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Root knot nematodes associated with eggplant in different localities of District Sargodha-Pakistan and impact of *Pasteuria* isolates on development of *Meloidogyne incognita*

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Key words: RKN, Incidence, *Pasteuria* isolates, Population dynamics

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Abstract

Root knot nematodes (*Meloidogyne* spp.) are sedentary, obligate parasites and considered a major pest of vegetables all over the world. A systematic survey was conducted to get a reliable estimate of nematode and their level of infestation in the field of eggplant plantation located in major vegetable production areas of district Sargodha. 150 soil and root samples were collected from 15 different localities. Nematode population in 10g of roots and 100 cm^3 of soil samples were determined by White Head and Hemming Tray method. Maximum population of nematode in case of root samples was found in Dharema (515) and lower population was found in Chak 103 (91). In case of soil samples maximum population of RKN was recorded in Chak 50 (454.33) and lower population of nematode was found in Chak 103 (69). The incidence of infestation was also assessed. Pot studies were carried out in green house (25 ± 2°C) to manage RKN by using two *Pasteuria* isolates PP-J and PP-3. The influence of different levels of endospore of each *Pasteuria* isolate was determined on root invasion, development and population dynamics of *M. incognita* in eggplant. Data were recorded after 7, 14, 21 and 28 days on nematode developmental stages (vermiform, developing J2, swollen, sausage, adult female and egg mass). The results revealed that by increasing endospore level/J2, the nematode development stages decreased. As biological control agent, the use of *P. penetrans* especially PP-3 isolate against *M. incognita* can be the most effective and environment friendly.

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Introduction

Vegetables play a significant role in human nutrition with the supply of most important elements minerals, vitamins, fibers and carbohydrates. Eggplant (*Solaman melongena* L.) is most important and common vegetable crops throughout the world. It belongs to Solanaceae family and extensively grown in Asia, Egypt, Middle East and U.S.A. The area under cultivation is9,044 ha area and its production is 88,148tonnes in Pakistan (Anonymous, 2010). Eggplants are rich source of vitamins such as C, K, B6, niacin, thiamin and nutrients like magnesium, phosphorous, copper, dietary fiber, folic acid, potassium, and manganese. There is almost no cholesterol in it and prevents from cancer.

Eggplant crop is attacked by fungi, bacteria, viruses and nematodes and cause extensive losses in yield. Root knot nematodes are important pest of vegetables around the globe (Kamran *et al.*, 2010). Five spp. of RKN namely, *Meloidogyne incognita, M. javanica,M. hapla, M. arenaria, and M. graminicola* have already been recorded in Pakistan (Anwar*et al.*,2007). The incidence of *Meloidogyne* species was found 85.10% in Punjab (Anwar and Mckenry, 2012) with occurrence of (76%) of this nematode infection in Faisalabad (Khan *et al.,* 2005). RKN are obligate parasites of vascular tissues of plant roots. Root lesions, reduction in plant growth and deformation are the additional symptoms of RKN. The infected plant shows reduced root system with less feeder roots. (Anwar *et al.,* 2010).Extensive galling and root damage is associated with nematode infection. Vegetable crops are among the most susceptible and worst affected by these nematodes (Sharma *et al.,* 2006).

Different management practices are adopted for the control of RKN i.e., resistant variety, solarization, crop rotation, chemical and biological control. Biological control can provide environment friendly and long-term solution to a pest problem. By using this method pests do not become resistant. Several microbial pathogens have been developed into commercial formulations against nematodes. These includes, the bacteria (*Pseudomonas, Azotobacter, Bacillus and Pasteuria penetrans* (formerly known as *Bacillus penetrans*) and fungi that includes *Trichoderma, Verticillium, Myrotheciym* are found to be highly effective in control of nematodes.

Pasteuria penetrans is an endospore-forming, gram-positive bacterium from Actinomycetes. It is an obligate parasite of RKN (*Meloidogyne* spp). *P. penetrans* is recognized as a potential biocontrol agent for root knot nematodes. Its obligate nature and life cycle has limitation in mass culturing and development. *Pasteuria* spp. has a variety of nematode hosts in different climates around the globe (Stirling, 1988; Sayre and Starr, 1988; Hewlett *et al.*, 1994).

The current research was planned to access the damage potential of RKN on eggplant in different localities of district Sargodha and to evaluate the impact of different endospore level of *Pasteuria* isolates on invasion and development of *M. incognita* in greenhouse conditions.

