproduction was decreased. The degree of nematode suppression was proportional to inoculum size of the bacterial culture added to the pots. Moreover, application of bacterial inoculants improved the growth of eggplant regardless to bacterial isolate or inoculum size.

Key Words: Nematode, *Meloidogyne incognita*, Rhizobacteria, Biological control.

INTRODUCTION

Plant parasitic nematodes have caused great losses to economic crops world wide. Attempts have been devoted to eliminate a such effect by using chemical nematicides. However, with the increasing of environmental hazards, it is necessary to develop safe alternative methods such as biological control, in which microorganisms are selected for their ability to antagonize pathogens.

A wide range of microorganisms including several fungi, bacteria, soil invertebrates and predatory nematodes have been regarded as potential bio-agents (Stirling, 1991).

The presence of specific naturally occurring or introduced rhizobacteria can significantly modify the rhizophere environment and affect the nematode or the host parasitic interrelationship directly or indirectly.

Therefore, rhizobacteria have been evaluated for their antagonistic effects on plant-parasitic nematodes including *Meloidogyne incognita* (Becker *et al.*, 1988 and Kloepper *et al.*, 1992); *M. hapla* (Honglin *et al.*, 1995); *M. javanica* (Al-Shalaby and Sedik, 2003); *Heterodera glycines* (Kloepper *et al.*, 1992 and Honglin and Riggs, 2000); *H. Schachtii* (Oostendrop and Sikora, 1989 and Neipp and Becker, 1999); *Globodera pallida* (Racke and Sikora, 1992); and *G. rostochiensis* (Cronin *et al.*, 1997).

Many strains of *Serratia* spp. and *Pseudomonas* spp. have been recorded as effective antagonists to plant parasitic nematodes (Becker *et al.*, 1989 and Zavaleta – Meija and VanGundy, 1989).

Also, *Bacillus* spp. have been regarded as antagonists against some plant parasitic nematode species belonging to the genera *Meloidogyne, Heterodera* and *Rotylenchulus* (Madamba *et al.*, 1999 and Li *et al.*, 2005).

In addition, the actinomycetes have been considered as one of the main groups of interest, which produce antibiotics with suppressive effects against other organisms (Omura, 1986). *Streptomyces* spp. were negatively effective on egg hatching and juvenile survival of *M. javanica* (El-Sherif *et al.*, 1994 and Ali, 1996).

Some bacterial metabolites, such as avermectins (Stretton *et al.*, 1987), valinomycin (Mishra *et al.*, 1987), and 2,4-diacetylphloroglucinol (Cronin *et al.*, 1997) as well as volatile metabolites such as various organic acids, hydrogen sulfide, and ammonia have adverse effects on nematode development (Stirling, 1991).

The objective of this research was to evaluate the antagonistic potential of seven bacterial isolates of *Mycobacterium phlei*, *Micrococcus* spp., *Escherichia coli, Bacillus subtilis, Serratia marcescens, Pseudomonas aeruginosa* and *Sarcina* spp. on development and reproduction of *M. incognita* parasitizing eggplant grown under greenhouse conditions.

MATERIALS AND METHODS

1- Bacterial isolates:

A number of bacterial isolates belonging to *M. phlei, Micrococcus* spp., *E. coli, B. subtilis, S. marcescens, P. aeruginosa* and *Sarcina* spp. previously isolated from Egyptian sandy soils by Sedik (1997), were studied to detect their efficacy in controlling *M. incognita.*

The isolates were purified and identified according to API microtube system; API 20B and API 20E (Logan and Berkeley, 1984).

2- Growth media and conditions:

A single colony of each representative previously mentioned isolates was transferred into CCM liquid culture medium (Hegazi *et al.,* 1998). Then, the sterilized flasks containing 100 ml of CCM liquid medium were individually inoculated with 1 ml of 24 hours old culture of the tested bacterial isolate. Inoculation took place at 25-28°C for 3-5 days with a continuous shaking at the rate of 140 rpm. The bacterial cells were harvested by centrifugation at 4000 rpm for 15 minutes after 48 hours. The bacterial inoculum was prepared for each isolate in a liquid culture containing $1x10^9$ cells ml^{-1}. The inoculum of each strain was added to the soil at the rate of 2.5, 5 and 10.0 ml/Kg soil/pot.

