"Plant growth promoting Bacillus species elicit defense against Meloidogyne incognita infecting tomato in polyhouse"

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Abstract

Background Plant mediated induced systemic resistance against the plant-parasitic nematode, M. incognita infecting tomato cv Pusa Ruby was evaluated on application of four nematicidal rhizobacterial isolates (Bacillus subtilis, B. pumilus, B. megaterium and B. cereus) as elicitors and compared with the application of a chemical nematicide, Velum Prime. Methods The bioefficacy trial was conducted in pots preinoculated with the above isolates followed by M. incognita inoculation tomato to observe the reduction in nematode infection at 60 days and effect on photosynthetic and transpiration rates. The mechanism of induced resistance was assessed using qRT-PCR for quantification of three key defense genes(PR-1b, JERF3 and CAT) at 0,2,4,8 and 16 days after inoculation (DAI). The defense enzymes viz., super oxide dismutase(SOD), polyphenol oxidase(PPO), peroxidase (PO), and phenylalanine ammonia lyase (PAL) were quantified. Results Significant reduction in per cent root galling viz. 84.21 in B. pumilus, 83.70 in B. megaterium, 91.95 in B. subtilis, 81.8 in B. cereus, was observed compared to control. The reproduction factor was the lowest (15.83) in B. subtilis, followed by B. pumilus (21.00), compared to 48.16 in control, with enhanced photosynthetic and transpiration rate. The defense genes, PR-1b, JERF3 and CAT were expressed at 2.5 to 7.5 folds in rhizobacterial treated plants, but not in Velum Prime treatment. The increase in enzyme levels (µmol/min/mg) for SOD was from 1.5 to 17.5, PPO from 2.1 to 7.8, PO from 1.8 to 10.2, and PAL from 1.8 to 8.7 during 0 to 16 DAI.

Plant growth promoting *Bacillus* species elicit defense against *Meloidogyne incognita* infecting susceptible tomato in polyhouse

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root galling viz. 91.95 in *B. subtilis*, 84.21 in *B. pumilus*, 83.70 in *B. megateriu* and 81.8 in *B. cereus*, was observed compared to control. The reproduction factor was the lowest (15.83) in *B. subtilis*, followed by *B. pumilus* (21.00), compared to 48.16 in control, with enhanced photosynthetic and transpiration rates. The defence genes, PR-1b, JERF3 and CAT were expressed at 2.5 to 7.5 folds in rhizobacterial treated plants, but not in Velum Prime treatment. The increase in enzyme levels (µmol/mg protein) for SOD was from 1.5 to 17.5, PPO from 2.1 to 7.8, PO from 1.8 to 10.2, and PAL from 1.8 to 8.7 during 0 to 16 DAI.**Abbreviations:**CAT,catalase; cfu/ml, colony-forming units per millilitre; J2 second stage juvenile, MEGA 6,Molecular Evolutionary Genetics Analysis version 6.0; NA, Nutrient agar; NCBI, National Centre for Biotechnology Centre; PAL, Phenylalanine ammonia lyase; PGPR, Plant growth promoting rhizobacteria; PPO, Polyphenol oxidase; PR, pathogenesis related; SOD, superoxide dismutase

1.INTRODUCTION

The root-knot nematode, *Meloidogyne incognita* is a serious constraint to crop production and causes significant damage to vegetable crops worldwide (Gowda *et al* ., 2019; Perry and Moens 2013). Under protected cultivation, losses in tomato range from 25-100 per cent (Phani *et al* ., 2021,Seid *et al* , 2015).The nematode is an endoparasite that feeds on the plant roots withdrawing nutrients from the hosts, leading to weakening of entire plant and reducing productivity. Chemical nematicides have been used for soil application; but these are gradually being withdrawn from the global market due to hazardous effects on human health and environment (Xiang *et al* ., 2018). There is a need for environment friendly and economically feasible strategies for nematode management. The rhizobacteria suppress the nematodes by release of toxins, antibiotics and enzymes that interfere with the recognition patterns of nematode with its host (Subedi *et al.,* 2020). Several rhizobacterial species such as*Bacillus* spp., *Pseudomonas* spp.*, Rhizobiumand, Burkholderia, Agrobacterium radiobacter* , *Streptomyces* spp. have been reported to suppress *M. incognita* by eliciting induced systemic resistance (Khanna *et al* ., 2019). The present study was undertaken to determine the potential of four rhizobacterial isolates (*Bacillus pumilus* , *B. megaterium* , *B. subtilis* and*B. cereus*) which were isolated from nematode infested polyhouses and found to be nematicidal (Devindrappa *et al* . 2022); in induced systemic resistance for suppression *M. incognita* in tomato, by observing upregulation in defense related genes. The validation was conducted using qRT-PCR at 4 time intervals, after rhizobacterial inoculations. The above parameters were also examined in plants treated with the chemical pesticide, Velum Prime.

