BIOLOGICAL CONTROL OF *MELOIDOGYNE INCOGNITA* ON TOMATO AND BANANA WITH RHIZOBACTERIA, ACTINOMYCETES, AND *PASTEURIA PENETRANS*

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ABSTRACT

Jonathan, E. I., K. R. Barker, F. F. Abdel-Alim, T. C. Vrain, and D. W. Dickson. 2000. Biological control of *Meloidogyne incognita* on tomato and banana with rhizobacteria, actinomycetes, and *Pasteuria penetrans*. Nematropica 30:231-240.

Glasshouse experiments were conducted to determine the efficacy of plant growth-promoting rhizobacteria, (*Bacillus cereus, B. subtilis, B. sphaericus, Agrobacterium radiobacter, Pseudomonas fluorescens, P. chlororaphis and Burkholderia cepacia*), uncharacterized actinomycetes (strains 29 and 45), and the nematode-parasitic bacterium *Pasteuria penetrans* (isolate 100) against *Meloidogyne incognita* race 1 on tomato and banana. All bacteria and actinomycetes enhanced the growth of both crops, and suppressed root-gall development on tomato as compared to control plants. Root-gall indices on tomato inoculated with *M. incognita* and bacteria ranged from 25 to 31% versus 94% for the nematode control. The bacteria also limited reproduction of *M. incognita* on both tomato and banana with the reproductive factor (Rf) for *M. incognita* on bacteria-treated tomato ranging from 9 to 24 versus 143 for the nematode-untreated control. The Rf for *M. incognita* on both tomato and banana ranged from 9 to 38 where bacteria were added versus 80 without bacteria. Significant suppression of associated root necrosis was observed in all bacteria-treated tomato in one of two tests. The root-galling pattern on tomato was radically different in all the rhizobacteria and actinomycetes-treated plants which showed minute round galls almost resembling *M. hapla* infection with a profuse root system.

Key words: Actinomycetes, banana, biological control, *Meloidogyne incognita*, *Pasteuria penetrans*, rhizobacteria, tomato.

RESUMEN

Jonathan, E. I., K. R. Barker, F. F. Abdel-Alim, T. C. Vrain y D. W. Dickson. 2000. Control biológico de *Meloidogyne incognita* en tomate y banano con rhizobacterias, actinomicetes y *Pasteuria penetrans*. Nematropica 30:231-240.

Se llevaron acabo experimientos de invernadero para determinar la eficacia de rhizobacterias promovedoras del crecimiento de las plantas (*Bacillus cereus, B. subtilis, B. sphaericus, Agrobacterium radiobacter, Pseudomonas fluorescens, P. chlororaphis* and *Burkholderia cepacia*), actinomicetes no caracterizados y la bacteria parasitica de nematodos, *Pasteuria penetrans* (aislamiento 100) sobre *Meloidogyne incognita* raza 1 en tomate y banano. Tanto las bacterias como los actinomicetes favorecieron el crecimiento de ambos cultivos y suprimieron el desarrollo de agallas en las raíces de tomate en comparación con las plantas testigo. Los índices de agallas en las plantas de tomate inoculadas con *M. incognita* y las bacterias se mantuvieron entre 25 y 31% mientras que el testigo inoculado presentó un índice de 94%. Las bacterias también disminuyeron la reproducción de *M. incognita* en ambos cultivos donde el factor de reproductividad (Fr) se mantuvo entre 9 y 24, mientras que el testigo no inoculado presentó un Fr de 143. El Fr de *M. incognita* en las plantas de banano inoculadas con bacterias se mantuvo entre 9 y 38, mientras que la no inoculadas presentaron un Fr de 80. En uno de dos experimentos realizados, se observó una supresión significativa de la necrosis radical en todas las plantas de tomate

tratadas con las bacterias. El patrón de formación de agallas en tomate fue muy diferente en todas las plantas tratadas con rhizobacterias y actinomicetes, presentando agallas muy pequeñas y redondas, parecido a una infección causada por *M. hapla* con un sistema radical profuso.

Palabras claves: Actinomycetes, banano, control biológico, *Meloidogyne incognita*, *Pasteuria penetrans*, tomate.

