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Management of Root Knot Nematode *Meloidogyne incognita* by Plant Growth Promoting Rhizobacteria on Tomato

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Abstract.- This study was planned to assess the efficacy of plant growth promoting rhizobacteria (PGPR) against *Meloidogyne incognita* infection on roots of tomato (*Lycopersicon esculentum* Mill.) in the green house at 30 $\pm$ 4 ^{o} C. Fifteen-days-old seedlings of tomato cultivar "Money Maker" were planted singly in 15-cm-diam pots filled with sterilized sandy soil. Two days after transplanting, 20-ml of 5% sugar solution containing 10^7 CFU/ml each of *Bacillus* spp., *Azotobacter* spp., *Pseudomonas putida* and *P. fluorescens*, were pipetted into three 3-cm deep holes surrounding the root zone of each plant. Five days after the application of PGPR, freshly hatched 2000 J_2 were applied at root zone. The experiment consisted of seven treatments; each with seven replicates and arranged in CRD. Pots with nematodes, without nematodes, and PGPR were kept as control for comparison. Sixty days after inoculations, data of plant growth parameters such as plant height, fresh and dry root and shoot weight and nematode reproduction in term of egg masses per root system, galls per root system, J2/ one gm of root and females per root system were recorded. The plants treated with *P. fluorescens* significantly (P = 0.05) suppressed females per root system (40.52%), J2/one gm of root (39.80%), galls per root system (41.50%) and egg masses per root system (43.23%) resulting in improved growth over control plants. The treatments having *P. putida*, *Bacillus* spp. and combination of PGPR showed intermediary effects on both nematode reproduction and plant growth. *Azobacter* spp. was least effective in suppressing only 28.10%, 15.87%, 29.38%, 29.29% females per root system, J2 per gm of root, galls per root system and egg masses per root system, respectively.

Key words: Rhizobacteria, PGPR, root knot nematode, tomato, management.

INTRODUCTION

Root knot nematodes are sedentary obligate endoparasitic nematodes that cause major economic damage to crops around the world (Williamson and Hussey, 1996). Plant parasitic nematodes cause global losses to crop plants with an estimated loss of $ 125 billion per year in the tropics (Chitwood, 2003). Four major species, namely *M. incognita M. javanica*, *M. hapla* and *M. arenaria* have been reported to infect tomatoes in Pakistan (Anwar, 1989; Maqbool *et al*., 1988) but *M. incognita* has been found dominant and major limiting factors in the tomato crop production in major production regions of Pakistan (Anwar, 1989; Maqbool *et al*., 1988). Second stage juveniles (J_2) penetrate the roots and migrate to the vascular cylinder, induce severe root galling ravage the utilization efficiency of water and nutrients and greatly affect the partitioning of photosynthetic products (McClure, 1977). Consequently the nematode infection of plants leads to foliage symptoms including stunted growth, wilting, and poor fruit yield.

Several control strategies, such as host plant resistance, rotation with non-hosts, sanitation and avoidance, destruction of residual crop roots, and judicious use of nematicides, have been reported to effectively control root-knot nematodes (Whitehead, 1998). Due to environmental concerns and increased regulations on use of chemical fumigants, more management strategies for management of root-knot (*Meloidogyne* spp.) nematodes are currently being investigated (Nico *et al*., 2004). Biological control using microbial antagonists is one potential alternative to chemical nematicides. Among the biological control agents that have been assessed are egg-parasitic fungi, nematode-trapping fungi, bacteria, and polyphagous predatory nematodes (Gray, 1988; Kerry, 1988; Kerry and Hidalgo-Diaz, 2004; Kiewnick and Sikora, 2005). Plant-growth promoting rhizobacteria (PGPR) have been identified as a biological control alternative to

