SUPPRESSION OF *MELOIDOGYNE JAVANICA* BY *PSEUDOMONAS AERUGINOSA* AND *BACILLUS SUBTILIS* IN TOMATO

I. A. Siddiqui

Soil Biology and Ecology Laboratory, Department of Botany, University of Karachi, Karachi-75270, Pakistan

Summary. The effects of two strains of *Pseudomonas aeruginosa* (IE-6 and Pa-7) and an isolate of *Bacillus subtilis* on egg hatching, mortality and infection of *Meloidogyne javanica* in tomato roots was evaluated under laboratory, greenhouse and field conditions. Cell-free culture filtrates of the bacterial isolates significantly reduced egg hatching and caused mortality of second stage juveniles of *M. javanica*. Better biocontrol was observed in 15 day old seedlings as compared to 30 and 45 day old plants. Culture filtrates of *P. aeruginosa* strains IE-6 and Pa-7 resulted in activity similar to that caused by viable and dead cells of *P. aeruginosa* whereas activity of the culture filtrate of *B. subtilis* was greater compared to the dead cells. Introduction of rhizobacteria in the root zone gave better results than stem injection or foliar application. Under field conditions, the isolates also significantly reduced nematode population densities and subsequent gall formation due to *M. javanica*.

Fluorescent pseudomonads and bacilli are a group of bacteria that have often been reported to occur in the rhizosphere of a wide range of plants (Curl and Truelove, 1986). Many isolates from these groups are known to possess antagonistic activity against other micro-organisms of the soil and rhizosphere and, therefore, have been intensively used in trials for the biological control of soil borne root-infecting fungi (Sharma and Nowak, 1998; Siddiqui *et al.*, 2000). Siderophore activity, suppression of deleterious rhizobacteria, antibiotic and toxin production, plant hormone production, and induced resistance are the mechanisms responsible for the control of fungal pathogens (Weller, 1988).

The use of rhizosphere bacteria for the biological control of plant-parasitic nematodes provides a different approach to control since, in contrast to many soil borne root-infecting fungi, plant-parasitic nematodes that are obligate parasites are dependent on the host plant for development and reproduction. The rhizobacteria based system utilizes microorganisms that alter hatch, attraction, or host recognition (Oostendorp and Sikora, 1990). Rhizosphere bacteria have been reported to produce nematicidal compounds that affect the vitality of the second stage juveniles of root-knot nematodes (Becker *et al.*, 1988). Rhizobacteria applied as a seed dressing reduce nematode penetration of the root system of potato and sugarbeet (Racke and Sikora, 1985; Oostendorp and Sikora, 1986, 1989).

Pseudomonas aeruginosa, a plant growth promoting rhizobacterium has potential as a biocontrol agent of *Meloidogyne javanica* in tomato (Siddiqui *et al.*, 2000). Similarly, *Bacillus subtilis* has also been reported to control *M. incognita* race-3 in chickpea (Siddiqui and Mahmood, 1993). In our recent study, two strains of *P. aeruginosa* (IE-6 and Pa-7) and *B. subtilis* isolate caused marked reduction of *M. javanica* population densities in soil and root and subsequent root-knot infection in mungbean in two years of field experiments (Siddiqui *et al.*, 2001). In the same study, generic and even strain-specific differences were evident among bacteria to suppress root-knot nematodes (Siddiqui *et al.*, 2001). The aim of the present study was to characterize this interaction and provide evidence that it involves a specific interaction between the bacteria and the nematode.