INTRODUCTION

Recent studies have demonstrated the ability of rhizobacteria to induce systemic resistance against microbial pathogens (Wei *et al.*, 1996). Antagonistic activity of rhizobacteria toward plant-parasitic nematodes have been observed in numerous crops (Becker *et al.*, 1988; Hackenberg and Sikora, 1994; Hackenberg *et al.*, 1999; Hoffman-Hergarten *et al.*, 1997). In field experiments, Kloepper *et al.* (1992) showed that a number of bacteria increased on velvet bean and other legumes in soybean rotations, and that those bacteria were associated with low numbers of *M. incognita* and *Heterodera glycines*. Whole cultures and supernatants (exotoxins) of *Bacillus thuringiensis* subsp. *brasiliensis* and *B. laterosporus* caused high mortality of *Meloidogyne javanica* in *in-vitro* bioassays and greenhouse tests on tomato (Carneiro *et al.*, 1998). In contrast, whole cultures of several strains of *B. sphaericus* and other *Bacillus* spp. as well as the pellets/spores (endotoxins) of *B. thuringiensis* subsp. *brasiliensis* and *B. laterosporus* had little effect on *M. javanica*. The efficacy against *Meloidogyne* populations of the obligate bacterial parasite *Pasteuria penetrans* has been demonstrated (Dickson *et al.*, 1994).

The present study, based on earlier reports of nematode suppressivenes of a range of bacteria (Becker *et al.*; Gherna and Piente, 1992; Dickson *et al.*, 1994; Hackenberg *et al.*, 2000; Hoffman-Hergarten et al., 1997), is an assessment of selected plant growth-promoting rhizobacteria, actinomycetes, and *P. penetrans* against *M. incognita* (Kofoid & White) Chitwood race 1 in tomato and banana plants.

MATERIALS AND METHODS

General procedures: The bacterial cultures *B. cereus* (Type Strain. Cat. No. 14579), *B. subtilis* (Type Strain, Cat. No. 6051), *B. sphaericus* (W. W. Ford 25 and Cat. No. 4525), *A. radiobacter* (Type Strain, Cat. No. 19358), and *Pseudomonas fluorescens* (Type Strain, Cat. No. 13525) were obtained from American-type culture collection, Manassas, Virginia, U.S.A. *Burkholderia cepacia* strain 5.5 #14 was provided by D. Benson, N.C. State University. Two uncharacterized actinomycetes (strains 29 and 45) and *P. chlororaphis* were contributed by T. Vrain, Agriculture and Agri-Food Canada, and *P. penetrans* was from D. Dickson, University of Florida, U.S.A.

The actionomycetes were increased for 48 h on actinomycetes broth (Gherna and Piente, 1992). A powder-like preparation of *P. penetrans* (crushed tomato roots infected with *M. incognita* that were parasitized with *P. penetrans*, isolate 100) was introduced at the rate of 5 g/pot. The inoculum potential of this material was assessed by incubating juveniles of *M. incognita* in 5 g of the preparation for 3 days and then extracting the nematodes by Baermann funnel. Juveniles had a range of 15 to 100+ spores of *P. penetrans* attached.

The other bacteria *viz.*, *B. cereus*, *B. subtilis*, and *B. sphaericus* were cultivated in trypticase soy broth, *A. radiobacter*, *P. fluorescens* and *P. chlororaphis* were cultivated in King's B medium broth, and *B. cepacia* was increased on nutrient broth (Gherna and Piente, 1992). These organisms were grown for 2 days at room temperature (25°C ± 2). The whole culture suspensions

of all the biocontrol agents were prepared by diluting with deionized sterile water to have a population of 2×10^9 colony-forming units (cfu)/ml. Twenty milliliters of material were added to a given plant. To preserve moisture, terasorb (a gelatinized starch hydrolized polyacrytonitrile copolymer—Industrial Services International, Inc., Bradenton, Florida) was included in the final bacterial suspensions as well as the water for control plants at a rate of 6.42 g/L. Terrasorb is used to minimize dessication of transplant roots and wilting which enhances plant survival.

Test plants—tomato and banana: Tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers 30-day-old seedlings, grown in steam-sterilized soil were carefully removed from plastic seedling trays. Banana tissue-culture plants Williams hybrid of uniform height of ca. 30 cm were used in the greenhouse tests.

Greenhouse inoculations: For the bacteria inoculations, the roots of both types of plants were dipped in 20 ml suspension of the biocontrol agents for 1 minute and immediately planted in 15-cm diameter pots filled with steam-sterilized sand soil (1:1 v/v) mixture (final composition: 85% sand, 10% silt and 5% clay). The remaining suspensions after dipping the roots, were carefully drenched around the plant roots.

