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Research Article

Protective and Curative Influence of Plant Growth Promoting Rhizobacteria on the Development of *M. incognita* Juveniles

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Abstract

In Pakistan, the low yield of eggplant is ascribed to legions of biotic constraints. Among biotic restraints, root-knot nematodes, *Meloidogyne* spp. are economically very important and cause losses to the tune of $ 125 billion per year throughout the world. In this experiment, efficacy of PGPRs was checked as protectant and curative against *M. incognita* under greenhouse conditions. Three weeks old seedlings of eggplant (cv. Dilnasheen) were transplanted singly in each 20cm-dia. earthen pots filled with two kg sterilized soil. Ten days after seedlings transplantation, each pot was inoculated with PGPRs (*B. subtilis*, *P. fluorescens*, *A. chroococcum*, *Azospirillum sp,* and *R. leguminosarum*) having 10^7 cfu/ml @ 30 ml per plant with 5% sugar solution. One week after of PGPR application, 2000 freshly hatched juveniles of *M. incognita* (contained in 15 ml of water) were inoculated in the root zone. While in curative application ten days after transplantation of seedlings, 2000 freshly hatched juveniles (contained in 15 ml of water) were inoculated in the root zones. One week of after J2s inoculation, PGPRs having 10^7 cfu/ml were applied to pots @ 30 ml per plant with 5% sugar solution. Plants without PGPR and J2s inoculation were kept as control. The experiment was arranged in Completely Randomized Design with ten replicates. The effectiveness of PGPRs was evaluated against *M. incognita*, as protective and curative applications, all the PGPRs caused significant reductions in nematode infestations. When the comparison was made between the protective and curative applications, it was found that the protective application was better than the curative application of PGPRs. Galls per root system on eggplant were significantly lower in protective application than the curative application over control. Similar trends were observed in other parameters viz. number of females, egg masses, soil and root population and reproduction factors of nematodes.

Keywords: Biocontrol agents; Eggplant; Root Knot Nematode; *Meloidogyne incognita*.

Introduction

Eggplant (*Solanum melongena*) belongs to nightshade family *Solanaceae* and is mostly

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cultivated for its edible fruit. It is closely related cousin of tomato and potato (Doijode, 2001; Doganlar *et al.*, 2002). It was cultivated on 8427 ha with production of 84255 tons annually in Pakistan (FAO, 2021). Eggplant is an excellent source of Vitamin B1, Dietary fiber, Cu and a good source of Mn, K, Vitamin B6, Niacin, Folate, Vitamin K (Ensminger *et al.*, 1983; Bliss and Elstein, 2004). It also contains water, carbohydrate, protein and low fat. Eggplant is also a rich source of phytonutrients i.e. Flavonoids (Nasunin) and Phenolic compounds (Chlorogenic acid and Caffeic acid) which act as antioxidants. Chlorogenic acid also has antiviral, antimicrobial, anticancer (antimutagenic) and anti-LDL activities. Nasunin being a free radical scavenger, saves the lipids of brain cell membranes from damage (Zambrano-Moreno *et al.*, 2015; Whitaker and Stommel, 2003; Wang and Stoner, 2008; Noda *et al.*, 2000; Kimura *et al.*, 1999; Jorge *et al.*, 1998; Cassidy *et al.*, 2013; Akanitapichat *et al.*, 2010; Ahmad *et al.*, 2022). Due to environment and genotype, a little change in nutritional composition of eggplant was observed (San José *et al.*, 2014).

Many abiotic (weather, fertilizers, water and temperature) and biotic constrains (seed, insect pests and pathogens) cause low production of eggplant in Pakistan (Oka *et al.*, 2000). Different diseases caused by several pathogens like fungi, bacteria, viruses and nematodes reduce the production and quality of fruit but root knot disease caused by root knot nematode (*Meloidogyne* spp.) is one of the most important and destructive maladies of eggplant (Roberts, 1987).

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 nematodes are polyphagous and more than 3000 plant species have been reported as hosts of these nematodes (Abad *et al.*, 2003; Agrios, 2005). Due to such wide host arrange, root knot nematodes cause major economic damage to vegetables, fruit plants and field crops and an estimated loss of 125 billion $ occurs annually worldwide (Dodzia *et al.*, 2012; Collange *et al.*, 2011; Chitwood, 2003; Williamson and Hussey, 1996; Koenning *et al.*, 1999). In Pakistan as well as worldwide, 10-100% yield losses on vegetables were reported by many scientists (Anwar and McKenry, 2012; Shahid *et al.*, 2007; Kamran *et al.*, 2010; Tariq-Khan *et al.*, 2020).

