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# Evaluating *Bacillus* spp. As Biocontrol Agents against *Meloidogyne incognita* infesting Tomato

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## ABSTRACT

Tomato is attributed as a global host for root-knot nematode (*Meloidogyne incognita*) soliciting ponderous damage. Using biocontrol agents to control plant parasitic nematodes is a well-established, green approach in advance of synthetic nematicides. The role of *Bacillus* spp. in inciting physiological and biochemical alterations in nematode infestation is discussed in the present study. The susceptible (PKM-1) and resistant (Hisar Lalit) tomato cultivars treated with *Bacillus pumilus* augmented the shoot length, root length and biomass of plants compared to the standard check, *Pseudomonas fluorescens*, followed by *B. megaterium.* Accordingly, all the biocontrol agent-treated susceptible plants showed reduced galling and exhibited a root gall index of 3 (moderately resistant) and reduced nematode population in soil and roots. Contrarily, all the resistant plants showed highly resistant reactions. *B. pumilus* showed the topmost expression of all the biochemical enzymes like peroxidase (PO), polyphenol oxidase (PPO), catalase (CAT), phenylalanine ammonia-lyase (PAL) and total phenols. Conclusively, *B. pumilus* was found to be the most potential in reducing nematode infestation by embellishing the plant growth and enhancing defense-related enzymes in tomatoes.

**KEYWORDS:** Root-knot nematode, Tomato, *Bacillus* spp., Biochemical enzymes.

## INTRODUCTION

Plant parasitic nematodes (PPNs) are one of the egregious biotic stresses that are a well-recognized threat to crop production and food security. PPNs are omnipresent, enabling them to attack all different kinds of crop plants, field crops, and vegetable and flower crops that cause annual economic losses of USD 173 billion globally (Elling *et al.,* 2013). They are also responsible for multiple pathogen infection that predisposes the susceptible crops to other pathogens like fungi, bacteria and thus indirectly contributing to reduced yield and crop productivity (Back *et al.,* 2002).

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There exist more than 4000 PPNs attacking crops. Of these, the most important and prominent one is the root-knot nematode (*Meloidogyne* spp.), which voraciously feeds all crops, especially vegetables (Koenning *et al.,* 1999). Global vegetable production is under threat due to the damage from four different species of *Meloidogyne viz., M. arenaria*, *M. javanica*, *M. incognita* and *M. hapla*. However, *M. incognita* is one of the important pests of solanaceous crops majorly in tomatoes.

Tomato is the second most paramount remunerable exigent solanaceous vegetable grown worldwide after potato. Infestation of *M. incognita* in tomatoes impedes production and lowers yield, making them vulnerable to other wilt and/or rot causing pathogens (Ogwulumba *et al.,* 2011). The habitual monitoring tactic employed by growers is the use of synthetic nematicides which create economic and environmental constraints. Under these circumstances, substitutive strategies are gaining preponderance. The current study explains one such alternative strategy to combat root-knot nematode infesting tomatoes.

Biocontrol or biological control is an economically and environmentally well-fit tactic against plant pathogens. Precisely, biocontrol of nematodes can be defined as the management of nematode populations and the damage caused by them through the action of antagonists either directly or indirectly by manipulating the environment favorable for nematodes (Poveda *et al.,* 2020) that includes many of fungi and bacterial species. The organism that shows antagonism against pathogens are called biocontrol agents (BCA) where, these organisms interact with pathogens through various mechanisms like competition, antibiosis and/or through inducing the plant resistance against pathogens.

Plant resistance is one of the defensive strategies used by most of the hosts against pest and pathogen attacks. Once the plant encounters the attack, the pathogen-associated molecular patterns (PAMPs) will be recognized by the pattern recognition receptors (PRRs) on the cell surface. Thereby it triggers a complex signaling network leading to defense responses (PAMP-triggered immunity) (Ramirez-Prado *et al.,* 2018; Hou *et al.,* 2019). When it fails, these biocontrol agents help the plant to trigger its innate immunity by producing microbial-associated molecular patterns (MAMPs) that act as elicitors for triggering the complex defense responses by the host against pathogens (Poveda *et al.,* 2020). It includes the production of reactive oxygen species, hypersensitive response, production of defensive enzymes (peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, catalase, etc.), phytohormones (salicylic acid, jasmonic acid and/or ethylene) and many signaling cascades.

In the current study, we investigated the nematicidal potential of a bacterial bioagent, *Bacillus* spp. against *M. incognita* in tomato and the level of defensive enzymes across different *Bacillus* spp. treated plants.

## MATERIALS AND METHODS

The experiments were laid out in a glasshouse at AICRP (Nematodes), Zonal Agricultural Research Station, GKVK, Bengaluru. Precedently, the species of *Meloidogyne* was validated as *M. incognita* by using the “Perineal pattern technique” (Chitwood, 1949).

### Collection of Bacterial Culture

Three distinct species of *Bacillus viz., B. subtilis* IIHR Bs-2, *B. megaterium* Bm-IIHR, *B. pumilus* IIHR Bp-2 cultures were obtained from ICAR-Indian Institute of Horticultural Research, Bengaluru whereas *Pseudomonas fluorescence* (standard check) was obtained from Pathogenomics Laboratory, Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bengaluru.

### Collection of Tomato Seeds

Tomato seeds of nematode susceptible variety, PKM-1 were collected from IIHR, Bengaluru and tomato seeds of nematode resistant variety, Hisar Lalit (NRT 8) were collected from Chaudhary Charan Singh Agricultural University, Hisar, Haryana. Tomato plants were raised in portrays and transplanted into pots (2 kg) filled with sterilized soil after 21 days.