Seven days later, the plants were inoculated with *M. incognita* race 1 cultures obtained from the populations maintained on tomato cv. Rutgers. The egg inoculum was prepared using the NaOCl method (Hussey and Barker, 1973) with an inoculum level of 10 000 eggs/plant. There were 11 treatments, which were replicated five times. The treatment details are furnished in Table 1. All plants were provided adequate nutrients for normal growth [20:20:20 Peter's (Grace-Sierra)] on a twice weekly basis.

Plants were harvested 12 weeks after nematode inoculation and plant-growth index (on a scale of 1 to 10 with the latter being optimum and 1 being very poor), plant height, shoot weight, and root weight were recorded. The roots were carefully washed with tap water, and the percentage galling and root necrosis were recorded (Barker *et al.*, 1986). The number of eggs per plant was estimated by extracting 5-g root sub-samples from each plant by the NaOCl method (Hussey and Barker, 1973). The data were subjected to analysis of variance and the Waller Duncan k-ratio t-test (where k = 50) was used to separate the means (Waller and Duncan, 1969). The experiment was repeated (staggered) in a 15-day interval during 1998. The banana test was repeated again during the summer of 1998. Because plant and nematode responses for the first two experiments per crop were generally similar, data for one greenhouse test per plant type are presented herein.

Microplot experiment with banana and Meloidogyne incognita: A microplot experiment in 1998 in a Fuquay sand, involved six of the bacteria and the nematode used in the greenhouse tests and a nematode-only control. The microplots had last been used for a *Heterodera glycines* test about 10 years earlier, and received no nematicide treatment. One liter of preparation of a given bacterium was added per 75-cm diam microplot. Bacterial concentrations per milliliter of medium were used in the range of 1×10^{10} CFU/ml. Plots were infested with 17 500 eggs of *M. incognita* per plot. The bacteria and nematodes were in the center 40-cm diam area of the plots to a depth of 20 cm. Single banana plants with a height of about 30 cm were placed in all plots and shaded for the first 3 to 4 days after transplanting. Peter's 20:20:20 nutrient solution was added, as needed, for normal plant growth.

Table 1. Plant growth, disease symptoms, and development of root-knot nematode *Meloidogyne incognita* race 1 on tomato and banana treated with rhizobacteria, actinomycetes and *Pasteuria penetrans*.

Treatments	Plant-growth index (1-10 rating)	Plant "size"[x]	Shoot weight (g)	Root weight (g)	Gall index[y]	Root necrosis[y]	Total eggs/ plant (in 1,000s)	R_f[y]
				TOMATO				
Actinomycetes 29	8.0 a[z]	150 ab	267 ab	47 c	25 b	8 bcd	90 d	9 d
Actinomycetes 45	7.9 ab	132 b	256 ab	58 bc	31 b	9 bc	108 cd	11 cd
Bacillus cereus	7.9 a	137 ab	230 b	53 bc	29 b	10 b	175 bcd	17 d
B. subtilis	8.0 a	133 b	251 ab	51 bc	29 b	5 d	236 b	24 b
B. sphaericus	7.9 ab	132 b	230 b	51 bc	28 b	9 bc	194 bc	19 bc
Agrobacterium radiobacter	7.6 b	133 b	266 ab	54 bc	31 b	8 bcd	225 b	23 b
Pseludomonaas fluorescens	7.9 ab	117 c	279 a	49 bc	29 b	6 bcd	125 cd	12 cd
P. chlororaphis	7.9 ab	125 bc	258 ab	47 c	30 b	7 bcd	130 cd	13 cd
Burkholderia cepacia	7.9 ab	136 ab	253 ab	52 bc	29 b	5 d	177 bcd	18 bcd
Pasteuria penetrans	8.0 a	138 ab	279 a	60 b	29 b	6 bcd	172 bcd	17 bcd
Control	5.5 c	102 d	107 c	85 a	94 a	19 a	1,418 a	143 a
				BANANA				
Actinomycetes 29	7.5 ab	3.2 abc	155 b	183 b	48 a-c	10 a	222 b	22 b
Actinomycetes 45	7.3 bc	3.0 bcd	142 b-d	188 b	36 c	7 a	267 b	26 b
Bacillus cereus	6.8 bc	2.5 f	113 c-e	150 c-e	55 ab	10 a	349 b	34 b
B. subtilis	6.8 c	2.7 ef	108 de	148 de	58 a	9 a	161 b	16 b
B. sphaericus	7.4 abc	2.9 de	131 b-d	180 bc	55 ab	10 a	383 b	38 b

For plant size, data for tomato are plant height (in cm), and for banana data are pseudostem diam (in cm).

