Nematol. medit. (1999), 27: 233-238

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EFFECTS OF FIELD APPLICATION OF VARIOUS MICRO-ORGANISMS ON *MELOIDOGYNE INCOGNITA* ON TOMATO

by
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Summary. Biocontrol potential of *Bacillus subtilis, B. thuringiensis, Pseudomonas stutzeri, Paecilomyces lilacinus* (soil and root-dip treatments) against *Meloidogyne incognita* on tomato was examined in field plots. Soil application of *B. subtilis* or *P. stutzeri* improved the plant growth, leaf pigments and yield of tomato in plots not inoculated with *M. incognita*. In inoculated plots yield was significantly greater in all plots with the bioagents applied in the soil, being greatest with *B. subtilis*. Significant yield enhancement due to root-dip treatments was recorded with *B. subtilis* and *P. stutzeri*. All the treatments, except *P. stutzeri*, decreased root galling and inhibited reproduction of *M. incognita*, being greatest with *B. subtilis*. *P. stutzeri*, however, increased nematode galling and egg mass production. Soil application of the bio-agents was more effective than the root-dip treatment.

Microorganisms with biocontrol ability are of two types. The first like *Paecilomyces lilacinus* (Jatala, 1986), *Verticillium chlamydosporium* (Morgan-Jones *et al.*, 1983) and *Pasteuria penetrans* (Sayre and Starr, 1985) directly parasitise nematodes. The second type antagonises nematodes indirectly by providing greater amounts of nutrients to a plant through their solubilisation (Gaur, 1990), producing certain toxic materials and/or competing for nutrients and space such as *Bacillus subtilis* (Broadbent *et al.*, 1977; Yuen *et al.*, 1985), *B. thuringiensis* (Mankau, 1981), *Clostridium butyricum* (Hollis and Rodriguez-Kabana, 1967).

The present investigation was undertaken as a field trial in which both types of micro-organisms, together with the chemical nematicide aldicarb were tested against the root-knot disease of tomato caused by *Meloidogyne incognita*.

Materials and methods

The bacterial bio-agents, *Bacillus subtilis* Cohn *amend.* Prazmowski (MTCC-2274, *B. thuringiensis* Berliner (MTCC 1953) and *Pseudomonas stutzeri* (Threvesan) Migula (MTCC 863) were obtained from the Institute of Microbial Technology, Chandigarh, India, and were cultured on nutrient broth. The fungus *Paecilomyces lilacinus* (Thoms.) Samsun was procured from the Indian Agricultural Research Institute, New Delhi, and mass-cultured on Richard's liquid medium. Nematode culture was prepared from egg masses, which were excised from glasshouse cultured eggplants.

Seventy two plots (three replicates per treatment), each of 3x1 m dimension were prepared to incorporate the treatments indicated in Tables I-IV. The treatments were applied as a soil appli-

cation or root-dip with three replicates of each. Before application a nematode suspension of *M. incognita* (Kofoid *et* White) Chitw. in water was added to soil where seedlings were to be planted (2000 second stage juveniles/plant).

Soil application of micro organisms was performed by adding 1 ml of bacteria suspension (around 10^8 cells/ml) or 1 ml liquid suspension of *P. lilacinus* (1 g spores + mycelium/ml) to soil around the roots of a seedling at the time of transplant. Aldicarb at 4 kg ai/ha was applied through irrigation which was broadcasted after the nematode inoculation but before seedling planting.

Root-dip treatments were given to seedlings by immersing their roots in the culture of bacteria (10^8 cells/ml) or fungus (1 g spore + mycelium/ml) for 3 hr or aldicarb (100 ppm) for 5 min. After treatment, tomato (*Lycopersicon esculentum* Mill) cv. Pusa Ruby seedlings were directly planted into soil. There were three rows of plants with ten plants per row in each of the 72 microplots. At harvest (three months after the start of the trial) four plants were uprooted (alternate) from each row of a plot. Hence, 12 plants were harvested for each plot (36 plants/treatment) and dry weights of shoots and roots, number of flowers and fruits per plant, mean fruit weight, total weight of fruits/plant, chlorophyll contents of leaves (MacKinney, 1941), number of galls and egg masses per root system and number of eggs per egg mass (fecundity) were determined.

The data on plant growth, yield and chlorophyll content were analysed by a two-facto-

TABLE I - *Effect of soil application of certain micro-organisms on dry matter production and yield of tomato plants inoculated with* Meloidogyne incognita.