MATERIALS AND METHODS

The technique as suggested by Bashan *et al.* (1993) was used for the isolation of *Pseudomonas aeruginosa* (Shroeter) Migula. Roots of sunflower were washed with running tap water and soaked for 10 min in sterile phosphate buffer saline (PBS) [10 m M K_2HPO_4], then chopped into small pieces and transferred in Petri dishes containing Sl medium ((g/l) agar, 18; sucrose, 10; glycerol, 10 ml; casamino acids (Difco), 5.0; $NaHCO_3$, 1.0; $MgSO_4.7H_2O$, 1.0; K_2HPO_4, 2.3; sodium lauroyl sarcosine (SLS), 1.2 and 20 mg of trimethoprim). Dishes were incubated for three days at room temperature and bacterial colonies that showed fluorescent pigment under ultra violet light at 366 nm were purified on King's B medium (King *et al.*, 1954). Biochemical tests were performed for the identification of *P. aeruginosa* (Krieg and Holt, 1984). *P. aeruginosa* strains were gram negative rods, produced blue green pigments on KB medium after three days and *Bacillus subtilis* Cohn was an endospore forming gram positive bacterium. The strains of *P. aeruginosa* isolated from sunflower and cotton were designated as IE-6 and Pa-7 respectively. *B. subtilis* strain used in the present study was isolated from sunflower and maintained on KB medium.

Aqueous suspension of 5 day old bacterial cultures, prepared by scrapping the surface with a sterilized bent glass rod after adding 10 ml sterile distilled water were maintained on KB medium at room temperature (28±2

°C). The suspension was stored at 4 °C before use. After making the dilution, one ml suspension was poured on KB medium and incubated at 28±2 °C for 3-5 days. Bacterial colonies growing on the plates were counted and multiplied by the dilution factor (10^{-3}, 10^{-4} or 10^{-5}) that gave cfu/ml of bacteria. Bacterial strains were grown in KB liquid medium at 30 °C for 48 hrs in dark and centrifuged twice at 2,800 x g for 20 mins. Pellets were discarded and the culture filtrate was collected in a beaker prior to use. The culture filtrate was tested for the presence or absence of the bacterium as described above; it did not produce any viable growth of either bacterium.

Meloidogyne javanica (Treub) Citw. was obtained from pure cultures maintained on roots of eggplants (*Solanum melongena* L.). The entire root system was dipped in water and soil was removed gently without detaching any egg sacs. Eggs were extracted by vigorous shaking of infested roots in a 1% sodium hypochlorite solution for three minutes. The resulting suspension was then passed through a range of different mesh sieves (Hussey and Barker, 1973). The eggs collected on a fine sieve (38 mm) were washed in tap water to remove all traces of sodium hypochlorite before use. Seeds of tomato (*Lycopersicon esculentum* Mill.), cv 'SUN 6002 (PVP)' after surface sterilization with 1% $Ca(OCl)_2$ were washed thoroughly with running tap water and allowed to dry under a laminar flow hood. The seeds were planted in a 35 cm diam., earthen pots containing steam-sterilized soil. Three-week-old seedlings were then used for the experiments.

To determine the effect of bacteria on egg hatching activity of *M. javanica*, two ml of the culture filtrate was transferred in watch glasses into which two medium size egg masses hand-picked from the knots of eggplant were placed. Egg masses kept in either distilled water or KB liquid medium without the bacterium served as control. After 72 hrs, the numbers of juveniles hatched were counted with the aid of a stereomicroscope (x 6). Treatments were quadruplicated and watch glasses were randomised at room temperature (28±2 °C). The egg masses from the culture filtrate were then transferred to sterile distilled water and their hatching in water was recorded to ascertain whether the egg masses kept in the culture filtrate had been permanently or temporarily inactivated. The emergence of juveniles was again recorded after 72 hrs. The experiment was repeated. To determine the nematicidal activity, one ml of the freshly hatched surface sterilized juvenile suspension (30-40 juveniles/ml) with two ml of either autoclaved or non-autoclaved culture filtrate of each bacterium were transferred separately to watch glasses. Juveniles kept in either 2 ml of autoclaved or non-autoclaved distilled water or KB broth with out the bacterium served as controls. There were four replicates of each treatment and watch glasses were incubated at room temperature (28±2 °C). After 48 hrs, the numbers of dead juveniles were counted with the aid of a stereomicroscope (x 6). Nematodes were considered to be dead if they did not move when probed with a fine needle (Cayrol *et al.*, 1989). The experiment was repeated once.