### Preparation of Bacterial and Nematode Suspension

The bacterial suspensions of respective bio-agents were prepared by growing a bacteria in 250 mL nutrient broth and incubating for 24 hours. The turbid broth was centrifuged at 5000 rpm for 5 min and cells were resuspended with phosphate buffer (pH=7). The concentration was maintained at the rate of $2.5\times10^7$ CFU/mL and the bacterial suspension was inoculated through the seedling dip method to 25-day-old seedlings before translation for 3 hours. Here, *Pseudomonas fluorescens* was used as a standard check.

Nematode inoculum was obtained from infected tomato roots. We extracted the nematode by following Combined Cobb’s sieving and Baermann’s funnel technique (Ayoub, 1977). 50 mL of nematode suspension was prepared at the rate of 20 juveniles per mL of water. After a week of transplanting, the nematode suspension of 50 mL per plant with 1000 juveniles was inoculated by making 4-5 holes around the seedling.

Nematicide-treated (carbofuran @ 1g/kg pot) plants were considered as a positive control. Complete random design (CRD) was maintained in a glass house (temperature: 20□) with three replications of seven treatments. The experiment was conducted twice.

**Studies on Physiological Alterations in Tomato**

The efficacy of *Bacillus* spp. on the incidence and establishment of root-knot nematode in tomatoes was studied through various parameters of plant growth and nematode infection under pot experiments. After 45 days of inoculation, observations *viz.,* shoot growth (cm), root growth (g) and root-knot index (1-5 scale), nematode population in roots and soil were taken (Narasimhamurthy *et al.,* 2017).

**Status of Defense-related Biochemicals in Tomato**

The biochemical levels in tomato-treated and untreated plants were assessed. One gram of fresh root was collected from susceptible and resistant plants after 28 days of nematode inoculation from each treatment for biochemical analysis. Fresh roots were washed gently in running tap water and homogenized in 1 mL of 0.1 M phosphate buffer (pH 7.0) at 4 °C in a pre-chilled pestle and mortar. The homogenate was centrifuged at 20,000 rpm at 4 °C for 15 min and the supernatant served as an enzyme source for further analysis (Anita *et al.,* 2004).

Here tomato plants were tested for biochemical levels of several defense-related enzymes like peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), catalase (CAT) and total phenols using spectrophotometer by following standard procedures (Prabhu *et al.,* 2019).

**Estimation of peroxidase (PO) activity**

Peroxidase activity was assayed spectrophotometrically (Chander, 1990). The reaction mixture consisted of 2.5 mL of 0.05 M potassium phosphate buffer, 0.2 mL of o- phenyl diamine (OPD), 0.2 mL of 0.3 per cent $H_2O_2$ and 0.03 mL of enzyme extract. The reaction mixture was incubated at room temperature (28±1$^0$C). The change in absorbance was recorded at 60 sec intervals for 5 min. The enzyme preparation without $H_2O_2$ served as blank. The enzyme activity was expressed as change in the absorbance at 450 nm min$^{-1}$ g$^{-1}$ on fresh weight basis.

**Estimation of polyphenol oxidase (PPO)**

PPO activity was determined as per the procedure given by Selvaraj and Kumar (1995). The reaction mixture consisted of 2.9 mL of 0.05 M potassium phosphate buffer (pH 6.8), 0.1 mL of 1.25 per cent pyrogallol and enzyme extract of 0.5 mL. The increase in absorbance was measured at 450 nm up to 5 min for 1 min interval. Polyphenol oxidase activity was expressed as absorbance/min/gm FW.

**Estimation of phenylalanine ammonia lyase (PAL)**

The PAL assay was conducted as per the method described by Whetten and Senderoff (1992). 0.4 mL of enzyme extract was incubated with 0.5 mL of 0.1 M borate buffer (pH 8.8) and 0.5 mL of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The reaction was arrested by adding 0.5 mL of 1 M TCA and incubated at 37 °C for 5 min. The blank was prepared, that contains 0.4 mL of crude enzyme extract and 2.7 mL of 0.1 M borate buffer (pH 8.8) and absorbance was measured at 290 nm in spectrophotometer. Standard curve was drawn with graded amounts of cinnamic acid dissolved in acetone. The enzyme activity was expressed as μM of trans-cinnamic acid $min^{-1}$ $g^{-1}$ fresh weight.

**Estimation of Catalase**

The catalase activity was estimated as per the procedure given by Masia (1998). The reaction mixture contains 2.6 mL of 0.067 M sodium phosphate buffer (pH 7.0), 0.3 mL of 3 per cent $H_2O_2$ and 0.1 mL of enzyme extract. The decrease in absorbance was measured at 240 nm up to 5 min for 1 min time interval. Catalase activity was expressed as μg $H_2O_2$/gm FW.

**Estimation of Total phenol (Singleton and Rossi, 1965)**

Total phenol content was estimated by spectrophotometric method using Folin Ciocalteu Reagent (FCR) at an absorbance of 700 nm by following procedure. Five grams of sample was homogenized with 20 mL of methanol (80%) in a pestle and mortar 2-3 times and volume was made to 50 mL. 0.5 mL of the extract was taken in test tubes, 0.2 mL of Folin-Ciocalteau's Phenol Reagent was added followed by 3.3 mL of distilled water and mixed well. After 2 min, 1 mL of sodium carbonate solution was added and mixed. Allowed to stand at room temperature for 30 min and blue colour was read in a spectrophotometer at 700 nm. Standard curve for phenols was prepared using gallic acid (GA) as standard. The content of the total soluble phenol was calculated according to a standard curve obtained from a Folin Ciocalteau reagent with a phenol solution ($C_6H_5OH$) and expressed as mg gallic acid equivalent/100 g fresh weight.

**Data Analysis**

The data so generated were analyzed using WASP – Web Agri Stat Software Package 2.0 developed by ICAR. Completely Randomized Design (CRD) was used in the present study.

