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Nematicidal potential of plant growth-promoting rhizobacteria against *Meloidogyne incognita* infesting tomato under protected cultivation

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Abstract

Background: In the protected cultivation of tomato (*Solanum lycopersicum* L.) crop, the severity of root-knot nematode, *Meloidogyne incognita,* incidence is alarming nowadays. To combat this, growers usually prefer using synthetic chemical pesticides, which in turn results in harming non-target beneficial microbes present in the soil micro-flora and indirectly toxic to human health. Therefore, attempts were made to find out the nematicidal potential of plant growth-promoting rhizobacteria (PGPR) against *M. incognita*, which could be used as an alternative solution to manage *M. incognita* incidence under protected cultivation.

Results: Nematicidal potential of three PGPR isolates and their consortium against *M. incognita* under laboratory, pots, and field experiments was studied. Juvenile mortality of 91.67% was recorded at 100% consortium, followed by 73.33–81.33% by individual isolates. Similarly, hatching inhibition of 84.26% was recorded at 100% PGPR consortium followed by 78.48–83.21% in individual isolates after 96 h. of incubation. In the pots' study, soil drenched with PGPR isolates consortium, followed by *Bacillus subtilis* DTBS 5, *Pantoea agglomerans*, and *Bacillus amyloliquefaciens* DSBA 11 recorded significant reductions in the nematode incidence. Whereas in the field study, PGPR isolates applied as soil drenching also significantly reduced nematode's incidence in consortium, followed by *B. subtilis* DTBS 5 and *B. amyloliquefaciens* DSBA 11-treated soil in both field experiments. On an average, the plant growth promotion and fruit yield were enhanced than untreated control and PGPR isolates applied as soil drenching gave a significant result than bare root dip treatment.

Conclusions: PGPR isolates, *B. amyloliquefaciens* DSBA 11, *B. subtilis* DTBS 5, and *P. agglomerans*, were found to be effective against *M. incognita*. This finding can be incorporated into the nematode management strategy in tomato crop grown under protected cultivation. Further to enhance the biocontrol efficacy of these PGPR isolates, suitable formulations of either individual or consortium need to be done.

Keywords: Plant growth-promoting rhizobacteria, Tomato, *Meloidogyne incognita*, Consortium, Protected cultivation, Nematode management

Background

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown and consumed all around the world. Annually, India produces about 16.38 MT of tomato, and which is low as compared with the developed countries because of its

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vulnerability to several fungal, viral, bacterial, and nematode diseases (Horna et al. 2006). Plant parasitic nematodes are reported to cause 21.3% of crop losses amounting to INR 102039.79 million (1.58 billion USD) annually (Kumar et al. 2020). The demand for high-quality tomatoes for domestic consumption as well as international market is reaching very high and to claim this several growers shifted from open field cultivation of tomato to protected cultivation especially during off season but plant pathogenic nematodes, specially, root-knot nematodes (*Meloidogyne incognita*) incidence started to increase under protected cultivation and later on became severe, which leads to huge crop losses.

Plant growth-promoting rhizobacteria (PGPR) are the varied group of free-living soil bacteria that colonize the plants rhizosphere and helps in enhancing the growth and yield of agriculture crops (Kumar et al. 2016). Among PGPR genera, *Azospirillum*, *Pseudomonas*, and *Bacillus* are the broadly explored bioagents commercially. These bioagents have the ability to boost the plant growth by production of several plant growth promoting substances and eradicate plant parasitic nematodes. PGPR were also stated to be the potential bio-agent to lessen damage caused by plant parasitic nematodes, and their interaction was studied widely for the effective management of plant parasitic nematodes (Tabatabaei and Saeedizadeh 2017). The rhizosphere bacterial isolates like *Bacillus pumilus, Paenibacillus castaneae, Pseudomonas fluorescens, Bacillus subtilis, Bacillus cereus*, *Arthrobotrys oligospora, Beauveria bassiana, Bacillus megaterium, Pseudomonas striata* and *Paenibacillus polymyxa* were directly and indirectly suppressed the nematode's infestation and promote plant growth (Alfianny et al. 2019). Biopesticides constitute a desirable component of pest management (Ntalli et al. 2010). Continuous use of these synthetic chemical nematicides, often at higher than recommended rates, bio-magnification, and environment deterioration due to their toxicity has rendered ecosystems unstable and non-profitable because of which they are withdrawn from the market (Xiang et al. 2018). In this situation, avoiding the use of chemical nematicides, use of biological control agents can limit the damage toll and less harmful to environment and other non-target organisms. Nematode management strategies comprising of biological control agents could more efficiently regulate nematodes' populations (Saad et al. 2022). Thus, a comprehensive study was planned to evaluate the potential of PGPR isolates under laboratory, pots and field conditions against root-knot nematode (*M. incognita*).

Methods

Nematode culturing

The *M. incognita* population used in the study was originally collected from the heavily infected tomato plants grown at Centre for Protected Cultivation Technology (CPCT), ICAR-IARI-New Delhi, India. The identification of the species was done morphologically based on the perineal pattern of mature females (Jepson 1987). The infected roots were washed, and egg masses were removed with sterile forceps and kept for hatching using the modified Baermann method. Second-stage juveniles (J_2s) were collected in the Petri plate containing water after 24 h. From infested soil samples, the juveniles were extracted by Cobb's decanting and sieving technique (Cobb 1918). Further, egg masses of uniform size were collected from the galled roots and inoculated (one egg mass/pot) into the root zones of susceptible Pusa Purple Long variety of brinjal and tomato cv. NS 4266. Pots were maintained in a greenhouse and growth chambers at 25–30 °C with a photoperiod of 12 h. For laboratory and pot experiments, egg masses from heavily galled roots were handpicked and transferred to vial containing 0.5% (v/v) sodium hypochlorite (NaOCl) and shaken for 3 min. The egg mass suspension was then passed through a series of filters with pore sizes of 74, 45, and 25 µm. Eggs that were retained on the 25-µm filter were collected with sterile distilled water (Hussey 1973) and allowed to hatch in modified Baermann setup at 28 °C to get freshly hatched second-stage juveniles (J_2s), which were used for subsequent experiments (Viglierchio and Schmitt 1983).

Preparation of soil for pot experiments

Field soil from CPCT-IARI, New Delhi, was used for all the experimental purpose. The soil was mixed with sand in the ratio of 3:1. The soil sand mixture was steam-sterilized at 1.0546 kg/cm^3 pressure for 4 h. and stored in polythene bags.