MATERIALS AND METHODS

2.1 Isolation of Rhizobacterial strains and their identification

The four bacterial isolates used in the experiments were isolated from tomato accessions at centre for protected cultivation and technology (CPCT), Indian Agricultural Research Institute (IARI), New Delhi (Devindrappa *et al* ., 2022). The isolates were found to cause more than 80 percent mortality in second-stage juveniles of *M. incognita* in laboratory bioassays (Devindrappa *et al* ., 2022).For identification of the isolates, the 24-h bacterial cultures were used for genomic DNA isolation by Zymo Research Crop quick DNA Fungal/Bacterial Miniprep kit (Cat. No. D6005) according to manufacturer's instructions and analyzed by 0.8% agarose gel. The PCR amplifications of 16S rRNA was performed using the universal primers; 27F (5-GTT TGA TCC TGG CTCAG-3) and 1494R (5-ACG GCT ACC TTG TTA CGA CTT-3). The PCR was carried out using standardized protocol, and the amplicons were sent for Sanger sequencing to ABA Biotech Pvt. Ltd. The nucleotide sequences obtained were aligned with the existing nucleotide database of NCBI GenBank, and the reference sequences were retrieved. The 16S rRNA gene sequences obtained were submitted under accession numbers MZ675428-MZ675431 and identified as *B. pumilus* , *B. megaterium* , *B. subtilis* and *B. cereus* .

The pure cultures were maintained on nutrient agar (NA) slants under refrigerated conditions and in 30per cent glycerol stocks at -80C.

2.2 Identification of *Meloidogyne incognita* and maintenance of its culture

The *Meloidogyne incognita* species was multiplied in culture pots on tomato cv Pusa Ruby, using a single

egg mass and identified based on the perineal pattern of an adult female (Taylor *et al* ., 1955). Root-knot nematode (RKN) species was confirmed by molecular method using DNA extracted from RKN J2s. Single eggmass was picked from culture plants and juveniles were allowed to hatch. Hatched juveniles were used for genomic DNA extraction using soil DNA extraction kit (Quagen) following manufactures protocol. Quality of DNA was checked and PCR amplification carried using universal primers specific to Internal Transcribed Region (ITS) of nematodes (Forward primer 5' TTGATTACGTCCCTGCCCTTT 3' and reverse primer 5' TTTCACTCGCCGTTACTAAGG 3') as described by Vrain *et al.,* 1992. PCR conditions and programme for amplification of ITS region was followed as per the method used by Gawade *et al.* , 2022. PCR product was subjected to 1.2% agarose gel electrophoresis and visualized under gel documentation system (G-Box, Syngene). PCR products were purified and sequenced using both the primers to know the gene sequences. The nucleotide sequence was analyzed and checked for sequence similarity in Genbank DNA database of NCBI using nucleotide Basic Local Alignment Search Tool (nBLAST) of National Center for Biotechnology Information (NCBI).

The culture of *M. incognita* was multiplied using J2s from a single eggmass on tomato maintained at greenhouse, Division of Nematology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. The egg masses of *M. incognita* were hand-picked from culture pots and kept for hatching at 21^0C in a BOD incubator to collect the juveniles.

