EFFICACY OF ANTAGONISTIC RHIZOBACTERIA ON THE CONTROL OF ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* IN TOMATO PLANTS

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Abstract

Three antagonistic bacteria, *Bacillus thuringiensis, Bacillus subtilis* and *Pseudomonas flourescens*, were evaluated throughout this investigation for the control of root-knot nematode, *Meloidogyne incognita*, on tomato plants. All antagonistic bacteria were used by adding 150 ml broth culture adjusted to 10^8 cfu/ml for each treatment.

Both *Bacillus thuringiensis* and *Pseudomonas fluorescens* isolates were most effective in nematicidal activity against hatched juveniles and adults of *M.incognita*. On the other hand, the mortality levels of juveniles and adults of *M.incognita* increased with the highest concentrations of bacterial cells (10^5, 10^8 and 5×10^8 cfu/ml).

Generally, the percentage of gall formation and root-gall index were decreased when the antagonistic bacteria were introduced prior to nematodes compared to the simultaneous introduction of both the nenatode and bacteria. Furthermore, it can be concluded from the obtained data that plant parasitic nematodes were subjected to a wide range of anutibiotic-producing antagonists *Ps. flourescens* or sporecrystal mixtures of *B.subtilius*, and *B.thuringiensis*, respectively.

INTRODUCTION

Tomato, *Lycopersicon esculentum*, is one of the major vegetable crops in Egypt. Root-knot nematodes, *Meloidogyne* spp., have been recognized as a potentially serious problem to the crop productivity (Oteifa and El-Gindi, 1956, Oteifa *et al.*, 1964 and Ibrahim *et al.*, 1986. *Meloidogyne* spp. normally exists in the newly reclaimed areas, which presently constitute the further expansion of agricultural lands in Egypt, cause serious economic losses to tomato plants (Salem *et al*, 1974; Franklin, 1979 and Lamberti, 1979).

In recent years, the awareness of the nematicides hazards to humans and environment has directed the attention towards soil-borne antagonists as an altemative method to chemical control.

Biological control is gaining increasing role throughout the world for nematode suppression. *Bacillus penetrans* (Thorne) Mankau is reported as a biocontrol agent

against root-knot nematodes (Mankau, 1980; Sayer, 1980; Sterling, 1984 and Shahzad *et al.*, 1990). The nematicidal activity of the spore-crystal mixtures of three isolates of *Baacillus thuringiensis* against hatched juveniles and adults of *Caenorhabditis elegans* was investigated by Frederik *et al.* (1995). Nematode mortality was observed from 8 hours incubation, and a concentration of at least 10^8 cfu/ml was necessary to cause nematode mortality higher than 30%.

The objective of this study was to evaluate the influence of some antagonistic bacteria as biocontrol agents on the root-knot nematode.

MATERIALS AND METHODS

1. Isolation of antagonistic bacteria

Rhizosphere-rhizoplane colonizing bacteria were isolated from fresh roots of tomato and cotton plants from different areas and soil types. After shaking 10 g root segments and adhering soil in 90 ml of sterile distilled water for 30 minutes, fluorescent pseudomonads were isolated using King's B medium, (King *et al.*, 1954). Different species of *Bacillus* were isolated on nutrient glucose agar medium (NGA) (Dowson, 1957).

All antagonistic bacteria were used throughout this investigation by adding 150 ml of 96 hr nutrient glucose broth culture, adjusted to $5x10^8$ cfu/ml, to each pot containing 5 kg sandy loam soil.

II. Preparation of nematode inoculum

The rot-knot nematode, *Meloidogyne incognita* was obtained from galled tomato roots collected from infected plants. The identified nematode was maintained and propagated on the sensitive tomato variety Supermarmande grown in greenhouse, 2nd stage active larvae were collected and prepared in water suspension. This suspension was adjusted to contain 100 larvae/ml .

III. Bioefficacy of antagonistic bacteria

To test the efficacy of bacterial isolates in inhibiting the activity of *M.incognita* juveniles *in vitro*, 1 ml of each bacterial isolate ($5x10^8$ cfu) was added to 1 ml of nematode suspension in Petri dishes. The plates were incubated at 25^oC for two periods e.g. 4 and 24 hours. The number of active and non active juveniles were counted microscopically.

IV. Greenhouse studies

Tomato seedlings (cv. Supermarmande), 35 days old were planted in 25 inch pots filled with 5 kg sandy loam soil each. Three sets of treatments were used:

1. Treatment of plants with antagonistic bacteria before adding the active nematode.
2. Treatment of plants with antagonistic bacteria at the same time of adding the nematode larvae.
3. Treatment of plants with nematode active larvae without antagonistic bacteria as control.

Active nematode larvae were used at the rate of 3000 newly hatched 2nd stage Juveniles/pot.