Raising, transplanting, and maintenance of tomato seedlings

Healthy susceptible seedlings' of tomato cv. NS 4266 were raised using sterilized mixture of cocopeat: vermiculite: perlite (3:1:1). After attaining 21 days, seedlings were transplanted into earthen pots (6 inches size) and arranged in a completely randomized design under polyhouse condition. During the polyhouse experiments, all agronomic practices like irrigation by drip at 3–4 days interval, weeding was done thrice throughout the crop period, nutrient management (N:P:K: 19:19:19 at 3 g/L, through fertigation at 2 months interval) and training of tomato plants after attaining particular stage was done. The average temperature during the pot and field

experiments under protected cultivation was 30 ± 2 °C, and the crop season was Kharif-Rabi.

Treatment details for laboratory, pots and field experiments

Ba: *Bacillus amyloliquefaciens* DSBA 11; Bs: *Bacillus subtilis* DTBS 5; Pa: *Pantoea agglomerans*; Ba + Bs + Pa (Consortium); NB: Nutrient broth; SDW: Sterile distilled water; VP: Velum Prime® (500 g a.i./ha) as positive control.

Laboratory experiments

Preparation of PGPR culture filtrates

All the PGPR isolates, *B. amyloliquefaciens* DSBA-11 (ITCC BJ-0013), *B. subtilis* DTBS 5 (ITCC BJ-0011) and *P. agglomerans* (ITCC BC-0001), used in this study were collected from the Bacteriology lab and Indian Type Culture Collection (ITCC), Division of plant pathology, ICAR-IARI, New Delhi, Delhi, India. A single colony from the pure cultures of PGPR isolates was taken from 24-h. old culture plates and inoculated into 50 mL of sterilized King's B broth in 100-mL Erlenmeyer flasks and incubated in a shaker incubator at 150 r.p.m at 37 °C for 24 h. The bacterial growth after 24 h. was tested. Culture filtrate obtained by centrifugation at 10,000 r.p.m for 15 min at 4 °C. The supernatant culture filtrate was collected and passed through syringe filter of 0.22 µm. Consequently, collected culture filtrate was tested for the absence of any viable cell and used for bioassay (Rompalli et al. 2016).

Juvenile's mortality bioassay

The nematode suspension of 100 J_2s/10 µL was poured into each well of 24-well culture plate, and 1 mL of different concentrations of cell-free culture filtrates of each PGPR isolates at 100, 50 and 25% was added and mixed thoroughly. Nutrient broth and sterile distilled water were taken as negative control, whereas Velum Prime® (Fluopyram 400 SC) was taken as positive control and 24 well plates were incubated at 28 ± 2 °C. Observation was recorded at 24, 48, 72 and 96 h. of exposure in each treatment; all dead and alive J_2s were counted with the aid of counting dish under stereoscopic binocular microscope. The ratio of dead nematodes/number of total nematodes expressed the percentage mortality. Mortality rates were calculated using Abbott's formula (Abbott 1925).

Egg hatching inhibition bioassay

The egg suspension of *M. incognita* (100 eggs/10 µL) was poured into 24-well tissue culture plate, and 1 mL of different concentrations of cell-free culture filtrates of each PGPR isolates at 100, 50 and 25% was added and mixed. Nutrient broth and sterile distilled water were taken as a negative control, whereas Velum Prime® was taken as a positive control and plates were incubated at 28 ± 2 °C. Observation on egg hatching was recorded at 2, 4, 6 and 8 days of exposure in each treatment. Hatching percentage was calculated by counting the number of hatched and unhatched eggs under stereoscopic binocular microscope. The percentage suppression in hatching of juveniles (J_2s) was calculated using the following formula: Percentage of hatching of eggs = $[1 - (Ht/Hc)] \times 100$, where Ht is the number of juveniles hatched in treatment, and Hc is the number of juveniles hatched in control.

Pot experiments

Application of PGPR isolates as bare root dip treatment prior transplanting

Twenty-one days old tomato seedlings' (cv. NS 4266) which are highly susceptible to *M. incognita* were raised in the nursery pro-trays. Healthy seedlings' were uprooted carefully, and the roots were dipped for 15–20 min. in each PGPR isolates at 10^8 CFU/mL concentration and immediately transplanted in the earthen pots (6 inch in size) containing 1500 cc soil. After 7 days of transplanting, each pot was inoculated with freshly hatched second-stage juveniles at 2 J_2s/cc soil. Plants treated with only water were taken as negative control and Velum Prime® (500 g a.i./ha) as positive control. The pots were arranged in completely randomized manner in the polyhouse. All the treatments were replicated four times. After 90 days, the plants were carefully uprooted, and observations were recorded.

Application of PGPR isolates as soil drenching

Earthen pots of 6 inches in size filled with steam-sterilized soil (1500 cc/pot) were inoculated with freshly hatched second-stage juveniles at the rate of 2 J_2s/cc soil and were arranged in completely randomized design under polyhouse condition. Before transplanting, each pot containing soil and nematode inoculum (2 J_2s/cc soil) was drenched with PGPR isolates at 50 mL/pot (10^8 CFU/mL). After 1 week, 21 days old tomato seedlings' (cv. NS 4266) which were highly susceptible to *M. incognita* were transplanted into each treated soil at one seedling/pot. Soil drenched with only water was taken as negative control and Velum Prime® (500 g a.i./ha) as positive control. All the treatments were replicated four times. After 90 days, the plants were carefully uprooted, and observations were recorded.

Field experiments under protected cultivation

Two field experiments were conducted during the year 2019–2020 and 2020–2021 in field plots (28.6281° N and 77.1606° E) naturally infested with *M. incognita* at CPCT, ICAR-IARI, New Delhi, Delhi, India.

Application of PGPR isolates as bare root dip treatment prior transplanting

The nematode-susceptible tomato seedlings' (21 days old) roots were dipped for about 15–20 min. in each PGPR isolates at 10^8 CFU/mL concentration and then transplanted in blocks (10 m^2) assigned as randomized block design on the selected naturally infested polyhouse beds with an average initial soil population of 6 J_2s/cc soil (2019–2020) and 4 J_2s/cc soil (2020–2021) were assessed using Cobb's decanting and sieving technique (Cobb 1918). The planting distance of (60 × 60 cm) was maintained in each block having 14 plants/block. Each block had 2 rows with 7 plants in each row separated by (0.5 m) distance. Plants treated with only water and nutrients were taken as a negative control and Velum Prime® (500 g a.i./ha) as a positive control. All the treatments were replicated five times. After 7 months at crop termination stage, observations on plant growth and nematode multiplication parameters were recorded.

Application of PGPR isolates as soil drenching

Each block (10 m^2) assigned on the selected naturally infested polyhouse beds with an average initial soil population of 6 J_2s/cc soil (2019–2020) and 4 J_2s/cc soil (2020–2021) was drenched with each PGPR isolates at 1 L/block (10^8 CFU/mL). After 1 week, tomato seedlings (21 days old), susceptible to *M. incognita* were transplanted into each block arranged in randomized complete block design. Throughout the crop period, soil was drenched three times with each PGPR isolates and consortium at 2 months interval. Soil drenched with only water and nutrients was taken as a negative control and Velum Prime® (500 g a.i./ha) as a positive control. All the treatments were replicated five times. After 7 months at crop termination stage, observations on plant growth and nematode multiplication parameters were recorded.