2.3 Bioefficacy of rhizobacteria against *M. incognita* infecting tomato

Three week old tomato cv Pusa Ruby seedlings were transplanted in 10 inch pre-sterilized plastic pots filled with sterilized soil individually mixed with 3% bacterial cultures (*B. pumilus. B. megaterium, B. subtilis* and *B. cereus*) @ ˜10^8cfu/mL. Freshly hatched second stage juveniles (J2) of *M. incognita* were inoculated @ 2J2/ cc soil after seven days of transplanting. The nematicide Velum prime (Bayer crop science) was taken as an additional treatment for comparison and applied @0.56µL/kg soil after mixing in 500mL sterilized water for uniform spread. The treatments were, T1: Water + *M. incognita*(Mi), T2: *B. pumilus* + Mi, T3: *B. megaterium* + Mi, T4:*B. subtilis* + Mi, T5: *B. cereus* + Mi, T6: Velum Prime +Mi. Six replications of the six treatments were maintained in a polyhouse for 75 days. The plants were uprooted and the number of root galls per plant, number of J2s per cc soil, number of egg masses per plant, and nematode reproduction factor.

2.3.1 Effect of rhizobacteria on rate of photosynthesis and transpiration

Photosynthesis related parameters *viz* ., photosynthetic rate; transpiration rate, stomatal conductance and leaf temperature were measured from the bioefficacy trial of rhizobacterial treatments at 45 days after the inoculation by using an infrared gas analyser (IRGA) (LI6400XT portable photosynthesis system, LI-COR, Inc, Nebraska, USA) between 11 to 12 AM.

2.3.2 Evaluation of defence gene expression against *M. incognita* infection in tomato

Three week old healthy seedlings of tomato cv Pusa Ruby were transplanted in six inch plastic pots filed with a mixture of sterilized soil and 3% bacterial cultures (*Bacillus pumilus* , *B. megaterium, B. subtilis* and *B. cereus*) in nutrient broth @ ˜10^8cfu/mL, individually. Freshly hatched second stage juveniles (J2s) of *M. incognita* were inoculated @ 2J2s / cc of soil after seven days of transplanting. The treatments were, T1: Sterilized water (SW), T2: SW +*M. incognita* (Mi), T3: *B. pumilus* , T4: *B. pumilus* + Mi, T5:*B. megaterium* , T6: *B. megaterium* + Mi, T7: *B. subtilis* ,T8: *B. subtilis* + Mi, T9: *B. cereus* , T10:*B. cereus* + Mi, T11: Velum prime (VP, Fluopyram, nematicide), T12: VP + Mi. Plant samples were collected at an interval of 0, 2, 4, 8 & 16 days after inoculation (DAI), carried to the laboratory in the liquid nitrogen and stored at -80@C until further use. Expression of three defense genes (PR1-1b, JERF3 and CAT) was evaluated to observe the effect of rhizobacteria inoculation on tomato plants.

2.3.3 RNA extraction and quantitative real-time PCR (qRT-PCR) reaction

Root samples (1 g) of tomato plants were fine powdered with the help of pre-sterilized and pre-chilled mortar and pestle using liquid nitrogen. An aliquot of macerated tissue (100 mg per sample) were used for RNA

extraction. An extraction of RNA was processed using RNA-easy Plant Mini Kit (Qiagen, Germany), according to the manufacturer's instructions. RNA quality was quantified using a Nano-drop spectrophotometer. QuantiTect Reverse Transcription Kit (Qiagen, Germany) with random hexamers were used for cDNA synthesis from 1 µg of total RNA. PCR mixtures (20-µl final volume) were prepared which contained RNAse free water, 0.2µM each of forward and reverse primers, 1.5µl cDNA template and 10 µlSYBR1. ThePCR programme used was of initial denaturation at 95@C (10 min); 40 cycles of- denaturation (95@C for 30 sec), annealing (58@C for 30 sec) extension (72@C, 30 sec) and final extension 72@C for 1 min). QRT-PCR were performed in triplicate using a Bio-Rad iQ5 Multi-coloured real time PCR detection system. Actin gene was used as reference gene due to its consistent expression in tomato which does not vary after the nematode infections. Genes and respective primers used in the study are given in **Table 1.** Relative fold changes of genes expression were calculated by the $2^{-\Delta\Delta}$CT method (Livak andSchmittgen 2001).