Sandy loam soil (sand:silt:clay, 70:19:11), pH 8.1 obtained from the experimental field of the Department of Botany, University of Karachi was used. The soil was screened through a 2-mm sieve to discard non-soil particles and 350 g samples were potted into 8 cm-diam., plastic pots. The soil was naturally infested with 100-150 juveniles of *M. javanica*/250 g soil. Four different experiments were conducted on the glasshouse bench of Soil borne Diseases Research Laboratory, Department of Botany University of Karachi. All the experiments were repeated at least once.

Experiment 1 was designed to determine the efficacy of bacteria on egg hatching of *M. javanica*. The soil was excavated to a depth of 3 cm and 25-ml cell suspension of the bacterial antagonist containing 2.5 x 10^8 cfu ml^{-1} was drenched in a pot. Soil drenched with 25 ml sterile distilled water or KB broth served as control. After treatment, the surface was covered with soil and three tomato seedlings were transplanted to each pot. Treatments were replicated four times and the pots were arranged in a randomised complete block design. One week after seedling transplantation, a 10 ml egg suspension of *M. javanica* containing 1500 surface sterile eggs/ml was pipetted into three holes made in the rhizosphere of the tomato seedlings. The experiment was terminated one week after the addition of eggs and numbers of hatched juveniles were extracted from soil using a modified Baermann funnel technique (Whitehead and Hemming, 1965).

Since little information is available on the duration of rhizobacteria-mediated root protection against root-knot nematode, the second experiment was designed to determine the efficacy of *P. aeruginosa* (IE-6 and Pa-7) and *B. subtilis* on root-knot nematode penetration into tomato roots at three different time intervals. Freshly hatched second stage juveniles were selected as the inoculum because they provide a greater rate of penetration than egg suspensions. The bacterial cell suspension (2.0-2.2 x 10^8 cfu ml^{-1}) prepared in sterile distilled water was drenched separately in each pot. Soil drenched with 25 ml sterile distilled water served as control. After drenching, the surface was covered with soil. Three seedlings were transplanted in each pot which were allowed to established for one week before 2000 freshly hatched second stage juveniles of *M. javanica* in 10 ml water were pipetted into three holes in the soil around the seedlings. The experiment was set out in a randomised complete block design with 15 replicates for each treatment. Five replicates from each treatment were harvested 15 days after nematode inoculation. The remaining pots were harvested after 30 and 45 days (5 replicates at each harvest). At each harvest, root system was carefully removed, excess soil was shaken off the roots which were then washed in running tap water. Fresh roots were cut into small segments, weighed,

wrapped in a muslin cloth and dipped for 3-5 minutes in boiling 0.25% acid fuchsine in lactic acid. Roots were then washed in running tap water to remove the excess stain and cooled in vials containing 1:1, glycerol: water with few drops of lactic acid. Roots were macerated in an electric grinder for 45 seconds and the macerate suspended in 100 ml water. *M. javanica* females + juveniles in 5 samples of 5 ml each were counted with the aid of low power microscope (x 6).

The third experiment was designed to examine the effects of live bacteria (2.0 x 10^8 cfu ml^{-1}), killed bacteria and their culture filtrates on root-knot activity. Heat-killed bacteria cells were produced by autoclaving bacteria 20 min at 121 °C at 15 lbs p.s.i. Sterility was checked by pouring the heat-killed cell suspension on KB medium. Heat-killed cells produced no viable growth of the bacteria. Twenty five ml of each treatment with 25 ml sterile distilled water or King's B liquid medium (control) was drenched separately in each pot. Two thousand second stage juveniles (< 5-day-old) were used to inoculate tomato seedlings. Each treatment was replicated four times and the pots were randomised. The experiment was terminated 21 days after the addition of nematodes and the number of juveniles/g root counted as described earlier.