Material and methods

Survey for assessment of root knot nematodes damage in different localities of Sargodha

Eggplant roots and soil samples were collected from the field located at different localities of Sargodha to estimate the RKN population associated with roots and soil. Samples of roots were collected upto 15-20cm depth of eggplant along with about 1 kg of adhering soil. Preliminary data e.g host, locality and soil type was recorded. Samples were brought in Plant Pathology Laboratory Department, University of Agriculture, Sargodha and were stored in refrigerator at 5°C until processed.

Processing of roots and soil samples

Root and soil samples were processed separately. The roots were separated from the soil, washed and weighed. Galling rating scale was used for root system as follow; where 0 = no gall or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = >100 galls per root system.

The incidence of infestation was assessed by following formula:

$$\text{Incidence} = \frac{\text{Number of samples infeected with RKN}}{\text{Total number of samples examined}} \times 100$$

The entire root system was diced, chopped and a 20g composite root sample was processed for 5 days to hatch the eggs (Whitehead and Hemming, 1965). Soil sample was thoroughly mixed and a composite 100 cm^3 samples were processed through Whitehead and Hemming tray method (1965) to collect nematodes. Nematode counting was done under stereo binocular microscope.

Perennial patterns

Perennial patterns of mature females were prepared for different RKN species (Jepson, 1987). The adult female was removed from root tissue by using forceps. The anterior region of the nematode was excised and posterior was treated with 45% lactic acid to remove all body tissues. Then, the perennial patterns was trimmed and transferred to a drop of glycerin. At least 10 perennial patterns were examined for identification of nematode species of each sample.

Nematode inoculum

Eggplant roots were used for mass rearing of RKN. The roots were cut into 2-3cm segments and shaken vigorously (manually) for 3-4 minutes in a coffee jar (1 litre) with a tightly fitting lid, containing 200 ml of 1% Sodium hypochlorite (NaOCl) solution (Chlorax) to dissolve the gelatinous matrix and to release the eggs from the egg masses (Hussy and Barker, 1973). This suspension was quickly passed through 200-mesh (75 µm) sieve nested over 500-mesh (25 µm) sieve to collect root fragments on the former and freed eggs on the latter. The eggs collected on the 500-mesh sieve were rinsed with tap water to remove the residual NaOCl. Rinsing of eggs was done for several minutes. Then these freed eggs were collected in a beaker. This process was repeated twice, for removing additional eggs and gets rid of residual Chlorax.

The egg suspension was poured onto 9 cm Petri dishes. Eggs were incubated for 2-3 days at 28 °C and the nematode suspension was collected after every 24 h.

Effect of endospore levels of different Pasteuria isolates on the invasion and development of M. incognita

Two isolates of *Pasteuria penetrans* PP-Japan and PP-3 were used. Air-dried roots were cut with scissors and ground 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 water and filtered through using mesh size of 38µm to remove debris. Suspension was collected in a plastic beaker and make the volume upto 100 ml (Stirling and Wachtel, 1980). Two levels of attachment (4-5 spores/J_2, 8-10 spores/J_2) for each isolates were obtained by leaving the J_2 in the suspensions for different periods of time prior to inoculations.

Three weeks old seedlings of eggplant were transplanted in 10 cm (top diameter) earthen pots and 500 encumbered J_2s of *M. incognita* were inoculated in each pot. Seven days after transplanting all the pots were inoculated with 500 juveniles per pot of *M. incognita* in the rhizosphere of each plant by making 3-4 holes (Campos and Campos, 2005) and then filled with soil. The pots were arranged in a completely randomized design and experiment was accomplished in four sets of treatments with fifteen replications in the green house and watered once every second day. The optimum temperature during the growth period ranged from 25±2°C.

Data were recorded after 7, 14, 21 and 28 days on nematode developmental stages (J2, developing (d) J2, J3/swollen, J4/ sausage, adult female and egg masses). Phloxine B was used to stain the egg masses of nematodes (Holbrook *et al.,* 1983).

Statistical analysis

Data were subjected to ANOVA and differences among the means were partitioned at P=0.05 according to least significant difference (LSD) test (MSTAT version 3.1). In pot experiment when the overall P was significant, post-hoc multiple comparisons were conducted with the Dunnett's test (versus control).

Results

Survey for assessment of root knot nematodes damage in different localities of Sargodha

During the survey 150 soil and root samples were collected from 15 different localities. Out of which 68 were infected with RKN. Eleven sites showed the presence of RKN and only four sites were devoid of RKN infestation.

Table 1. Assessment of root knot nematode damage in different localities of Sargodha, its population and gall index.

