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Research Article

Biological Management of Root Knot Nematode, *Meloidogyne incognita* through Bacterial Antagonists Infecting Tomato

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Abstract

Root-knot nematodes have wide host range, causing damage to many annual and perennial crops. More than 100 species of *Meloidogyne* are known in which *Meloidogyne incognita* (Kofoid and White) Chitwood is one of the most common and important plant parasitic nematode in tropical and subtropical regions of the world including Pakistan. This study was planned to investigate the interaction of Plant Growth Promoting Rhizobacteria and *Pasteuria penetrans* for the management of root knot nematode *M. incognita* on tomato in green house at 30 ± 4 ^{0}C. Earthen pots 20 cm dia. having sterilized soil was mixed with *Pasteuria penetrans* root powder @ 10^3 spores per gm of soil. Three week old seedlings of tomato Money Maker were transplanted singly in pots. One week after transplanting, 30 ml of bacterial (*Bacillus subtilis, Pseudomonas fluorescens* and *Enterobacter cloacae*) suspension were applied in root zone. Seven days after application of PGPR, 2000 J_2 were applied at root zone. The experiment was arranged in Completely Randomized Design and six treatments were replicated tenfold. *Pasteuria penetrans* alone or in different combinations with PGPR were applied. Pots with nematode and without PGPR, *Pp* and nematode were kept as control. Sixty days after nematode application, plants were harvested carefully. Data were recorded on plant growth parameters and nematode reproduction in terms of number of egg mass, number of females, number of galls and galling index (0-10). The plant treated with *Enterobacter cloacae* + *Pp* significantly suppressed the number of egg mass 69.38%, number of galls 68%, number of females 66.21% and galling index 56.82% compared to control resulting in improved growth over control. The treatments *B. subtilis* + *Pp* and *P. fluorescens* + *Pp* showed intermediary effect on both nematode reproduction and plant growth..*Pasteuria penetrans* was least observed effective in suppressing number of egg mass 57.53 %, number of females 55%, number of galls 55.73% and galling index 47.73%.

Keywords: PGPR; Pp; RKN; Tomato.

Introduction

Meloidogyne spp. are obligate sedentary endoparasites of host plants which attack plant roots. Five root-knot species viz. *M. arenaria, M. graminicola, M. hapla, M. incognita,* and *M. javanica* out of more than 100 known *Meloidogyne* spp. are found more frequently in

Pakistan as well as all over the world as major pests of vegetables, fruit plants and field crops (Sasser and Freckman, 1987; Hunt and Handoo, 2009; Eisenback *et al.*, 1981; Anwar and McKenry, 2012; Anwar *et al.*, 1991; Sasser, 1980, 1979; Moens *et al.*, 2009; Menjivar *et al.*, 2011; Mateille *et al.*, 2000; Maqbool, 1986; Maqbool *et al.*, 1988; Fourie and McDonald, 2000; Anwar and Khan, 1992; Anwar, 1989). Root knot nematode particularly *M. incognita* has been found the most damaging and economically important nematode of eggplant and other vegetables in Pakistan and worldwide (Siddiqui and Shaukat, 2003; Mukhtar *et al.*, 2013a; Hussain *et al.*, 2017; Tariq-Khan *et al.*, 2017; Sikora and Fernandez, 2005; Sasser, 1980; Mukhtar *et al.*, 2013c, 2013d; Mukhtar *et al.*, 2014; Mukhtar *et al.*, 2017b; Mukhtar *et al.*, 2013b; Mukhtar *et al.*, 2017a; Kayani *et al.*, 2013; Fourie and McDonald, 2000; Anwar *et al.*, 2007). Root knot nematode infection on host provides opportunity to other soil borne pathogens to attack plants by breaking the resistance and cause more severe damage and yield losses by providing entry sites for weak soil borne pathogens like bacteria and fungi (Javed, 2000; Shahbaz *et al.*, 2015).

Many management strategies i.e. host plant resistance; cultural practices, physical, biological and chemical methods are used for the management of root knot nematodes but chemicals have given relatively quick and better results to farmers. Therefore, some microbial antagonists were potentially used in the replacement of chemical nematicides against root knot nematodes (Siddiqui and Shaukat, 2003; Jairajpuri *et al.*, 1990; Whitehead, 1998; Nico *et al.*, 2004; Mukhtar *et al.*, 2017b; Mukhtar *et al.*, 2013a; Hussain *et al.*, 2014; Brand *et al.*, 2010; Barker and Koenning, 1998; Veremis and Roberts, 1996; Sikora and Fernandez, 2005). Plant growth promoting rhizobacteria (PGPR) have the potential as bio-control agents to substitute chemicals because they are ecofriendly and significantly reduce the disease.

