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Field Evaluation of *Pasteuria* Isolates for the Control of Root-Knot Nematodes, *Meloidogyne javanica* on Tomato

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Abstract: In a mirco-plot field experiment four isolates of *Pasteuria* (one from UK and three local isolates viz., PK 1, PK2 and PK3) and a mixed inoculum of all four isolates (blend) were tested against *Meloidogyne javanica* on tomato to understand the pathogenic variability of different bacterial isolates. Yield of tomato and plant growth as plant height, fresh shoot and root length was improved significantly by blend inoculum of bacterial antagonists compared to control. Nematode development in roots and soil was suppressed by blend applications followed by individual *Pasteuria* applications i.e. UK > PK 1 > PK2 and >PK3 isolates compared to untreated control plots.

Key words: Biological control, *Pasteuria penetrans, Meloidogyne javanica*, tomato.

Introduction

Root-knot nematodes are obligate parasites and therefore obtain nourishment for their development and reproduction from living plant cell. The root damage from phytoparasitic nematode parasitism leads to stunted growth, and crop producing low yields. Severe infestations of crop field with nematode such as *Meloidogyne* spp., often can result in annual yield losses of 10 to 50% (Sasser, 1980). *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* have been encountered on over 50 hosts in Pakistan including more than 33 vegetable crops (Maqbool and Shahina, 2001). Losses caused by the root-knot nematode in chickpea are 31-37% in Pakistan and 28% in mungbean in Brazil (Anwar *et al.*, 1995). To prevent losses caused by nematode several control methods are used. Growers in developing countries rely on commercial available nematicides. Because of wide host range of knot nematodes, the use of crop rotation is limited. Similarly nematode resistant varieties are few, interest is developing in the method of biological control (Trivino and Gowen, 1996). Of the nematode biocontrol organisms, *P. penetrans* is an obligate parasite and it can not grow on synthetic media, current methods of mass culturing of this bacterium are limited to the multiplication of the parasite in its nematode host (Stirling and Wachtel, 1980). *P. penetrans* which parasitizes root knot nematodes (*M. incognita* and *M. javanica*) (Sayre and Starr, 1985), and the diseased nematodes do not reproduce and at maturity are filled with spores of the pathogens (Sayre and Wergin, 1977). Infected nematodes usually fail to invade roots of crop plants (Davies *et al.*, 1990). The bacterium was successfully tested in greenhouse and showed promising results in soil infested with root knot nematodes (Dickson *et al.*, 1990) whereas its field application is scared. The present work is aimed to assess the potential of different *Pasteuria* isolates against root-knot nematodes and impact of bacterial inoculum on root-knot nematode on tomato under field conditions.

Materials and Methods

Four isolates of *Pasteuria* sp. one from UK, designated UK I (provided by Dr. S. R. Gowen, Reading Univ., UK.) and three local isolates PK 1 (University Garden, Karachi) PK 2 (Shahfaisal Colony, Karachi) and PK 3 (Memon Goth, Malir) (Zaki, 2000) were selected for this comparative study. All test isolates were multiplied on *M. javanica* and *M. incognita* growing on tomato (*Lycopersicon esculentum* Mill cv. Roma VF) in a greenhouse. *Pasteuria* spore attached J2 (2-3 days old) were collected by centrifugation attachment method (Hewlett and Dickson, 1994). Two-week-old tomato seedlings inoculated with bacterial spore encumbered juveniles @ 1000 J2/pot, 3.4 times at one-week interval. A month after inoculation, root systems were chopped and powdered in electric grinder. Root powder containing *Pasteuria* spores was used as bacterial inoculum.

Study was conducted in the experimental field of Department of Botany, University of Karachi in September 1999. The microplots (20 cm cemented rings) were arranged in 6 rows (each row has four microplots) and filled with fine sand (93% sand, 4% silt, 3% clay and 1% organic matter, pH 8.1). Root-knot nematode, (*M. javanica*) population was established in experimental micro plots. Three weeks old tomato seedlings were transplanted in microplots (4 seedlings/plot) and after 10 days freshly hatched root-knot juveniles @2000 J2 per plot were inoculated in the root zone of each plant (8000 J2 per plot). The plots without nematode served as control. Plots were watered, weeded, sprayed for three month and harvested. After nematode establishment on tomato, microplots were amended with all test isolates of *P. penetrans* (UK 1, PK 1, PK 2, PK 3 and a blend inoculum) and 3 week old seedlings @ 4 seedlings/plot were transplanted according to experimental plan. There were nematode and nematode free controls. All the replicates were randomized complete block design. Tomato crop was harvested after three months. Plots were watered when needed and sprayed with insecticides twice a month. The experiment was terminated after 3 month of bacterial inoculation. Growth parameters (as plant height, shoot weight, root length, root weight and yield), nematode development in soil (200g) and root (one g) (Franklin and Goodey, 1949; Schindler, 1961) and bacterial infection in juveniles and females was recorded. For the estimation of % female infested with *P. penetrans*, root samples were collected from the bacterial applied pots. Ten mature females/root system, were teased out randomly, put on a glass slide, crushed under cover slip and confirmed the presence of bacterial endospores under compound microscope (x40). Percentage of the female infection by *P. penetrans* was calculated. Soil samples from bacterial applied pots were sub-sampled. Nematodes extracted from soil by modified Baermann method (Schindler, 1961) and *P. penetrans* spore attachment on 25 juveniles/ sample was recorded using (0-3) scale described by Striling (1984).