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).

M. incognita is one of the most important key nematodes in *Meloidogyne* genus which is difficult to be managed because of high rate of reproduction. *Meloidogyne* spp. completes their life cycle within 25 to 30 days at 25 to 35°C and females lay egg masses which contain

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400 to 2000 eggs (Ploeg and Maris, 1999; Chitwood, 2002; Hirunsalee *et al.*, 1995). It is mainly concerned to find suitable and efficient solution or strategy for the management of root knot nematodes. Plant growth promoting rhizobacteria (PGPR) have the potential as bio-control agents to substitute chemicals because they are ecofriendly and significantly reduce the disease. Plant growth promoting rhizobacteria also enhance the defense mechanisms through root colonization, production of sideropores, antibiosis and induced systemic resistance (ISR) which are cost effective, efficient and ecofriendly to control plant diseases of vegetables, fruit plants and field crops (Yildirim *et al.*, 2006; Van Loon *et al.*, 1998; Kloepper *et al.*, 2004; Johnsson *et al.*, 1998; Anwar-ul-Haq *et al.*, 2011; Ahmad *et al.*, 2014). Keeping in view the aforementioned facts about root knot nematodes and plant growth promoting rhizobacteria, the present studies were planned with the following objective: Evaluation of the potential of PGPRs for the management of *M. incognita*.

Methodology

Sterilization of soil

The soil used in the trials was mixed thoroughly with 1:1:1 ratio (loam, sand and well rotten sugarcane molasses). The mixed soil was sieved to remove stones, pebbles and root fragments. For sterilization, the soil was treated with formalin (1:320 parts) and covered with polythene sheet for three days to kill the pathogens present in the soil. The polythene sheet was removed from heap and the soil was disturbed intermittently for 2 days to dispense the fumes of formalin. The sterilized soil was filled in earthen pots for experiments.

Collection of germplasm and raising of nursery

The seeds of tomato cultivar Money Maker and eggplant cultivar Dilnasheen, collected from Vegetable Research Institute, Faisalabad for experiment and multiplication and maintenance of nematode culture.

The nurseries of Money Maker and Dilnasheen were raised throughout the experimental period for continue supply of seedlings for both experiment and culture maintenance purpose.

Single egg mass culture of *M. incognita*

Eggplants showing characteristic root knot symptoms (poor and stunted growth, and galled roots) were selected during surveys of vegetable fields. Plants were uprooted carefully along with rhizosperic soil, put in polythene bags, labeled properly and brought to laboratory. Soil was detached from the roots and roots were washed under tap water carefully. After washing, roots were cut into small pieces. Healthy light brown egg masses were picked singly with the help of needle and inoculated individually the five-week-old seedlings of tomato in the root zone. After 50 days of inoculation, tomato plants were harvested carefully, and root systems were cut into pieces. From each root system, fifteen mature whitish females were randomly isolated under stereoscope, put in 40% lactic acid and perineal patterns were made (Taylor, 1967). The perineal patterns were compared with standard diagrams and females were confirmed to be *M. incognita* (Eisenback et al., 1981).

Mass culturing of *M. incognita*

For mass culturing, three-week-old tomato seedlings (Money Maker) were transplanted

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singly in 15-cm-diam. pots having formalin sterilized soil. Two weeks after transplantation, two thousand eggs of *M. incognita* contained in 10 ml of water were inoculated in each plant in the root zone by making three holes around the stems of plants grown in pots containing 2 kg of sterilized soil. The holes were filled with soil to avoid vaporization of water. The earthen pots were watered according to their requirement. For the continuous supply of culture throughout the experiments, the same procedure was repeated regularly.

Collection of bio-control agents

Bio-control agents, plant growth promoting rhizobacteria (PGPRs) were collected from Plant Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad. Five PGPRs (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Azospirillum* sp., *Rhizobium leguminosarum*) were used for experimental purpose. These PGPRs were multiplied on Nutrient Broth for their culture maintenance.