The difference between treatment means was compared with the critical difference values to know a significant difference.

## RESULTS

### Physiological Transfiguration Incited by *Bacillus* spp. in Tomato

*Bacillus* spp. had shown its potential to embellish the shoot and root growth in relevance with control treated with only nematode (Table 1). The effectuation of *Bacillus pumilus* was premier among different *Bacillus* spp. and a standard check, *Pseudomonas fluorescence*.

### Effects on tomato growth

In the case of susceptible variety, despite the fact that carbofuran-treated plants showed overhead expression of shoot growth, *Bacillus pumilus-treated* plants showed the highest shoot length (57.27 cm) and shoot weight (Fresh weight: 179.18 g and Dry weight: 81.72 g) among different bioagents followed by *B. megaterium* (shoot length: 55.34 cm; Fresh weight: 175.90g and Dry weight: 77.67g). Conversely, in the case of resistant variety, regardless of the bioagents, the nematode-challenged plants exhibited the highest shoot growth which was comparable with healthy plants but then carbofuran-treated plants showed the least shoot growth.

A similar trend was noticed with respect to root growth as that of shoot growth in both root-knot nematode susceptible and resistant tomato varieties, respectively. In the case of susceptible variety, the root growth of the bacterial bio-agents treated-*Meloidogyne incognita*-infested plants showed higher values than only nematode-challenged plants, among which B. pumilus showed maximal root length (30.73 cm) and root weight (Fresh weight: 7.67 g and Dry weight: 4.89 g). Even when the healthy resistant variety showed the topmost expression of root growth witnessing the fact that the bioagent treatment was non-significant.

### Effects on nematode infestation

The root-knot index was calculated by considering the number of galls produced per root system (Table 2). Except for the fact that the resistant variety showed highly resistant to resistant reactions with a root-knot index of 1-2, the bioagents-treated plants revealed a lesser number of galls (23.33-28.33) in susceptible variety and a root-knot index of 3 imposing a moderately resistant reaction. Of these, *B. pumilus* eliminated at most the production of galls on the roots (Fig. 1a & 1b). Further number egg masses per root system (Table 2) and nematode population was evaluated both in soil and roots. The nematode population was significantly reduced in the *Bacillus* spp. treated plants compared to control showing the significance of biocontrol treatment (Table 3).

**Biochemical levels after *Bacillus* spp. treatment in Tomato**

In this study, biochemicals like peroxidase (PO), polyphenol oxidase (PPO), catalase (CAT), phenylalanine ammonia-lyase (PAL) and total phenols were analyzed in both susceptible and resistant varieties. The inoculation of bio-agents to *M. incognita* infested plants showed increased activity of all tested enzymes (peroxidase, polyphenol oxidase, phenylalanine lyase, catalase and total phenols) over control in both susceptible and resistant varieties (Fig. 2a & 2b). Among bioagents, *B. pumilus* showed the topmost expression of all the enzymes. However, the resistant variety showed higher enzymatic activity than the susceptible variety except for the catalase enzyme.

**Studies on peroxidase (PO) activity**

The activity of peroxidase was assayed spectrophotometrically at 450 nm as per procedure given by Chander (1990). The increased activity of peroxidase was recorded in *Meloidogyne incognita* inoculated samples of susceptible variety along with resistant tomato variety (0.487 and 1.461 abs $min^{-1}$ $g^{-1}$, respectively) compared to respective healthy plants (0.187 and 0.561 abs $min^{-1}$ $g^{-1}$), but enzymatic activity was higher in resistant variety (Fig. 2aA).

The bacterial bio-agents treated-*Meloidogyne incognita* infested plants showed the highest enzymatic activity (susceptible: 0.697-0.793 and resistant: 2.537-2.056 abs $min^{-1}$ $g^{-1}$) compared to all other treatments in susceptible as well as in resistant varieties. Among them, *Bacillus pumilus* treated plants (susceptible: 0.793 and resistant: 2.537 abs $min^{-1}$ $g^{-1}$) showed significantly supreme activity of peroxidase, followed by *Pseudomonas fluorescens* (susceptible: 0.733 and resistant: 2.482 abs $min^{-1}$ $g^{-1}$) and *B. subtilis* (susceptible: 0.730 and resistant: 2.125 abs $min^{-1}$ $g^{-1}$).

**Studies on polyphenol oxidase estimation (PPO) activity**

The polyphenol oxidase activity was assayed spectrophotometrically at 450 nm by following the procedure given by Selvaraj and Kumar (1995). The activity of polyphenol oxidase was increased upon bacterial bio-agents treatment to *Meloidogyne incognita* inoculated susceptible and resistant tomato varieties (0.076 to 0.094 and 0.171 to 0.216 abs $min^{-1}$ $g^{-1}$, respectively) compared to respective *M. incognita* infested plants (0.060 and 0.108 abs $min^{-1}$ $g^{-1}$) however, enzymatic activity was higher in resistant variety compared to susceptible variety (Fig. 2aB).

Among bacterial bio-agents treated-*Meloidogyne incognita* infested plants, *Bacillus pumilus* treated plants showed highest activity (susceptible: 0.094 and resistant: 0.216 changes in absorbance $min^{-1}$ $g^{-1}$) and it was on par with *Pseudomonas fluorescens* (susceptible: 0.090 and resistant: 0.207 changes in absorbance $min^{-1}$ $g^{-1}$).