Experiment 4 was designed to compare the level of root protection against root-knot nematode resulting from the application of bacteria via leaf application, stem injection and soil drench. The experimental design was a 3 x 4 factorial with three replicates, each with three plants, arranged in a randomised complete block manner. Factors were three bacterial application methods and four treatments including two strains of *P. aeruginosa*, one *B. subtilis* isolate and an untreated control. For leaf application, one week after transplanting the seedlings, a 1 ml aqueous cell suspension of *P. aeruginosa* or *B. subtilis* (2.0 x 10^8 cfu ml^{-1}) with 1% carboxymethyl cellulose was spread over the leaves. Control leaves were spread with 1% carboxymethyl cellulose solution. To avoid bacterial contamination, after application the plants were covered with plastic wrap which was secured around the stem. For stem injection, a 0.25 ml of bacterial suspension was injected into the stem. This method was previously reported to differentiate among strains for endophytic colonization potential (Chen *et al.*, 1995). Control plants were inoculated with sterile distilled water. A 25 ml aqueous cell suspension was drenched in each pot and soil drenched with sterile distilled water was the control. There were three replicates for each treatment and pots were randomised. After one week of the bacteria application, a suspension containing 2000 juveniles (< 5-day-old) was pipetted onto the soil near the roots. The experiment was terminated 21 days after nematode inoculation and the numbers of juveniles that had penetrated the roots were counted.

An experiment was carried out in 2 m x 1m microplots at the experimental field located near the Department of Botany, University of Karachi during September 1998. The soil characteristics were the same as described for the glasshouse experiments. For the bare root-dip treatment, tomato roots were dipped for 15 minutes in bacterial cell suspensions containing 2.1-2.4 x 10^8 cfu ml^{-1} and then planted at 6 seedlings/furrow. Roots dipped in sterile distilled water served as control. In another set, soil was removed to a depth of 12 cm and the bacterial cell suspension prepared in 300 ml sterile distilled water was drenched in each row before transplantation of seedlings. Soil treated with 300 ml sterile distilled water served as control. Treatments were arranged in a randomised complete block design and replicated thrice. One week after transplanting the seedlings, roots of each seedling was inoculated with 1000 freshly hatched second stage juveniles of *M. javanica*. The juveniles were suspended in 25 ml sterile distilled water that was poured into three holes made around the tomato seedlings. Plants were harvested 60 days after nematode inoculation and root-knot nematode populations in soil and root was estimated. The soil from which the roots were taken was dug to a 15 cm depth before 250 g soils were taken from all the treated and untreated plots. The soil was used for the extraction of nematodes using a modified Baermann funnel technique.

Data were analysed using analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) in case of more than one factor. Since the error variances between repeated experiments were similar, the analysis was performed on the pooled data. Treatment means were compared by Duncan's multiple range test (Sokal and Rohlf, 1995).

RESULTS

Egg-hatch of *M. javanica* was significantly ($P<0.001$) less in the cell-free culture filtrates of *P. aeruginosa* and *B. subtilis* than in untreated KB broth or sterile distilled water (Table I). The culture filtrate of *P. aeruginosa* strain IE-6 had the greatest effect (>95%) on egg hatching as compared to *B. subtilis* that caused > 86% inactivation in eggs of *M. javanica*. Strain Pa-7 of *P. aeruginosa* also inhibited egg hatch by more than 93%. Juveniles continued to emerge from egg masses transferred from culture filtrate to sterile distilled water in all treatments but their numbers were significantly ($P<0.05$) less in the treatments that were previously treated with filtrates of *P. aeruginosa* strains as compared to *B. subtilis* or KB broth Strains IE-6, Pa-7 and *B. subtilis* resulted in >83, 78 and 56% inactivation in egg hatching. Unboiled cell-free culture filtrate of the bacterial antagonists had nematicidal effects ($P<0.001$), on the second stage juveniles of *M. javanica* (Table II). Strain IE-6 of *P. aeruginosa* was most toxic, killing 75% of the juveniles; strain Pa-7 killed 65% juveniles compared with untreated KB broth or sterilized distilled water. The culture

Table I. Effect of the cell-free culture filtrate of *Pseudomonas aeruginosa* (IE-6 and Pa-7) and *Bacillus subtilis* on egg hatching of *Meloidogyne javanica* in vitro.