2.3.4Estimation of defense enzymes in tomato

Plant samples (roots) were collected from same treatments of gene expression trial at an interval of 0, 2, 4, 8 & 16 days after inoculation and immediately placed in the liquid nitrogen and stored at -80°C until processing of the sample. The activities of enzymes were determined using assay kit (Himedia Pvt. Ltd) by following the manufacturer's protocol. Fresh 1 g tissue of tomato roots were powdered using liquid nitrogen and suspended in 0.9 mL of 10 mM chilled phosphate-buffered solution (PBS) with a pH of 7.4 and homogenate was centrifuged 10,000 rpm for 10 min at 4°C. The 200 µl of supernatant was collected and loaded in 96 well micro-titre plates along with blank solution. Absorbance was recorded at 450 nm wavelength in GEN5 microplate reader (Molecular Devices) for determination of enzymes activities *viz* ., super oxide dismutase (SOD), Polyphenol oxidase (PPO), Phenylalanine ammonia lyase (PAL) and Peroxidase (PO) as for standardized protocols (Rajan and Pushpa, 2015).

2.4 **Statistical data analysis**

Data were analysed for significant mean differences by one-way ANOVA using statistical software Statistical Tool for Agricultural Research (STAR).The multiple mean comparisons were performed using Tukey's Honestly Significant Difference test (Tukey's HSD).

3.RESULTS

3.1 Bioefficacy of rhizobacteria against *M. incognita* infecting tomato

Nematode infection was significantly reduced in rhizobacterial treatments as compared to untreated. An average of 265.80 root galls per root was observed in control treatment which reduced significantly ($P<0.05$) in presence of rhizobacteria isolates *B. pumilus* (42.60), *B. megaterium* (41.80), *B. subtilis*(22.50) and *B. cereus* (48.75) (**Table 2**). Thus the reduction was significant on application of rhizobacteria. The average number egg mass per plant were 165.80 in untreated control which reduced in presence of *B. pumilus* (36.70), *B. megaterium* (34.25),*B. subtilis* (15.45) and *B. cereus* (44.60). The effect of at for *B. pumilus* , *B. megaterium* , *B. subtilis* and*B. cereus* , but was significantly more for *B. subtilis* . This was also evident in the number of eggs per egg mass and reproduction factor on nematode in *B. subtilis* treatments. The effect of *B. pumilus* , *B. megaterium* , *B. subtilis*and *B. cereus* was for with Velum Prime with respect to reduction in average number of galls, egg mass per plant, eggs per egg mass and reproduction factors of nematode. However *B. subtilis* was found to result in significantly ($P < 0.05$) higher reduction in the above mentioned parameters of nematode infection (Table 2).

3.2 Effect of rhizobacteria on rate of photosynthesis and transpiration

Photosynthesis parameters were computed using an infra-red gas analyser (IRGA) and presented in **Table 3** . The average rate of photosynthesis, transpiration, stomatal conductance increased significantly ($P<0.05$) in rhizobacterial treated plants and showed an average decrease in nematode inoculated plants, as compared to untreated control at 45 days of nematode inoculation. The average rate of photosynthesis in the rhizobacterial treatments was in the range of 26.07- 28.13 µmol $CO_2m^{-2}s^{-1}$,in the absence of nematode, 23.90 to 25.47 in the presence of nematode, as compared to a low of 16.27 in nematode inoculated control. The average rate

of transpiration declined significantly (7.23 mmol H_2O $m^{-2}s^{-}1$) on nematode inoculation but enhanced in the range of 7.72-9.83 mmol H_2O $m^{-2}s^{-1}$ in rhizobacterial treatments in presence of the nematode. This effect of enhanced transpiration was also evident in rhizobacterial treatments without nematode. No significant difference was observed in the stomatal conductance and leaf temperature in any rhizobacterial treatment as compared to control.