**Studies on phenylalanine ammonia lyase (PAL) activity**

The polyphenol oxidase activity was assayed according to the procedure given by Ross and Senderoff (1992) spectrophotometrically at 290 nm. There was an increased enzymatic activity in *Meloidogyne incognita* infested plants (susceptible: 0.337 and resistant: 0.741 µM trans-cinnamic acid $min^{-1}$ $g^{-1}$) compared to healthy plants (0.147 and 0.323 µM trans-cinnamic acid $min^{-1}$ $g^{-1}$, respectively). However, the activity was significantly higher in bacterized-*M. incognita* infested plants.

The susceptible plants treated with *Bacillus pumilus* along with *Meloidogyne incognita* (0.510 µM trans-cinnamic acid $min^{-1}$ $g^{-1}$) showed higher activity of phenylalanine ammonia lyase, followed by *Pseudomonas fluorescens* treated- *M. incognita* infested susceptible plants (0.503 µM trans-cinnamic acid $min^{-1}$ $g^{-1}$). Resistance plants followed a similar course (*B. pumilus*: 1.275 and *P. fluorescens*: 1.156 µM trans-cinnamic acid $min^{-1}$ $g^{-1}$), but they were on par with each other (Fig. 2aC).

**Studies on catalase (CAT) activity**

The activity of catalase was assayed spectrophotometrically at 240 nm by following the procedure given by Masia (1998). The increased activity of catalase was recorded in *Meloidogyne incognita* inoculated samples of susceptible and resistant tomato varieties (32.01 and 21.340 µg $H_2O_2$ $g^{-1}$, respectively) compared to respective healthy plants (24.964 and 16.643 µg $H_2O_2$ $g^{-1}$), but enzymatic activity was higher in susceptible variety (Fig. 2bA). The activity of catalase got significantly decreased in bacterized nematode challenged tomato varieties (susceptible: 15.251- 18.614 and resistant: 9.500-11.740 µg $H_2O_2$ $g^{-1}$).

Among bacterial bio-agents, *Bacillus pumilus* treated plants showed least catalase activity (susceptible: 15.251 and resistant: 9.500 µg $H_2O_2$ $g^{-1}$), followed by *Pseudomonas fluorescens* (susceptible: 16.754 and resistant: 10.503 µg $H_2O_2$ $g^{-1}$) and both were on par and no significant difference was found.

### Studies on total phenols

Total phenols were estimated by spectrophotometric method using Folin Ciocalteu Reagent (FCR) at an absorbance of 700 nm. Higher accumulation of phenol was observed in bacterial-bio-agents treated plants than untreated plants. In general, the inoculation of *Meloidogyne incognita* and bacterial-bio-agents simultaneously recorded significantly higher phenol (susceptible: 65.987-68.593 and resistant: 79.184-82.311 mg gallic acid equivalent/100g fresh weight) content than control with *M. incognita* inoculation (susceptible: 48.747 and resistant: 58.496 mg gallic acid equivalent/100g fresh weight).

Among the bacterial bio-agents treated-*Meloidogyne incognita* infested tomato plants, the plants treated with *Bacillus pumilus* showed the highest phenol content (susceptible: 68.593 and resistant: 82.311 mg gallic acid equivalent/100g fresh weight) and it was on par with *Pseudomonas fluorescens* (susceptible: 67.413 and resistant: 80.895 mg gallic acid equivalent/100g fresh weight) (Fig. 2bB).

## DISCUSSION

The influence of different *Bacillus* spp. on the physiology and biochemical status of *Meloidogyne incognita*-infested tomato plants was investigated. The significant increment in the shoot and root growth of tomato plants proves that the biocontrol agents do act as plant growth-promoting agents. From the above observations, it is evident that bacterial bio-agents have a positive effect on root and shoot growth. This may be attributed to one or more of the following factors; production of phosphatases by *Bacillus* spp. facilitates the conversion of insoluble phosphorus to available one for the use of plants (Abdelmoteleb and Gonzalez-Mendoza, 2020), production of growth-promoting phytohormones *viz.,* indole acetic acid (IAA), gibberellic acid (GA), cytokine (Calvo *et al.,* 2010), improvement of water and nutrients uptake, production of antibiotic metabolites effective against soil-borne pathogens and production of B-group vitamins that promote rooting capacity and affect the population of the microbial community (Wu *et al.,* 2005; Rai, 2006).

Furthermore, the reduced galling in bacterial bio-agents treated plants might be owing to the ability of the bio-agents to modify root exudates, thus hindering the feeding site recognition by the nematodes in the soil (Siddiqui and Mahmood, 1999; Zhou *et al.,* 2019). Thus, affected the gall formation. In addition, the bacterial bioagents produce nematicidal acids that decrease the nematode population in the soil and reduce the level of infection and hence the galling of roots (Iatsenko *et al.,* 2014; Lee and Kim, 2016).

However, when nematode-susceptible and resistant varieties are compared, the average number of galls formed per root system and root-knot index were less in the case of the resistant variety. These results were adjacent to the results obtained by Kumari *et al.* (2016), who made a comparative study on *M. graminicola* susceptible (Pusa 1121) and resistant (Vandana) cultivars of rice. It was found that after 15 dpi, all the growth parameters of nematode were recorded low in the resistant variety and were significantly different from the susceptible variety. However, while comparing the response of susceptible versus resistant varieties upon infection with *M. incognita*, many contradictions arose may be due to nematode effects (nematode biology varies with resistant and susceptible hosts), systemic hormone signaling effects or tissue-specific differential expression of selected genes (Cabasan *et al.,* 2012).

The bio-agent activity was clearly noticed in susceptible plants infested with *M. incognita*. The genetic background of the resistant variety (Hisar Lalit) contains *Mi 1.2* gene which shows resistance to *M. incognita* (Reddy *et al.,* 2016) and this might be the reason for variations observed in nematode infection in the resistant genotypes and it might attract nematodes meagerly less than susceptible ones (Peacock, 1959). Because of the ability of a resistant variety to overcome nematode infection on its own, the treatments were established to be non-significant in many of the nematode growth parameters.