Treatments	No. of eggs hatched in Culture filtrate	Distilled Water [a]	Total number of eggs hatched	Percentage reduction over untreated control
Control (KB broth)	183a	70ab	253	–
P. aeruginosa (IE-6)	8 b	35 c	41	83.79
P. aeruginosa (Pa-7)	12 b	42 bc	54	78.65
B. subtilis	26 b	85a	111	56.12

[a] After a 48 hrs hatching period in the culture filtrate, egg masses were transferred to sterile distilled water. Data represent mean of 8 values obtained in two experiments each with 4 replicates. Figures followed by same letters in each column are not significantly different (P≤0.05) according to the Duncan's multiple range test.

Table II. Effect of boiled and unboiled culture filtrate of *P. aeruginosa* (IE-6 and Pa-7) and *B. subtilis* on mortality of *M. javanica* juveniles *in vitro*.

Treatments		Mortality %
KB broth	Unboiled	2 e
	Boiled	2 e
P. aeruginosa (IE-6)	Unboiled	75a
	Boiled	12 d
P. aeruginosa (Pa-7)	Unboiled	65 b
	Boiled	7 de
B. subtilis	Unboiled	52 c
	Boiled	11 d

Data represent mean of 8 values obtained in two experiments each with 4 replicates. Figures followed by same letters in each column are not significantly different (P≤0.05), according to the Duncan's multiple range test.

Table III. Effect of *P. aeruginosa* (IE-6 and Pa-7) and *B. subtilis* on egg-hatching of *M. javanica* in the rhizosphere of tomato under greenhouse conditions.

Treatments	Juveniles/250g soil
KB broth	581a
P. aeruginosa (IE-6)	321 c
P. aeruginosa (Pa-7)	496ab
B. subtilis	457 b

Data represent mean of 8 values obtained in two experiments each with 4 replicates. Figures followed by same letters in each column are not significantly different (P≤0.05), according to the Duncan's multiple range test.

filtrate of *B. subtilis* also caused 52% juvenile mortality compared to the untreated KB broth or distilled water. Culture filtrates of the bacteria lost their nematicidal activity when boiled.

Strain IE-6 of *P. aeruginosa* and *B. subtilis* reduced (P<0.05) egg-hatching activity in the rhizosphere of tomato whereas strain Pa-7 did not (Table III). Strain IE-6 reduced egg hatch of *M. javanica* by 44% whereas *B. subtilis* caused a 21% inhibition in egg hatch compared to the controls. Aqueous cell suspension of *P. aeruginosa* and *B. subtilis* significantly (P<0.001) reduced nematode penetration into tomato roots compared to untreated controls. The efficacy of the bacterial antagonists gradually (p<0.001) declined with increasing time. Strain IE-6 caused 72, 66 and 50% reduction and Pa-7 resulted in 64, 42 and 37% reduction in nematode penetration in 15, 30 and 45-day-old samplings respectively. More than 60, 30 and 40% reduction in nematode penetration was achieved in 15, 30 and 45 day-old samplings, respectively, when *B. subtilis* was used (data not presented). Viable cells, heat-killed cells and the cell-free culture filtrates of *P. aeruginosa* and *B. subtilis* provided significant (p<0.05) protection to tomato roots from invasion of root-knot nematode (Table IV). Application of live cells of IE-6, Pa-7 and *B. subtilis* caused a 53, 43 and 49% reduction in nematode penetration respectively. Dead cell of *P. aeruginosa* (IE-6 and Pa-7) demonstrated similar effects as those caused by viable cells whereas marked (P<0.05) differences on the activity between live and dead cells of *B. subtilis* was observed. Heat-killed cells of IE-6 and Pa-7 caused 48 and 40% reduction in nematode penetration, respectively while dead cells of *B. subtilis* reduced nematode penetration by up to 30%. Activity of the culture filtrate of *B. subtilis* was better compared with the dead cells while *P. aeruginosa* dead cells exhibited better results as com-