3.3 Evaluation of defence gene expression against *M. incognita* infection in tomato

Significant ($P < 0.05$) differences were observed in expression levels of the three defense related genes (PR1-1b, JERF3 & CAT) in rhizobacteria (*B. subtilis* ,*B. pumilus. B. megaterium,* and *B. cereus*) treated tomato (cv Pusa Ruby) against*M. incognita* infection at intervals of 0, 2, 4, 8 & 16 days after inoculation (DAI) when compared with untreated control (**Fig 1)** . The expression of PR1-1b, JERF3 and CAT genes increased gradually in rhizobacterial treated roots at 2, 4 & 8 DAI, while the Velum prime treated plants did not show any enhanced expression. On day 0, the expression of CAT gene was most enhanced; 1.0 to 1.94 folds enhanced expression, as compared to untreated control; the maximum was observed in *B. subtilis* treatment. On day 2, PR1-1b was most enhanced with 5.7 fold expression in *B. subtilis*treatment. The expression of JERF-3 enhanced in the range of 2.4 to 3 folds. The expression of CAT was also the maximum in *B. subtilis*(3.9 fold), and about 3.0 folds in other rhizobacterial treatments. On day 4, the enhancement for PR1-1b was 6.94 folds for *B.subtilis*and it was close to 5 folds in *B cereus* , *B. megaterium* and*B. pumilus* treatments in presence of the nematode. The JERF-3 expression was 5.9 folds in *B. subtilis* and about 5.0 folds in other rhizobacterial treatments. The CAT expression was in the range of 4.02 to 4.49 folds in all rhizobacteria. On day 8, the enhanced gene expression was the maximum in all rhizobacterial treatments, for PR1-1b, it was 7.94 fold in *B.subtilis* treatment and close to 7 folds in other rhizobacterial treatments, in presence of the nematode. The gene JERF-3 enhanced 6.94 fold in *B.subtilis* and close to 6.0 folds in the other 3 rhizobacterial treatments. The expression of CAT was close to 3.0 folds in all rhizobacterial applications. On day 16, the enhancement in CAT expression was the maximum 4.3 folds in *B. subtilis* and was at par with JERF3 in the other three rhizobacteria. The expression of PR1-1b and JERF-3 declined in the range of 3.0 to 4.0 fold, as compared to earlier days of observations. The gene expression in Velum Prime treatment was at par with control on all days of observation, indicating there was no induction of systemic resistance in the plants

3.4 Defence enzyme activity

The expression of the four defense enzymes *viz* ., super oxide dismutase (SOD), polyphenol oxidase (PPO), peroxidase (PO), and phenylalanine ammonia lyase (PAL) were found significantly ($P<0.05$) enhanced in the rhizobacteria (*B. pumilus. B. megaterium, B. subtilis* and *B. cereus*) treated plants but not in Velum prime treatment, as compared to nematode inoculated control. The quantified data on the enzymes evaluated shows a gradual increase from 2 to 16 DAI in rhizobacteria pre-treated plants**(Table 4**). In nematode infected plants, pre-treated with*B. pumilus* , the SOD activity was 2.1, 3.2, 6.2, 8.3 & 14.5 µmol/min/mg, in *B. megaterium* it was 1.5, 2.8, 6.5, 7.3, & 15.6 µmol/min/mg, in *B. subtilis* treated plants it was 2.3, 3.5, 6.8, 8.6 & 17.5 µmol/min/mg at 0, 2, 4, 8 & 16 DAI and in *B. cereus*treated plants it was 1.4, 3.1, 5.5, 6 & 13.6 µmol/min/mg at 0, 2, 4, 8 & 16 DAI; respectively. In Velum prime treated plants, the SOD activity was 0.2, 2.3, 3.0, 3.9, & 8.2 µmol/ min/mg at 0, 2, 4, 8 & 16 DAI, respectively .The levels of these enzymes were at par in the four rhizobacterial treatments, but were significantly enhanced wrt nematode inoculated control and velum prime treatment. The PPO enzyme activity in*B. pumilus* pre-treated plants was 2.1, 2.2, 3.3, 4.1 & 6.5 µmol/min/mg, *B. megaterium* treated plants 1.9, 2.3, 2.9, 4.2 & 7.1 µm/ min/mg , *B. subtilis* treated plants it was 2.5 ,2.5, 3.8, 4.5, & 7.8 µmol/ min/mg and in *B. cereus* treated plants it was 2.0, 2.8, 3.9, 3.2 & 7.5 µmol/ min/mg at0, 2, 4, 8 & 16 DAI. In Velum prime treated plants the PPO enzyme was significantly low at 0.3, 2.1, 2.7, 2.9, & 3.3 µ mol/min/mg at 0, 2, 4, 8 & 16 DAI, respectively. The PAL enzyme activity in *B. subtilis* treated plants was 2.4, 2.8, 4.5, 5.5 & 8.7 µmol/min/mg, in *B. pumilus* pre-treated plants, it was 2.1, 2.4, 4.1, 5.2 & 7.5 µmol/min/mg at0, 2, 4, 8 & 16 DAI, in*B. megaterium* pre-treated plants it was 1.8, 2.2, 3.9, 5.3 & 7.8 µm/min/mg, and in *B. cereus* treated plants it was 1.9, 2.0, 4.9, 4.3, & 6.8 µmol/min/mg at0, 2, 4, 8 & 16 DAI, respectively. In Velum prime treated plants, the levels