Observations on plant growth parameters (shoot length, root length, fresh root weight, fresh shoot weight) and nematode multiplication parameters (No. of galls/root system, No. of egg masses/root system, No. of eggs/egg mass, and reproduction factor (RF)) were recorded in both the pot and field experiments, and fruit yield (kg/10 m^2) was estimated only in field experiments. Nematode RF was calculated using the formula, $RF = P_f/P_i$, where P_f = final nematode population and P_i = initial nematode population in soil. P_i is determined by soil sampling from the selected nematode-infested polyhouse beds, around 25 subsamples were collected, pooled, and processed using Cobb's decanting and sieving method (Cobb 1918), similarly P_f is calculated at the time of harvest with respect to each treatment, *M. incognita* infective juveniles were counted, and the RF was calculated.

Experimental designs and statistical analysis

The experiments were carried out using completely randomized and randomized block designs in pots and field beds, respectively, under polyhouse conditions. The experimental data obtained were statistically analysed using Web Agri Stat Package (WASP) version 2.0 (at 5%).

Results

Effect of PGPR isolates on juveniles (J_2s) mortality of root-knot nematode (*M. incognita*)

The effect of different concentrations of cell-free culture filtrates of three individuals and one consortium of all three PGPR isolates on the juvenile mortality of *M. incognita* revealed that among the three isolates, all of them proved to be effective. The higher concentration (100%) of consortium of all three isolates was more effective than individual isolates (100%) and was significantly ($P < 0.05$) effective. After 96 h. of exposure, juvenile mortality of 91.67% was recorded at 100% consortium and 73.33–81.33% was recorded in individual isolates (100%) and they were also effective at 50 and 25% concentrations of both individual as well as consortium after 96 h. Juvenile mortality increased with the time of exposure and concentration. Among all, consortium of PGPR isolates proved highly effective at its 100% concentration as compared to individual isolates at 100% after 96 h. of exposure. No juvenile mortality was recorded in control containing nutrient broth and distilled water (Table 1).

Effect of PGPR isolates on egg hatching inhibition of root-knot nematode (*M. incognita*)

In egg hatching inhibition bioassay, among three isolates tested against *M. incognita*, all of them proved to be effective. There was a significant ($P < 0.05$) reduction in the egg hatching and was recorded in all the three isolates consortium as compared with individual isolates (100%). After 8 days of incubation, hatching inhibition of 84.26% was recorded at 100% consortium and 78.48 to 83.21% was recorded at 100% of individual isolates and they were also effective at 50 and 25% concentrations of both individual as well as consortium after 8 days. Hatching inhibition of *M. incognita* was affected by the concentration and time of exposure. Among all, consortium of PGPR isolates proved highly effective at its 100, 50, and 25% concentration than individual isolates. No hatching inhibition was recorded in control containing distilled water, and 7.26% hatching inhibition was observed in nutrient broth (Table 2).

Pots' experiments under polyhouse conditions

Effect of different PGPR isolates applied as soil drenching and bare root dip treatment on *M. incognita* infested tomato plants was studied on plant growth and nematode

Table 1 Bio-efficacy of plant growth-promoting rhizobacterial isolates with different concentrations of their cell-free culture filtrates on J_2s mortality of *M. incognita*

Treatments	100%				50%				25%			
	24 h. Mean ± SE	48 h. Mean ± SE	72 h. Mean ± SE	96 h. Mean ± SE	24 h. Mean ± SE	48 h. Mean ± SE	72 h. Mean ± SE	96 h. Mean ± SE	24 h. Mean ± SE	48 h. Mean ± SE	72 h. Mean ± SE	96 h. Mean ± SE
Ba	23.33 ± 1.45^{b}	42.00 ± 2.08^{b}	78.67 ± 1.20^{c}	81.33 ± 0.88^{c}	16.00 ± 1.15^{b}	35.00 ± 1.00^{b}	70.67 ± 1.20^{b}	70.00 ± 0.58^{c}	13.33 ± 1.45^{b}	25.67 ± 1.33^{c}	59.00 ± 1.15^{c}	64.00 ± 1.73^{c}
Bs	19.00 ± 1.15^{c}	41.33 ± 0.88^{b}	71.00 ± 1.15^{d}	73.33 ± 1.45^{d}	15.00 ± 0.58^{b}	30.33 ± 0.67^{c}	64.33 ± 1.67^{c}	70.00 ± 0.58^{c}	12.00 ± 0.58^{b}	21.00 ± 0.58^{d}	52.67 ± 0.88^{d}	58.00 ± 1.53^{d}
Pa	18.00 ± 0.58^{c}	24.67 ± 1.20^{c}	67.00 ± 2.08^{d}	77.00 ± 2.31^{d}	11.67 ± 0.67^{c}	19.33 ± 0.88^{d}	54.00 ± 2.08^{d}	63.33 ± 1.45^{d}	9.33 ± 0.33^{c}	14.33 ± 0.88^{e}	45.67 ± 0.88^{e}	51.67 ± 0.67^{e}
Ba + Bs + Pa	23.33 ± 1.45^{b}	43.00 ± 3.06^{b}	87.33 ± 2.33^{b}	91.67 ± 2.33^{b}	16.33 ± 1.45^{b}	35.33 ± 1.20^{b}	75.00 ± 4.16^{b}	80.67 ± 5.04^{b}	12.33 ± 0.67^{b}	30.67 ± 0.33^{b}	67.00 ± 2.31^{b}	76.00 ± 2.65^{b}
NB	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}								
SDW	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}								
VP	95.67 ± 0.88^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}								
F value	1108.39	508.828	931.897	915.385	1544.341	2180.515	394.113	382.509	2101.676	2721.309	1107.009	770.496
CV	6.598	7.278	4.019	4.002	6.625	3.989	6.406	6.340	6.243	4.141	4.051	4.685
CD (0.05)	2.960	4.570	4.063	4.239	2.564	2.195	5.834	6.091	2.228	1.986	3.288	4.098

Data shown correspond to the mean of three replicates ± SE. Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different

CD critical difference, *CV* coefficient of variation, *hrs*. hours, *SE* standard error, Treatment details: *Ba Bacillus amyloliquefaciens* DSBA 11; *Bs Bacillus subtilis* DTBS 5, *Pa Pantoea agglomerans*; Ba + Bs + Pa (Consortium), *NB* nutrient broth, *SDW* sterile distilled water, *VP* Velum Prime® (500 g a.i./ha) as positive control

Table 2 Bio-efficacy of plant growth-promoting rhizobacterial isolates with different concentrations of their cell-free culture filtrates on egg hatching inhibition of *M. incognita*