Table IV. Effect of viable cells, heat-killed cells and the culture filtrate of *P. aeruginosa* (IE-6 and Pa-7) and *B. subtilis* on penetration of *M. javanica* in tomato roots.

Treatments		Juveniles/g root
KB broth		116a
P. aeruginosa (IE-6)	Viable	54 d
	Heat-killed	60 bcd
	Culture filtrate	68 bc
P. aeruginosa (Pa-7)	Viable	66 bcd
	Heat-killed	69 bc
	Culture filtrate	73 b
B. subtilis	Viable	59 cd
	Heat-killed	81 b
	Culture filtrate	63 cd

Data represent mean of 8 values obtained in two experiments each with 4 replicates. Figures followed by same letters in each column are not significantly different (P≤0.05), according to the Duncan's multiple range test.

pared to cell-free culture filtrate. Cell-free culture filtrate of IE-6, Pa-7 and *B. subtilis* reduced nematode penetration up to 41, 37 and 46% respectively. Factorial analysis revealed no interaction between delivery method (soil drench, stem injection or leaf application) and treatments on root-knot nematode penetration in tomato roots. However, soil application with *P. aeruginosa* (IE-6 and Pa-7) and *B. subtilis* significantly ($P<0.05$) reduced nematode penetration compared with untreated controls. Maximum reduction in nematode penetration (more than 52% compared with untreated controls) was achieved with application of strain IE-6. Strain Pa-7 of *P. aeruginosa* and *B. subtilis* caused 50 and 34% reductions in nematode penetration, respectively. Stem injection and leaf application of the bacterial isolates did not reduce nematode penetration (data not presented).

In a microplot experiment, galling rates due to *M. javanica* were significantly ($P<0.001$) reduced following application with rhizosphere bacteria (Table V). There was a significant ($P<0.001$) difference between the delivery system of the isolates whereas interaction between treatment and application technique of the isolates was non-significant. *P. aeruginosa* strain IE-6 applied as a bare root-dip treatment (39%) and *B. subtilis* used as a soil drench (37%) caused the greatest reduction in root-knot development due to *M. javanica* in tomato. Rhizobacterial strains significantly reduced nematode populations in soil ($P<0.001$) and root ($P<0.05$) as compared to untreated controls. There was no significant difference between application method of the isolates in lowering the nematode population densities in the soil and roots. *B. subtilis* used as a bare root-dip treatment (39%) or as a soil drench (28%) resulted in the greatest reduction in nematode populations in soil whereas strain IE-6 used as either bare root-dip treatment or as a soil drench reduced nematode penetration by up to 33% and 40%, respectively, compared with untreated controls.

DISCUSSION

Cell-free culture filtrate of *P. aeruginosa* and *B. subtilis* caused significant inhibition in egg hatching of *M. javanica* both *in vitro* and under glasshouse conditions. Culture filtrate of the bacterial antagonists also caused mortality of *M. javanica* juveniles. The nematicidal activity was heat labile hence might be proteinaceous or glycoproteinaceous in nature. Bacteria are known to produce metabolites that have nematicidal activity. Rhizosphere bacteria have been reported to produce nematicidal compounds that affect the vitality of second stage juveniles of *M. incognita* (Becker, 1988). Similarly, culture filtrates of *Bacillus sphaericus* induced systemic resistance in tomato against the potato cyst nematode *Globodera pallida* (Hasky-Günther *et al.*, 1998).