were much lower at 0.2, 1.8, 2.5, 2.6, & 3.6µ m/min/mg at0, 2, 4, 8 & 16 DAI, respectively as compared to nematode inoculated control. The PO enzyme activity shown in *B. pumilus* treated plants was 2.3, 2.2, 3.6, 4.8 & 8.5 µmol/ min/mg, in*B. megaterium* treated plants it was 1.8, 2.4, 3.8, 4.7 & 9.5 µmol/ min/mg, in *B. subtilis* treated plants, it was 2.6, 2.5,4.6, 5.3 & 10.2 µmol/min/mg and in *B. cereus* treated plants shown it was 2.8, 2.0, 3.6, 4.5 & 8.5 µm/ min/mg at0, 2, 4, 8 & 16 DAI. In Velum prime treated plants, the levels of PO were at a low of 0.3, 2.4, 2.6, 3.0 & 3.7 µmol/ min/mg at0, 2, 4, 8 & 16 DAI, respectively as compared to control. Among the above defense enzymes the activity of SOD was found highest followed by PAL, PO and PPO. In all the rhizobacterial treatments, a gradual increase in enzyme levels were observed with an increase in time period from 2 to 16 DAI, as compared to control. Thus application of all the four rhizobacteria increased the levels of defense enzymes in the nematode free plants which further increase upon nematode inoculation. The levels of all four enzymes were at par on respective 5 days of observation and were significantly higher than that observed in velum prime treated plants. The rhizobacteria thus up regulated the defense mechanism in plants, unlike the chemical nematicide Velum Prime.