Treatments	100%				50%				25%			
	2 DAT Mean ± SE	4 DAT Mean ± SE	6 DAT Mean ± SE	8 DAT Mean ± SE	2 DAT Mean ± SE	4 DAT Mean ± SE	6 DAT Mean ± SE	8 DAT Mean ± SE	2 DAT Mean ± SE	4 DAT Mean ± SE	6 DAT Mean ± SE	8 DAT Mean ± SE
Ba	93.50 ± 0.86^{b}	88.61 ± 0.50^{c}	87.00 ± 1.15^{c}	78.48 ± 0.53^{c}	86.51 ± 0.42^{c}	84.29 ± 1.42^{c}	80.49 ± 1.45^{c}	74.81 ± 0.75^{d}	72.10 ± 0.59^{e}	70.11 ± 0.77^{d}	62.90 ± 2.59^{d}	55.79 ± 2.35^{d}
Bs	94.40 ± 1.43^{b}	88.19 ± 0.64^{c}	88.15 ± 0.52^{c}	83.21 ± 1.34^{bc}	87.91 ± 1.23^{c}	86.56 ± 1.31^{c}	78.63 ± 1.63^{c}	78.11 ± 0.43^{c}	76.73 ± 1.76^{d}	72.31 ± 2.46^{d}	66.39 ± 1.50^{d}	64.96 ± 0.49^{c}
Pa	93.94 ± 1.90^{b}	91.37 ± 0.86^{b}	88.52 ± 0.85^{bc}	80.60 ± 2.80^{bc}	87.45 ± 0.70^{c}	83.50 ± 0.76^{c}	79.73 ± 1.09^{c}	78.10 ± 0.60^{c}	82.80 ± 0.87^{c}	78.01 ± 1.15^{c}	72.03 ± 2.57^{c}	70.09 ± 0.67^{b}
Ba + Bs + Pa	96.27 ± 0.50^{b}	94.87 ± 0.43^{a}	91.58 ± 1.10^{b}	84.26 ± 2.78^{b}	93.94 ± 0.98^{b}	92.51 ± 0.80^{b}	87.38 ± 1.27^{b}	86.86 ± 0.61^{b}	88.38 ± 1.66^{b}	87.06 ± 1.01^{b}	79.01 ± 1.28^{b}	72.98 ± 1.44^{b}
NB	16.26 ± 1.12^{c}	19.59 ± 1.88^{d}	12.51 ± 2.09^{d}	7.26 ± 2.15^{d}								
SDW	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}								
VP	100.00 ± 0.00^{a}	96.05 ± 0.42^{a}	95.78 ± 0.46^{a}	92.31 ± 1.33^{a}								
F value	1641.125	2169.703	1467.670	451.943	2691.128	1235.637	866.110	1332.493	1250.492	719.534	405.441	578.716
CV	2.603	2.204	2.815	5.273	2.033	2.917	3.674	3.085	3.014	3.862	5.497	4.831
CD (0.05)	3.220	2.639	3.264	5.622	2.401	3.376	3.995	3.222	3.290	4.088	5.344	4.393

Data shown correspond to the mean of three replicates ± SE. Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different

CD critical difference, *CV* coefficient of variation, *SE* standard error. Treatment details: *Ba Bacillus amyloliquefaciens* DSBA 11; *Bs Bacillus subtilis* DTBS 5, *Pa Pantoea agglomerans*; Ba + Bs + Pa (Consortium), *NB* Nutrient broth, *SDW* Sterile distilled water, *VP* Velum Prime® (500 g a.i./ha) as positive control

multiplication parameters like: shoot length, fresh shoot weight, root length, fresh root weight, number of galls/root system, number of egg masses/root, number of eggs/egg mass and RF.

Effect of PGPR isolates on plant growth promotion

Data presented in the (Table 3) revealed that there was a significant ($P<0.05$) effect on plant growth treated with PGPR isolates in both soil drenching as well as bare root dip treatments. There was enhanced in shoot length, fresh shoot weight, and root length recorded in consortium (165.00 cm, 226.50 g and 28.00 cm), followed by *B. subtilis* DTBS 5 (165.00 cm, 227.75 g and 27.25 cm), *B. amyloliquefaciens* DSBA 11 (158.50 cm, 223.75 g and 27.50 cm) and *P. agglomerans* (153.50 cm, 224.25 g and 25.50 cm) as compared with bare root dip treatment and untreated control (120.25 cm, 167.50 g and 21.75 cm). Whereas minimum fresh root weight was observed in *P. agglomerans* (28.60 g), followed by consortium (29.31 g), *B. amyloliquefaciens* DSBA 11 (29.73 g) and *B. subtilis* DTBS 5 (30.04 g) treated plants as compared with bare root dip treatment and untreated control (31.10 g). Fresh root weight was directly related to the number of galls, more the number of galls more the fresh root weight. Overall, the efficacy of PGPR isolates was more effective when applied as soil drenching than seedling dip treatment prior transplanting.

Nematicidal potential of PGPR isolates

There was a significant ($P<0.05$) reduction (Fig. 1) in the gall formation, egg mass formation, eggs/egg mass and RF, which were recorded in consortium (7.00 galls/root, 4.25 egg mass/root, 174.75 eggs/egg mass and 0.19 RF), followed by *B. subtilis* DTBS 5 (7.25 galls/root, 9.25 egg masses/root, 207.50 eggs/egg mass and 0.25 RF), *P. agglomerans* (9.00 galls/root, 5.75 egg masses/root, 222.50 eggs/egg mass and 0.20 RF) and *B. amyloliquefaciens* DSBA 11 (9.00 galls/root, 4.75 egg masses/root, 180.75 eggs/egg mass and 0.26 RF) treated plants when applied as soil drenching as compared with seedling dip treatment and untreated control plants (24.75 galls/root, 31.25 egg masses/root, 369.75 eggs/egg mass and 3.10 RF). However, consortium of all the above three isolates was found to cause significantly higher reduction in the nematode incidence and multiplication. The efficacy of these PGPR isolates was at par with the synthetic chemical nematicide, Velum Prime® (Table 4).

Field experiments under protected cultivation

Effect of PGPR isolates on *M. incognita* infested tomato plants under protected cultivation was studied in two tomato growing season (2019–2020 and 2020–2021) on plant growth and nematode multiplication parameters like: shoot length, fresh shoot weight, root length, fresh root weight, fruit yield, number of galls/root system, number of egg mass/root, number of eggs/egg mass, and RF.