Studies on biochemical levels suggest that defense-related enzymes (peroxidase, phenylalanine ammonia-lyase, polyphenol oxidase and catalase) and biomolecules (phenols) get enhanced when the plants were subjected to biotic stress and abiotic stress. The above observations on biochemical studies revealed that there was a significant accumulation of all tested enzymes in bacterized tomato plants (susceptible and resistant) challenged with *Meloidogyne incognita*. Among different *Bacillus* spp., *B. pumilus* treated-*M. incognita* infested plants showed supreme activity of biochemicals, followed by *B. subtilis* in susceptible as well as in resistant varieties compared to *Pseudomonas fluorescence* (standard check).

When compared to susceptible and resistant varieties, the activities of enzymes were superior in the resistant variety (except catalase) probably due to avoidance of nematode invasion (Rao *et al.,* 2017) which was because of its early and enhanced accumulation of enzymes in response to invading pathogens. Enhanced levels of defense enzymes and phenolic content in plants treated with bio-agents may contributed to resistance development, safeguarding the plants without causing harm.

The present investigation revealed the potentiality of *Bacillus* as a bio-agent that could reduce gall formation, root-knot index and nematode population, thus improving the shoot and root growth under pot experiments. *Bacillus* spp. could be included in integrated disease management strategies to reduce the damage caused by *M. incognita* in tomatoes. These findings revealed that *B. pumilus* was the most potential *Bacillus* bio-agent in reducing *M. incognita* infection by embellishing the plant growth and strengthening the plants by enhancing defense-related enzymes, followed by *B. subtilis* and *B. megaterium.*

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**Table 1.** Physiological alterations incited by Bacillus spp. in *M. incognita*-infested tomato plants.

| Treatments | Susceptible variety | | | Resistant variety | | | Susceptible variety | | | Resistant variety | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot length (cm) | Shoot weight (g) | | Shoot length (cm) | Shoot weight (g) | | Root length (cm) | Root weight (g) | | Root length (cm) | Root weight (g) | |
| | | Fresh | Dry | | Fresh | Dry | | Fresh | Dry | | Fresh | Dry |
| *M. incognita*+ Carbofuran | 60.75 (±0.08)$^{b}$ | 185.25 (±0.69)$^{b}$ | 86.28 (±0.29)$^{b}$ | 59.71 (±0.35)$^{e}$ | 163.24 (±0.28)$^{f}$ | 71.86 (±0.37)$^{e}$ | 33.82 (±0.36)$^{b}$ | 8.90 (±0.22)$^{b}$ | 5.08 (±0.08)$^{ab}$ | 33.68 (±0.28)$^{e}$ | 9.03 (±0.27)$^{e}$ | 4.56 (±0.14)$^{d}$ |
| *M. incognita+ B. pumilus* | 57.27 (±0.51)$^{c}$ | 179.18 (±0.33)$^{c}$ | 81.72 (±0.33)$^{c}$ | 67.65 (±0.43)$^{bc}$ | 208.09 (±0.34)$^{b}$ | 97.54 (±0.28)$^{ab}$ | 30.73 (±0.24)$^{c}$ | 7.67 (±0.27)$^{c}$ | 4.89 (±0.20)$^{ab}$ | 39.30 (±0.41)$^{b}$ | 11.74 (±0.20)$^{b}$ | 5.89 (±0.12)$^{abc}$ |
| *M. incognita+ B. megaterium* | 55.34 (±0.29)$^{d}$ | 175.90 (±1.36)$^{d}$ | 77.67 (±2.68)$^{d}$ | 67.38 (±0.33)$^{cd}$ | 206.78 (±0.31)$^{c}$ | 96.37 (±0.65)$^{b}$ | 29.30 (±0.33)$^{d}$ | 7.29 (±0.22)$^{cd}$ | 4.53 (±0.25)$^{b}$ | 37.86 (±0.2)$^{c}$ | 11.28 (±0.35)$^{bc}$ | 5.51 (±0.33)$^{bc}$ |
| *M. incognita+ B. subtilis* | 53.63 (±0.69)$^{e}$ | 171.01 (±0.89)$^{f}$ | 70.89 (±0.28)$^{e}$ | 66.54 (±0.3)$^{d}$ | 203.19 (±0.30)$^{e}$ | 92.63 (±0.53)$^{d}$ | 27.39 (±0.38)$^{e}$ | 6.30 (±0.24)$^{ef}$ | 4.29 (±0.53)$^{b}$ | 36.51 (±0.72)$^{d}$ | 10.34 (±0.18)$^{d}$ | 5.11 (±0.08)$^{cd}$ |
| *M. incognita+ P. flourescens* | 54.08 (±0.37)$^{de}$ | 173.30 (±9.74)$^{e}$ | 71.09 (±5.16)$^{e}$ | 66.87 (±0.18)$^{cd}$ | 204.51 (±0.31)$^{d}$ | 94.34 (±0.70)$^{c}$ | 27.82 (±0.37)$^{e}$ | 6.81 (±0.26)$^{de}$ | 4.53 (±0.32)$^{b}$ | 36.03 (±0.19)$^{d}$ | 10.93 (±0.32)$^{cd}$ | 5.19 (±0.47)$^{cd}$ |
| *M. incognita* only | 43.21 (±0.46)$^{f}$ | 144.52 (±0.59)$^{g}$ | 56.06 (±0.36)$^{f}$ | 68.61 (±0.32)$^{b}$ | 210.03 (±0.46)$^{a}$ | 97.71 (±0.2)$^{ab}$ | 21.57 (±0.61)$^{f}$ | 5.75 (±0.02)$^{f}$ | 3.36 (±0.16)$^{c}$ | 40.04 (±0.22)$^{ab}$ | 11.92 (±0.19)$^{b}$ | 6.25 (±0.27)$^{ab}$ |
| Healthy control | 66.48 (±0.33)$^{a}$ | 200.70 (±0.52)$^{a}$ | 93.75 (±0.33)$^{a}$ | 69.84 (±0.22)$^{a}$ | 210.34 (±0.59)$^{a}$ | 98.70 (±0.32)$^{a}$ | 37.36 (±0.32)$^{a}$ | 10.50 (±0.31)$^{a}$ | 5.50 (±0.27)$^{a}$ | 40.96 (±0.199)$^{a}$ | 12.83 (±0.14)$^{a}$ | 6.47 (±0.29)$^{a}$ |
| **SEm ±** | **0.55** | **0.69** | **0.43** | **0.32** | **0.44** | **0.69** | **0.46** | **0.17** | **0.26** | **0.42** | **0.18** | **0.23** |
| **CD @ 1 %** | **1.81** | **2.02** | **1.60** | **1.39** | **1.62** | **2.03** | **1.65** | **1.01** | **1.24** | **1.59** | **1.05** | **1.16** |