[y]Root-gall and root necrosis indices on a scale of 0 (healthy) to 100 (100% of root affected); R_f (reproductive factor) = final population (P_f)/ initial population (PI).

[z]Means followed by the same letter are not significantly different according to Waller Duncan k-ratio *t*-test (k ratio = 50).

Table 1. (Continued) Plant growth, disease symptoms, and development of root-knot nematode *Meloidogyne incognita* race 1 on tomato and banana treated with rhizobacteria, actinomycetes and *Pasteuria penetrans*.

Treatments	Plant-growth index (1-10 rating)	Plant "size"[x]	Shoot weight (g)	Root weight (g)	Gall index[y]	Root necrosis[y]	Total eggs/ plant (in 1,000s)	R_f[y]
Agrobacterium radiobacter	7.6 ab	3.1 bcd	138 b-d	182 b	50 a-c	11 a	334 b	33 b
Pseudomonas fluorescens	8.0 a	3.4 a	192 a	235 a	52 a-c	9 a	249 b	24 b
P. chlororaphis	7.3 bc	2.9 cde	128 b-d	184 b	48 a-c	10 a	197 b	19 b
Burkholderia cepacia	7.6 ab	3.1 bcd	144 bc	162 b-d	58 a	12 a	382 b	38 b
Pasteuria penetrans	7.6 ab	3.3 ab	159 ab	165 b-d	34 c	15 a	262 b	26 b
Control	5.5 b	1.9 g	88 e	128 e	50 a-c	13 a	805 a	80 a

For plant size, data for tomato are plant height (in cm), and for banana data are pseudostem diam (in cm).

[y]Root-gall and root necrosis indices on a scale of 0 (healthy) to 100 (100% of root affected); R_f (reproductive factor) = final population (P_f)/ initial population (PI).

[z]Means followed by the same letter are not significantly different according to Waller Duncan k-ratio *t*-test (k ratio = 50).

RESULTS AND DISCUSSION

Tomato greenhouse tests: Significantly enhanced plant growth was observed in all the bacteria-treated tomato plants as compared to the *M. incognita* untreated control. The control plants showed the lowest growth index of 5.5; whereas, in other treated plants the growth index ranged from 7.6 to 8.0 (Table 1). Significant increases in shoot height and weight also occurred in all bacteria-treated plants with shoot weight being more than 2-fold greater than those for *M. incognita*-alone plants. Highest root weight of 85 g was observed in control plants, and this increase in root weight was due to severe root galling.

Compared to the nematode-alone control, all bacteria tested restricted root-gall development and reproduction of *M. incognita* (Table 1). Gall indices ranged from 25 to 31 for the bacteria-treated plants compared to 94 for the nematode control. The reproductive factor for nematodes alone on tomato was 143 versus a range of 9 to 24 for plants that received one of the test bacteria. Associated root necrosis was slightly but significantly restricted on plants receiving bacteria and nematodes (Table 1), but this response was not significant in the second tomato test (data not included).

Banana-greenhouse test: Significant increases in plant growth indices and pseudostem diameter were observed for all microbe-treated bananas in the first experiment (Table 1). Shoot and root weights were enhanced by all bacteria treatments except *B. cereus* and *B. subtilis*. Significant suppression in root-galling generally was not observed in bacteria-treated plants, but *P. penetrans*- and actinomycetes-45-treated plants had lower gall indices than plants receiving *B. cereus, B. subtilis*, or *B. sphaericus*. Root necrosis was not significantly affected by microbe-treatments. All bacteria treatments restricted numbers of eggs per plant and thereby the reproductive factor as compared to *M. incognita* alone. The relative suppression of nematode reproduction by the bacteria was much less on banana than on tomato (Table 1). Trends in the responses of banana to *M. incognita* were similar in the first two greenhouse tests (data for only one test presented). However, a third run of the banana test conducted during the summer of 1998, which encountered very high temperatures, failed to have any significant treatment effects.