Effect of PGPR isolates on plant growth promotion

There was a significant ($P<0.05$) effect on plant growth (Fig. 1) observed when the PGPR isolates applied as soil drenching as well as bare root dip treatment in both the field experiments (2019–2020 and 2020–2021). Plants with enhanced shoot length, fresh shoot weight, root length, and fruit yield were recorded in consortium, followed by *B. amyloliquefaciens* DSBA 11, *B. subtilis* DTBS 5 and *P. agglomerans* as compared with bare root dip treatment and untreated control in both the field experiments. Whereas minimum fresh root weight was observed in *B. amyloliquefaciens* DSBA 11, followed by *B. subtilis* DTBS 5, *P. agglomerans* and consortium treated plants as compared with bare root dip treatment and untreated control in both the field experiments. Fresh root weight was directly related to the number of galls, more the number of galls more the fresh root weight. Overall, the efficacy of PGPR isolates was more effective when applied as soil drenching than seedling dip treatment prior transplanting (Tables 5, 6 and 8).

Nematicidal potential of PGPR isolates

There was a significant ($P<0.05$) reduction in the nematode infestation and multiplication (Fig. 1) in the plants treated with PGPR isolates. PGPR isolates applied as soil drenching significantly reduced root galling (40.40 and 33.80 galls/root) and egg mass formation (28.20 and 22.20 egg masses/root) in consortium, followed by least eggs/egg mass (218.60 and 219.20) in *B. subtilis* DTBS 5 and minimum RF (0.54 and 0.47) in *B. amyloliquefaciens* DSBA 11 treated soil was recorded in both the field experiments, respectively. Whereas the PGPR isolates applied as bare root dipping prior transplanting also showed significant effect on nematode incidence in terms of reduced gall (54.60 and 40.40 galls/root) and egg mass formation (32.60 and 30.20 egg masses/root) in consortium, followed by least eggs/egg mass (190.40 and 230.80) in *P. agglomerans* and minimum RF (0.69 and 0.53) in *B. amyloliquefaciens* DSBA 11 treated plants as compared with untreated control (764.40 and 532.60 galls/root, 501.60 and 456.20 egg masses/root, 327.80 and 302.60 eggs/egg mass, and 3.28 and 2.93 RF) in the field experiments conducted during 2019–2020 and 2020–2021, respectively. However, all the three individual isolates and their consortium proved to be significantly condensed the nematode infestation and the results were at par with the synthetic chemical nematicide, Velum Prime® (Tables 7 and 8).

Table 3 Effect of PGPR isolates applied as soil drenching and bare root dipping prior transplanting on tomato plant growth infested by *M. incognita* in pot experiment under polyhouse condition

Treatments	Shoot length (cm)		Fresh shoot weight (g)		Root length (cm)		Fresh root weight (g)	
	Soil drenching Mean ± SE	Bare root dip Mean ± SE	Soil drenching Mean ± SE	Bare root dip Mean ± SE	Soil drenching Mean ± SE	Bare root dip Mean ± SE	Soil drenching Mean ± SE	Bare root dip Mean ± SE
Ba	158.50 ± 4.03^{a}	148.75 ± 2.10^{b}	223.75 ± 2.63^{ab}	218.50 ± 1.32^{a}	27.50 ± 0.65^{b}	24.25 ± 1.18^{bc}	29.73 ± 0.94^{a}	30.15 ± 0.38^{a}
Bs	165.00 ± 4.42^{a}	151.75 ± 4.07^{ab}	227.75 ± 5.02^{a}	214.75 ± 3.07^{a}	27.25 ± 1.44^{b}	26.00 ± 0.82^{b}	30.04 ± 1.03^{a}	29.98 ± 0.18^{a}
Pa	153.50 ± 6.38^{a}	144.25 ± 4.40^{b}	224.25 ± 2.06^{ab}	220.00 ± 2.89^{a}	25.50 ± 0.96^{b}	23.00 ± 0.71^{c}	28.60 ± 1.39^{a}	30.64 ± 1.13^{a}
Ba + Bs + Pa	165.00 ± 3.49^{a}	158.50 ± 2.40^{a}	226.50 ± 2.90^{a}	217.00 ± 3.94^{a}	28.00 ± 1.08^{b}	29.25 ± 1.18^{a}	29.31 ± 0.78^{a}	30.48 ± 0.32^{a}
VP	154.25 ± 3.20^{a}	152.25 ± 1.89^{ab}	210.75 ± 1.25^{b}	208.75 ± 3.75^{ab}	30.75 ± 0.85^{a}	26.75 ± 1.11^{ab}	30.40 ± 0.85^{a}	31.29 ± 0.77^{a}
UTIC	120.25 ± 3.35^{c}		167.50 ± 8.29^{d}		21.75 ± 0.75^{c}		31.10 ± 0.82^{b}	
F value	16.652	17.328	23.298	16.459	8.616	7.655	9.062	20.618
CV	5.458	4.329	4.351	4.494	6.961	7.279	6.533	4.542
CD (0.05)	12.054	9.189	13.507	13.630	2.742	2.719	2.771	1.969

Data shown correspond to the mean of four replicates ± SE. Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different

CD critical difference, *CV* coefficient of variation, *SE* standard error. Treatment details: *Ba Bacillus amyloliquefaciens* DSBA 11; *Bs Bacillus subtilis* DTBS 5, *Pa Pantoea agglomerans*; Ba + Bs + Pa (Consortium), *UTIC* Untreated nematode inoculated control, *VP* Velum Prime® (500 g a.i./ha) as positive control

Fig. 1 **1** Effect of PGPR isolates on gall formation in tomato cv. NS 4266 infected with *M. incognita* (Mi). **1a** *Bacillus subtilis* DTBS 5 + Mi; **1b** *Bacillus amyloliquefaciens* DSBA 11 + Mi; **1c** *Pantoea agglomerans* + Mi; **1d** Consortium + Mi; **1e** Untreated control (only Mi); **2:** Enhanced plant growth promotion in pot study; **3**, **4** PGPR treated tomato crop in polyhouse; **5** Heavily infested tomato root in protected cultivation of tomato; **6** Above ground symptoms of heavily infected tomato crop

Discussion

Plant growth-promoting rhizobacterial isolates known to possess different modes of action which suppress plant parasitic nematodes in the plants rhizosphere. The mechanisms exhibited by PGPR on nematode suppression like: direct antagonism by producing enzymes, releasing toxins and other metabolic products and indirect effect by nematode behaviour regulation, root

Table 4 Effect of PGPR isolates applied as soil drenching and bare root dipping prior transplanting on nematode multiplication in tomato plants infested by *M. incognita* in pot experiment under polyhouse condition