Nematode penetration into tomato roots was considerably reduced within 15 days of bacterial application whereas the penetration rate was higher after 30 and 45 days. Presumably, metabolites released by the bacteria were initially toxic to the nematodes and made them less able to penetrate their host. The level of protection provided by bacteria against plant diseases correlates with the rhizosphere population density of these bacteria (Bull *et al.*, 1991; Raaijmakers *et al.*, 1995). It is speculated that after 15 days the bacterial population declined thereby reducing their biocontrol potential. Oostendorp and Sikora (1990) observed a decline in the population of *Pseudomonas fluorescens* in the rhizosphere during the first two weeks after sowing sugarbeet seeds. Aqueous cell suspensions and the cell-free culture filtrate of *P. aerations* and *B. subtilis* are able to cause a reduction in nematode penetration. The biological activity of *P. aeruginosa* is not destroyed by autoclaving, which indicates that the causal factor is thermostable. The culture filtrate of *P. aeruginosa* strain IE-6 and Pa-7 demonstrated similar activity as that caused by viable and dead cells whereas activity of culture filtrate of *B. subtilis* was greater compared with the dead cells. This indicates the activity of surface structures of the bacterial cell wall as well as the production of inter-

Table V. Effects of bare-root-dip treatment or soil drench with *P. aeruginosa* (IE-6 and Pa-7) and *B. subtilis* on the development of root-knot infection, nematode population in tomato under field conditions.

Treatments	Galls/root system		Root-knot nematodes/ 250 ml soil		Juveniles/g root	
	B.R.D.T.	S.D.	B.R.D.T.	S.D.	B.R.D.T.	S.D
Control	61	50	2210	2223	89	97
P. aeruginosa (IE-6)	42	36	1453	1690	60	59
P. aeruginosa (Pa-7)	46	36	1783	1676	65	59
B. subtilis	48	35	1353	1606	66	70
Significance level (P)						
Treatments	<0.001		<0.001		<0.05	
Application method	<0.001		Non-significant		Non-significant	
Treatment x method	Non-significant		Non-significant		Non-significant	

B.R.D.T. = bare-root-dip treatment; S.D. = soil drench.

cellular metabolites by *P. aeruginosa* whereas *B. subtilis* lack extracellular activity but produced nematicidal components in the culture filtrate involved in the suppression of root-knot nematode.

In the present study, application of *P. aeruginosa* and *B. subtilis* in the root zone caused significant reductions in nematode penetration whereas leaf application and stem injection of the bacteria had no such effects. This is in agreement with the findings of Husky-Günther *et al.* (1998), who observed no basipetal activity in inducing resistance to nematode root infestation after inoculation of rhizobacterial strains on the leaf surface. However, it has been reported that application of some plant growth promoting rhizobacteria (PGPR) strains on foliage could increase growth and protect plants from foliar diseases (Mei, 1989; Mei *et al.*, 1990). Similarly, seed inoculation and cotyledon injection with PGPR induced resistance against bacterial angular leaf spot caused by *Pseudomonas syringae* pv *lachrymans* (Liu *et al.*, 1995).

LITERATURE CITED

Bashan Y., Holguin G. and Lifshitz R., 1993. Isolation and characterization of plant growth promoting rhizobacteria. Pp. 331-344. *In*: Methods in Plant Molecular Biology and Biotechnology (Glick B.R. and Thompson J.E. Eds), FCRS Press, London.

Becker J.O., Zavaleta-Mejia E., Colbert S.F., Schroth M.N., Weinhold A.R., Hancock J.G. and Van Gundy S.D., 1988. Effects of rhizobacteria on root knot nematode and gall formation. *Phytopathology, 78*: 1466-1469.

Bull C.T., Weller D.M. and Thomashow L., 1991. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. *Phytopathology, 81*: 954-958.