**Table 2.** Effect of *Bacillus* spp. on egg masses and number of galls.

| Treatments | Susceptible variety | | | | Resistant variety | | | |
|---|---|---|---|---|---|---|---|---|
| | **No. of galls per root system** | **Root-knot Index** | **No. of egg masses per root system** | **Per cent reduction over control** | **No. of galls per root system** | **Root-knot index** | **No. of egg masses per root system** | **Per cent reduction over control** |
| *M. incognita*+ Carbofuran | 14.00 (±0.57)[e] | 3 | 20.67[e] | 78.16 | 1.67(±0.33)[ab] | 2 | 1 | 50 |
| *M. incognita*+ *B. pumilus* | 23.33(±0.33)[d] | 3 | 24.33[d] | 74.30 | 1(±0.57)[b] | 1 | 1 | 50 |
| *M. incognita*+ *B. megaterium* | 25.33(±0.33)[c] | 3 | 27.33[c] | 71.13 | 1.33(±0.33)[ab] | 2 | 1.33 | 33.50 |
| *M. incognita*+ *B. subtilis* | 28.33(±0.33)[b] | 3 | 31.33[b] | 66.90 | 1(±0)[b] | 1 | 0.67 | 66.50 |
| *M. incognita*+ *P. flourescens* | 27.33(±0.33)[b] | 3 | 30.00[b] | 68.31 | 1(±0)[b] | 1 | 0.67 | 66.50 |
| *M. incognita* only | 103.00(±0.57)[a] | 5 | 94.66[a] | - | 2(±0)[a] | 2 | 2 | - |
| Healthy control | 0.00(±0)[f] | 1 | 0.00[f] | - | 0.00(±0)[c] | 1 | 0 | - |
| **SEm ±** | **0.47** | | **0.66** | | **0.23** | | **NS** | |
| **CD @ 1 %** | **1.67** | | **1.98** | | **1.18** | | | |

**Table 3.** Effect of bacterial bio-agents on final nematode population.

| Treatments | Susceptible variety | | | | Resistant variety | | | |
|---|---|---|---|---|---|---|---|---|
| | **Soil (200 cc)** | **Per cent reduction over control** | **Root (5g)** | **Per cent reduction over control** | **Soil (200 cc)** | **Per cent reduction over control** | **Root (5g)** | **Per cent reduction over control** |
| $T_1$: *Meloidogyne incognita* + carbofuran 3G (Positive control) | $253.33^{f}$ | 35.86 | $50.00^{e}$ | 64.36 | $15.00^{c}$ | 16.67 | $5.33^{c}$ | 33.37 |
| $T_2$: *M. incognita* + *Bacillus pumilus* | $268.33^{e}$ | 32.06 | $54.33^{d}$ | 61.28 | $15.67^{a}$ | 12.94 | $6.67^{ab}$ | 16.62 |
| $T_3$: *M. incognita* + *B. megaterium* | $269.67^{d}$ | 31.72 | $57.33^{c}$ | 59.14 | $16.33^{b}$ | 9.27 | $7.00^{ab}$ | 12.50 |
| $T_4$: *M. incognita* + *B. subtilis* | $273.33^{b}$ | 30.80 | $61.33^{b}$ | 56.29 | $17.67^{bc}$ | 1.83 | $7.67^{b}$ | 4.12 |
| $T_5$: *M. incognita*+ *Pseudomonas fluorescens* | $271.67^{c}$ | 31.22 | $61.67^{b}$ | 56.05 | $16.00^{bc}$ | 11.11 | $7.33^{ab}$ | 8.37 |
| $T_6$: Control with *M. incognita* inoculation | $395^{a}$ | - | $140.33^{a}$ | - | $18.00^{a}$ | - | $8.00^{a}$ | - |
| $T_7$: Absolute control without *M. incognita* inoculation | $0^{g}$ | - | $0^{f}$ | - | $0^{d}$ | - | $0^{d}$ | - |
| **SEm ±** | **0.52** | | **0.38** | | **0.57** | | **0.47** | |
| **CD @ 1 %** | **1.75** | | **1.50** | | **1.83** | | **1.67** | |