Protective effectiveness of PGPRS on the development of *M. incognita*

In this experiment, efficacy of PGPRs was checked as protectant against *M. incognita* under greenhouse conditions. Three weeks old seedlings of eggplant (cv. Dilnasheen) were transplanted singly in each 20cm-dia. earthen pots filled with two kg sterilized soil. Ten days after seedlings transplantation, each pot was inoculated with PGPRs (*B. subtilis*, *P. fluorescens*, *A. chroococcum*, *Azospirillum sp,* and *R. leguminosarum*) having 10^7 cfu/ml @ 30 ml per plant with 5% sugar solution. One week after of PGPR application, 2000 freshly hatched juveniles of *M. incognita* (contained in 15 ml of water) were inoculated in the root zone. Plants without PGPR and J2s inoculation were kept as control. The experiment was arranged in Completely Randomized Design with ten replicates.

Sixty days after J2s inoculation, the plants were harvested from the earthen pots and the soil was removed carefully to avoid the damage to roots and egg masses. In laboratory, the roots were gently washed under tap water to avoid loss of egg masses. Data were recorded on nematodes reproduction parameters viz. number of galls, egg masses, females, J2s/100 cm^3 of soil and reproduction factor. Roots of eggplant were stained with Phloxine B for counting of egg masses per root system (Southey, 1986; Holbrook *et al.*, 1983). For counting the number of females, roots were stained with acid fuchsin.

Curative effectiveness of PGPRS on the development of *M. incognita*

In this experiment, the efficacy of PGPRs was tested as curative against *M. incognita* under greenhouse conditions. Three weeks old seedlings of eggplant (cv. Dilnasheen) were transplanted singly in each 20cm-dia. earthen pots filled with two kg sterilized soil. Ten days after transplantation of seedlings, 2000 freshly hatched juveniles (contained in 15 ml of water) were inoculated in the root zones. One week of after J2s inoculation, PGPRs (*B. subtilis*, *P. fluorescens*, *A. chroococcum*, *Azospirillum sp.* and *R. leguminosarum*) having 10^7 cfu/ml were applied to pots @ 30 ml per plant with 5% sugar solution. Plants without PGPRs and J2s inoculation were kept as control. The pots were arranged in Completely Randomized Design replicating tenfold.

Sixty days after J2s inoculation, the plants were harvested from earthen pots and removed the soil carefully to avoid the damage of roots and egg masses. In lab, roots were carefully washed under tap water to avoid loss of egg masses. Data was recorded

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on nematodes reproduction parameters as described pervious section.

Statistical analysis

All the data were subjected to Analysis of Variance (ANOVA) using Statistix 8.1 package. The means were compared by Tukey Honestly Significant Difference Test HSD at 0.05%.

Results

Protective effect of PGPRS on the development of *M. incognita*

Efficacy of PGPRs as protective was examined on root knot nematodes reproduction on eggplant. Efficiency of PGPRs varied significantly over control (P= 0.05) on the *M. incognita* J2s development (Table 1).

Number of galls per root system

All the PGPRs as protective treatments significantly reduced the development of galls on eggplant root systems over control. The maximum number of galls (195.60) was found in control (without PGPR) treatment while the minimum galls (19.70) were observed in *Bs* treatment which showed *Bs* was the most effective treatment as compared to other treatments i.e. *Pf* (29.70), *Azoto* (35.10), *Azo* (46.70) and *Rhiz* (51.00) (Table 1).

Number of females per root system

The protective treatments of PGPRs caused significant reductions in the development of females on eggplant root systems. *Bs* as protective treatment resulted in the minimum production of females (20.10) followed by *Pf*, *Azoto* and *Azo* producing 31.00, 37.30, and 49.30 females respectively while the maximum females were produced in the treatment with *Rhiz* (54.60) (Table 1).

Number of egg masses per root system

All the rhizobacteria showed significant reductions in eggmasses as protective treatments. The maximum number of egg masses (162.00) was found in the control (without PGPR) treatment while the minimum eggmasses (10.90) were observed in *Bs*. The rest of the treatments i.e. *Pf*, *Azoto*, *Azo* and *Rhiz* (14.40, 19.30, 25.50, and 28.50 respectively) showed intermediary results (Table 1).

Juveniles per 100 cc of soil

PGPRs as protective treatments varied significantly (P=0.05) regarding number of juveniles in the soil as compared to control treatment. The minimum J2s (77.20) recovered from the soil where *Bs* was applied while the maximum J2s (127.90) recovered in the *Rhiz* treatment. The remaining PGPR treatments showed intermediary effects (Table 1).

Juveniles per root system

The minimum no. of J2s (2289.0) recovered from the root system of eggplant in *Bs* treatment as protective which proved the most effective treatment as compared to other treatments *Pf*, *Azoto*, *Azo* and *Rhiz* (3168.0, 4342.5, 5814.0, and 6555.0 respectively). On the other hand, the maximum J2s were observed in the control treatment (Table 1).