Cayrol J.C., Djian C. and Pijarowski L. 1989. Study on the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Revue de Nematologie, 12*: 331-336.

Chen C., Bauske E.M., Musson G., Rodríguez-Kábana R. and Kloepper J.W., 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. *Biological Control, 5*: 83-91.

Curl A.E. and Truelove B., 1986. *The Rhizosphere*. SpringerVerlag, Berlin, Heidelberg, 283 pp.

Devidas P. and Rehberger L.A. 1992. The effects of exotoxin (thuringiensin) from *Bacillus thuringiensis* on *Meloidogyne incognita* and *Caenorhabditis elegans*. *Plant & Soil, 145*: 115-120.

Hasky-Günther K., Hoffmann-Hergarten S., and Sikora R.A., 1998. Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43). *Fundamental and Applied Nematology, 21*: 511-517.

Hussey R.S. and Barker K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter, 61*: 328-331.

King E.O., Ward M.K. and Raney D.E., 1954. Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine, 11*: 441-449.

Krieg N.R. and Holt J.G., 1984. *Bergey's Manual of Systematic Bacteriology. Vol. I.* William & Wilkins, Baltimore. 964 pp.

Liu L., Kloepper J.W. and Tuzun S., 1995. Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. *Phytopathology, 85*: 843-847.

Mei R., Chen B., Lu S. and Chen Y., 1990. Field application of yield-increasing bacteria (YIB). *Chinese Journal of Microecology, 2*: 45-49.

Oostendorp M. and Sikora R.A. 1986. Utilization of antagonistic rhizobacteria as a seed treatment for the biological control of *Heterodera schachtii* in sugarbeet. *Revue de Nematologie, 9*: 304 [Abstract].

Oostendorp M. and Sikora R.A. 1989. Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugarbeet. *Revue de Nematologie, 12*: 77-83.

Oostendorp M. and Sikora R.A. 1990. *In vitro* interrelationship between rhizosphere bacteria and *Heterodera schachtii*. *Revue de Nematologie, 13*:269-274.

Racke J. and Sikora R.A. 1985. Einfluss von Rhizosphärenbakterien auf *Rhizoctonia solani* und den befall der Kartoffelsorte hansa mit *Globodera pallida*. *Biotechnologische Vorträge zur Resistenzselektion; Vorträge für Pflanzenzüchtung Statusseminar Grünback, 9*: 21-28.

Raaijmaker J.M., Leeman M., van Oorschot M.M.P., van der Sluis I., Schipper B. and Baker P.A.H.M. 1995. Dose-response relationship in biological control of *Fusarium* wilt of radish by *Pseudomonas* spp. *Phytopathology, 85*: 1075-1081.

Sharma V.K. and Nowak J., 1998. Enhancement of *Verticillium* wilt resistance in tomato transplant by *in vitro* co-culture of seedlings with a plant growth promoting rhizobacterium (*Pseudomonas* sp. strain PsJN). *Canadian Journal of Microbiology, 17*: 67-75.

Siddiqui I.A., Qureshi S.A., Sultana V., Ehteshamul-Haque S. and Ghaffar A., 2000. Biological control of root rot-root knot disease complex of tomato. *Plant & Soil, 227*: 163-169.

Siddiqui I.A., Ehteshamul-Haque S. and Shaukat S.S. 2001. Use of rhizobacteria in the control of root rot-root knot disease complex of mungbean. *Journal of Phytopathology, 149*: 337-346.

Siddiqui Z.A. and Mahmood I., 1993. Biological control of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* by *Paecilomyces lilacinus* and *Bacillus subtilis* alone and in combination on chickpea. *Fundamental and Applied Nematology, 16*: 215-218.

Sokal R.R. and Rohlf F.J., 1995. *Biometry: The Principles of Statistics in Biological Research*. Freeman, New York, 887 pp.

Weller D.M., 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology, 26*: 379-407.

Accepted for publication on 2 April 2002