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Soil-borne Diseases Research Laboratory,
Department of Botany, University of Karachi, Karachi-75270, Pakistan

USE OF *PSEUDOMONAS AERUGINOSA* FOR THE CONTROL OF ROOT ROT-ROOT KNOT DISEASE COMPLEX IN TOMATO

by
I. A. SIDDIQUI and S. EHTESHAMUL-HAQUE

Summary. The potential of two *Pseudomonas aeruginosa* isolates (Pa-7 and IE-6) as biological control agents against *Meloidogyne javanica* at five inoculum densities (0, 500, 1000, 2000 and 4000 juveniles/plant) was tested on tomato in earthen pots. The effects of root-knot nematodes and the root-infecting fungi *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani* on bacterial endophytic colonization (root and shoot) as well as the effect of the bacteria on nematode multiplication, control of the root rot-root knot disease complex and the growth of tomato plants were investigated. The two *P. aeruginosa* isolates significantly reduced nematode populations and root invasion by the nematode and colonization by the root-infecting fungi on tomato. Both the isolates showed similar results at low nematode densities (500 and 1000 J2) but isolate IE-6 was also effective at the higher nematode densities (2000 and 4000 J2). Bacteria survived well both in roots and stem but increased nematode densities were associated with decreased bacterial populations. A negative correlation between *F. oxysporum* infection and bacterial root and shoot colonisation was also observed. A progressive decrease in plant growth was observed with an increase in nematode density both in bacteria-treated and untreated plants but damage caused by *M. javanica* was less in treated plants.

Root-knot nematodes (*Meloidogyne* spp.) and root-infecting fungi viz., *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani* are associated with serious losses in tomato production (Mai, 1985; Ehteshamul-Haque *et al.*, 1994). As soil applied pesticides are questionable because of toxicological and environmental concerns, the use of microorganisms in the biological control of disease is an alternative method of plant disease control (Lumsden and Lewis, 1989). Of the potential antagonistic rhizobacteria, pseudomonads are aggressive colonizers of roots and shoots (Pillay and Nowak, 1997) and have a broad spectrum of antagonistic activity against phytopathogens (Sharma and Nowak, 1998). Of the *Pseudomonas* species, *P. aeruginosa* is a plant growth promoting rhizobacterium that has been found to be an effective biocontrol agent against root-infecting fungi (Siddiqui *et al.*, 1999). An experiment was, therefore, carried out to examine the effect of two strains of *P. aeruginosa* (Schroeter) Migula used as a soil drench in the control of the root rot-root knot disease complex of tomato.

Materials and methods

Five-day-old bacterial cultures maintained on King's B medium were scraped from the surface using a sterile bent glass rod after adding 10 ml sterile distilled water. Sandy loam soil (pH 8.1) was obtained from the experimental field of the Department of Botany, University of Karachi. The soil had a natural infestation of 6% colonization

of *Rhizoctonia solani* Kuehn on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu g^{-1} of soil of a mixed population of *Fusarium oxysporum* Schlecht emend. Snyd *et* Hans., and *Fusarium solani* (Mart) Appel *et* Wollenw. emend. Snyd. *et* Hans., as assessed by a soil dilution method (Nash and Snyder, 1962). Earthen pots (21 cm. diam.) were each filled with 2 kg soil and planted with two 3-week-old tomato (*Lycopersicon esculentum* Mill.) seedlings raised in sterilized soil. Before transplanting the seedlings, the soil was drenched with a cell suspension of *P. aeruginosa* isolates (Pa-7, $2.4x10^8$ cfu ml^{-1} and IE-6, $2.0x10^8$ cfu ml^{-1}) in 200 ml water. In another similar set of pots, one week after transplanting, the seedlings were inoculated with 0, 500, 1000, 2000, 4000 freshly hatched second stage juveniles (J2) of *Meloidogyne javanica* (Treub) Chitw. cultured on egg plant (*Solanum melongena* L.). A third set of pots was similarly treated with nematodes but without the bacterial inoculum. A set without the bacterium and nematodes was used as control. There were three replicates of each treatment and pots were randomized on a screen house bench at the Soil-borne Disease Research Laboratory, Department of Botany, University of Karachi. Plants were watered daily.