Reproduction factor

As protective treatment of PGPRs, significant reductions were observed in reproduction factor of root knot nematodes on eggplant. The minimum reproduction factor of *M. incognita* (1.19) was found with *Bs* application while all the other PGPR treatments

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showed intermediary effects on reproduction factor (Table 1).

Table 1: Protective effect of PGPRs on the development of *M. incognita*.

*Treatments	No. of Galls	No. of Females	No. of Egg Masses	J2 from soil	J2/Root systems	**Reproduction Factor
Pf	29.70 cd	31.00 cd	14.40 d	87.100 e	3168.0 d	1.63 d
Bs	19.70 d	20.10 d	10.90 d	77.200 f	2289.0 d	1.19 d
Azto	35.10 bcd	37.30 bcd	19.30 cd	97.300 d	4342.5 cd	2.23 cd
Azo	46.70 bc	49.30 bc	25.50 bc	108.10 c	5814.0 bc	2.98 bc
Rhiz	51.00 b	54.60 b	28.50 b	127.90 b	6555.0 b	3.36 b
Control	195.60 a	205.50 a	162.00 a	297.00 a	40500 a	20.50 a

Means within a column sharing the same letters are not significantly different from each other at P =0.05 according to Tukey HSD Test *Bs= *Bacillus subtilis*, *Pf = Pseudomonas fluorescens*, *Azoto = Azotobacter chroococcum*, *Azo = Azospirillum sp*., *Rhiz = Rhizobium leguminosarum*; **Reproduction Factor = ***Final Population / Initial Population; ***Final Population= Number of females + J2 from soil + J2 from Root

Curative effects of PGPR on the development of *M. incognita*

Curative application of PGPRs also caused significant reductions in the development of nematode on eggplant. All the nematode reproduction parameters were significantly lower as compared to control treatment.

Number of galls per root system

The minimum galls (52.00) were observed in *Bs* treatment which showed that application of *Bs* was better treatment as compared to other PGPR treatments viz. *Pf* (59.00), *Azoto* (65.00), *Azo* (71.00) and *Rhiz* (83.00). Contrarily, the maximum number of galls (200.00) were found in the control (without PGPR) treatment (Table 2).

Number of females per root system

Curative treatments caused significant reductions in the development of females on eggplant root system. In the treatment where *Bs* was applied, the minimum females (63.00) were produced followed by *Pf*, *Azoto* and *Azo* producing 77.00, 88.00, and 94.00 females respectively. On the other hand, the maximum females (98.00) were produced with the application of *Rhiz* as compared to control (Table 2).

Number of egg masses per root system

All the PGPRs showed significant reductions in eggmasses on eggplant root system. The maximum number of eggmasses was observed in control (191.50) while the minimum eggmasses (25.20) were found in *Bs* treatment. The treatments *Pf, Azoto, Azo* and *Rhizo* with eggmasses of 33.10, 38.70, 44.10, and 56.90 showed intermediate results (Table 2).

Juveniles per 100 cc of soil

PGPRs as curative treatments varied significantly regarding their soil population over control. The minimum number of J2s (96.80) was found in the soil treated with *Bs* while the maximum J2s (138.10) were recovered from the *Rhiz* treatment (Table 2).

Juveniles per root system

The minimum no. of J2s (5846.4) was obtained from the root system of eggplant in *Bs* treatment as compared to other treatments *Pf*, *Azoto*, *Azo* and *Rhiz* (7778.5, 9442.8, 10805,

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14225 respectively). On the contrary, the maximum J2s were observed in the control treatment (49790) (Table 2).

Reproduction factor

Among all PGPRs as curative treatments, significant reduction was observed in reproduction factor of the nematode on eggplant. The minimum reproduction of *M. incognita* (3.00) was observed in *Bs* treatment while all other PGPR treatments showed intermediary effects on reproduction factor as compared to control with reproduction factor of 25.19 (Table 2).