Microplot-banana-M. incognita test: Of the bacteria tested with *M. incognita* in micro–plots, *B. subtilis, P. fluorescens*, *A. radiobacter*, and *B. cepacia* effected the greatest increases in growth of banana (Table 2). The shoot weights for plants receiving either of these bacteria were more than 2-fold greater than those of the *M. incognita* controls. All bacteria treatments tended to result in greater plant growth, but not at a significant level. Fernández-Falcón *et al.* (1998) found inoculation of banana with a commercial mixture of microorganisms (RET-FLO PX357) to suppress *Helicotylenchus* numbers while generally enhancing plant growth, maturation, yield, and nutrient status under field conditions. Thus, banana appears to be very responsive to a number of rhizosphere microflora.

The restriction of root-gall development by the bacteria on banana in microplots also was much less than that observed on tomato under greenhouse conditions. Only *A. radiobacter* had a significant suppressive effect on gall development on banana in the microplots (Table 2). Numbers of eggs per gram of root tended to be lower than the *M. incognita* alone, but usually not at a significant level.

The experimental data show the potential of rhizobacteria, actinomycetes, and *P. penetrans* in repressing nematode infec-

Table 2. Effects of selected bacteria on *Meloidogyne incognita*-banana interactions in microplots.

Treatments	Plant-growth index (0-10)	Stem diam. (cm)	Shoot weight (g)	Root weight (g)	Root-gall index (0-100)[y]	Eggs per g root (log_{10})
Agrobacterium radiobacter	6.8 a[z]	5.6 ab	678 a-e	355 ab	24.7 b	1.92 ab
Bacillus cereus	5.0 bc	4.3 c	399 cd	200 cd	35 ab	1.73 b
B. subtilis	6.7ab	6.3 a	828 a	283 a-d	25 ab	1.88 ab
Burkholderia cepacia	6.3 ab	5.6 ab	523 ed	263 b-d	31 ab	1.89 ab
Pseudomonas chlororaphis	6.7 a	4.8 bc	571 a-d	322 a-c	33 ab	1.78 ab
P. fluorescens	6.9 a	6.0 a	759 ab	400 a	27 ab	1.81 b
Nematode control	3.2 c	3.8 c	294 d	155 d	37 a	2.20 a

[y]Root-gall indices on a scale of 0 (healthy) to 100 (100% of root affected).
[z]Means followed by the same letter are not significantly different according to Waller Duncan *k*-ratio *T*-test (*k* ratio = 50).

tion and reproduction. These organisms also resulted in much enhanced shoot growth in two greenhouse experiments for tomato and banana. In both experiments with tomato, the rhizobacteria- and actinomycetes-treated plant roots resulted in minute, uniformly rounded galls resembling *M. hapla* infection with profuse secondary and tertiary roots. In contrast, the control plants had larger galls typical of *M. incognita* infection (Fig. 1). This altered tomato root-gall size and shape may be due to induced systemic resistance or multiple potential defense mechanisms due to the interaction between the host, the rhizobacteria, and the nematode (Wei *et al.*, 1996).

Since whole culture preparations were used in our experiments, further research is needed to delineate the nature of the nematode antagonism associated with the rhizobacteria. Recent research by Carneiro *et al.*, (1998) indicated that repression of *M. javanica* by selected *Bacillus* spp. involved materials in the supernatants (possibly exotoxins) rather than with the pellets/spores (endotoxins). Lyophilized bacterial preparations for inocula should minimize the potential for any nutritional effects of culture media on plant responses. In our tests, however, many plants exhibited phytotoxic reactions to some of the media-inocula, but they overcame that damage. Also, the third tests included all media-only controls as well as bacteria inocula for tomato, and provided no evidence of a nutritional benefit from the media. The prevailing high temperatures during those tests apparently prevented a positive plant-growth response to the bacteria and resulted in poor nematode control (data not included). Dr. Jerry Feitelson (Akkadix, San Diego, CA) kindly used his *Caenorhabditis elegans* screen for testing several of the bacteria employed in this study for the production of potentially nematicidal compounds, and detected none. Based on the earlier work of Sayre *et al.* (1965), *C. elegans* may be tolerant to some compounds that suppress the activity of *M. incognita.* Earlier research by Hoffman-Hergarten *et al.* (1997) showed *B. cereus* to significantly suppress *M. incognita* on tomato. A number of growth-promoting rhizobacteria also may induce systemic acquired host resistance to a number of plant pathogens (DeMeyer and Hofte, 1997; Wei *et al.*, 1996). Cropping systems that favor the build-up of a range of these bacteria may suppress the activity of nematodes as well as other plant pathogens (Kloepper *et al.*, 1988, 1992; Wei *et al.*, 1996). The use of legumes or other cover crops that foster the buildup of plant growth promoting rhizobacteria, as utilized by Klopper *et al.,* (1992), has great potential for practical biocontrol of nematodes. Still, further research is needed to determine the magnitude of the potential benefits of each of the rhizobacteria, especially under a wide range of field conditions.

