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Department of Plant Protection, Rafi Ahmad Institute of Agriculture Science, Aligarh Muslim University, Aligarh 202 002, India

EFFECTS OF CERTAIN ANTAGONISTIC FUNGI AND RHIZOBACTERIA ON WILT DISEASE COMPLEX OF TOMATO CAUSED BY *MELOIDOGYNE INCOGNITA* AND *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

by

M. R. KHAN and M. AKRAM

Summary. An investigation was carried out to examine the effect of soil application of *Paecilomyces lilacinus*, *Gliocladium virens*, *Pseudomonas fluorescens* PRS-9, *Bacillus polymyxa* and pesticides (aldicarb + thiram) against a disease complex caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* on growth and yield of tomato in field plots. The nematode and fungus, acting alone caused characteristic root galling and shoot wilting, respectively and significantly reduced plant growth and yield. In concomitant inoculation, severity of fusarial wilt was significantly increased and plant growth and yield reductions were also considerably greater compared to the sum of individual effects of the pathogens. On such plants, pathogenesis and reproduction of the nematode was, however, suppressed. Application of *P. lilacinus* and *G. virens* significantly enhanced the plant growth and yield of nematode and fungus inoculated plants, respectively. Greatest enhancement of plant growth and yield of nematode-fungus infected plants occurred with *P. fluorescens*, followed by the pesticides and *G. virens* or *P. lilacinus*. Hatching of larvae, gall formation, egg mass production, fecundity and soil population of *M. incognita* and wilting index and rhizospheric population of *F. oxysporum* f. sp. *lycopersici* were decreased due to application of the control agents except *B. polymyxa*, which caused increase in the dry matter production and yield of uninfected tomato plants and soil population and fecundity of the nematode and rhizosphere population of the fungus.

In dealing with a *Meloidogyne-Fusarium* disease complex, the bioagent should be selected with reference to specific targets. *Paecilomyces lilacinus* is a strong parasite of eggs of root-knot nematodes (Jatala, 1986). *Gliocladium virens* is a mycoparasite of *Fusarium* species (Cipriano *et al.*, 1989). Certain isolates of *Pseudomonas fluorescens* may suppress both the nematode and fungus (Leeman *et al.*, 1995).

The growth promoting rhizobacteria, such as *Bacillus polymyxa*, may also suppress nematode and fungal diseases in an indirect way by making healthier and stronger host, which may resist pathogens (Khan and Khan, 1998).

An investigation was carried out to evaluate efficacy of certain biocontrol organisms and pesticides against the wilt disease complex caused by *M. incognita* (Kofoid *et* White) Chitw. and *F. oxysporum* f. sp. *lycopersici* (Schlecht) on tomato in field plots.

Materials and methods

Seventy two plots, each of 2x3 m, were prepared in a sandy loam soil and farm yard manure was added (10 kg/plot). Treatments were applied as indicated in Table I. The cultures of *F. oxysporum* f. sp. *lycopersici*, *P. lilacinus* (Thom.) Samson and *G. virens* Corda., obtained from the Indian Agricultural Research Institute, New Delhi were reared on Richard's liquid me-

TABLE I - *Effects of biocontrol agents and pesticides on the plant growth biomass and yield of tomatoes in soil inoculated with* Meloidogyne incognita *and/or* Fusarium oxysporum *f. sp.* lycopersici.