Table 2: Curative effect of PGPRs on the development of *M. incognita.*

*Treatments	No. of Galls	No. of Females	No. of Egg Masses	J2from soil	J2/Root systems	**Reproduction Factor
Pf	59.00 c	77.00 bc	33.10 cd	106.60 e	7778.5 cd	3.98 cd
Bs	52.00 c	63.00 c	25.20 d	96.80 f	5846.4 d	3.00 d
Azto	65.00 bc	88.00 b	38.70 cd	118.10 d	9442.80 cd	4.82 cd
Azo	71.00 bc	94.00 b	44.10 bc	127.20 c	10805 bc	5.51 bc
Rhiz	83.00 b	98.00 b	56.90 b	138.10 b	14225 b	7.23 b
Control	200.00 a	261.00 a	191.50 a	337.30 a	49790 a	25.19 a

Means within a column sharing the same letters are not significantly different from each other at P =0.05 according to Tukey HSD Test *Bs= *Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp., Rhiz = Rhizobium leguminosarum;* **Reproduction Factor = ***Final Population / Initial Population; ***Final Population= Number of females + J2 from soil + J2 from Root

Comparison between protective and curative application of PGPRS

When comparison was made between the protective and curative applications, it was found that the protective application was better than the curative application of PGPRs. Galls per root system on eggplant were significantly lower in protective application than the curative application over control. Similar trends were observed in other parameters viz. number of females, egg masses, soil and root population and reproduction factors of nematodes as shown in figures 1, 2, 3, 4, 5 and 6.

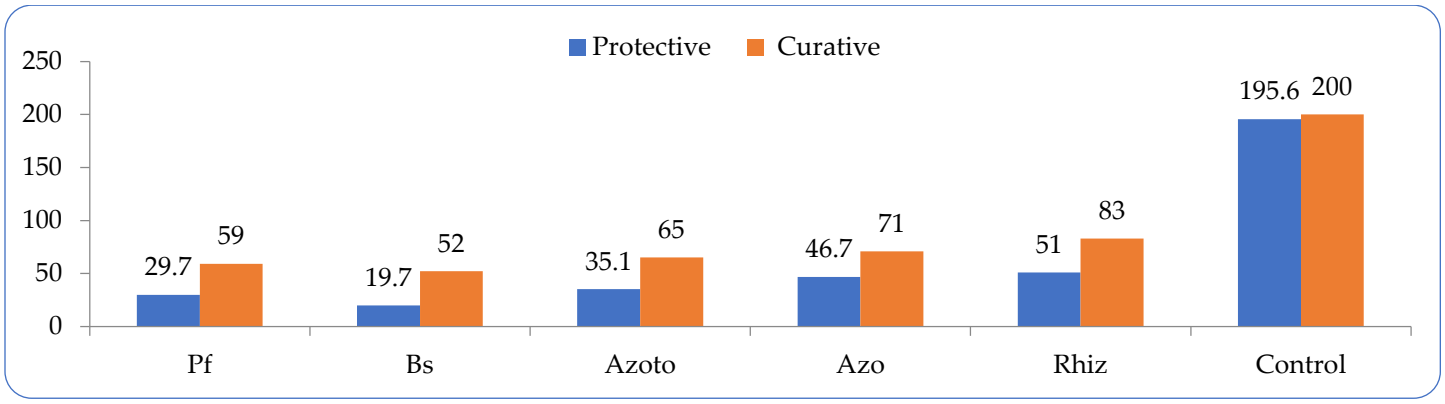

Figure 1: Comparison of Protective and curative effects of PGPRs on number of galls

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp, Rhiz = Rhizobium leguminosarum*

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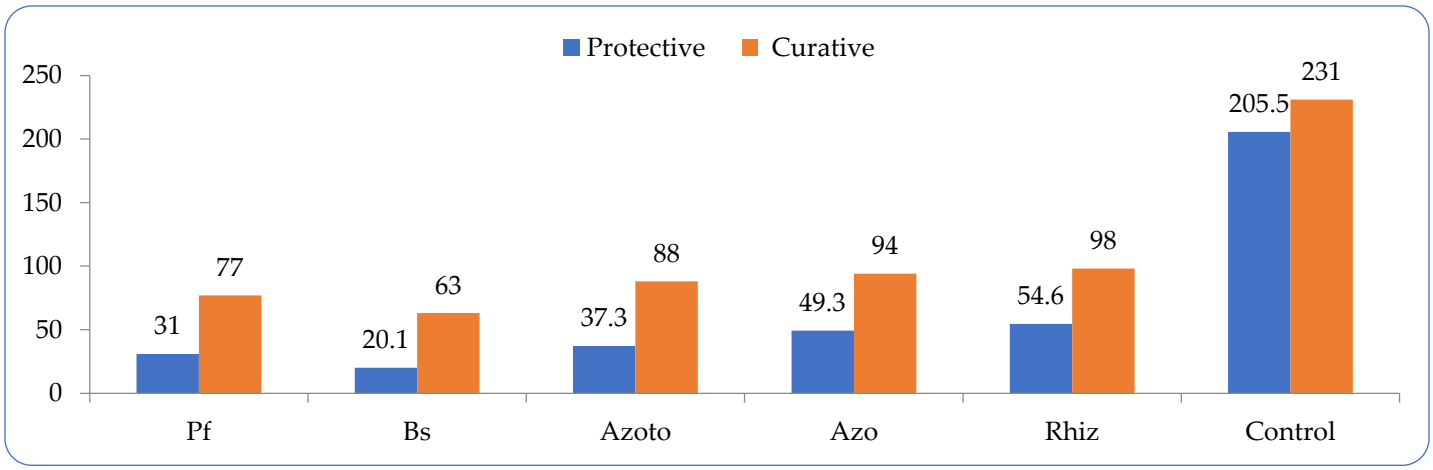