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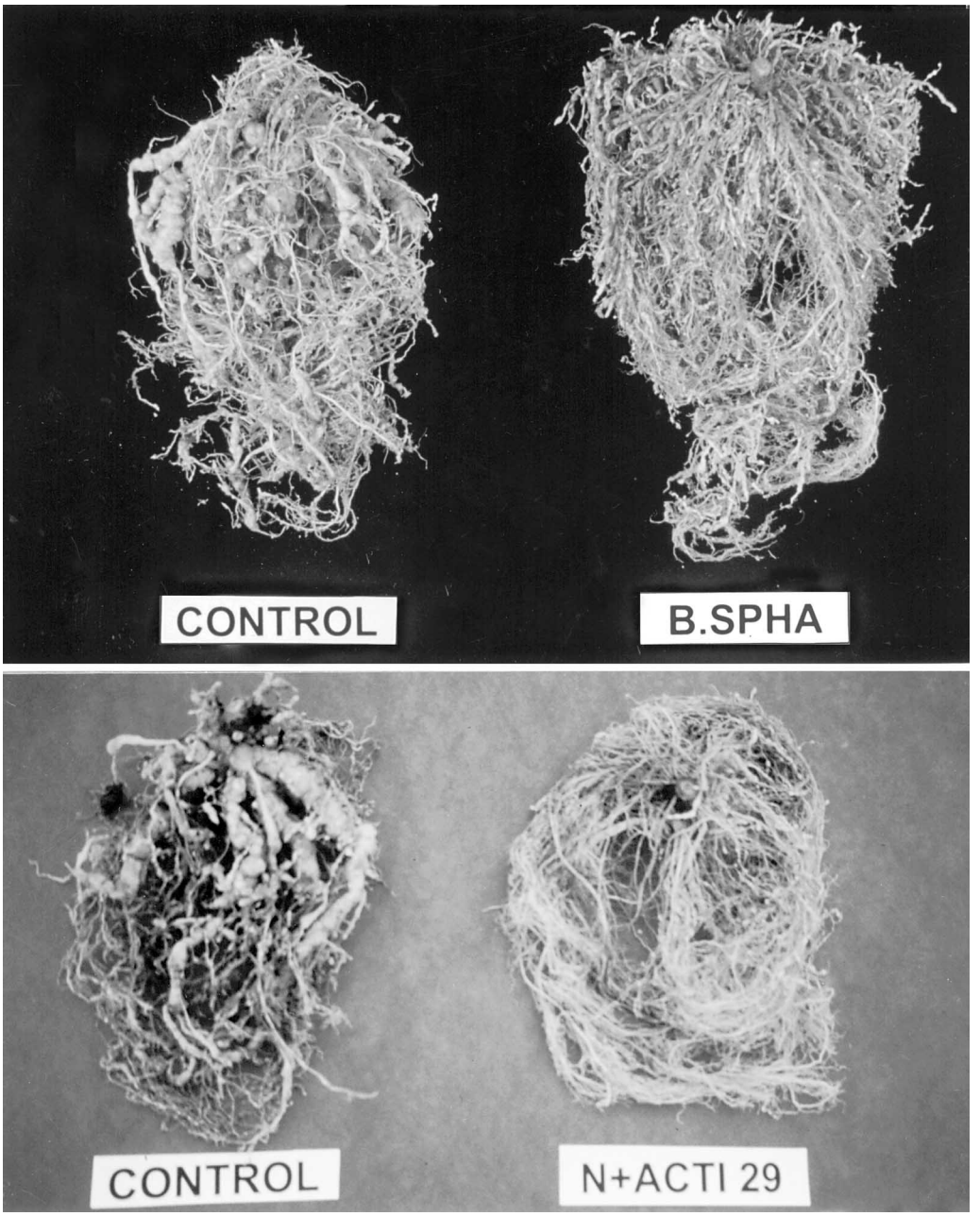

Fig. 1. Control tomato plant roots showing severe galling due to the infection by *Meloidogyne incognita* race 1. *Bacillus sphaericus*- and actinomycetes 29-treated roots showing significant difference in root galling pattern with profuse root system having very small galls.

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