Locality	RKN spp.	Root knot nematode population[1]		Galls Index[2]	Incidence* %	Soil type
		Roots [20g]	Soil [100- cm3]			
Dharema	*M.incognita +M.javanica*	515 a	369.67 b	5	80	Sandy Loam
Chak # 70	*M. incognita*	328.33 e	176.67 e	5	70	Sandy Loam
Silanwali	*M. incognita*	218 g	118 g	4	50	Clay Loam
Jhaal 85	*M. incognita*	0 k	0 j	0	0	Clay Soil
Chak # 51	*M. incognita+ M. Javanica*	130.67 h	119.33 g	0	60	Sandy Loam
Chak # 104	*M. incognita*	0 k	0 j	0	0	Clay Soil
Chak # 103	*M. incognita + M. javanica*	91 j	69 i	4	50	Clay Loam
Chak # 50	*M. incognita*	482 b	454.33 a	5	70	Sandy Loam
Chak # 85 NB	*M. incognita + M. javanica*	136 h	155 f	4	60	Loamy Soil
Bhagta wala	*M. incognita + M. javanica*	119.67 i	90.667 h	3	40	Clay Soil
49 Tails	*M. incognita*	0 k	0 j	0	0	Clay Soil
Chak # 48	*M. javanica*	290.33 f	246.67 d	4	60	Loamy soil
Chak # 74 NB	*M. incognita*	0 k	0 j	0	0	Clay Soil
Chak# 8NB	*M. javanica+ M.incognita*	348 d	176.67 e	5	70	Sandy Loam
Chak #6NB	*M.incognita*	457.77c	280.67c	4	50	Clay Loam

[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. [2] Gall indices: 0-5 scale; where 0 = no galls, 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls, and 5 = > 100 galls per root system (Quesenberry *et al.,* 1989). * Incidence = Number of infected samples with RKN ÷ Total Samples × 100.

RKN population in root and soil samples

Root knot population was assessed from roots and soil samples. Maximum population of nematode in case of root samples was found in Dharema (515a) and lower population was found in Chak 103 (91j) respectively (Table 1).

In case of soil samples maximum population of RKN was recorded in Chak 50 (454.33a) and lower population of nematode was found in Chak 103 (69i). Four sites (Jhaal 85, Chak 104, 49 Tail and Chak 74 NB) showed no infestation in roots and soil samples.

RKN incidence and galling index

The incidence of *M. incognita* was 80% in Dharema and minimum incidence of 40% was recorded in Bhagtawala, respectively.

In six locations (Dharema, Chak 51, Chak 103, Chak 85NB and Bhagtawala Chak 8NB) *M. incognita* and *M. javanica* were found in combination and in eight locations (Silanwali, Chak 70, Jhaal 85, Chak 104, Chak 50,49 Tail, Chak 74NB and Chak 6NB) *M. incognita* was found as dominant species. *M. javanica* was found in only in one location (Chak 48).

Table 2. Population of nematodes other than root knot nematode and their feeding habits.

District	Nematodes	Nematode population		Feeding habit
		Roots/20g	Soil/100 cm^3	
Dharema	*Longidorus*	0	4	Ectoparasites
Chak # 70	*Helicotylenchus*	0	11	Ectoparasites
Silanwali	*Helicotylenchus*	0	3	Ectoparasites
Jhaal 85	*Criconema*	0	5	Ectoparasites
Chak # 51	*Helicotylenchus*	0	4	Ectoparasites
Chak # 104	*Hoplolaimus*	2	6	Endoparasites
Chak # 103	*Xiphinema*	0	3	Ectoparasites
Chak # 50	*Longidorus*	0	2	Ectoparasites
Chak # 85 NB	*Helicotylenchus*	0	5	Ectoparasites
Bhagta wala	*Hoplolaimus*	0	7	Ectoparasites
49 Tails	*Pratylenchus*	3	4	Ectoparasites
Chak # 48	*Xiphinema*	0	5	Ectoparasites
Chak # 74 NB	*Criconema*	0	3	Ectoparasites
Chak# 8NB	*Xiphinema*	0	6	Ectoparasites
Chak#6 NB	*Hoplolaimus*	0	7	Ectoparasites

These sites (Chak 51, Chak 85 NB and Chak 48) have intermediate level of incidence (60%) and four sites showed zero incidences (Jhaal 85, Chak 104, 49 Tail and Chak 74 NB).

The gall index was rated from 0 to 5 with a mean of 2.86.Maximum gall index was recorded in location Dharema,Chak 70, Chak 50 and Chak 8NB. Lower gall index was recorded in Bhagtawala, respectively.

Other than RKN nematodes

The root and soil analysis also verified the presence of six other plant parasitic nematode genera, *Longidorus, Helicotylenchus, Criconema, Hoplolaimus, Xiphinema* and *Pratylenchus*. Their root and soil population was very low ranging from 0 to 3 in roots and 2 to 11 in soil (Table 2). There nematode genera are of minor importance.