Methodology

Soil Sterilization

Soil with 1:1:1 ratio (clay loam, farm yard manure and sand) thoroughly mixed and sterilized with 37% formalin (1:320 ratio) and then covered with polythene sheet for 72 hours. Then polythene sheet was removed to overcome the effect of formalin for 48 hours and mixed the soil thoroughly. After dry, pots were filled with sieved soil to avoid the contamination of plant material and large stone.

Collection of seeds

Tomato seeds of susceptible variety CV. Moneymaker were collected from Vegetables Research Institute (AARI) Faisalabad. Care was required for seed germinating capacity, health and purity. Seed were not treated with any kind of chemical. Tomato susceptible variety CV. Moneymaker was raised in sterilized soil in plastic trays. They were allowed to grow for 30-days.

Maintenance and Transplanting of seedlings

Tomato plants grown in plastic trays were shifted to 15 cm diameter earthen pots. Stop watering nursery plants one day before transplanting due to hardening of seedling. Next day seedling were transplanted in earthen pots by making a hole with the sharp pointed wooden sticks. Seedling transplantation was done carefully to avoid the leaves and roots damage.

Single egg mass culture of *Meloidogyne incognita*

Infected plants with KN were collected during survey. Plant roots were washed under tap water and cut in to large pieces to avoid egg masses from damage. Seven weeks old

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singly grown plants were inoculated with an egg mass in root zone which was isolated from infected roots with the help of needle under stereo microscope. The plants root were covered again with soil and watered.

Identification of *Meloidogyne spp*.

An egg mass inoculated plant was harvested & -12 weeks after inoculation of egg. mass, Some root pieces were select having egg masses. Under microscope egg were removed and female were isolated by teasing with the help of inoculating needle and placed the mature females in 45% lactic acid to Harding cuticles of females. Adult female was placed on cavity slide having one drop of water. The interior part of female was cut and removed the debris from female body with the help of fine camel hair brush and then shifted the cut portion of female to the next cavity slide which had only one drop of glycerin and placed a cover slip. Under microscope pattern was examined and identified on base of perineal pattern of M. incognita (Eisenback et al., 1981; Jepson, 1987). Cover slip was sealed with wax for further use in feature.

Mass culturing of root-knot nematode (*Meloidogyne incognita*)

The susceptible egg plant variety Dilnasheeen was used for RKN mass culturing. 42 days old eggplant singly transplanted seedling were inoculated with 3000 juveniles in root zone by making three holes with sharp pointed wooden stick to avoid the root damage by stick (Campos and Campos, 2005). The holes were enclosed with steam sterilized soil to avoid drying. After inoculation, watered the pots carefully and avoided excessive watering and pots were kept at 30 + 4 ^{0}C temperature in green house.

Collection of PGPR

The cultures of PGPR were collected from Institute of Mycology and Plant Pathology. Plant growth promoting rhizobacteria included *B. subtilis, E. cloacae, E. aerogenes and P. fluorescens*.

Multiplication of PGPR

Plant growth promoting rhizobacteria were multiplied on LB broth. Prepared the LB broth and picked the pure healthy colony of each PGPR with the help of inoculating needle. The LB broth was inoculated and the flask was placed on shaker at 100 rpm at 25 °C for 24hrs.

Sources of *P. penetrans* utilized in experiments

Pasteuria penetrans J (a commercial product of Japan) was utilized in experiment. It was received from Dr. S. R. Gowen, School of Agriculture, Policy University of Reading U.K.

Biological Management of Root Knot Nematode, *Meloidogyne incognita* through Bacterial Antagonists

This study was planned to investigate the interaction of Plant Growth Promoting Rhizobacteria and *Pasteuria penetrans* for the management of root knot nematode *M. incognita* on tomato in green house at 30 $\pm$ 4 ^{0}C. Earthen pots 20 cm dia. having sterilized soil was mixed with *Pasteuria penetrans* root powder @ 10^3 spores per gm of soil. Three week old seedlings of tomato Money Maker were transplanted singly in pots. One week after transplanting, 30 ml of bacterial (*Bacillus subtilis, Pseudomonas fluorescens* and *Enterobacter cloacae*) suspension were applied in root zone. Seven days after application of PGPR, 2000 J_2 were applied at root zone. The experiment was arranged in Completely Randomized Design and six treatments were replicated tenfold. *Pasteuria penetrans* alone or in different combinations with PGPR were applied. Pots with nematode and without PGPR, *Pp* and nematode were kept as control. The pots were watered carefully to reduce

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the risk of juveniles leached due to over watering. Sixty days after nematode application, plants were harvested carefully. Data were recorded on plant growth parameters and nematode reproduction in terms of number of egg mass, number of females, number of galls and galling index (0-10).