Figure 2: Comparison of Protective and curative effects of PGPRs on number of female.

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp, Rhiz = Rhizobium leguminosarum*

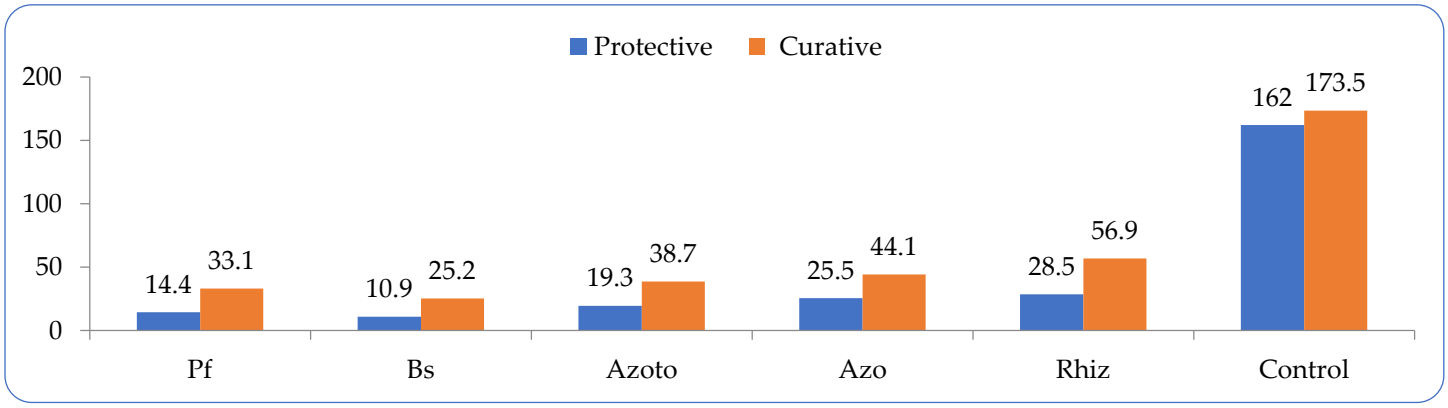

Figure 3: Comparison of Protective and curative effects of PGPRs on number of egg masses.

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp, Rhiz = Rhizobium leguminosarum*