The experiment was terminated 45 days after the addition of nematodes when plant height and fresh weight of shoot and root were recorded. Number of galls and egg masses produced on the entire root system were counted using a stereoscope microscope. To determine the nematode population, soil from the replicates of each treatment was mixed thoroughly in a plastic container and five samples of 50 g were used for nematode extraction using the modified Baermann funnel technique. To determine nematode invasion of the roots, a one g root sample from each plant was stained in 0.25% acid fuchsin in lactic acid and comminuted in an electric grinder for 45 seconds. The macerate was suspended in 100 ml water and *M. javanica* females and juveniles in five samples of 5 ml were counted with the aid of a low power microscope.

To determine the incidence of fungi, roots were washed with running tap water and after surface disinfection with 1% $Ca(OCl)_2$, four 5-mm-long root pieces were plated on to PDA medium supplemented with penicillin (100,000 units/l.) and streptomycin sulphate (0.2 g/l.). After incubation for five days at 28 °C, the incidence of root-infecting fungi was recorded.

To assess the endophytic population of bacteria, the method used by Pillay and Nowak (1997) was modified where one g fresh roots and shoots were surface disinfected in 1% $Ca(OCl)_2$ for 1 (roots) or 2 min. (shoots), rinsed twice with sterilized distilled water, submerged for 30 s (roots) or 1 min (shoots) in 15% H_2O_2 and rinsed twice again in sterile distilled water. The tissue was then macerated in 10 ml of 0.1 M $MgSO_4$ (buffered to pH 6.5) plus 0.05% Tween 20. Ten fold serial dilutions of the suspension were prepared and 50 µl aliquots from the appropriate dilution were plated on to KB medium. Plates were incubated at room temperature (25-30 °C) for 48 hours and the number of cfu was recorded. Plants grown in non-bacterized soil were also checked for the presence of contaminants. Data were analysed and subjected to bifactorial Analysis of Variance (FANOVA) according to Gomez and Gomez (1984). The experiment was repeated.

Results and discussion

P. aeruginosa isolates significantly suppressed root-rot and root-knot diseases and nematode population densities in tomato. An increase in nematode inoculum resulted in increased nematode population density and subsequent gall formation induced by *M. javanica*. *P. aeruginosa* isolates significantly suppressed the nematode population in soil ($p<0.001$), root-knot formation ($p<0.01$) and egg production ($p<0.001$). Treatment with both the bacterial isolates resulted in similar effects on *M. javanica* at lower populations (500 and 1000 J2/plant), whereas only isolate IE-6 was found ef-

fective at the higher nematode densities i.e. 2000 and 4000 J2/plant (Table I).

Soil treatments with *P. aeruginosa* isolates provided significant protection to tomato roots against *F. oxysporum* ($p<0.05$) and *F. solani* ($p<0.001$) but no significant effects on *R. solani* were recorded. Both the isolates showed more than 75% suppression in *F. oxysporum* infection in the presence of low nematode populations (500, 1000 and 2000 J2/plant). Occurrence of *F. oxysporum* was greater in the absence of nematode and/or the bacterium. *P. aeruginosa* isolates were found effective against *Fusarium* spp., in the absence of root-knot nematodes, whereas in the presence of the nematode, isolate Pa-7 was effective at low nematode densities (500, 1000 and 2000 J2/plant) and isolate IE-6 was also found effective at the larger nematode populations (2000 and 4000 J2/plant). Isolate Pa-7 provided complete control of *R. solani* infection at low inoculum densities (0, 500, 1000 and 2000 J2/plant) whereas isolate IE-6 completely prevented root infection by *R. solani* at high population densities of *M. javanica* (2000 and 4000 J2/plant). *R. solani* was not isolated from roots without nematodes and/or the bacterium presumably due to the greater infection of *F. oxysporum*. However, soil treatment with bacterial isolate IE-6 resulted in some colonisation by *R. solani* in the absence of nematodes (Table I).