3- Biological activity:

Plastic pots of 12 cm diameter each was filled with 1 kg sandy clay soil (2: 1, v:v) and four weeks old seedlings of eggplant cv. Black Beauty were transplanted into pots. The bacterial inoculants were added to the soil seven days before nematode inoculation. Thereafter, the pots were inoculated with 1000 newly hatched juveniles (J_2) of *M. incognita* per pot.

A set of pots was inoculated with the nematode only at the above-mentioned inoculation rate (check 2); and other one received the nematode inoculation and plain media (check 1). Each treatment was replicated five times and all the pots were arranged in a completely randomized design on a green house bench. All pots received normal agricultural practices.

After 45 days, the experiment was terminated. Plant growth parameters based on length and weight of shoots and roots were recorded. In addition, the number of galls, nematode counts in soil and roots were determined to calculate the nematode final population and rate of reproduction.

Statistical analysis:

The least significant difference using MSTAT microcomputer statistical program (Power *et al.,* 1982) performed the statistical analysis.

RESULTS AND DISCUSSION

1- Effect of bacterial isolates on development and reproduction of *M. incognita*:

The nematicidal activities of bacterial isolates on development and reproduction of *M. incognita* infecting eggplants are presented in Table (1). Data indicated that all bacterial isolates were effective in controlling *M. incognita.* In general, all tested inoculum levels of all isolates significantly reduced the number of galls on egg plant roots comparing with those of the two checks. Evidently, no significant differences in all values were detected between both check treatments. The number of galls was gradually decreased by increasing the bacterial cell concentration.

In addition, all bacterial inoculants greatly reduced the nematode counts either in soil or in roots. Likely, they all significantly reduced counts of the nematode developmental stages and egg-laying females in roots as well as those of (J_2) in soil. In addition, fecundity of the nematode based on number of eggs/egg mass was also affected by the bacterial strains.

Consequently, the nematode final population and its rate of build-up were reduced in all treatments when compared with those of the checks. A negative correlation was detected between bacterial inoculation rate and the nematode build-up in all treatments.

Comparatively, treatments of *Sarcina* sp., *B. subtilis, M. phlei, S. marcescens* and *P. aeruginosa* achieved the highest adverse effect on nematode reproduction where the rates of build up attained 2.15, 2.40, 2.40, 2.76 and 2.80% respectively in treatments of the highest level of bacterium inoculum (10 ml) followed by those of *Micrococcus* spp. and *E. coli* 3.15 & 4.03%, respectively.

2- Effect of bacterial isolates on growth parameters of eggplant:

Application of the tested bacterial isolates had positive effect on growth of the nematode-infected plants. Growth response of those plants as measured by length and fresh weight of shoots and roots is shown in Table (2).

In general, pronounced improvement was obtained in shoot length of those plants treated with *E. coli, Sarcina* spp. and *S. marcescens.*

Also, application of most bacteria isolates improved shoot weight. On the other hand, the highest percentage of increase in root weight was achieved in treatment of *Sarcina* spp. which was 53.3% on the highest inoculation rate of the bacterial isolate. At the same inoculation level treatment of *P. aeruginosa* achieved 43.3% followed by that of *B. subtilis* (40%). As for root length, *S. marcescens* and *E. coli* caused the highest percentage of increase (19.5%), while the lowest one was obtained by *Sarcina* sp. (10.9%) In general, most tested bacterial isolates did improve growth parameters, regardless to levels of the inoculation rate.

Nematodes in soil are naturally subjected to infections by other microorganisms like bacteria and fungi. This creates the possibility of using these soil microorganisms to control these pests and minimize the excepted damage to their hosts (Jatala, 1986).

A variety of nematophagous bacteria groups have been isolated from soil, host-plant tissues and nematodes and their eggs or cysts (Stirling, 1991 and Kerry, 2000). Bacteria affect nematodes by a variety of modes for example; by parasitizing and/or producing toxins, antibiotics, enzymes; interfering with

Table (1): Effect of some bacterial isolates on nematode development and reproduction of *M. incognita* infecting plants of eggplant.