Treatments	No. of galls/root		No. of egg masses/root		Eggs/egg mass		Reproduction factor (RF)	
	Soil drenching Mean ± SE	Bare root dip Mean ± SE	Soil drenching Mean ± SE	Bare root dip Mean ± SE	Soil drenching Mean ± SE	Bare root dip Mean ± SE	Soil drenching Mean ± SE	Bare root dip Mean ± SE
Ba	9.00 ± 0.41^{b}	10.75 ± 0.63^{c}	4.75 ± 0.85^{cd}	7.50 ± 0.65^{c}	180.75 ± 6.94^{c}	194.25 ± 7.56^{bc}	0.26 ± 0.02^{b}	0.37 ± 0.02^{b}
Bs	7.25 ± 0.48^{bc}	11.25 ± 0.63^{c}	9.25 ± 0.63^{b}	9.50 ± 0.29^{b}	207.50 ± 5.75^{b}	215.75 ± 7.88^{b}	0.25 ± 0.02^{bc}	0.35 ± 0.01^{b}
Pa	9.00 ± 0.82^{b}	14.00 ± 0.91^{b}	5.75 ± 0.63^{c}	7.75 ± 0.48^{c}	222.50 ± 3.01^{b}	222.50 ± 4.03^{b}	0.20 ± 0.02^{bc}	0.25 ± 0.02^{c}
Ba + Bs + Pa	7.00 ± 0.41^{bc}	10.75 ± 0.85^{c}	4.25 ± 0.48^{cd}	6.50 ± 0.65^{c}	174.75 ± 4.27^{c}	195.75 ± 14.77^{b}	0.19 ± 0.02^{c}	0.22 ± 0.02^{c}
VP	6.50 ± 0.29^{c}	9.25 ± 0.48^{c}	3.75 ± 0.48^{d}	4.75 ± 0.48^{d}	131.00 ± 9.17^{d}	167.00 ± 11.71^{c}	0.08 ± 0.01^{d}	0.12 ± 0.01^{d}
UTIC	24.75 ± 1.75^{a}		31.25 ± 0.63^{a}		369.75 ± 12.85^{a}		3.10 ± 0.07^{a}	
F value	91.290	66.188	320.570	396.518	238.854	125.411	1292.626	1282.402
CV	17.430	15.570	13.822	10.471	7.784	9.930	9.201	8.415
CD (0.05)	2.326	2.642	1.713	1.480	21.037	28.480	0.094	0.093

Data shown correspond to the mean of four replicates ± SE. Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different. CD: Critical difference, CV: Coefficient of variation, SE: Standard error. Treatment details: *Ba Bacillus amyloliquefaciens* DSBA 11; *Bs Bacillus subtilis* DTBS 5, *Pa Pantoea agglomerans*; Ba + Bs + Pa (Consortium), *UTIC* Untreated nematode inoculated control, *VP* Velum Prime® (500 g a.i./ha) as positive control

Table 5 Effect of PGPR isolates applied as soil drenching and bare root dipping prior transplanting on tomato plant growth infested by *M. incognita* under protected cultivation

Treatments	Shoot length (ft)				Fresh shoot weight (kg)			
	Soil drenching		Bare root dip		Soil drenching		Bare root dip	
	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE
Ba	13.40 ± 0.29^{b}	11.40 ± 0.58	11.80 ± 0.46^{bc}	11.70 ± 0.34^{b}	1.42 ± 0.03^{bc}	1.49 ± 0.04^{b}	1.68 ± 0.17^{a}	1.48 ± 0.07^{b}
Bs	12.90 ± 0.43^{b}	11.80 ± 0.41	12.70 ± 0.37^{ab}	11.00 ± 0.52^{bc}	1.32 ± 0.03^{c}	1.24 ± 0.04^{c}	1.29 ± 0.04^{b}	1.18 ± 0.05^{c}
Pa	13.40 ± 0.24^{b}	12.20 ± 0.51	11.20 ± 0.25^{c}	13.20 ± 0.37^{a}	1.29 ± 0.05^{c}	1.26 ± 0.06^{bc}	1.22 ± 0.06^{b}	1.27 ± 0.02^{c}
Ba + Bs + Pa	13.20 ± 0.72^{b}	12.00 ± 0.65	12.10 ± 0.46^{abc}	13.00 ± 0.35^{a}	1.67 ± 0.18^{b}	1.35 ± 0.12^{bc}	1.29 ± 0.05^{b}	1.19 ± 0.02^{c}
VP	15.20 ± 0.51^{a}	12.80 ± 0.34	12.00 ± 0.35^{abc}	13.10 ± 0.37^{a}	2.12 ± 0.06^{a}	1.97 ± 0.04^{a}	1.84 ± 0.11^{a}	1.82 ± 0.08^{a}
Control	12.80 ± 0.25^{b}	10.60 ± 0.19			1.31 ± 0.05^{c}	1.14 ± 0.10^{c}		
F value	4.428	2.225	3.190	9.565	13.727	15.270	9.206	17.015
CV	6.914	9.506	6.138	6.886	12.916	12.170	13.079	10.476
CD (0.05)	1.230	NS	0.980	1.099	0.260	0.226	0.248	0.186

Data shown correspond to the mean of four replicates ± SE. Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different

CD critical difference, *CV* coefficient of variation, *SE* standard error. Treatment details: *Ba Bacillus amyloliquefaciens* DSBA 11, *Bs Bacillus subtilis* DTBS 5, *Pa Pantoea agglomerans*; Ba + Bs + Pa (Consortium), *VP* Velum Prime® (500 g a.i./ha) as positive control

diffusates alteration and encouraging the production of repellents by the host plant that unfavourably distresses the host recognition, alteration in the nematode feeding site development or sex ratio inside the root tissue, endorsing plant growth, competing for essential nutrients, inducing systemic resistance and have gained widespread courtesy due to their beneficial effects in defending the host plants against biotic and abiotic stresses (AbdelRazek and Yaseen 2020). Antagonistic property of PGPR can prevent egg hatch, the growth and reproduction of plant parasitic nematodes, through different mechanisms like predation, release of toxins/enzymes including hydrogen cyanide, 2,4-diacetylphloroglucinol, glucanases, chitinases, proteases, and lipases (Sayre and Starr 1985). Furthermore, Abd-Elgawad (2020) demonstrated how to strengthen their beneficial effects via synergistic or additive interaction with compatible agricultural inputs, for example, organic manure and/or chemicals. Those authors stressed the need to optimize the delivery of such biocontrol agents as well as their interaction and persistence under actual conditions.