Plant growth progressively decreased with an increase in nematode density in both treated and untreated plants. Plant height and fresh weight of shoot in the treated plants was significantly

TABLE I - *Effects of* Pseudomonas aeruginosa *and different population levels of* Meloidogyne javanica *on the development of root-rot and root-knot infection, nematode population densities, plant growth and bacterial endophytic colonization in tomato.*

Treatment	Galls per root system	Egg mass per root system	Nematodes in		Infection%			Plant height (cm)	Shoot weight (g)	Root weight (g)	Bacterial colonization (log cfu g^{-1} fresh) +1	
			250 g soil	g^{-1} root	*F. oxysporum*	*F. solani*	*R. solani*				root	shoot
Control	0	0	0	0	83	83	0	25.6	7.9	4.0	0	0
M. javanica (MJ) 500	13	11	560	21	50	67	17	28.5	5.6	2.7	0	0
MJ 1000	17	18	1690	45	33	50	33	29.8	5.4	2.9	0	0
MJ 2000	49	45	4070	107	50	100	0	27.8	6.8	4.6	0	0
MJ 4000	70	66	8730	194	50	100	33	22.8	5.5	6.5	0	0
Control + *P. aeruginosa* (Pa7)	0	0	0	0	0	50	0	32.3	10.0	4.5	4.67	4.53
MJ 500 + Pa7	6	3	470	15	0	50	0	28.5	7.8	3.9	4.46	4.44
MJ 1000 + Pa7	16	11	1570	35	17	17	0	29.8	9.2	4.9	4.62	4.49
MJ 2000 + Pa7	35	36	3500	100	0	50	0	27.8	7.1	2.8	4.46	4.29
MJ 4000 + Pa7	49	46	4300	146	33	100	17	22.8	5.7	2.0	3.89	3.55
Control + *P. aeruginosa* (IE-6)	0	0	0	0	0	17	17	32.3	8.8	4.7	5.19	4.86
MJ 500 + IE-6	8	7	450	16	0	67	17	32.5	12.1	3.3	5.05	4.77
MJ 1000 + IE-6	15	10	1510	30	0	67	17	28.3	9.2	3.4	5.06	4.95
MJ 2000 + IE-6	31	26	1790	55	17	17	0	29.3	10.5	5.4	4.95	4.43
MJ 4000 + IE-6	37	27	3260	123	33	67	0	27.8	9.8	4.6	4.86	4.35
F value: Nematode	30.5	37.0	187.7	50.2	2.6	0.8	0.8	34.3	4.7	0.7	11.5	15.7
Bacteria	5.7	11.2	16.0	3.2	4.6	107	1.1	140.0	55.4	0.7	116.5	31.6
Nematode x Bacteria	1.3	2.2	3.6	0.2	1.7	0.8	0.6	9.6	6.2	2.1	2.9	2.3

greater compared to untreated plants grown in either nematode-infested or non-infested soils. Maximum plant height and fresh weight of shoot were recorded in the treatment where isolate IE-6 was used in soil infested with 500 *M. javanica* J2/plant. Greater root weight was observed where soil was infested with 4000 J2/plant in the absence of the biocontrol agent (Table I).

Both the isolates of *P. aeruginosa* were found to colonize root and shoot tissue. Bacterial populations declined significantly ($p<0.05$) at high nematode densities (2000 and 4000 J2/plant). With the decrease in bacterial population a decrease in plant growth promotion and biocontrol potential of *P. aeruginosa* was observed. At the highest inoculum level of *M. javanica*, the population of isolate Pa-7 declined drastically whereas isolate IE-6 was less affected by the nematode. A negative correlation between *F. oxysporum* infection and bacterial colonization was also observed (Table I).

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