Result and Discussion

Management of *M. incognita* with plant growth promoting rhizobacteria and *P. penetrans* on tomato

Effectiveness of PGPR and Pp on plant growth variables

The results revealed that four PGPR strains varied in response in increasing plant growth variables. This indicates that each PGPR strain has different potential to increase plant growth variables. The PGPR increased the root and shoot length and weight in a variable range.

Root weight

The PGPR + Pp increased the root weight in a variable range. The maximum root Weight was in the treatment where *Pp* (12.32) was applied, which showed least effect. *P.florescence+Pp* (6.98) and *Bacillus subtilis + Pp* (*6.33)* showed moderate effect on root weight. The minimum root weight was observed in *E. cloacae + Pp* (5.69) treatment because RKN ability to produce gall on roots which increase the root weight due to malfunction of root, the PGPR which effectively decreased the weight was *P. florescence* (Table 1). Maximum increase in root weight 76.52 % was found in *Pp* and minimum increase in root weight 23.69 % was found in *E. cloacae + Pp*.

Table 1. Effectiveness of PGPR and *Pp* on plant growth variables.

Treatment	Root Length	Shoot Length	Root Weight	Shoot Weight
E. cloacae + Pp	34.34 a	45.52 b	5.69 e	24.52 b
Bacillus subtilis + Pp	31.5 b	42.32 c	6.33 d	23.05 c
P.florescence+Pp	28.25 c	47.49 a	6.98 c	25.45 a
Pp	24.8 d	39.7 d	8.12 b	20.52 d
Control	22.92 e	36.11 e	4.6 f	18.04 e
Control	20.77 f	30.3 f	12.32 a	16.92 f

Root length

The PGPR was increased the root length in a variable range. The maximum root length was observed in *E. cloacae + Pp* (34.34) treatment, *P. florescence + Pp* (28.25) and *B. subtilis + Pp* (31.50) showed moderate effect on root growth. The minimum root length was observed in *Pp* (24.80) treatment which showed least effect. Maximum increase in root length 49.82 % was found in *E. cloacae + Pp* and minimum increase in root length 8.20 %was found in *Pp* (Figure 1).

Shoot weight

The PGPR was increased the shoot weight in a variable range. The maximum shoot weight was observed in *P. florescence + Pp* (25.45) treatment. *E. cloacae + Pp* (24.52) and *B. subtilis + Pp* (23.05) showed moderate effect shoot growth. The minimum shoot weight was observed in *Pp* (20.52) treatment which showed least effect. Maximum increase in shoot weight 41.07 % was found in *P. florescence + Pp* and minimum increase in shoot

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weight 13.74 % was found in *Pp*.

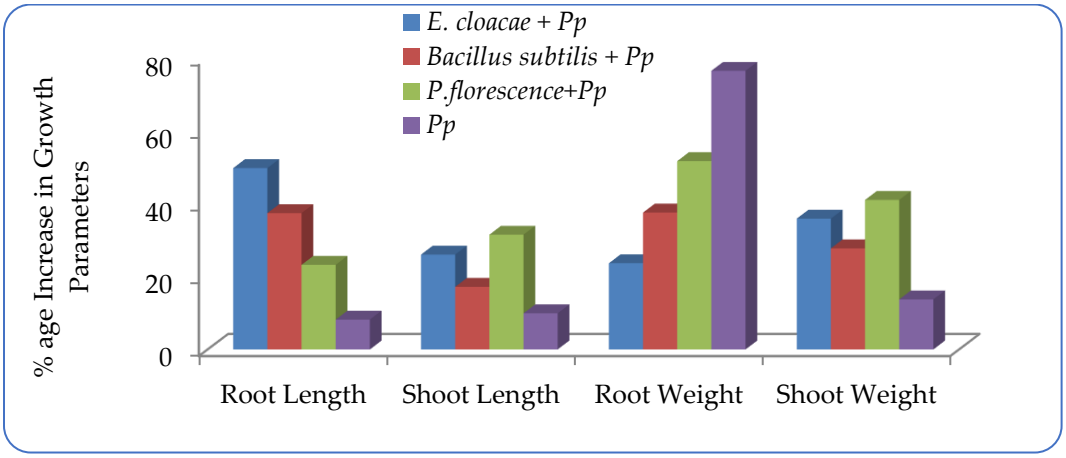

Figure 1. Effectiveness of PGPR and Pp on %age increase in plant growth variables.

Shoot Length

The PGPR was increased the shoot length in a variable range. The maximum shoot length was observed in P. fluorescens + Pp(47.49) treatment. *E. cloacae + Pp* (45.52) and *B. subtilis + Pp* (42.32) showed moderate effect on shoot growth. The minimum shoot length was observed in *Pp* (39.70) treatment which showed least effect. Maximum increase in shoot length 31.51 % was found in *P. florescence + Pp* and minimum increase in shoot length 9.94 % was found in *Pp*.