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pesticide use for disease suppression without negative effects on the user, consumer or the environment (Johnsson *et al*., 1998). The knowledge that agricultural production depends on complex biological equilibrium in soil will ultimately aid in modifying agro-ecosystems and obtaining more favorable conditions for plant growth and health. One practical challenge to implementing this approach is establishing beneficial microbial communities, such as PGPR to promote soil ecosystem health that contributes to suppression of plant pathogens and other pests. PGPR have shown positive effects in plants on such parameters as germination rate, tolerance to drought, weight of shoots and roots, yield, and plant growth under salt stress (Yildirim *et al*., 2006; Kloepper *et al*., 2004; Kokalis-Burelle and Dickson, 2003; Van Loon *et al*., 1998). PGPR based inoculants include formulations containing a singles strain, a mixture of two strains, or complex mixtures of strains of *Bacillus* spp. (Kloepper and Ryu, 2006; Lucy *et al*., 2004; Martinez-Ochoa, 2000; Zehnder *et al.*, 2001; Ryu *et al*., 1999; Raupach and Kloepper, 1998). Another major benefit of PGPR is their use as biological control agents for plant disease-causing organisms (Ji *et al*., 2006; Zehnder *et al*., 2001). Therefore this study was planned with this objective to test the efficacy of PGPR against *M. incognita* infection.

MATERIALS AND METHODS

Nematode inoculum

Meloidogyne incognita population, originally isolated from eggplants was multiplied and maintained on susceptible eggplant cv. Dilshan in a greenhouse. Eggplants were uprooted carefully; roots were washed gently and cut in to small pieces. The roots were shaken vigorously for four minutes in a beaker containing 200 ml 1% NaOCl to release the eggs from egg-masses (Hussey and Barkar, 1973). Eggs were collected on 400 sieves and poured on extraction dish. Eggs were allowed to hatch for 48 hours at 30±2°C in incubator to obtain second stage juveniles (J_2) for inoculation of tomato seedlings.

Multiplication of PGPR

The PGPR was supplied by Soil Bacteriology section, Ayub Agriculture Research Institute, Faisalabad. PGPR included *Bacillus* spp., *Azotobacter* spp., *Pseudomonas putida* and *Pseudomonas fluorescens*. They were multiplied on nutrient broth. For making the stock solution, their culture was mixed in 100 ml 5% sugar solution to a have the concentration of 10^7 CFU/ml of each PGPR.

The experiment was conducted in Plant Pathology Institute, Ayub Agriculture Research Institute, Faisalabad. Clay pots of 15-cm-diam were filled with formalin sterilized sandy soil. Fifteen-day-old seedlings of tomato cv. Money Maker were planted singly in pots. Two days after transplanting, 20 ml of 5% sugar solution containing PGPR 10^7 CFU/ml was pipetted into three, 3-cm deep holes surrounding the root zone of each plant. Five days after the application of PGPR, freshly hatched 2000 J_2 were introduced at root zone. Inoculation holes were re-filled with steam-sterilized soil and pots were watered immediately to moisten the soil. Pots with nematodes only as well as without nematodes and PGPR were kept as control. The experiment was arranged in a completely randomized design with seven treatments, each with seven replication laid out in green house.

The plants were allowed to grow for 60 days and then harvested to determine the plant growth parameters consisting of height, fresh and dry root and shoot weight and number of egg masses per root system. Plants were carefully removed from the pots, and the root systems washed free of soil. The root systems were rated for galling on a 0 to 10 scale (Bridge and Page, 1980). The roots were stained with Pheloxin B (Southey, 1986) and number of egg masses were counted.

Data analysis

Data was analyzed using ANOVA by using SAS statistical software (SAS Institute, Cary, NC, USA). The significance of differences within treatments was separated by using Least Significant Difference test at 5%.

RESULTS

The result of the experiment revealed that four

Table I.- Effect of PGPR on nematode reproduction.

Treatments	Egg masses per root system	Females per root system	J2 per g root	Galls per root system
Bacillus spp.	430.00 d	480.00 d	14330 c	456.00 d
Azotobacter spp.	502.00 b	550.00 b	15680 b	524.00 b
P. putida	420.00 e	470.00 e	12850 e	451.00 e
P. fluorescens	396.00 f	455.00 f	11220 f	434.00 f
Bacillus spp. + *Azotobacter* spp.+*P. Putida*+ *P. Fluorescens*	465.00 c	498.00 c	13630 d	486.00 c
Control (only RKN)	710.00 a	765.00 a	18640 a	742.00 a
Control	0.0000 g	0.0000 g	0.000 g	0.0000 g

Numbers followed by different letters in the same columns are significantly different from each other at 5% probability level. Data is mean of seven replications

Table II.- Effectiveness of PGPR on the plant growth.