Bacterial species and inoculum level (ml)	No. of Galls	No. of egg-masses	Developmental stages	Average Eggs/egg mass	No. of J_2	Final pop. (Pf)	Rate of build up Pf/Pi
Mycobacterium phlei							
2.5	201^{d}	147^{d}	66^{b}	340^{c}	5634^{b}	5847^{b}	5.847
5.0	124^{e}	100^{e}	36^{d}	300^{c}	3640^{cd}	3776^{cd}	3.776
10.0	58^{g}	45^{g}	31^{e}	284^{c}	2326^{e}	2402^{g}	2.402
Micrococcus spp							
2.5	364^{b}	311^{b}	87^{a}	414^{b}	5442^{b}	5840^{b}	5.840
5.0	214^{d}	184^{cd}	67^{b}	320^{cd}	4911^{bc}	5162^{bc}	5.162
10.0	146^{de}	124^{de}	49^{c}	318^{cd}	2973^{de}	3146^{e}	3.146
Bacillus subtilis							
2.5	155^{de}	129^{de}	45^{c}	219^{d}	308^{de}	3255^{e}	3.255
5.0	102^{ef}	82^{ef}	36^{d}	197^{e}	2593^{de}	2711^{ef}	2.711
10.0	80^{f}	65^{f}	30^{e}	174^{ef}	2309^{e}	2404^{g}	2.404
Serratia marcescens							
2.5	129^{e}	104^{e}	34^{d}	193^{e}	4334^{c}	4472^{cd}	4.472
5.0	93^{ef}	74^{ef}	30^{e}	179^{e}	3478^{d}	3582^{e}	3.582
10.0	63^{g}	50^{fg}	23^{ef}	164^{f}	2682^{e}	2755^{ef}	2.755
Pseudomonas aeruginosa							
2.5	219^{cd}	192^{cd}	56^{c}	316^{cd}	4603^{c}	4850^{bc}	4.850
5.0	117^{e}	97^{e}	44^{d}	278^{c}	2746^{de}	2888^{ef}	2.888
10.0	58^{g}	41^{g}	31^{e}	241^{d}	2729^{de}	2801^{ef}	2.801
Sarcina spp.							
2.5	281^{c}	250^{bc}	53^{c}	283^{cd}	3246^{d}	3549^{e}	3.549
5.0	148^{de}	127^{de}	40^{d}	252^{d}	2332^{e}	2498^{f}	2.498
10.0	86^{ef}	70^{f}	32^{d}	246^{d}	2047^{f}	2148^{g}	2.148
Escherichia coli							
2.5	275^{c}	248^{bc}	63^{b}	408^{b}	5522^{b}	5832^{b}	5.832
5.0	177^{de}	153^{d}	53^{c}	344^{c}	4877^{bc}	5033^{bc}	5.033
10.0	115^{e}	94^{e}	40^{d}	329^{cd}	3898^{cd}	4032^{cd}	4.032
Check-1 (nematode + media)	488^{a}	460^{a}	89^{a}	521^{a}	8074^{a}	8623^{a}	8.623
Check-2 (nematode only)	490^{a}	465^{a}	92^{a}	524^{a}	8140^{a}	8696^{a}	8.696

Data with the same letters(s) within a column are not significantly different according to New L.S.D. (P=0.05).

Table (2): Effect of some bacterial isolates on growth parameters of plants of eggplant infected with *Meloidogyne incognita.*