In the present research work, the effect of three PGPR isolates and their consortium against *M. incognita* under laboratory conditions followed by pots and field

Table 6 Effect of PGPR isolates applied as soil drenching and bare root dipping prior transplanting on tomato plant growth infested by *M. incognita* under protected cultivation

Treatments	Root length (cm)				Fresh root weight (g)			
	Soil drenching		Bare root dip		Soil drenching		Bare root dip	
	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2019–2020 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE
Ba	34.30 ± 0.66^{b}	36.50 ± 0.50^{b}	31.70 ± 0.30^{ab}	33.50 ± 0.50^{ab}	60.37 ± 0.98^{e}	55.41 ± 1.49^{e}	66.07 ± 2.26^{d}	59.58 ± 1.49^{e}
Bs	31.00 ± 0.63^{c}	32.20 ± 0.72^{c}	30.10 ± 0.46^{bcd}	29.90 ± 0.40^{cd}	66.32 ± 1.88^{d}	63.08 ± 1.57^{d}	61.93 ± 1.41^{d}	61.80 ± 1.50^{e}
Pa	35.40 ± 0.70^{ab}	34.70 ± 0.94^{bc}	30.20 ± 0.60^{bc}	31.10 ± 1.21^{bc}	70.82 ± 0.82^{d}	69.63 ± 1.29^{d}	78.95 ± 2.16^{c}	75.17 ± 4.32^{d}
Ba + Bs + Pa	34.70 ± 0.66^{b}	35.90 ± 1.35^{b}	28.70 ± 0.62^{cd}	31.50 ± 0.95^{bc}	78.75 ± 2.49^{c}	79.00 ± 3.68^{c}	81.69 ± 0.31^{c}	85.67 ± 2.09^{c}
VP	37.40 ± 1.13^{a}	40.20 ± 1.89^{a}	32.90 ± 0.51^{a}	34.90 ± 0.84^{a}	85.70 ± 2.34^{b}	94.69 ± 4.03^{b}	90.85 ± 0.47^{b}	94.88 ± 3.05^{b}
Control	28.50 ± 1.01^{c}	27.90 ± 0.60^{d}			106.07 ± 3.14^{a}	112.20 ± 2.13^{a}		
F value	12.988	14.611	9.436	8.656	66.711	76.039	85.750	56.816
CV	5.972	7.072	4.092	6.037	5.760	6.879	4.842	7.361
CD (0.05)	2.643	3.225	1.639	2.506	5.928	7.170	5.170	7.920

Data shown correspond to the mean of four replicates ± SE. Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different

CD critical difference, *CV* coefficient of variation, *SE* standard error. Treatment details: *Ba Bacillus amyloliquefaciens* DSBA 11, *Bs Bacillus subtilis* DTBS 5, *Pa Pantoea agglomerans*; Ba + Bs + Pa (Consortium), *VP* Velum Prime® (500 g a.i./ha) as positive control

Table 7 Effect of PGPR isolates applied as soil drenching and bare root dipping prior transplanting on nematode multiplication in tomato plants infested by *M. incognita* under protected cultivation

Treatments	No. of galls/root				No. of egg masses/root			
	Soil drenching		Bare root dip		Soil drenching		Bare root dip	
	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE
Ba	55.00 ± 2.45^{b}	46.40 ± 2.14^{b}	69.20 ± 2.29^{b}	63.80 ± 1.16^{b}	34.00 ± 1.10^{b}	26.80 ± 1.36^{b}	38.80 ± 1.71^{b}	33.80 ± 0.86^{b}
Bs	57.40 ± 1.69^{b}	48.60 ± 2.09^{b}	67.20 ± 2.20^{b}	59.40 ± 1.86^{b}	35.60 ± 0.93^{b}	29.60 ± 2.04^{b}	42.20 ± 1.11^{b}	37.00 ± 2.02^{b}
Pa	49.20 ± 1.28^{bc}	35.20 ± 2.50^{bc}	53.80 ± 2.35^{bc}	42.00 ± 3.65^{bc}	30.00 ± 1.00^{bc}	26.40 ± 2.46^{b}	35.40 ± 1.81^{bc}	31.80 ± 1.39^{b}
Ba + Bs + Pa	40.40 ± 1.03^{bc}	33.80 ± 2.27^{bc}	54.60 ± 2.32^{bc}	40.40 ± 3.57^{bc}	28.20 ± 0.97^{bc}	22.20 ± 1.46^{b}	32.60 ± 0.93^{bc}	30.20 ± 1.02^{b}
VP	17.40 ± 1.44^{c}	14.00 ± 1.34^{c}	27.80 ± 1.24^{c}	23.00 ± 1.52^{c}	8.60 ± 0.75^{c}	8.20 ± 1.16^{b}	12.40 ± 1.12^{c}	10.40 ± 0.87^{b}
Control	764.40 ± 27.74^{a}	532.60 ± 22.99^{a}			501.60 ± 20.54^{a}	456.20 ± 27.43^{a}		
F value	645.419	455.393	683.276	423.822	528.105	242.355	517.122	244.577
CV	15.809	17.984	14.363	17.063	17.742	26.814	17.075	25.019
CD (0.05)	34.198	28.100	32.749	28.560	24.889	33.572	24.893	32.974

Data shown correspond to the mean of four replicates ± SE. Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different

CD critical difference, *CV* coefficient of variation, *SE* standard error. Treatment details: *Ba Bacillus amyloliquefaciens* DSBA 11, *Bs Bacillus subtilis* DTBS 5, *Pa Pantoea agglomerans*; Ba + Bs + Pa (Consortium), *VP* Velum Prime® (500 g a.i./ha) as positive control

experiments was studied. Juvenile mortality of 91.67% was recorded at 100% consortium followed by 73.33–81.33% by individual isolates after 96 h. of exposure. Similarly, hatching inhibition of 84.26% was recorded at 100% PGPR consortium followed by 78.48–83.21% in individual isolates after 96 h. Similar results were recorded by Popal (2020). Bacteria are ubiquitous in nature and destroy the plant parasitic nematodes present in rhizospheric soil, *Pasteuria penetrans* destroyed nematodes by their parasitic behaviour, whereas the non-parasite rhizobacteria reduced nematode populations by colonizing the host plant rhizosphere (Davies 2009). Notably, a bacterial species used herein (*B. subtilis*) was additionally recommended as protective treatments against soil-borne plant pathogens; both nematode and fungal species (Abd-Elgawad et al. 2010). A large number of rhizobacteria (*Agrobacterium*, *Alcaligenes*, *Bacillus*, *Clostridium*, *Desulfovibrio*, *Pseudomonas*, *Serratia* and *Streptomyces*) were reported to possess nematicidal potential (Siddiqui and Mahmood 1999). In pot study, soil drenched with PGPR isolates consortium, followed by *B. subtilis* DTBS 5, *P. agglomerans*, and *B. amyloliquefaciens* DSBA

Table 8 Effect of PGPR isolates applied as soil drenching and bare root dipping prior transplanting on *M. incognita* multiplication and fruit yield in tomato plants under protected cultivation