Treatments	Fresh weight (g)		Height (cm)		Dry weight (g)	
	Root	Shoot	Root	Shoot	Root	Shoot
Bacillus spp.	7.67 c	23.54 d	38.60 d	33.75 d	0.80 c	1.83 d
Azotobacter spp.	8.30 b	22.55 e	37.68 e	30.25 e	0.93 b	1.73 e
P. putida	6.39 e	25.35 b	40.54 b	37.50 b	0.70 e	1.96 b
P. fluorescens	5.46 f	27.26 a	43.43 a	40.45 a	0.60 f	2.06 a
Bacillus spp.+*Azotobacter* spp.+*P. Putida*+ *P. fluorescens*	6.87 d	24.65 c	39.46 c	35.65 c	0.76 d	1.90 c
Control [only RKN]	9.85 a	16.85 g	30.93 g	21.95 g	1.05 a	1.53 g
Control	4.81 g	20.20 f	36.82 f	26.66 f	0.48 g	1.66 f

Numbers followed by different letters in the same columns are significantly different from each other at 5 % probability level. Data is mean of seven replications

strains of PGPR varied in response for controlling root knot nematode (Tables I and II). This indicates that each PGPR strain has different potential to check RKN populations. The PGPR strains increased the root and shoot length in a variable range. The maximum root and shoot length was observed after treatment with *P. fluorescens*. *Bacillus* spp., *P. putida* and combined treatments showed moderate effect on root and shoot growth. The minimum root and shoot length was observed in *Azotobacter* spp. (Table II).

Root knot nematode ability to produce gall on roots which increased the root weight due to malfunction of root, the bacteria which effectively decreased the weight was *P. fluorescens.* The other treatments have moderate effect. The minimum effect was show by *Azotobacter* spp. As compared to control the same result was obtained when root and shoot was oven dried at 70°C for 72h. The *P. fluorescens* proved best, while *Azobacter* spp. had minimum effect compared to the control (Table II).

The nematode reproduction was assessed by females per root system, J2/one g of root, galls per root system and production of egg masses per root system. The minimum females per root system (455.00), J2 per g of root (11220), galls per root system (434.00) and egg masses per root system (396) was shown by *P. fluorescence. P. putida*, *Bacillus* spp. and combined application moderately reduced egg masses, while *Azobacter* spp. produced maximum females per root system (550.00), J2 per g of root (15680), galls per root system (524.00) and egg masses per root system (502.00) as compared to all other PGPR and include least potential in controlling RKN population (Table I).

DISCUSSION

The results showed that damage of root knot nematode was reduced by using PGPR, a single strain or two strains or complex mixtures of PGPR (Kloepper and Ryu, 2006; Lucy *et al.*, 2004; Martinez-Ochoa, 2000; Zehnder *et al.*, 2001; Raupach and Kleopper, 1998). The plant growth

promoting rhizobacteria significantly reduced galling and egg masses on the roots by root-knot nematodes in tomato crops and resulted in increased yield (Kokalis-Burelle and Dickson, 2003; Siddiqui *et al*., 2001). The plant growth promoting rhizobacteria have been reported to improve plant growth either through direct stimulation by the synthesis of phytohormones (Xie *et al*., 1996) or by decreasing the effect of pathogens (Weller, 1988; Weller *et al*., 2002). It has been reported that *Bacillus* spp. produces lipopeptides, surfactins, bacillomycin D, and fengycins, which are secondary metabolites mainly with antifungal activity (Chen *et al*., 2006). *Pseudomonas* spp. is aggressive colonizers of the rhizosphere of various crop plants and has broad spectrum antagonistic activity against plant pathogens (Weller *et al*., 2002; Li *et al*., 2002; Raajimakers and Weller, 2001; Parveen *et al*., 1998). Some species of *Pseudomonas* and *Bacillus* are reported to induce systemic resistance in plants against invading pathogens and antagonists to root-knot nematodes (Kloepper and Ryu, 2006; Kloepper *et al*., 2004; Siddiqui *et al*., 2001; De Meyer *et al*., 1999; Wei *et al*., 1996; Zhou and Paulitz, 1994).

The reduction of galls and number of egg masses by PGPR, as found in our study, agrees with Kloepper *et al*. (1991), Kokalis-Burelle and Dickson (2003), Kloepper *et al*. (1999), Siddiqui *et al*. (2001), Ali *et al*. (2002), Siddiqui and Shaukat, (2002) and Li *et al*. (2005).

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