***M. incognita* + *Bacillus pumilus*** **Control (only *M. incognita*)**

**(a)**

***M. incognita* + *Bacillus pumilus*** **Control (only *M. incognita*)**

**(b)**

**Figure 1.** Effect of *Bacillus pumilus* galling of susceptible (a) and resistant (b) tomato plants infested with *Meloidogyne incognita.*

| Treatments | PO (absorbance min$^{-1}$ g$^{-1}$ of fresh tissue) | | PPO (absorbance min$^{-1}$ g$^{-1}$ of fresh tissue) | | PAL (µM trans-cinnamic acid min$^{-1}$ g$^{-1}$ of fresh tissue) | | CAT (µg $H_2O_2$ g$^{-1}$) | | Total phenols (mg gallic acid equivalent/100g fresh weight) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Susceptible | Resistant | Susceptible | Resistant | Susceptible | Resistant | Susceptible | Resistant | Susceptible | Resistant |
| $T_1$: *Meloidogyne incognita* + carbofuran 3G (Positive control) | 0.377$^{d}$ | 1.074$^{d}$ | 0.05$^{c}$ | 0.092$^{cd}$ | 0.28$^{e}$ | 0.507$^{f}$ | 26.149$^{b}$ | 17.433$^{b}$ | 43.403$^{c}$ | 52.083$^{c}$ |
| $T_2$: *M. incognita* + *Bacillus pumilus* | 0.793$^{a}$ | 2.537$^{a}$ | 0.094$^{a}$ | 0.216$^{a}$ | 0.51$^{a}$ | 1.275$^{a}$ | 15.251$^{e}$ | 9.5$^{e}$ | 68.593$^{a}$ | 82.311$^{a}$ |
| $T_3$: *M. incognita* + *B. megaterium* | 0.697$^{b}$ | 2.056$^{b}$ | 0.076$^{ab}$ | 0.171$^{b}$ | 0.443$^{c}$ | 0.941$^{d}$ | 18.614$^{c}$ | 11.74$^{c}$ | 65.987$^{a}$ | 79.184$^{a}$ |
| $T_4$: *M. incognita* + *B. subtilis* | 0.73$^{b}$ | 2.125$^{b}$ | 0.084$^{a}$ | 0.181$^{b}$ | 0.473$^{b}$ | 1.045$^{c}$ | 17.825$^{cd}$ | 11.217$^{cd}$ | 66.71$^{a}$ | 80.052$^{a}$ |
| $T_5$: *M. incognita* + *Pseudomonas fluorescens* | 0.733$^{b}$ | 2.482$^{a}$ | 0.09$^{a}$ | 0.207$^{a}$ | 0.503$^{a}$ | 1.156$^{b}$ | 16.754$^{de}$ | 10.503$^{de}$ | 67.413$^{a}$ | 80.895$^{a}$ |
| $T_6$: Control with *M. incognita* inoculation | 0.487$^{c}$ | 1.461$^{c}$ | 0.06$^{bc}$ | 0.108$^{c}$ | 0.337$^{d}$ | 0.741$^{e}$ | 32.01$^{a}$ | 21.34$^{a}$ | 48.747$^{b}$ | 58.496$^{b}$ |
| $T_7$: Absolute control without *M. incognita* inoculation | 0.187$^{e}$ | 0.561$^{e}$ | 0.048$^{c}$ | 0.083$^{d}$ | 0.147$^{f}$ | 0.323$^{g}$ | 24.964$^{b}$ | 16.643$^{b}$ | 29.637$^{d}$ | 35.564$^{d}$ |