Treatments	Nematode inoculation (juveniles/ plant)	Dry weight g		Number/plant		Fresh weight g	
		Shoot	Root	Flowers	Fruits	Mean fruit	Total fruit
Plant alone (control)	0	41.2	12.5	17.4	17.3	63.5	1099
Bacillus subtilis	0	45.1[a]	14.3[a]	18.1	18.0	66.1	1190[a]
B. thuringiensis	0	41.0	12.7	16.9	16.9	63.7	1077
Pseudomonas stutzeri	0	43.2	13.8[a]	18.3	18.1	65.5	1186[a]
Paecilomyces lilacinus	0	41.5	13.0	17.8	17.8	63.4	1129
Aldicarb	0	40.7	12.0	17.1	17.1	63.3	1083
Plant+Nematode (control)	2000	36.6[a]	11.2[a]	16.3	15.9[a]	60.0[a]	954[a]
B. subtilis	2000	39.5[a]	12.3[a]	17.2	17.1[a]	64.1[a]	1096[a]
B. thuringiensis	2000	37.0	11.6	16.5	16.0	60.9	974
P. stutzeri	2000	36.0	12.4[a]	16.7	16.7	63.4[a]	1059[a]
Paecilomyces lilacinus	2000	38.0	12.4[a]	17.2	17.2[a]	62.7	1078[a]
Aldicarb	2000	39.5[a]	12.1[a]	17.1	17.0	62.4	1061[a]
LSD (P=0.05)		2.7	0.8	1.6	1.2	3.3	852
F=value							
Nematode (df=1)		62.1[b]	39.7[b]	NS	11.7[b]	9.2[b]	26.9[b]
Control agents (df=5)		3.4[b]	8.56[b]	NS	NS	NS	5.36[b]
Interaction (df=5)		NS	3.08	NS	NS	NS	NS

[a] significantly different from the control at P=0.05; [b] significant at P=0.05; NS not significant at P=0.05.

TABLE II - *Effect of root-dip treatments with certain micro-organisms on the dry matter production and yield of tomato inoculated with* M. incognita.

Treatments	Nematode inoculation (juveniles/ plant)	Dry weight (g)		Number/plant		Fresh weight (g)	
		Shoot	Root	Flowers	Fruits	Mean fruit	Total fruit
Plant alone (control)	0	41.2	12.5	17.4	17.3	63.5	1098
Bacillus subtilis	0	43.8	13.5[a]	17.7	17.5	65.2	1141
B. thuringiensis	0	41.7	12.0	17.0	17.0	63.6	1081
Pseudomonas stutzeri	0	42.0	13.1	17.5	17.4	64.9	1129
Paecilomyces lilacinus	0	40.7	12.3	17.4	17.4	63.2	1100
Aldicarb	0	39.6	11.5[a]	16.9	16.7	63.6	1062
Plant+Nematode (control)	2000	36.6[a]	11.2[a]	16.3	15.9[a]	60.0	954[a]
B. subtilis	2000	40.4[a]	13.0[a]	17.4	17.4[a]	64.7[a]	1126[a]
B. thuringiensis	2000	37.2	11.4	16.3	16.2	62.5	1013
P. stutzeri	2000	38.0	12.7[a]	17.2	17.1[a]	63.8[a]	1091[a]
Paecilomyces lilacinus	2000	38.9	12.3[a]	17.2	17.2[a]	63.0	1083[a]
Aldicarb	2000	37.5	11.7	16.5	16.3	61.2	998
LSD (P=0.05)		2.8	0.8	1.4	1.2	3.6	85.0
F=value							
Nematode (df=1)		35.8[b]	NS	NS	7.35[b]	NS	6.74[b]
Control agents (df=5)		10.4[b]	12.0[b]	NS	6.8[b]	NS	5.51[b]
Interaction (df=5)		3.9[b]	NS	NS	NS	NS	NS

[a] significantly different from the control at P=0.05; [b] significant at P=0.05; NS not significant at P=0.05.

rial analysis of variance (ANOVA) and the galling, eggmass production and fecundity by a single factor ANOVA and least significance differences (LSDs) were calculated at a probability level of 0.05 (Dospekhov, 1984).