After 60 days, all plants were carefully uprooted and root gall index (RGI) was determined in each treatment according to Taylor and Sasser (1978). The treatments were designed in four replicates. Data obtained in this study were statistically analyzed and the LSD at 5% level was calculated.

RESULTS AND DISCUSSION

Nematicidial Bio-assay

Different Ps. *fluorescens, B.subtilis* and *B.thuringiensis* isolates caused low percentage of mortality of the juveniles and adult of *M.incognita*. In the first experiment, it is clear from data in Table (1) that no mortality was observed during the first 4 hours. After 24 hours, the mortality caused by *B.subtilis*, Ps. *fluorescens* and B.thuringiensis increased to 71.9 - 86.1% compared with control (3.3%). In the second experiment (Table 2) data generally indicate that mortality increased with increasing the concentration of antagonistic bacteria to reach a range of 72.0 - 93.5% at $5x10^8$ cfu/ml.

At a concentration of 10^8 cfu/ml data show difference in mortality percentage with antagonistic bacteria, *B.thuringiensis*, Ps. *fluorescens* and *B.subtilis* being 75.0, 67.8 and 60.0 respectively, while the percentages of mortality were 93.5, 84.8 and 75.0 at $5x10^8$, respectively. Data obtained are in agreement with those obtained by Hanna *et al.* (1995) who mentioned that *B.subtilis* and *B.thuringiensis* were effective against root-knot nematode *M.incognita* on tomato plants.

The present data confirm the nematicidal activity of *B.thuringiensis* and *Ps. fluorescens* isolates against hatched juveniles and adults of *M.incogneta* expressed in terms of galling indices.

Table 1. Effect of incubation time on nematicidal activity during the first 4 and 24 hours incubation period.

Treatment	No. of larvae/ml					
	During the first 4 hours			During 24 hours		
	Dead	Alive	%Mortality	Dead	Alive	%Mortality
Ps.fluorescens+M.Incognita	1	311	0.3	256	56	82
B.subtilis+M.incognita	0	282	0.0	203	79	71.9
B.thuringiensis + M.incognita	2	279	0.7	242	39	86.1
M.incognita (Control)	0	327	0.0	11	316	3.3

Table 2. Effect of colony-forming units (cfu) per ml of antagonistic bacterial isolates on nematicidal activity at 24 hours.

Treatment	No. of larvae/ml								
	10^5 (cfu)			10^8 (cfu)			$5x10^8$ (cfu)		
	Dead	Alive	% Mortality	Dead	Alive	% Mortality	Dead	Alive	% Mortality
Ps.fluorescens+M.incognita	1	308	0.3	207	98	67.8	263	47	84.8
B.subtilis+M.incognita	0	295	0.0	186	124	60.0	225	75	75.0
B.thuringiensis + M.Incognita	3	292	1.0	240	80	75.0	290	20	93.5
M.incognita (Control)	0	330	0.0	3	320	0.9	5	315	1.5

A differential susceptibility between nematode stages for nematicidal activity of different antagonistic bacterial isolates was observed, where only hatched juvenile and adult nematodes were killed. The ovicidal activity was not tested. A similar differential susceptibility was also reported by Bottjer *et al.* (1985) who observed activity of *B.thuringiensis israelensis* against the 1st and 2 nd stage juvenile and adult of *Trichostrongylus colubriformis* and *Nippostrongylus brasiliensis*. In contrast, no such different susceptibility was observed by Bone *et al.* (1988) and Meadows *et al.* (1989) who reported the *B.thuringiensis israelensis* and *B.thuringinesis kurstaki* were lethal to the eggs as well as the 1st, 2nd and 3rd stages juveniles of *T.colubriformis*.

On the other hand, the mortality levels of juvenile and adult of *M.incognita* increased with increasing cfu/ml and the highest mortality levels were obtained at concentration 5 x 10^8 cfu/ml within 24 hour with all antagonistic isolates used. Similar results were reported by Bone *et al* (1988). The data also showed that nematode mortality never reached 100% at the highest concentration of antogonists employed. This may be due to the decrease of toxin levels produced by the bacteria after 24 hour incubation, such results were approximately in line with what was reported by Meadows *et al.* (1990) who noticed that the highest mortality levels were observed after 10 days of exposure.

Furthermore, it could be suggested that the source of nematicidal activity may be the spores and crystal toxins formed in bacterial cells, as endotoxins are reported to be the origin of the nematicidal activity (Narva *et al.*, 1991).

Effect of antagonists on the development of root-knot nematodes

In this experiment, root-knot nematodes, *M.incognita* were exposed to the antagonistic bacteria mentioned in table (3) and incorporated into soil prior to and at the same time of nematodes infestation. Experiment was terminated after 60 days and the severities of galling were recorded.