DISCUSSION

The rhizosphere harbours many species of bacteria, many of which are known to induce resistance in plants against soil borne pests and pathogens. Indirect antagonism of PGPR against PPN that occurs by ISR, also referred to as PGPR-mediated priming, systematically equips the plants to cope with environmental constraints, actuating faster and/or stronger defense responses (adaption) to a subsequent exposure to various biotic and abiotic stresses. ISR is nonspecific in nature, and provides plants defense responses against various pests and pathogens. The rhizosphere bacterial isolates like *Bacillus pumilus, Paenibacillus castaneae, Pseudomonas fluorescens, Bacillus subtilis, Bacillus cereus* , *Arthrobotrys oligospora, Bacillus megaterium, Pseudomonas striata* and *Paenibacillus polymyxa* directly and indirectly suppress nematode infestation and promote plant growth (Subedi *et al* ., 2020). ISR induced by PGPR typically employs jasmonic acid (JA) and ethylene (ET) hormone signaling (Ahmed *et al* ., 2022). In the present investigation, the rhizobacteria elicited enhancement in defense gene expression and subsequently the enzymes that confer resistant reactions in tomato against *M. incognita*infection. The qRT-PCR results exhibited that the expressions of resistance genes viz., PR1-1b, CAT, and JERF3 in tomato plants were different, in the four rhizobacteria (*B. pumilus. B. megaterium, B. subtilis* and *B. cereus*) treatments at intervals of 0, 2, 4, 8 & 16 days DAI as compared with untreated control (**Fig. 1)** . Among the three genes, the maximum upregulation was observed in PR-1b, followed by CAT and JERF3. PR1-1b gene has been referred as an immune marker and the target gene for salicylic acid (Spoel & Dong, 2012; Fu & Dong, 2013).The hypersensitive response of PR-1b has been reported to be induced by activation of Jasmonic acid (JA) signalling pathway and ethylene (ET) in tomato (Mollinari and Leonatti, 2019). Furthermore, the rhizobacterial treated plants expressed JERF3 gene at 2- 6 folds and CAT gene at 3.5-6.0 folds, compared to untreated control. JERF3 and CAT genes encode for ERF proteins, a trans-acting factor responding to both ET and JA. Besides, the expression of CAT gene encodes for catalase, which neutralizes the toxic hydrogen peroxide produced in plant defense against pathogens and parasites. The defense gene expression during rhizobacterial-root interactions showed an initial overexpression to*M. incognita* infection followed by a defense repression. The process shares similarities with root mycorrhized plants where mycorrhizia induced resistance (MIR) (Pozo *et al* ., 2007). Comparably, our bacterial treated plants were primed for activation of defensive genes against *M. incognita* infection in tomato. The primed plants respond faster to biotic attacks and make stronger defense activation (Molinari S, Leonetti ,2019). The activities of defense enzymes *viz* ., SOD, PPO, PO, and PAL were found significantly ($P<0.05$) expressed in rhizobacteria (*B. pumilus. B. megaterium, B. subtilis* and *B. cereus*) treated tomato plants infected with *M. incognita* as compared to untreated plants. The activity of the above enzymes showed a gradual increased from 2 to 16 days after nematode inoculation in rhizobacteria treated roots. Among the enzymes, the activity of SOD was found highest followed by PAL, PO and PPO. Nematode infestation leads to the production of superoxide anions which are highly reactive. SOD is a constitutive enzyme produced by aerobic organisms in response to biotic stresses. The enzyme scavenges reactive oxygen species (ROS) by producing hydrogen peroxide and alongwith catalase maintains the cellular levels of ROS below the threshold levels that trigger cell death due to necrosis and hypersensitive reaction

leading to reduced nematode infection. Peroxidases (PO) catalyze the generation of ROS. PPO can inhibit the pest by producing quinones, reduce bioavailability of proteins and nutrients for the pest/pathogen, create lignin-like physical barriers and participate in the production of ROS. Similar upregulation of defense enzymes by rhizobacteria treated tomato against root knot nematode infection has been reported earlier (Ramazan *et al* ., 2018; Sharma and Sharma, 2016). The nematicide (Velum prime) treated plants did not show any enhancement in gene expression or enzyme activities as compared to untreated control.

The bioefficacy trials in pots revealed significant higher reduction in root galling (81-91%), average number of egg masses per root (16-43 %) and reproduction factor (51-67%) in rhizobacterial treatments as compared to control (**Fig 2-4**). Besides, the four rhizobacterial treatments also resulted in a significant enhancement in the photosynthetic and transpiration rate, though no significant difference was found with respect to stomatal conductance and leaf temperature, as compared to untreated control. Thus the rhizobacteria are environment friendly substitutes to agrochemicals for pest management. Among rhizobacteria, the *Bacillus* spp. are considered to be good options because they can quickly replicate and colonize plants, tolerate harsher environments, and easily form endospores. They are documented to affect a broad spectrum of plant pathogens including PPN and can promote plant growth and help the plant adapt abiotic stresses, enhancing yield potential (Ahmed at al. 2022).

The present study revealed that the pretreatment of tomato plants with nematicidal rhizobacterial isolates resulted in the activation of defense response by enhancing the expression of genes and enzymes governing resistance, reducing the nematode infection as indicated by gall index and reproduction factor of the nematode. The photosynthetic and transpiration rate of plants also increased, as compared to untreated control. Therefore, the tested rhizobacterial isolates are a promising approach for management of *M. incognita* and for improving the growth and productivity of plants.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing financial interests or conflicting personal relationships that influence the research work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

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TABLE 2 NEMATODE PARAMETERS.docx available at https://authorea.com/users/600832/articles/632257--plant-growth-promoting-bacillus-species-elicit-defense-against-meloidogyne-incognita-infecting-tomato-in-polyhouse

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