Treatments	Eggs/egg mass				Reproduction factor				Fruit yield (kg/10 m^2)			
	Soil drenching		Bare root dip		Soil drenching		Bare root dip		Soil drenching		Bare root dip	
	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE	2019–2020 Mean ± SE	2020–2021 Mean ± SE
Ba	219.40 ± 4.80^{b}	192.20 ± 6.63^{b}	229.60 ± 10.93^{b}	213.80 ± 11.10^{b}	0.54 ± 0.04^{b}	0.47 ± 0.03^{b}	0.69 ± 0.09^{bc}	0.53 ± 0.03^{b}	68.11 ± 3.02^{a}	62.16 ± 1.72^{d}	58.73 ± 2.00^{c}	66.29 ± 2.08^{cd}
Bs	218.60 ± 5.66^{b}	219.20 ± 9.40^{b}	229.40 ± 4.76^{b}	214.80 ± 7.23^{b}	0.63 ± 0.02^{b}	0.53 ± 0.03^{b}	0.80 ± 0.03^{b}	0.63 ± 0.06^{b}	59.96 ± 2.50^{bc}	66.95 ± 3.51^{cd}	66.91 ± 2.71^{ab}	69.10 ± 3.08^{bc}
Pa	223.40 ± 7.12^{bc}	229.60 ± 14.65^{b}	190.40 ± 42.14^{bc}	230.80 ± 11.15^{b}	0.55 ± 0.01^{b}	0.48 ± 0.02^{b}	0.83 ± 0.05^{b}	0.57 ± 0.02^{b}	58.65 ± 2.48^{c}	70.66 ± 1.15^{bc}	60.86 ± 2.96^{bc}	68.12 ± 2.56^{bc}
Ba + Bs + Pa	222.20 ± 5.48^{bc}	219.40 ± 4.41^{b}	232.60 ± 2.80^{bc}	220.6 0 ± 3.23^{b}	0.61 ± 0.02^{b}	0.55 ± 0.01^{b}	0.62 ± 0.05^{c}	0.61 ± 0.02^{b}	67.43 ± 2.74^{ab}	81.14 ± 1.58^{a}	72.23 ± 4.29^{a}	77.60 ± 1.39^{a}
VP	162.40 ± 5.25^{c}	144.80 ± 4.41^{b}	234.20 ± 6.81^{c}	223.00 ± 5.30^{b}	0.25 ± 0.01^{c}	0.17 ± 0.03^{c}	0.34 ± 0.04^{d}	0.28 ± 0.03^{c}	65.50 ± 2.62^{abc}	74.23 ± 2.81^{b}	67.20 ± 4.49^{ab}	74.62 ± 1.95^{ab}
Control	327.80 ± 8.52^{a}	302.60 ± 8.95^{a}			3.28 ± 0.07^{a}	2.93 ± 0.09^{a}			52.37 ± 0.72^{d}	50.37 ± 0.28^{e}		
F value	528.105	242.355	517.122	244.577	1213.267	515.875	494.708	384.059	5.760	26.328	6.795	7.109
CV	17.742	26.814	17.075	25.019	5.787	9.178	7.731	9.502	8.223	6.122	6.752	6.568
CD (0.05)	24.889	33.572	24.893	32.974	0.103	0.143	0.154	0.160	7.683	6.235	6.512	6.866

Data shown correspond to the mean of four replicates ± SE. Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different

CD critical difference, *CV* coefficient of variation, *SE* standard error. Treatment details: *Ba Bacillus amyloliquefaciens* DSBA 11, *Bs Bacillus subtilis* DTBS 5, *Pa Pantoea agglomerans*; Ba + Bs + Pa (Consortium), *VP* Velum Prime® (500 g a.i./ha) as positive control

11 recorded minimum root galling, egg mass formation, eggs/egg mass and RF. Consortium of rhizobacterial isolates was effectively suppressed nematode population in grapevine cultivation due to their diverse control mechanisms than individual isolates and could ensure the desired effects under varying environmental conditions (Aballay et al. 2020). Whereas in both the field studies, PGPR isolates applied as soil drenching also significantly reduced root galling and egg mass formation in consortium, followed by least eggs/egg mass in *B. subtilis* DTBS 5 and minimum RF in *B. amyloliquefaciens* DSBA 11 treated soil. On an average the plant growth promotion and fruit yield were enhanced than untreated control. The consortium of PGPR isolates (*Pseudomonas fluorescens*, Pf128 + *B. subtilis*, Bbv 57; *Bacillus* consortium) was reported to induce defence enzyme activities such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), peroxidase (PO), superoxide dismutase (SOD), lipoxygenase (LOX), catalase (CAT), chitinase, ascorbate peroxidase (APX), β-1,3-glucanase, and proteinase inhibitors associated with systemic resistance, which reduced the nematode infestation in tomato and papaya crop than individual isolates (Alfianny et al. 2017).

However, in this study, PGPR isolates applied as soil drenching gave a significant result in terms of its plant growth promotion and nematicidal potential than with bare root dip treatments. The decline in *M. incognita* infestation might be due to the immobilization, mortality, poor penetration, resistance inferred by activation of defence enzymes and obstruction of reproduction caused by PGPR isolates. From the present work, it would appear that PGPR had the potential to suppress the *M. incognita* infesting tomato crop and obtained results are in conformity with the work done by Alfianny et al. (2019), where they found different PGPR isolates like *Bacillus pumilus, Paenibacillus castaneae, Pseudomonas fluorescens, B. subtilis, P. agglomerans* MK-29, *Pseudomonas putida* MT-19, *Cedecea davisae* MK-30, *Enterobacter* spp. MK-42, *B. cereus*, *Arthrobotrys oligospora, Beauveria bassiana, B. megaterium, Pseudomonas striata,* and *Paenibacillus polymyxa* were effectively suppressed the *M. incognita* and *M. javanica* infestation in tomato and cucumber crop, respectively, with enhanced plant biomass and yield.

Conclusions

It has been concluded from the present study that PGPR isolates, *B. subtilis* DTBS 5, *B. amyloliquefaciens* DSBA 11 and *P. agglomerans*, were effective in the management of *M. incognita* in tomato crop. This finding can be used as one of the strategy to manage root-knot nematode incidence in tomato crop grown under protected cultivation by avoiding the use of toxic synthetic nematicides which are harmful to non-target organisms and environment.

Abbreviations

PGPR: Plant growth-promoting rhizobacteria; RKN: Root-knot nematode; J_2s: Second stage juveniles; CFU: Colony forming unit; NB: Nutrient broth; VP: Velum Prime®; SC: Suspension concentrate; SDW: Sterile distilled water; UTIC: Untreated nematode inoculated control; Ba: *Bacillus amyloliquefaciens* DSBA 11; Bs: *Bacillus subtilis* DTBS 5; Pa: *Pantoea agglomerans*; CPCT: Centre for Protected Cultivation Technology; WASP: Web Agri Stat Package; PAL: Phenylalanine ammonia lyase; PPO: Polyphenol oxidase; PO: Peroxidase; SOD: Superoxide dismutase; LOX: Lipoxygenase; CAT: Catalase chitinase; APX: Ascorbate peroxidase.

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Author contributions

APAG was involved in methodology, data curation, formal analysis, writing—original draft, review and editing. Pankaj contributed to methodology, conceptualization, supervision, writing—review and editing. DS and AKS were involved in methodology, conceptualization, supervision. SR assisted in laboratory experiments. All authors read and approved the final manuscript.

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Availability of data and materials

The data and materials that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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