Treatment	Inoculation		Shoot length cm	Fresh weight g		Dry weight g		Weight of fruits/plant g
	Nematode	Fungus		Shoot	Root	Shoot	Root	
Control	0	0.0	52.8	48.1	21.9	14.2	5.10	1177
Control	2000	0.0	47.1*	40.8*	20.8	12.7*	4.62*	980*
Control	0	2.0	48.7*	38.5*	20.0*	12.2*	4.61*	1021
Control	2000	2.0	41.9*	37.3*	18.1*	10.3*	4.0*	745*
P. lilacinus	0	0.0	53.5	47.6	22.0	14.6	5.07	1163
	2000	0.0	50.7*	43.7*	21.6	13.5*	5.34*	1085*
	0	2.0	48.5	39.1	19.9	12.3	4.67	1056
	2000	2.0	46.0*	39.5	19.0	11.9*	4.45*	912*
G. virens	0	0.0	52.3	47.7	22.1	14.0	5.11	1141
	2000	0.0	47.8	41.0	20.7	13.0	4.97	1008
	0	2.0	51.9*	41.1*	21.2*	13.1*	4.9*	1095*
	2000	2.0	43.4	40.8*	19.0	11.0	4.21	807*
P. fluorescens	0	0.0	53.3	48.4	22.3	14.4	5.26	1202
	2000	0.0	49.8*	42.9*	21.8	13.5*	4.96*	1056*
	0	2.0	51.0	40.7*	21.2*	13.0*	4.87*	1095*
	2000	2.0	49.2*	42.1*	21.0*	11.9*	4.69*	1008
B. polymyxa	0	0.0	53.5	48.9	22.1	15.5*	5.47*	1372*
	2000	0.0	47.9	41.3	21.0	13.0	4.78	1018
	0	2.0	49.2	39.0	20.3	12.5	4.70	1011
	2000	2.0	42.6	37.7	18.2	10.9	4.13	788
Aldicarb+	0	0.0	52.4	47.5	21.5	14.0	5.02	1138
Thiram	2000	0.0	51.9*	43.7*	22.2*	13.8*	5.00	1034*
	0	2.0	51.7*	42.9*	21.5*	13.1*	4.86*	1082*
	2000	2.0	49.0*	44.7*	21.2*	12.5*	4.65*	967*
L.S.D. (P=0.05)			2.35	1.95	1.18	0.76	0.24	48.5

* Significantly different from the respective controls at P=0.05.

dium. The mycelial mat collected from the flasks was blended in a known volume of distilled water. *P. fluorescens* (Threvesan) Migula (strain PRS-9) and *B. polymyxa* Cohn were procured from G. B. Pant University of Agriculture and Technology, Pant Nagar, India and Institute of Microbial Technology, Chandigarh, India and mass cultured in King's liquid medium and nutrient broth, respectively. For field application, the suspension containing 2 g mycelium + spores or 1 ml pure broth was spread to soil (10 cm diameter, 10 cm depth) where seedlings were to be planted. Inoculation was done at 30 spots in a microplot. Inoculum of *F. oxysporum*

f. sp. *lycopersici* and/or *M. incognita* (2000 juveniles/spot) was added a day before seedling planting, whereas the other organisms were applied at the time of planting. The mixture of carbofuran and thiram at the rate of 2.5 kg a.i./ha and 5 kg a.i./ha, respectively, was applied to the soil before the planting of the seedlings but after the inoculation of the pathogens.

Two-week old seedlings of tomato, *Lycopersicon esculentum* Mill. cv. Pusa Ruby, raised in sterilized soil were planted in exactly the locations where the various treatments had been given. Three rows were maintained in each microplot with 10 plants/row. Each of the 24 treatments was replicated three times; treatments were randomly distributed. Plots were irrigated with channel irrigation fortnightly. Three and half months after planting, five alternate plants were uprooted from each row and shoot length and dry weights of shoots and roots, number of flowers and fruits/plant, mean fruit weight, weight of fruits/plant, number of galls and egg masses/root system, number of eggs/egg mass and rhizospheric population of *F. oxysporum* f. sp. *lycopersici* (dilution plate method) and *M. incognita* (Cobb's decanting and sieving method) were determined. The entire root system was examined to count galls and egg masses.

The effect of *G. virens* on the radial growth of *F. oxysporum* f. sp. *lycopersici in vitro* was studied on potato dextrose agar in Petri plates. The plates were inoculated with *G. virens* and/or *F. oxysporum* f. sp. *lycopersici* 1 cm apart from each other in the centre. Ten plates were maintained for each of the three treatments viz. *F. oxysporum* f. sp. *lycopersici*, *G. virens* and *F. oxysporum* f. sp. *lycopersici* + *G. virens*. After inoculation the plates were incubated at 25±2 °C for 5 days in a B.O.D. incubator and radial growth of the fungi was measured.