Effectiveness of PGPR and *Pp* on nematode reproduction parameters

The results revealed that four PGPR strains varied in response for controlling RKN This indicates that each PGPR strain has different potential to check that RKN populations. The nematode reproduction was assessed by gall index (0-10), no. of galls, no. of females and no. of egg masses.

Galling Index

The PGPR was suppressed the *galling index* in a variable range. The minimum galling index was produced by *E. cloacae + Pp* treatment. The *P. florescence + Pp* and *B. subtilis + Pp* moderately reduced galling index while Pp produced maximum galling index as compared to all other PGPR and resulted in the least potential of RKN controlling. Maximum decrease in galling index 56.82 % was found in *E. cloacae + Pp* and minimum decrease in galling index 47.73 % (Table 2) was found in *Pp*.

Number of Galls per root system

The PGPR was prepossessed the galls in a variable range. The minimum galls were produced by *E. cloacae + Pp* treatment. The *P. florescence + Pp and Bacillus subtilis + Pp* reduced galls while *Pp* produced maximum galls as compared to all other PGPR and resulted in the least effective on RKN controlling. Maximum decrease in galls was 68 %found *in E. cloacae + Pp* and minimum decrease in galls was 5.73 % (Table 2) found in *Pp*.

Number of Females per root system

The PGPR was prepossessed the females in a variable range. The minimum females were produced by *E. cloacae + Pp* treatment. The *P. florescence + Pp* and *Bacillus subtilis + Pp* moderately reduced females while *Pp* produced maximum females as compared to all

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other PGPR and resulted in the least potential of RKN controlling. Maximum decrease in females 66.21 % was found in *E. cloacae* + *Pp* and minimum decrease in females 55 % was found in Pp.

Number of Egg mass

The PGPR was decreased the egg masses in a variable range. The minimum egg masses were produced by *E. cloacae* + *Pp* treatment. The P. florescence + Pp and Bacillus subtilis + Pp moderately reduced egg masses while *Pp* produced maximum egg masses as compared to all other PGPR and resulted in the least potential of RKN controlling. Maximum decrease in egg masses 69.38% was found in *E. cloacae* + *Pp* and minimum decrease in egg masses 57.53% (Table 2) was found in *Pp*.

Table 2. Effectiveness of PGPR and *Pp* on nematode reproduction parameters.

Treatment	No. of Females	No. of Galls	No. of Egg masses	Galling Index (0-10)
E. cloacae + *Pp*	249 e	231.2 d	198.6 d	3.8 c
B. subtilis + *Pp*	272 d	254.6 cd	233.4 c	4.4 b
P.florescence + *Pp*	301 c	278.8 c	250.6 bc	4.6 b
Pp	339 b	313.6 b	274.8 b	4.8 b
Control	737 a	707.2 a	647.4 a	8.8 a
Control	0 f	0 e	0 e	0 d

The two-thirds of the total fauna of the earth is represented by nematodes which are he multi cellular animals (Khan, 1993). Root-knot nematodes are widely distributed in vegetable crops and cause significantly yield losses in Pakistan. Interestingly, the PGPRs showed a significant influence on growth parameters viz. root and shoot (length and weight) when applied at one week before of nematode inoculation. *P. fluorescens, A. brasilense, A. chroococcum* and *B. megaterium* are well known as plant growth promoters (Fortes et al., 2007; Resende et al., 2004). Many microbes (fungi, bacteria and nematodes) could be used as bio-control agents to protect plants from pathogens. Rhizobacteria are able to colonize the roots and, therefore, can improve plant vigor against root-knot nematode (Sikora et al., 2007). Rhizobactera increase the uptake of nutrients and improve plant health; therefore increase plant resistance against soil borne pathogens (Compant et al, 2005; Liu et al., 2012). Our Results also agree with those obtained by Kalinovskaya et al. (2002), and Tian et al. (2007) who reported that the suppression of root knot nematodes by microbes is through competition for food and nutrients, root colonization, parasitism and production of antibiotics and enzyme like surfactin, Chitinase and lipopeptides. According to Silveira and Freitas (2007), the inoculation of microbes in soil must be as early as possible because the dynamics of the ecosystem that they face difficultly to establish in soil. Similarly, Oliveira et al. (2009) confirmed that *B. megaterium* strains produced secondary metabolites which caused a significant reduction in *M. exigua* reproduction on coffee. Youssef et al. (2017) stated that rhizobacteria that *B. subtilis, B. megaterium* and *B. pumilus* showed the nematicidal activity against *M. incognita* in addition to ameliorating suger beet parameters of growth. The bacterial strains tested in the current study may be promising biocontrol agents as PGPR for the future nematode management strategies.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

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