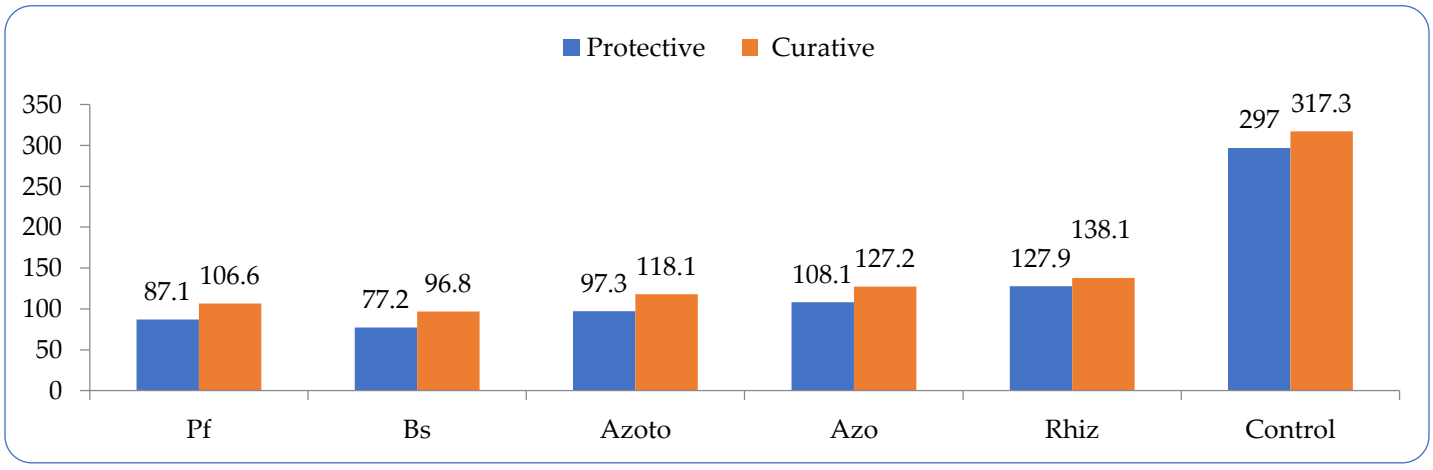

Figure 4: Comparison of Protective and curative effects of PGPRs on number of juveniles from soil.

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp, Rhiz = Rhizobium leguminosarum*

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In this study, the protective and curative effects of PGPRs were assessed against the most destructive nematode *M. incognita*. It was found that the protective application was better than the curative application of PGPRs. Number of galls, females, egg masses, soil and root populations and reproduction factors of nematodes on eggplant were significantly lower in protective application than the curative application over control (Figures 1, 2, 3, 4, 5 and 6).

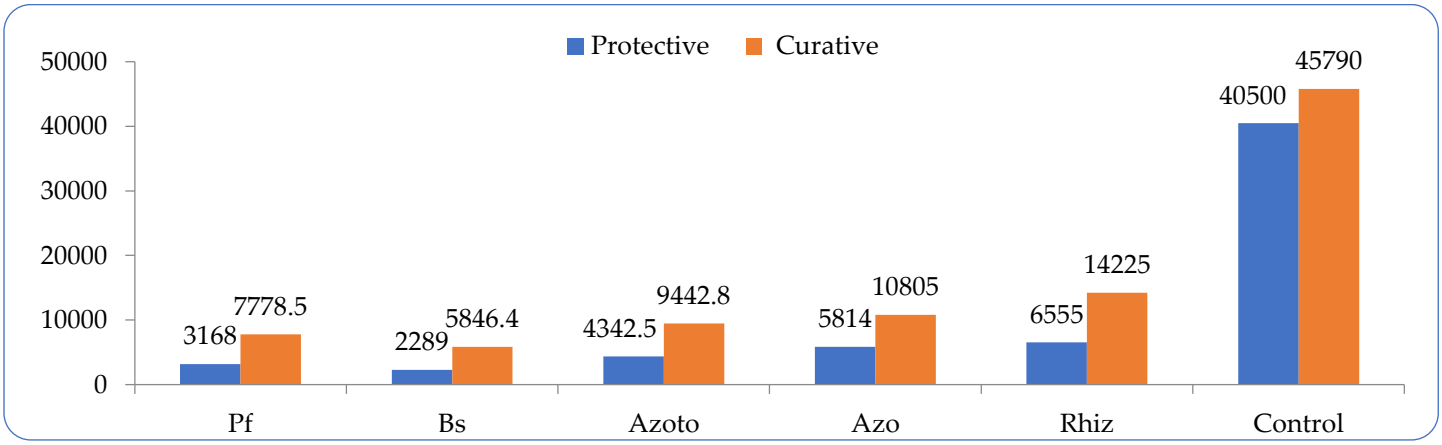

Figure 5: Comparison of Protective and curative effects of PGPRs on number of juveniles per root system.

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp, Rhiz = Rhizobium leguminosarum*

