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Efficacy of *Pasteuria penetrans* on *Meloidogyne incognita* Reproduction and Growth of Tomato

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Abstract.- Increase in plant growth responses of tomato including fresh and dry weight of shoot, shoot length, and yield of nematodes inoculated tomato plants increased when challenged with endospores of *Pasteuria penetrans* (Thorne). It increased the tomato yield 43.79 % over that of nematode inoculated plants. Nematode reproduction parameters *i.e.*, root galls, egg masses, and J2/100 ml was also variable between plants nematodes inoculated and that of nematode + *Pasteuria* treated plants. There was 88.54% reduction in nematode reproduction factor was recorded between nematode inoculated and that of nematode + *Pasteuria* treated plants.

Key words: *Pasteuria penetrans, Meloidogyne incognita*, tomato growth.

INTRODUCTION

Meloidogyne incognita (Kofoid & White) Chitwood is a damaging pest of vegetable crops (Anwar and McKenry, 2010; Kamran *et al*., 2012) and *Pasteuria penetrans* (Thorne, 1940) is a hyper parasite with potential as biological control agent of root knot nematodes (Dong and Zhang, 2006; Tian *et al*., 2007). Significant reduction in nematode population in vegetables were attained when *P. penetrans* were incorporated in potted soil, containers or tunnel soils (Gowen and Ahmad, 1990; Tzortzakakis and Gowen, 1994).

These bacteria either act as restraining the nematode migration toward the roots or by reducing their reproduction (Brown and Smart, 1985; Brown and Nordmeyer, 1985; Davies *et al*., 1988). *Pasteuria penetrans* was the most studied organism in producing *Meloidogyne* suppressive soils (Oostendorp *et al*., 1991; Stirling, 1991). The parasitism of *Meloidogyne* sp. by *P. penetrans* has two main steps: first, an external adhesion of free spores on the nematode cuticle in the soil which depends on attachment compatibility between bacterial spores and nematodes (Davies and Redden, 1997) and on the population densities of each organism (Stirling, 1991), second, an internal infection when nematodes after reaching the roots and start feeding spores germinate, penetrate through the nematode cuticle, colonize the nematode coelome (Sayre and Starr, 1985; Chen and Dickson, 1998) and produce new spores which are released in soil after nematode death.

In Pakistan work on *P. penetrans* has been done on their *in vitro* studies (Zareen *et al*., 2002), management of *M. javanica* (Treub) in combination with other biocontrol agents (Mukhtar *et al*., 2002) and their mass production (Ahmad and Mukhtar, 2007a). The objective of this work was to evaluate the potential of exotic *P. penetrans* isolate on local population of *M. incognita* reproduction and plant growth responses of tomato.

MATERIALS AND METHODS

Preparation of micro plots

Micro plot measuring 210 x 90 x 60 cm was constructed with bricks and cement in experimental fields at Department of Plant Pathology, University of Agriculture, Faisalabad (Pakistan). The foundation was sealed with concrete and there was no chance of leakage between the micro plots. The plots were filled with formalin sterilized well mixed sandy loam soil (70% sand, 22% silt, 8% clay). Two ridges/microplat were made, on which 10 three weeks old seedlings of tomato were transplanted in two rows.

Transplants were spaced 30-cm apart in the

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row and 45-cm between rows with 5 plants per row. Trails were laid out in a completely randomized design with following treatments *i.e.* nematode + *P. penetrans*-3 (PP-3), nematode only, PP-3 only and control. Clean hand pump water was used for irrigation and weeds were manually removed.

Preparation of P. penetrans *spore suspension for inoculation*

P. penetrans isolate PP-3 (South Africa isolate) was provided by Dr. S.R. Gowen, School of Agriculture, Policy and Development, University of Reading, UK. Air-dried roots were cut with scissors and milled in a small electric grinder, 100 mg of the powder was then ground with few drops of water with a pestle and mortar. The slurry was diluted with tap water and filtered through a 38µm sieve to remove debris. This suspension was collected in a plastic beaker and leveled at 100 mL (Stirling and Wachtel, 1980).

The spore suspension of *P. penetrans* was mixed with an air-pump and a sample of this suspension was taken with a one mL pipette and placed on a haemocytometer 0.1 mm deep and 1 mm wide, the spores were counted on a compound microscope at 400X magnification and expressed at 10^4 spores/mg. An average of ten counts was taken.

From the suspension of second-stages *Meloidogyne* juveniles (J2) obtained one ml of the spore suspension was pipetted to each Petri dish containing one mL of nematode suspension. Suspension was diluted by adding three mL of water before counting. The Petri dishes were examined after 45 min of exposure time and the number of spores attached/J2 on first 10 nematodes was counted under the microscope. The mean of ten counts was calculated as one observation for each replication

Inoculations

In treatment 3, only PP-3 was mixed thoroughly before planting in the upper 7.5 cm of soil @ 100 mg/kg soil. About 3,000 J2 encumbered with 7-10 *Pasteuria* spore/J2 were inoculated around rhizosphere (Stirling and Wachtel, 1980).

Data recording

Data were recorded after three month of inoculation. At final harvest, a sample consisted of five soil cores (2.5 cm diam. x 30 cm deep) was taken from each row. Soil cores were mixed thoroughly and a 100-cm^3 sub sample was processed (Whitehead and Hemming, 1965).