Bacterial species and inoculum level (ml)	Shoot				Root			
	Length (cm)	Increase %	Weight (g)	Increase %	Length (cm)	Increase %	Weight (g)	Increase %
Mycobacterium phlei								
2.5	26.25^{bc}	--	7.75^{de}	6.9	21.00^{de}	2.4	6.50^{geh}	--
5.0	29.50^{b}	10.3	8.25^{cd}	13.8	21.50^{de}	4.9	8.50^{def}	13.3
10.0	30.00^{b}	12.1	9.25^{bc}	27.6	23.00^{bc}	12.2	9.50^{cd}	26.7
Micrococcus spp.								
2.5	26.75^{be}	0	6.25^{f}	--	21.75^{de}	6.1	7.00^{fge}	--
5.0	28.00^{bc}	4.7	7.25^{de}	0	21.25^{de}	3.7	8.00^{ef}	6.7
10.0	32.25^{ab}	20.6	9.50^{bc}	31	23.00^{bc}	12.2	8.50^{def}	13.3
Bacillus subtilis								
2.5	25.00^{bc}	--	6.50^{ef}	--	21.25^{de}	3.7	7.25^{fg}	--
5.0	27.50^{b}	2.8	7.50^{de}	3.4	22.50^{cd}	9.8	10.00^{bcd}	33.3
10.0	29.25^{bc}	9.3	8.50^{cd}	17.2	24.00^{ab}	17.1	10.50^{abc}	40
Serratia marcescens								
2.5	28.50^{bc}	6.5	8.00^{cd}	10.3	22.50^{cd}	9.8	8.75^{cde}	16.7
5.0	30.25^{bc}	13.1	10.50^{b}	44.8	24.00^{ab}	17.1	8.50^{def}	13.3
10.0	33.50^{a}	25.2	11.00^{ab}	51.7	24.50^{a}	19.5	9.00^{cde}	20
Pseudomonas aeruginosa								
2.5	27.00^{b}	0.9	8.25^{cd}	13.8	23.00^{bc}	12.2	7.50^{fg}	0
5.0	30.00^{b}	12.1	9.25^{bc}	27.6	23.00^{bc}	12.2	10.00^{bcd}	33.3
10.0	31.25^{ab}	16.8	10.25^{bc}	41.4	24.00^{ab}	17.1	10.75^{abc}	43.3
Sarcina spp.								
2.5	29.50^{bc}	10.3	9.00^{c}	24.1	19.75^{ef}	--	7.00^{fge}	--
5.0	31.50^{bc}	17.8	11.00^{ab}	51.7	21.75^{de}	6.1	8.75^{cde}	16.7
10.0	34.25^{a}	28.0	11.25^{ab}	55.2	22.75^{cd}	10.9	11.50^{ab}	53.3
Escherichia coli								
2.5	31.25^{ab}	16.8	8.00^{cd}	10.3	21.25^{de}	3.7	6.75^{ge}	--
5.0	33.50^{ab}	25.2	10.50^{b}	44.8	23.25^{bc}	13.4	8.00^{ef}	6.7
10.0	34.50^{a}	29	12.50^{a}	72.4	24.50^{a}	19.5	9.00^{cde}	20
Check-1 (nematode + media)	26.75^{bc}	0	7.00^{e}	--	21.00^{def}	2.4	7.00^{fge}	--
Check-2 (nematode only)	26.75^{bc}	--	7.25^{e}	--	20.50e^{fg}	--	7.50^{fg}	--

Data with the same letters(s) within a column are not significantly different according to New L.S.D. (P=0.05).

Nematode-plant-host recognition; competing for nutrients, including systemic resistance of plants; and promoting plant health (Siddiqui & Mahmood, 1999).

The majority of the tested bacterial isolates in this research was suppressive to *M. incognita* on eggplant, when added to the soil one week before the nematode inoculation and this is in agreement with that of Al-Shalaby and Sedik (2003).

This positive effect was noticeable in reducing gall formation as well as the nematode rate of build-up. Evidently, the degree of nematode suppression was proportional to the bacterial inoculum level added to the soil. *B. subtilis* was, however, the most effective nematode antagonist bacterium and this is matching with results of many investigators (Gokta & Swarup, 1988; Kloepper *et al.,* 1992; Madamba *et al.,* 1999 and Li *et al.,* 2005). Such bacteria produce antibiotics like Bacillomycin D (Moyne *et al.,* 2001), Iturin A (Kloepper *et al.,* 2004) and Mycosubtilin (Leclere *et al.,* 2005).