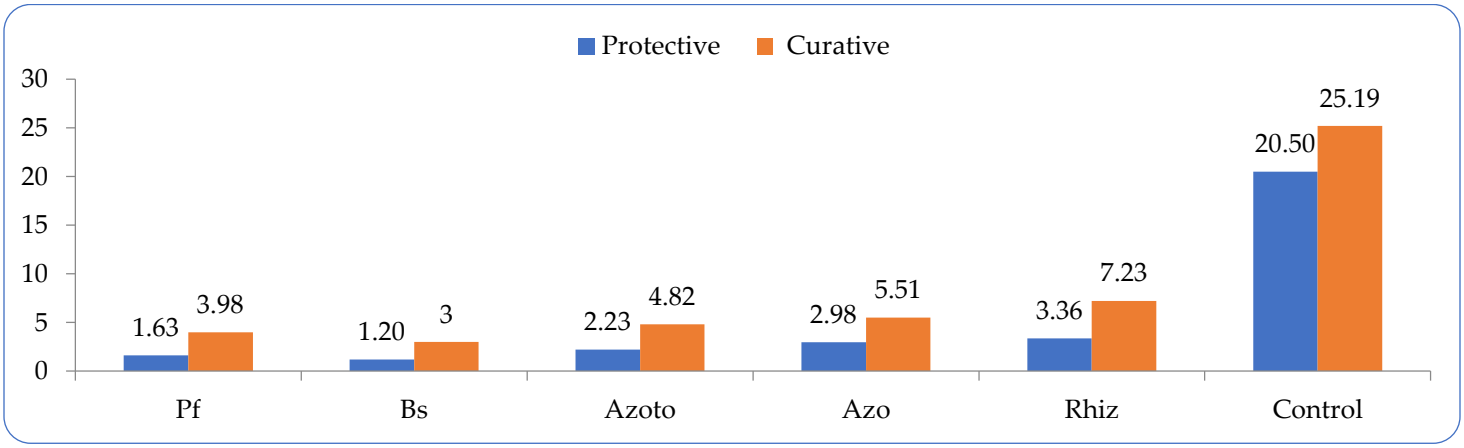

Figure 6: Comparison of Protective and curative effects of PGPRs on reproduction factor.

**Bs= Bacillus subtilis, Pf = Pseudomonas fluorescens, Azoto = Azotobacter chroococcum, Azo = Azospirillum sp, Rhiz = Rhizobium leguminosarum*

Discussion

Many researchers reported similar findings using PGPRs. Mousa et al. (2021) found that rhizobacteria (*P. fluorescens, A. brasilense, A. chroococcum* and *B. megaterium*) significantly reduced *Meloidogyne* spp. egg hatching and juveniles' mortality compared to control. Huang et al. (2010) revealed that the rhizobacteria *B. megaterium* YFM3.25 significantly inhibited *M. incognita* egg hatching and juveniles' mortality under *in vitro* conditions as well as showed a significant reduction in number of egg masses, galls and number of

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eggs from single egg mass over control under *in vivo* conditions. The reductions in these parameters could be attributed to the production of different chemicals (3, 5-dimethoxy toluene, 1-ethenyl-4-methoxy-benzene, benzene ethanol, 2-nonanone, 2, 6, 10-trimethyl-dodecane decanal, propyl-benzene 2-undecanone, 2-pentylfuran, dimethyl disulphide, phenyl ethanone, benzene acetaldehyde, nonane, hexadecane, propanone, phenol and 2, 3-dimethyl-butanedinitrile) which acted as nematicidal as well as nematostatic. Aballay et al. (2013) stated that the inhibition of egg hatching due to the secondary metabolites produced by the rhizobacteria which caused egg lysis and affected the egg viability.

The most important thing for the efficacy of biocontrol agents are rate of applications and time. In a study, Mousa et al. (2021) applied four PGPR (*P. fluorescens, A. brasilense, A. chroococcum* and *B. megaterium*) against root-knot nematodes under greenhouse conditions at three application times (one week before, at the same time and one week after) and found that repeated applications with one week before showed improved performance than those applied one week after and at the same time. Khyami-Horani and Al-Banna (2006) revealed that application of the bacterium (*B. thuringiensis jordanica*) one week before the transplantation of tomato nursery in nematode infested soil reduced the significant galling on roots (51-59%). 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. Sansinenea and Ortiz (2011) indicated that *Bacillus* spp. produced some substances i.e. antimicrobial compounds (antibiotics) such as zwittermicin produced which served as antifungal, antibiotic and also had nematicidal properties. Insunza et al. (2002) stated that rhizobacterial strains of *A. brasilense, A. chroococcum* and *P. fluorescens* inhibited egg hatching and juveniles mortality by producing different kinds of compounds likes protease, antibiotics, siderorhores, organic compounds, hydrolytic enzymes, HCN and phenol oxidation.

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. All plants treated with *B. cereus* strain (S18) combined with RKN showed plant growth improvement when compared with control treatment (Burkett-Cadena et al., 2008).

Conflict of Interest

The authors have not declared any conflict of interest.

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Authors Contributions

All the authors contributed equally in the manuscript.

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