Effect of endospore levels of different Pasteuria isolates on the invasion and development of M. incognita

After seven days

Two parameters vermiform and developing J_2 were studied. All the treatments were statistically significant.

The results showed that minimum number of J_2 invaded through PP-3 isolates. Maximum number of vermiform juvenile (134.20 a) were observed in PP-J and by increasing level of 8-10 spores/j2less vermiform (114.60 b) were invaded in both treatments. Maximum Dj_2 nematodes observed in PP-J (50a) while minimum in PP-3 isolate as compared to control (Table 3).

Table 3. Effect of *Pasteuria* isolates after 7 days on invasion and development of *M. incognita* on eggplant.

Treatments	Levels	Developmental stages	
		Vermiform	Developing J_2
PP-J	4-5 spores/J2	134.20 a	50 a
	8-10 spores/J2	114.60 b	44.20 b
PP-3	4-5 spores/J2	99 c	31.40 c
	8-10 spores/J2	77.60 d	24.20 d
Control		188.4	80.6

Mean with in a column sharing the same letter are not significantly different from each other at *P= 0.05* according to LSD Test. When the overall *P* was significant, post-hoc multiple comparisons were conducted with the Dunnett's test (versus control), which proved that all the treatments were significantly different from control.

After 14 days

Four stages of nematode were observed that were vermiform, developing j2, swollen and sausage. The results showed that minimum number of $DJ_2$was observed in PP-3 while maximum in PP-J and by increasing level of 8-10 spores/j_2 less DJ_2were invaded in both treatments as compared to control. Swollen and Sausage stages also varied with increasing endospore level/J2 (Table 4).

Table 4. Effect of *Pasteuria* isolates after 14 days on invasion and development of *M. incognita* on eggplant**.**

Treatments	Levels	Developmental stages			
		Vermiform	Developing J_2	Swollen	Sausage
PP-J	4-5 spores/J_2	47.40a	73.40a	49.80 a	69.60a
	8-10 spores/J_2	39.40b	65.60b	40.20 b	61.20 b
PP-3	4-5 spores/J_2	28.00c	46.80c	26.20 c	47.40 c
	8-10 spores/J_2	21.20 d	38.60 d	18.40 d	40.40 d
Control		66.6	88.6	59.2	97.4

Mean with in a column sharing the same letter are not significantly different from each other at *P= 0.05* according to LSD Test. When the overall *P* was significant, post-hoc multiple comparisons were conducted with the Dunnett's test (versus control), which proved that all the treatments were significantly different from control.

After 21 days

Developmental stages i.e. developing J_2, swollen, sausage and adult females were observed. The results showed that maximum number of DJ_2 were observed in PP-J while minimum in PP-3 and by increasing level of 8-10 spores/j_2 less DJ_2were invaded through both treatments as compared to control (Table 5).

Table 5. Effect of *Pasteuria* isolates after 21 days on development of *M. incognita* on eggplant.

Treatments	Levels	Developmental stages			
		Developing J_2	Swollen	Sausage	Adult female
PP-J	4-5 spores/J_2	33.40 a	39.80a	118.40a	38.40a
	8-10 spores/J_2	29.60 b	31.60 b	104.60 b	33.40 b
PP-3	4-5 spores/J_2	23.60 c	20.40 c	76.60c	27.20 c
	8-10 spores/J_2	20.40 d	14.60 d	54.0 d	24.20 d
Control		39.6	54.6	152.2	60.6

Mean with in a column sharing the same letter are not significantly different from each other at *P= 0.05* according to LSD Test. When the overall *P* was significant, post-hoc multiple comparisons were conducted with the Dunnett's test (versus control), which proved that all the treatments were significantly different from control.

After 28 days

Three developmental stages of nematode i.e., sausage, adult female and egg masses were observed after 28 days. The results showed that maximum number of sausage stage was observed in PP-J while minimum in PP-3 and by increasing level of 8-10 spores/j_2 less sausage stage were observed in both treatments as compared to control. The adult females and egg masses were also variable by increasing endospore density (Table 6).

Discussion

Survey for assessment of root knot nematodes damage in different localities of Sargodha

A systematic survey was conducted to observe the RKN infestation which was highly variable on eggplant in different localities of District Sargodha. Roots and soil samples were collected from field to estimate RKN population and data was recorded on host, locality and soil type. *Meloidogyne* spp. was predominant species in all surveyed localities. In this survey most nematodes were considered as serious pests (Anwar *et al.,* 2007; Anwar and Mckenry, 2012). *Meloidogyne* spp. are common in vegetable soil around the globe where they parasitize vascular root tissues and induce their familiar roots galls.