In addition, *Serratia* spp. is proved to be an effective bio-agent against *M. incognita* due to production of antibiotics and the induction of systemic resistance (Spiegel *et al.,* 1991; Siddiqui & Shaukat, 2003). *Pseudomonas* spp. are among the dominant populations in the rhizosphere that are able to antagonize nematodes (Krebs *et al.,* 1998). For example, *P. fluorescens* controls cyst nematode juveniles by producing several secondary metabolites such as 2, 4-diacetylphoroglucinol (DAPG) (Cornin *et al.,* 1997). Rhizosphere *Pseudomonas* isolates also exhibited diverse pathogenic mechanisms upon interaction with nematodes (Kerry, 2000, Siddiqui *et al.,* 2005).

The mechanisms employed by some *Pseudomonas* isolates to reduce the plant parasitic nematodes populations have been studied. Thus, the role of such antagonists depends on the production of antibiotics and the induction of systemic resistance (Spiegel *et al.,* 1991; Siddiqui & Shaukat, 2002, 2003). Moreover, most rhizobacteria act against plant parasitic nematodes by means of metabolic by-products, enzymes and toxins (Zukerman & Jasson, 1984 and Siddiqui & Mahmood, 1999).

Ammonia produced by ammonifying bacteria during decomposition of nitrogenous organic materials can result in reduced nematode population in soil (Rodriguez-Kabana, 1986). Some other rhizobacteria reduce deleterious organisms and create an environment more favorable for plant growth by producing compounds such as antibiotics or hydrogen cyanide (Zuckerman & Jasson, 1984).

Recently, rhizobacteria-mediated induced systemic resistance (ISR) in plants has been shown to be active against nematode pests (Ramamoorthy *et al.,* 2001). Plant growth-promoting rhizobacteria (PGPR) can bring about ISR by fortifying the physical and mechanical strength of the cell wall by means of cell-wall thickening, deposition of newly formed callose, and accumulation of phenolic compounds. They also change the physiological and biochemical ability of the host to promote the synthesis of defense chemicals against the challenge pathogen *e.g.* by the accumulation of pathogensis-related proteins, increased chitinase and peroxidase activity, and synthesis of phytoalexin and other secondary metabolites (Ramamoorthy *et al.,* 2001).

In conclusion, this study assured the potential activity of some isolates of bacteria against the root-knot nematode, *M. incognita* and can offer a promising bio-control tool in nematode management programs.

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الملخص العربى

التأثير البيولوجى لبعض العزلات البكتيرية فى مكافحة نيماتودا تعقد الجذور *Meloidogyne incognita*

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تم تقويم تأثير سبع عزلات مختلفة من البكتريا هى: *Mycobacterium phlei, Micrococcus* spp., *Escherichia coli*, *Bacillus subtilis*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Sarcina* spp. فى المكافحة الحيوية ضد نيماتودا تعقد الجذور *Meloidogyne incognita* على نباتات الباذنجان تحت ظروف الصوبه وذلك لمعرفة تأثيرها على نمو وتكاثر النيماتودا. وقد أضيفت العزلات البكتيرية السابقة بثلاث معدلات مختلفة وهى ٢,٥، ٥,٠، ١٠,٠ ملل/أصيص لقاح يحتوى على ١×١٠^{٩} خلية بكتيرية/ملل. وقد أوضحت النتائج المتحصل عليها أن التلقيح بغالبية الأنواع البكتيرية المختبرة أدى إلى خفض عدد العقد النيماتودية المتكونة على جذور نباتات الباذنجان وكذلك أعداد الأطوار النيماتودية المختلفة وأعداد كتل البيض المتكونة على الجذور وأعداد الطور اليرقى الثانى الموجود فى التربة. ونتيجة لهذا أنخفض معدل تكاثر النيماتودا مقارنة بالنباتات غير الملقحة بالبكتريا. كذلك وجد هناك ارتباط عكسى بين حجم اللقاح البكتيرى المضاف ومعدل تكاثر النيماتودا. وقد وجد تحسن ملحوظ فى نمو نباتات الباذنجان الملقحة بالبكتريا من خلال تقدير كل من الأطوال والأوزان لكل من السيقان والجذور لنباتات الباذنجان.