Measurements on 15 plants (5 plants/row) collected from a single plot were averaged and considered as one replicate. The data (three replicates) were analysed by one factor analysis of variance (ANOVA) and least significance difference (LSD) was calculated at a probability level of 0.05 (Dospekhov, 1984). To identify a significant treatment, nematode and/or fungus inoculated plants were compared with the uninoculated control, whereas nematode or fungus inoculated plants and treated with antagonistic fungi or rhizobacteria were compared with nematode or fungus inoculated plants not treated with the microorganisms. Similarly nematode + fungus inoculated and treated plants were compared with nematode + fungus inoculated and untreated plants.

Results and discussion

Inoculation of plants with *F. oxysporum* f. sp. *lycopersici* or *M. incognita* caused significant suppression of length and dry weight of shoots and roots and yield of tomato plants (Table I). Concomitant inoculations with the nematode and fungus resulted in greater reductions. Aplication of *B. polymyxa* significantly promoted plant growth and yield of uninoculated plants compared to the control (Table I). Growth and biomass production of nematode infected plants were significantly promoted due to the application of pesticides or *P. lilacinus*. Pesticide treatment also produced the greatest enhancement (significant) in the growth and biomass of pathogenic fungus inoculated plants in comparison to fungus inoculated untreated plants, next were *G. virens* and *P. fluorescens*. Pesticide treatments significantly improved the plant growth variables (14-22%) of nematode + fungus inoculated plants compared to the nematode + fungus inoculated untreated plants (Table I). Aplication of *P. fluorescens* also induced similar effects but growth enhancements were smaller ie., 10-17%. *G. virens* significantly enhanced the fresh weight of shoot of nematode + fungus infected plants.

Treatments with *P. fluorescens* (28%) and pesticides (24%) significantly increased the yield of nematode + fungus inoculated plants com-

pared to inoculated-untreated plants (Table I). Significant increase in mean fruit weight of nematode + fungus inoculated plants was associated with the application of *P. fluorescens*. The yield of concomitantly inoculated plants was, however, increased by all the microorganisms except *B. polymyxa*. Application of *P. lilacinus* and *G. virens* led to significant increase in fruit weight/plant of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* inoculated plants, respectively, whereas *P. fluorescens* increased the yield of both group of plants. Application of fungicides also resulted in an increase in the yield of nematode and/or fungus infected plants, but greater enhancement occurred due to *P. fluorescens*.

M. incognita at 2000 juveniles/plant caused extensive galling on the root system (Table II). Gall formation and egg mass production were, however, significantly decreased in the presence of *F. oxysporum* f. sp. *lycopersici*. Pesticide treatment suppressed gall formation by 71%, followed with 29 and 24% by *P. lilacinus* and *P. fluorescens*, respectively, in comparison to untreated plants. Reproduction of the nematode (number of egg masses/root system) was signif-

TABLE II - *Effects of biocontrol agents and pesticides on the intensity of root-knot and fusarial wilt and reproduction of the nematode and soil population of the wilt fungus on tomato plants inoculated with* M. incognita *and/or* F. oxysporum *f. sp.* lycopersici.

Treatment	Inoculation		Number/root system		Fecundity (Eggs/egg mass)	Wilting index (0-5 scale)	Soil population of *F. oxysporum* f. sp. *lycopersici* (CFUs/g of soil)
	Nematode	Fungus	Galls	Egg masses			
Control	2000	0.0	137	132	261	–	–
Control	0	2.0	–	–	–	2.1	17.5×10^4
Control	2000	2.0	89*	85*	181*	2.4*	18.3×10^4
P. lilacinus	2000	0.0	97*	62*	154*	–	–
	0	2.0	–	–	–	2.1	17.2×10^4
	2000	2.0	64*	40*	116	2.2*	17.7×10^4
G. virens	2000	0.0	132	125	247	–	$4.1 \times 10^{3*}$
	0	2.0	–	–	–	1.3*	$4.2 \times 10^{3*}$
	2000	2.0	86	80	173	1.8*	–
P. fluorescens	2000	0.0	104*	81*	122*	–	–
	0	2.0	–	–	–	1.6*	$7.9 \times 10^{3*}$
	2000	2.0	52*	32*	72*	1.7*	$12.0 \times 10^{3*}$
B. polymyxa	2000	0.0	141	138	293*	–	–
	0	2.0	–	–	–	2.0	17.6×10^4
	2000	2.0	82	70*	195	2.3	$20.5 \times 10^{4*}$
Aldicarb+	2000	0.0	39*	25*	57*	–	–
Thiram	0	2.0	–	–	–	1.0*	$12.3 \times 10^{2*}$
	2000	2.0	24*	17*	42*	1.1*	$14.7 \times 10^{2*}$
L.S.D. (P=0.05)			8.5	7.2	13.6	0.15	0.41×10^2