Table 6. Effect of *Pasteuria* isolates after 28 days on development of *M. incognita* on eggplant.

Treatments	Levels	Developmental stages		
		Sausage	Adult female	Egg Masses
PP-J	4-5 spores/J_2	30.60 a	134.60 a	109.60 a
	8-10 spores/J_2	27.60 b	117.60 b	96.40b
PP-3	4-5 spores/J_2	22.60 c	106.6 c	83.40 c
	8-10 spores/J_2	19.20 d	84.40 d	69.60 d
Control		35.4	233.8	225.2

Mean with in a column sharing the same letter are not significantly different from each other at *P= 0.05* according to LSD Test. When the overall *P* was significant, post-hoc multiple comparisons were conducted with the Dunnett's test (versus control), which proved that all the treatments were significantly different from control.

It is reported that plant parasitic nematodes were affected by the planting of cover crops, the use of alternate crop sequences, soil type and length of fallow in cultivated soil (Brodie *et al.*,1970; Brodie and Murphy, 1975).An effective control measure to control the nematodes emphasizes their economic importance. Nematodes are generally costly and toxic to the nature and have health hazards to human being and livestock directly or indirectly. Therefore developments of eco- friendly measures were the need of time.

Disease incidence of RKN was recorded in different localities of District Sargodha. Among localities maximum incidence 80% was recorded in Dharema which was due to sandy loam soil. Sandy soil having favorable pore size for the nematode penetration and movement through the soil. As more continuous cultivation increased soil borne diseases and nematodes have become an important pathogens in vegetable production (Anwar *et al.,* 1992). However, more farmers are continuously growing same vegetables in the same field which enhanced infestation level in soil (Hussain *et al.*, 2012).

In Pakistan, RKN problem is favored by high temperature and sandy soil. Maqbool (1987) reported population of *Meloidogyne incognita* 52 %, *M. arenaria* 8 %, *M. javanica*31 % and *M. hapla* 7 %in Pakistan and *Meloidogyne incognita* and *M. javanica* were found in most vegetables and ornamental crops (Maqbool and Shahina, 2001). In some localities of Sargodha nematode infestation was recorded low which was due to cropping pattern or fallowing of land. Farmers are unaware about this hidden pathogen in most localities and no management is being done by them. The result of this study showed that RKN are widely distributed on vegetable crops. This information will be helpful for the farmers through proper planning and following nematode management strategies to reduce nematode population below their threshold level.

Effect of endospore levels of different Pasteuria isolates on the invasion and development of M. incognita

In second experiment efficacy of *Pasteuria* isolates (PP-J and PP-3) on the development of *M. incognita* have been observed on eggplant and nematode developmental stages were differentially variable. In our study, minimum invasion and development was observed in PP-3 isolates because by increasing number of endospores level results in reduced invasion and minimum number of J_2 invaded in roots of eggplant. The vermiform stage was less observed because of nematodes passed to the next developmental stage.

Increase in attachment of endospores to cuticle decrease the number of J_2 in roots. Attachment plays important role in specificity of *P. penetrans* isolates (Davies *et al.*, 1991). During a study J_2 population was found to be reduced when 15 or more endospores were attached (Davies *et al.*, 1988).

Striling and White (1982) reported that a juvenile having larger number of *Pasteuria* spores become less moveable in the rhizosphere and can't easily invade plant tissues. *Pasteuria* not onlyreduce nematode populations by inhibiting the infected RKN females from producing eggs (Mankau, 1980; Sayre, 1980) but also through decrease number of roots (Mankau and Prasad, 1977;Brown and Smart, 1985). Stirling (1984)found that soil infestation with spore decrease the penetration upto70 %.

Pasteuria spores reduce motility and the longer second-stage juveniles encumbered with spores are active in the soil the greater is the chance that they will prohibit invasion. Previous studies had suggested that a minimum of 15 spores were required to affect invasion (Davies *et al.*,, 1988).

The results suggest that less numbers of 5-10 spore/J_2 of *Pasteuria* were appropriate way for reducing nematode parameters. By increasing spore numbers from 20-45 spore/J2 showed no significant differences in reducing nematode reproduction in comparison with the control treatment with uninfected juveniles.

These results are in agreement with other workers (Minton and Sayre, 1989; Espanol *et al.*, 1997; Chand and Gill, 2003; Darban *et al.*, 2005). As biological control, the use of *P. penetrans* especially PP-3 isolate against *M. incognita* can be the most effective and environment friendly.

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