Experimental data recorded after *Pasteuria* applications were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). Standard error difference (SED) was also calculated (Gomez and Gomez, 1984).

Results and Discussion

Tomato seedlings were transplanted in microplots and observations were taken after harvest of crop. Tomato plants growing in bacterial treated plots respond to *Pasteuria* isolates and plant health was enhanced. Varying degree of plant growth was

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Table 1: Effect of *Pasteuria* isolates on the growth and yield of tomato in nematode infested soil

Treatment	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Yield(g)
Isolate UK 1	33.48	52.64	15.42	6.55	478
Isolate PK 1	35.25	48.45	12.55	7.12	460
Isolate PK 2	29.64	36.65	15.46	5.58	415
Isolate PK 3	25.75	38.55	13.65	6.52	375
Blend	41.82	61.83	18.75	7.25	550
Control (+N)	18.65	30.12	10.25	9.25	275
Control (-N)	27.42	37.64	15	5.58	285
SED	6.25	5.12	2.25	2.74	130.41
Significant level (P < 0.001)	***	***	***	***	***

Table 2: Effect of *Pasteuria* isolates on nematode population and developed of *Meloidogyne* javanica on tomato

Treatments	Galls/root system	Eggs/eggs mass (x10)	Nematode density /g root	Nematode density /200 g
Isolate UK 1	48	51	212	1201
Isolate PK 1	64	49	188	1449
Isolate PK 2	56	55	195	1562
Isolate PK 3	68	58	212	1245
Blend	51	49	153	986
Control (+N)	114	76	324	2115
Control (-N)	-	-	-	-
SED	25	35.45	75.55	175.72
Significant level	**	***	**	***

** = P < 0.05 *** = P < 0.001

Table 3: Development and infection of *Pasteuria penetrans* in ematodes

Treatment	Female Infection (%)	No.of spore /juveniles	Spore attachment level (0-3)
Isolate UK 1	45	5.25	1.25
Isolate PK 1	58	5.75	1.2
Isolate PK 2	38	6.45	1.35
Isolate PK 3	41	7.31	1.41
Blend	70	8.55	1.52
Control (+N)	-	-	-
SED	4.81	0.89	0.05
Sign. (P<0.001)	***	***	***

Female infection (%) is mean of 10 replicates, For attachment of endospores 20 juveniles for each replicate were observed.

improved in each treatment compared to untreated crop plant. Plant height, fresh shoot weight and root length was enhanced significantly by blend inoculum compared to other treatments and untreated control (Table 1). All the bacterial isolates provided varying degree of control against root knot nematodes. Root knot nematode development was significantly reduced by combined (blend) inoculum of *Pasteuria* isolates. Galls formation, egg mass production, egg per egg mass were reduced effectively in treatments receiving blend of bacterial isolates. Similarly root invasion and soil density of root knot nematodes were also minimized by blend inoculum whereas lower degree of control was provided by alone application by bacterial isolates compared to untreated control (Table 2).

Higher number of root knot females were found infested by *P. penetrans* in blend inoculum where 70% root knot females were found infested by bacterium followed by PK 1 > UK 1 > PK 3 and PK 2. Maximum number of spores (9 spores/juvenile) were observed in blend application with spore attachment level (0-3) 1.52. Spore bioassay provided more vivid out put about the bacterial establishment in tomato rhizosphere in treated plots. Maximum number of juveniles encumbered with spores were obtained from blend inoculum plots i.e. > 10 spores per juveniles followed by PK 2 (7.25 spores/J2) > UK 1 (7.15 spores/J2) > PK 1 (6.48 spores/J2) and PK 3 (5.25 spores/J2) compared to untreated control (Table 3).

Pasteuria spp. an obligate parasite of plant parasitic nematodes have great potential as economically and environmentally friendly biological control agents (Nishizawa, 1984). As continuos and or even sudden decline of *Meloidogyne* spp. and other plant parasitic nematode populations, that were previously above economic thresholds have been found to have high population of *Pasteuria* spp. (Bird and Brisbane, 1988; Dickson *et al*., 1994; Minton and Sayre, 1989). The natural suppression of root-knot nematode in West Africa (Mankau, 1980) and on wines in South Australia (Stirling and White, 1982) have been associated a large proportion of spore encumbered juveniles in soil. It has been reported pathogenic variability of *Meloidogyne* spp. in field populations where more than one species, race of biotype may occur (Channer and Gowen, 1992). In this study growth of host plant and disease suppression of root-knot nematode is observed in plots receiving blend inoculum of *Pasteuria* compared to individual applications by each bacterial isolates and control. These results are in accordance to the previous finding that blending of bacterial population from different sources increase the consistency of infection and may circumvent the problem of resistance naturally developing in the field (Channer and Gowen, 1992).

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