* Significantly different from the respective controls at P=0.05.

icantly decreased by pesticides (81%), followed by *P. lilacinus* (53%) and *P. fluorescens* (39%) (Table II). Significant suppression of fecundity (number of eggs/egg mass) was recorded with pesticides (78%), *P. fluorescens* (53%) and *P. lilacinus* (41%) (Table II). The pesticides used were systemic (Johnson, 1985; Nene and Thapliyal, 1990) and remained active enough in the plant system even at a late stage and affected the nematode reproduction leading to a decrease in fecundity. Lower fecundity may also have resulted from some late residual effect of the pesticide that accumulated in the nematode body. Application of *B. polymyxa*, however, significantly increased the fecundity. Numbers of nematode eggs and juveniles in the soil were considerably greater in the rhizosphere of nematode-inoculated untreated plants (Table II). In the presence of *F. oxysporum* f. sp. *lycopersici* the population, however, declined by 26%. The pesticide treatment decreased the soil population of nematode infected plants by 60%, followed by 38% and 34% due to *P. fluorescens* and *P. lilacinus*, respectively. Application of *B. polymyxa* appeared to be favourable for nematode population leading to a significant increase in the soil population compared to nematode inoculated untreated plants (Table II). Maximum decline in the population of nematode + fungus infected plants was recorded with the pesticide treatment (52%) followed by *P. lilacinus* (24%) and *P. fluorescens* (23%) compared to the plants inoculated with both nematode and fungus but not treated. Application of *B. polymyxa* or *G. virens* resulted to a significant increase in the soil population of *M. incognita*, of nematode + fungus inoculated plants.

The pathogenic fungus (2 g mycelium/plant) induced moderate wilting of tomato foliage. *M. incognita*, however, exacerbated the wilting by 14.3% (Table II). The control agents checked the wilting to a varied degree. A decrease of 52% in wilting occurred on nematode-fungus inoculated plants due to the application of pesticides, compared to the control. Tomato plants developed 38.1 and 23.8% less wilting when treated with *G. virens* and *P. fluorescens*, respectively. *P. lilacinus* did not influence the fusarial wilt in the absence of nematodes. On the nematode-fungus inoculated plants, *P. lilacinus* application, however, suppressed the wilting significantly. Modest wilt symptoms on nematode + fungus infected plants were observed with pesticide (54% decrease), *P. fluoresens* (29%), and *G. virens* (25%), compared to the control (Table II).

The soil population of *F. oxysporum* f. sp. *lycopersici* in terms of the number of colony forming units (CFU)/g of soil was considerably greater (significant) in the rhizosphere of the fungus-inoculated plants (Table II). The nematode infection led to a significant increase in CFUs of the fungus. Application of bioagents induced various effects on the CFU count. Treatments with *G. virens*, *P. fluorescens* and pesticides caused a significant decline in CFUs in rhizosphere of fungus-infected plants. The growth of *F. oxysporum* f. sp. *lycopersici* was relatively slower than *G. virens* and the average colony size was 2.6 cm in diameter (data not given). Dual inoculated plates showed restricted colony growth of the wilt fungus, whereas growth of the mycoparasitic fungus was unaffected. Mycelium of *G. virens* restricted the growth of *Fusarium* colony on PDA in Petri plates.

Treatments with *P. fluorescens* decreased the soil population of *F. oxysporum* f. sp. *lycopersici* (41%) and *M. incognita* (38%) to a practically exploitable level. Suppression in the fungal and nematode pathogenesis led to 31% increase the yield of concomitantly inculated plants.

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