Results

Plants in plots inoculated with *M. incognita* were stunted and had smaller and chlorotic leaves. The plants usually wilted during the hotter period of the day but recovered at night. Plants in plots inoculated with *M. incognita* and treated with the various control agents exhibited normal growth and vigour but with some variation.

Soil application of *B. subtilis* or *P. stutzeri* (root growth) significantly improved plant growth and increased the yield of tomato by about 8% compared with the untreated control (Table I). Other treatments did not affect growth or yield. Root-knot nematode infection led to a significant decrease in the dry weights of shoots and roots and yield of tomato but the treatments, however, checked the pathogenic effects of the nematode (Table I). Aldicarb application was relatively more effective than the micro-organism treatments. *P. lilacinus* or *B. subtilis* significantly increased the number of fruits/plant (inoculated). All the control agents except *B. thuringiensis* checked the adverse effect of *M. incognita* on yield (weight of

fruits/plant). Greatest increase in the yield, however, occurred with *B. subtilis* (14.8%), *P. lilacinus* (12.9%), aldicarb (11.2%) and *P. stutzeri* (11%) compared with inoculated-untreated plants (Table I).

Root-dip treatment was relatively less effective in enhancing the plant growth and yield of tomato compared with soil application (Table II). *B. subtilis* significantly improved the dry weight of root of uninoculated plants. Reduction caused by *M. incognita* in plant growth and yield was considerably less in the plants treated with bacteria/fungus (Table II). Fruit formation was significantly greater in the presence of *B. subtilis*, *P. stutzeri* or *P. lilacinus* on nematode inoculated plants. Greatest enhancement in the yield (weight of fruits/plant) was obtained with *B. subtilis* (18%), *P. stutzeri* (14.3%) and *P. lilacinus* (13.5%). Mean fruit weight was increased by 7.8% (*B. subtilis*) and 6.3% (*P. stutzeri*) compared with inoculated untreated plants (Table II).

Leaves of uninoculated plants treated with *B. subtilis* contained significantly greater amounts of chlorophylls (Table III). Inoculation with *M. incognita*, however, caused a significant decrease in chlorophyll a, b and total chlorophyll. All the control agents through soil application countered the suppressive effects of the nematode on the pigments, but this effect was signifi-

TABLE III - *Effect of certain micro-organisms on the leaf chlorophyll contents of tomato inoculated with* M. incognita.

Treatments	Nematode inoculation (juveniles/ plant)	Chlorophyll (µg/g fresh leaf)					
		Soil application			Root-dip treatment		
		a	b	Total	a	b	Total
Plant alone (control)	0	692	254	946	692	254	946
Bacillus subtilis	0	746[a]	274[a]	1020[a]	733[a]	268[a]	1001[a]
B. thuringiensis	0	680	257	937	685	249	934
Pseudomonas stutzeri	0	721	266	987	711	260	971
Paecilomyces lilacinus	0	687	260	947	681	258	938
Aldicarb	0	675	251	925	669	246	915
Plant+Nematode (control)	2000	609[a]	235[a]	844[a]	609[a]	235[a]	844[a]
B. subtilis	2000	651[a]	246[a]	897	663[a]	250[a]	913[a]
B. thuringiensis	2000	620	238	858	617	235	852
P. stutzeri	2000	642	243	885	652[a]	247	899[a]
Paecilomyces lilacinus	2000	649[a]	241	896	659[a]	243	902[a]
Aldicarb	2000	650[a]	239	889	632	237	869
LSD (P=0.05)		37.6	14.9	56.9	31.0	12.8	49.6
F=value							
Nematode (df=1)		72.7[b]	24.1[b]	78.9[b]	70.9[b]	18.18[b]	15.25[b]
Control agents (df=5)		4.28[b]	3.61[b]	7.64[b]	5.86[b]	15.8[b]	22.16[b]
Combined effect (df=5)		NS	NS	NS	NS	NS	15.15[b]

[a] significantly different from the control at P=0.05; [b] significant at P=0.05; NS not significant at P=0.05.

cant for only chlorophyll a in treatments with *P. lilacinus* or aldicarb. Root-dip treatments with *P. stutzeri* or aldicarb significantly increased chlorophyll a and total chlorophyll of nematode inoculated plants (Table III). Application of *B. subtilis* increased chlorophyll a, b and total chlorophyll of inoculated as well as uninoculated plants.