Fresh weight of root and shoot , dry weight of root and shoot, shoot length, yield and root galls/ plant, egg masses, eggs/egg mass, J2 /100 cm^3 of soil and reproduction factor was recorded.

Data analysis

Data were subjected to ANOVA and differences among the means were partitioned test at P=0.05 according to least significant difference (LSD) test (MSTAT version 3.1).

RESULTS AND DISCUSSION

Efficacy of *P. penetrans* on plant growth responses of tomato and nematode reproduction parameters were differentially variable. A significant (P=0.05) increase in fresh and dry weight of shoot, shoot length and yield of tomato plants was observed when nematodes were treated with spores of *P. penetrans* (Table I). Nematode feeding resulted in the increase of root weight and it significantly varied between plants inoculated with nematodes and nematode + *P. penetrans* treated plants. Galls/plant, egg masses, J2/100 ml^3 and reproduction factor were significantly (P=0.05) variable between plants inoculated with nematodes and nematode + *Pasteuria* treated plants (Table II). These results are in agreement with those of Jonathan *et al*. (2000) and Mukhtar *et al*. (2002, 2003, 2005) who reported greater plant growth in the presence of different isolates of *Pasteuria*. Root knot nematode development was significantly restricted by combination of various *P. penetrans* populations compared to that of alone application of *Pasteuria* isolates (Zareen *et al*., 2002; Ahmad and Mukhtar, 2007b).

Our results differed with these of Cho *et al*. (2000) in which growth characteristics of the tomato and oriental melon did not show significant difference between the endospore treated pots and *M. arenaria* only treated pots. This might be due to experiment difference in experimental conditions. However, their results showed significant reduction

Table I.- Efficacy of *Pasteuria penetrans* on plant growth responses of tomato.

Treatments	Plant growth				Shoot length (cm)	Yield (g)
	Shoot	Root	Shoot	Root		
Nematode + PP-3	171.3 c[1]	22.57 b	33.16 c	6.54 b	50.33 c	882.5 c
Nematode only	90.77 d	24.68 a	23.78 d	7.46 a	48.79 d	496 d
PP-3 only	193.3 a	19.62 c	39.06 a	4.51 c	52.34 a	936.5 a
Control	191.4 b	19.74 c	37.16 b	4.63 c	51.29 b	916.0 b
Percent increase/ decrease between T1 over T2	47.01	9.34	28.28	14.06	3.05	43.79
LSD	1.621	0.2781	0.78	0.18	0.36	7.39

[1] Means with in a column sharing the same letter are not significantly different from each other at *P = 0.05* according to LSD Test.
Percent increase/decrease between T1 over T2 =T1-T2/T1x100

Table II.- Efficacy of *Pasteuria penetrans* on nematode reproduction.

Treatments	Nematode reproduction parameters				
	Galls/plant	Galls with egg masses	No. of eggs/ egg mass	J2 /100 ml[3] of soil	Reproduction factor
Nematode + PP-3	395.5 b[1]	105 b	130.5 b	335.5 b	0.11 b
Nematode only	527 a	474 a	293.5 a	718.8 a	0.96 a
PP-3 only	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c
Control	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c
Percent decrease T2 over T1	24.95	77.84	55.53	53.32	88.54
LSD	7.82	5.53	4.59	5.04	0.0009

[1] Means with in a column sharing the same letter are not significantly different from each other at *P = 0.05* according to LSD Test.
Percent decrease T2 over T1=T2-T1/T2x100

in root galling and egg mass numbers in short periods of 10-14 weeks both in tomato and oriental melon when 1x 10^5 and 2x 10^5 endospores/g medium was treated. Similarly, at least 1x 10^5 endospores/g of soil are required for significant suppression of *M. incognita* on lettuce and oriental melon (Yu *et al*., 2003). *P. penetrans* provided a continuous suppression on a mixed population of *M. incognita* and *M. javanica* in 7 years monoculture of tobacco (Dickson *et al*., 1994). Therefore, it appears that endospores of *P. penetrans* persistent in soil and consequently suppressive soil over time (Dickson *et al*., 1992; Oostendorp *et al*., 1991).

P. penetrans suppressive soil to *M. arenaria* on peanut showed reduction of the J2 penetration into the root and root galling (Minton and Sayre, 1989). In microplot test, peanut root galls and pod galls by *M. arenaria* race 1 were reduced and the yields increased in the endospore treated plots with 1x 10^5 endospores/l g soil (Chen *et al*., 1996). Root galling and egg mass production by *M. incognita* and *M. javanica* on tomato and cucumber were reduced in the plots treated with a combination of *P. penetrans* and oxamyl (Tzortzakakis and Gowen, 1994). When dried roots from an infested field site were transferred to another field site, three years after infestation of the new field site, root galling on peanut was reduced to the same level as in plots fumigated with 1,3-dichloropropene (Kariuki and Dickson, 2007).

The increased yield of tomato in current research demonstrated that *P. penetrans* could decrease the pathogenicity of *Meloidogyne* sp. for successive crops. The initial population of the nematode had greater impact on the development of host plant and their increase in soil.

CONCLUSIONS

If parasitized juveniles are prevented from feeding or if feeding is hampered, the rate of nematode population built-up is decreased. A high initial infestation of juveniles may damage a host to such a degree that subsequent lack of food limits the

increase in nematode numbers. On the other hand, if juveniles in some way are inhibited from root penetration or developing once within the roots, damage on the host may be less and non-infected juveniles that do successfully reproduce may produce more offspring due to less competition.

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