Data in Table (3) pointed out that the incorporation of biocontrol agents into the soil infested with the nematode resulted in a marked decrease in root-gall index and the number of galls were suppressed compared to the control. Data also revealed that introducing antagonistic bacteria prior to nematodes was more effective than when introduced simultaneously with the nematodes. The reduction was as much as 95.7, 97.5 and 98.4% in the treatments of *Ps. fluorescens*, *B.thuringiensis*, respectively, compared with the simultaneous introduction of both organisms, being 70.4, 69.6 and 96.3%, respectively.

It is known that some bacteria and actinomycetes have antagonistic effect on plant parasitic nematodes (Mousa *et al.*, 1989 and Yujioka *et al.*, 1993). In the

present study, the influence of rhizobacteria on nematode invasion and their ability to reduce gall formation by *M.incognita* is clearly demonstrated. The mechanisms were explained by the production of nematicidal compounds (Becker *et al.*, 1988) or by the host root exudate to prevent the penetration of host tissues by nematodes (Oostendrop and Sikora, 1989).

On the other hand, many soil bacterial species are capable of decomposing plant residue, and the products released by the metabolic activity of the bacteria vary from the complex to the simplest of molecules. Some of these products accumulate in soils and may be toxic, antibiotic or inhibitory to parasitic nematodes.

The present results are in agreement with those obtained by Ignoffo and Dropkin, (1977), who noticed that the toxin of *B.thuringiensis* was toxic to the populations of *Meloidogyne, Panagrellus* and *Aphelenchida* and prevented *M.incognita* larvae from forming gall on tomato roots.

In general, plant parasitic nematodes are subjected to a wide range of antagonists-antibiotic producers or antibiotic synthesizing micro-organisms such as *Bacillus, Pseudomonas, Streptomyces* and *Aspergillus.* Furthermore, it can be concluded from the present investigation that *B.thuringiensis, B.subtilis* and Ps. *fluoresence* are a potentially useful biological nematicides.

Table 3. Effect of antagonistic bacteria on root-knot nematode, *M.incognita* in tomato plants.

Treatment	Mean No. of galls/plant	% Reduction	Root gall index (RGI)
Ps. fluorescens prior to *M.incognita*	8.3	95.7	2.0
B.subtilis prior to*M.incognita*	4.8	97.5	2.0
B.thuringiensis prior to *M.incognita*	3.0	98.4	1.7
Ps.fluroescens+M.incognita at the same time	57.4	70.4	4.0
B.subtilis+M.incognita at the same time	59.0	69.6	4.0
B.thuringiensis+M.incognita at the same time	7.0	96.3	2.0
M.icognita (Control)	194.3	0.0	5.0
L.S.D. at 5%	5.0		

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كفاءة البكتريا المضادة من الريزوسفير في مقاومة نيماتودا تعقد الجذور، ميلودوجين انكوجينيتا، علي نباتات الطماطم

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تم تقييم ثلاث أنواع من البكتريا المضادة وهي باسيلس ثيرنجنس *Bacillus thurin-giensis* باسيلس ستلس *Bacillus subtillus* وبسيدوموناس فلوريسنس *Pseudomonas fluores-cens* لمقاومة مرض تعقد الجذور النماتودية المتسبب عن نيماتودا ميلودوجين انكوجنيتا *Meloidogyne incognita* علي نباتات الطماطم. تم إضافة ١٥٠ سم٢ من المعلق البكتيري المحتوي علي تركيز ٨ ١٠ خلية بكتيرية لجميع أنواع البكتريا المضادة السمتعملة لكل معاملة.

اتضح أن بكتريا ثيروجنسس وبكتريا باسيدوموناس فلوريسنس كانت أكثر تأثيراً علي اليرقات والطور الكامل لنيماتودا الميلودوجين أنكوجينيتا. ومن ناحية أخري كانت نسبة موت اليرقات والطور الكامل لليملودوجين تزداد عند زيادة تركيزات الخلايا البكتيرية (١٠ ^{٥}، ١٠ ^{٨}، ٥ x ١٠ ^{٨} خلية بكتيرية / سم٣). وبصفة عامة وجد أن إضافة البكتيريا المضادة قبل عدوي التربة بالنيماتودا كان لها تأثير أفضل في إختزال تكوين العقد النيماتودية علي الجذور بالمقاونة عند معاملة التربة بالبكتريا المضادة والنيماتودا في نفس الوقت.

ويمكن تفسير نتائج هذا البحث من خلال فعل البكتريا المضادة والمستعملة علي النيماتودا عن طريق المضادات الحيوية المفرزة أو المخلقة بواسطة البكتريا باسيلس ستلس وباسيدوموناس فلوروسنس وأيضاً من خلال البلورات التي تتكون داخل أجسام اليرقات بواسطة بكتريا باسيلس ثيروجنسس.