M. incognita at the inoculum level of 2000 J_2/plant caused extensive galling on tomato roots (Table IV). Reproduction of the nematode in terms of egg mass production and fecundity (number of eggs/egg mass) was also better on these plants. Soil application of *B. subtilis* and *P. lilacinus* suppressed gall formation ($P=0.05$), whereas *P. stutzeri* increased galling by 7.1%. The greatest decrease in the number of galls occurred in the aldicarb application (56.6%). All the treatments except *P. stutzeri* suppressed the egg mass production (Table IV). The reproduction of *M. incognita* was lowest with aldicarb (63.4%), followed by *P. lilacinus* (48.7%), *B. subtilis* (25.6%) and *B. thuringiensis* (4.9%).

Root-dip treatment with aldicarb, *B. subtilis* or *P. lilacinus* decreased gall formation by 39.7, 15.7 and 13.3%, respectively (Table IV). Significant decrease in egg mass production was recorded with aldicarb (52.4%), followed by *P. lilacinus* (18.3%) and *B. subtilis* (17.1%). *P. stutzeri* significantly enhanced the fecundity but the remaining treatments were found suppressive.

Discussion

Application of *B. subtilis* or *P. stutzeri* improved the growth and yield of uninoculated tomato plants, probably by solubilizing phosphates and other nutrients and making them available to the plants (Gaur, 1990). Soil application was relatively more effective than root-dip treatment in promoting growth and yield. Soil application may have resulted in a higher and more uniform distribution of bacterial cells in the root zone during the entire growth period, whereas with root-dip treatment, fewer cells may have remained available to the entire root-system.

Treatments with *B. subtilis* decreased the disease severity through its growth promoting effect (Broadbent *et al.*, 1977) or by producing balbiformii toxin (Vasudeva *et al.*, 1958). The nematicidal effect of *B. subtilis* on root-knot and cyst forming nematodes has also been noticed by other researchers (Gokte and Swarup, 1988; Gautam *et al.*, 1995). Reduction in galling and egg mass production of *M. incognita* due to application of *P. lilacinus* was due to parasitism of the nematode by the fungus as it is an established parasite of root-knot and cyst nematodes (Jatala, 1986). Greater decrease was observed in fecundity. Eggs are more susceptible for the infection by *P. lilacinus* (Morgan-Jones *et al.*, 1984).

Pseudomonas stutzeri promoted nematode disease as well as growth and yield of infected tomato plants. This indicates that yield enhancement was entirely due to growth promoting effects. *B. thuringiensis* synthesizes delta endotoxin (DET), which may inhibit the parasitism of *M. javanica*, *Heterodera glycine* and *Tylenchulus semipenetrans* (Osman *et al.*, 1988; Noel, 1990). The strain of the bacterium used either did not produce the toxin or it was not toxic to *M. incognita.*

Application of *B. subtilis* in soil induced the greatest enhancement in the growth and yield of tomato plants inoculated or not with *M. incognita* and the root-knot disease was also much suppressed. *P. lilacinus* decreased the galling and improved the yield only of nematode inoculated plants. Hence, *B. subtilis* can be used as a biofertilizer-cum-pesticide for the management of root-knot disease of tomato.

Acknowledgements. Authors are grateful to Prof. M. S. Jairajpuri and Dr. G. N. Bhat, then Directors (during 1995 and 1996, respectively), Institute of Agriculture, AMU, for providing necessary facilities to carry out the research.

TABLE IV - *Effect of certain micro-organisms on root-knot disease and reproduction of* M. incognita *on tomato.*

Treatments	Root-dip treatment			Soil application		
	Galls	Egg masses	Eggs	Galls	Egg masses	Eggs
Plant+Nematode (control)	83	82	219	83	82	219
Bacillus subtilis	70[a]	68[a]	172[a]	61[a]	61[a]	180[a]
B. thuringiensis	82	78[a]	215	80	79	210
Pseudomonas stutzeri	84	81	237[a]	89[a]	89[a]	231
Paecilomyces lilacinus	72[a]	67[a]	147[a]	66[a]	42[a]	132[a]
Aldicarb	50[a]	39[a]	181[a]	36[a]	30[a]	162[a]
LSD (P=0.05)	5.3	3.5	14.8	3.83	3.3	9.8
F=value						
Control agents (df=5)	106.6[b]	283.3[b]	141.8[b]	104.1[b]	272.9[b]	124.6[b]

[a] significantly different from the control at P=0.05; [b] significant at P=0.05.

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Accepted for publication on 3 May 1999.