**Table 4:** Biochemical levels in bacterial bio-agents treated *Meloidogyne incognita*-infested tomato plants.

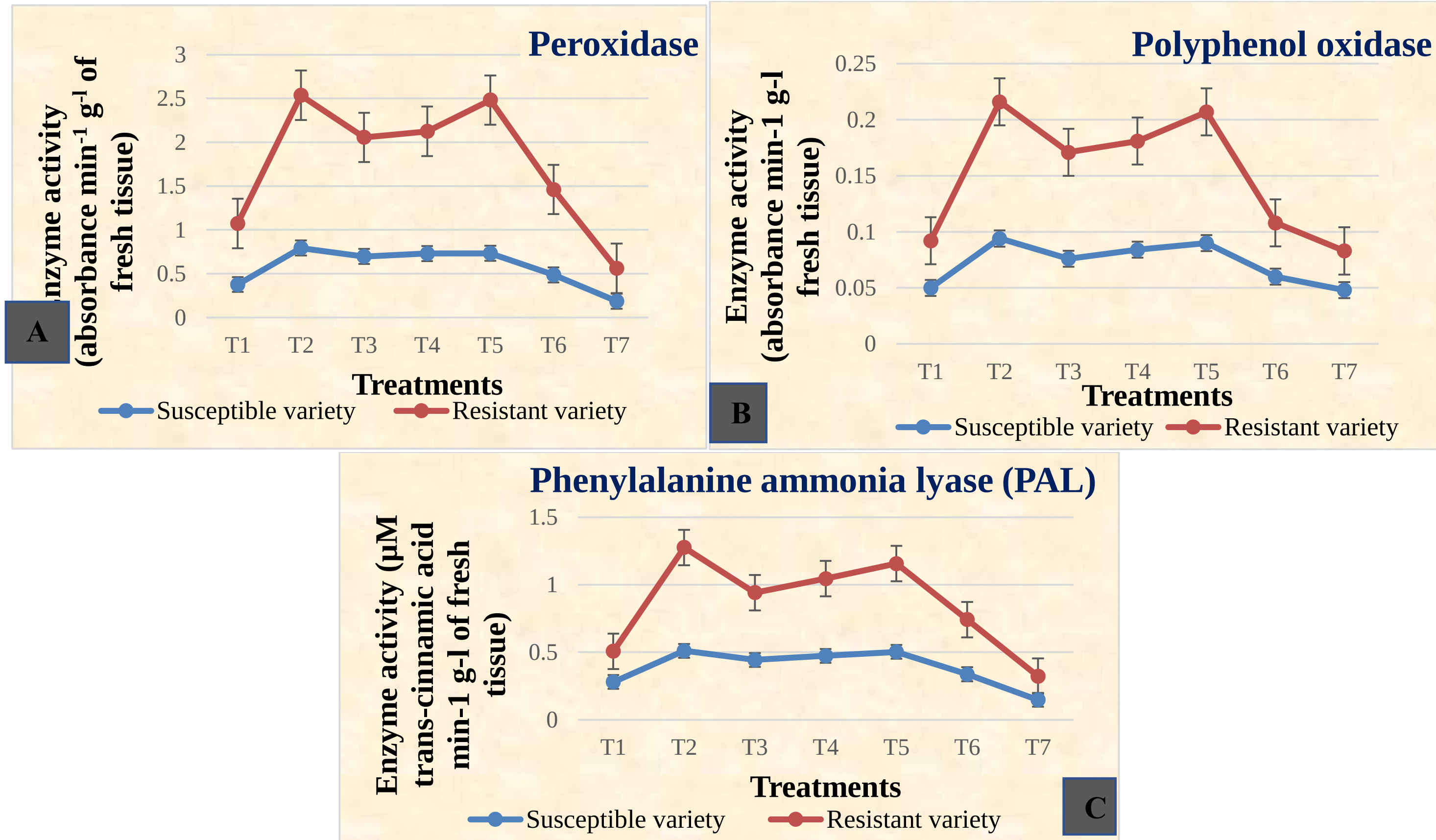
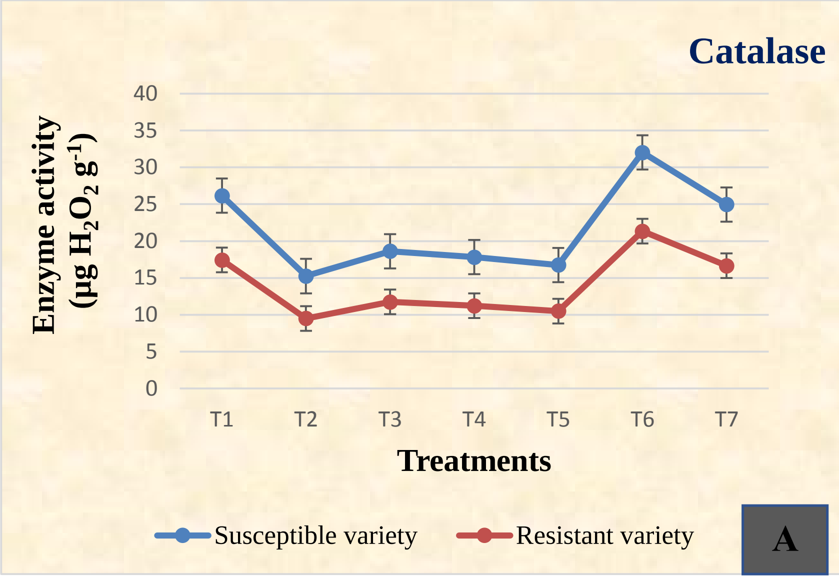



**Figure 2a.** Status of biochemicals incited by bacterial bio-agents on *Meloidogyne incognita*-infested tomato plants (A) peroxidase (PO) activity, (B) polyphenol oxidase (PPO) activity and (C) phenylalanine ammonia-lyase (PAL) activity. **Note:** $T_1$ = *M. incognita* + carbofuran 3G, $T_2$ = *M. incognita* + *B. pumilus*, $T_3$ = *M. incognita* + *B. megaterium*, $T_4$ = *M. incognita* + *B. subtilis*, $T_5$ = *M. incognita* + *P. fluorescens*, $T_6$ = *M. incognita* only and $T_7$ = Healthy control.



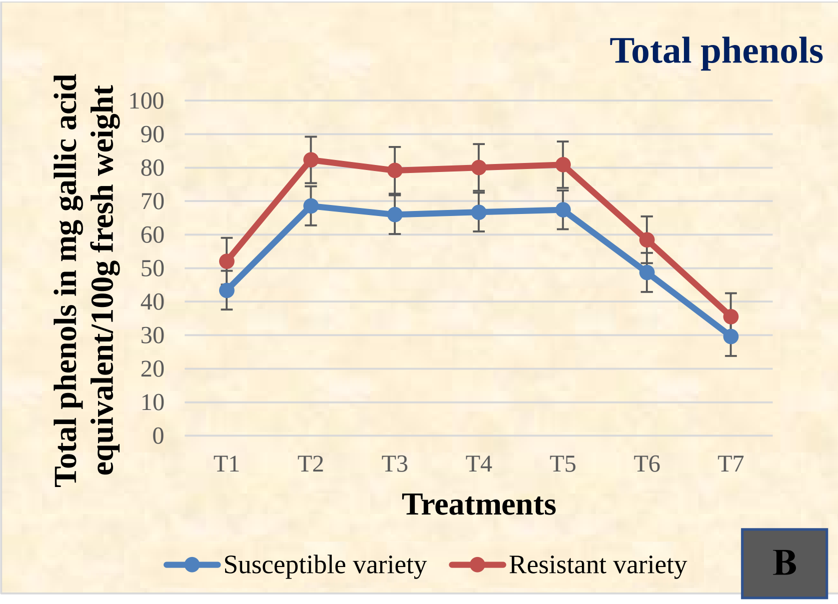




**Figure 2b.** Status of biochemicals incited by bacterial bio-agents on *Meloidogyne incognita*-infested tomato plants (A) catalase (CAT) activity and (B) total phenols content. **Note**: $T_1$ = *M. incognita* + carbofuran 3G, $T_2$ = *M. incognita* + *B. pumilus*, $T_3$ = *M. incognita* + *B. megaterium*, $T_4$ = *M. incognita* + *B. subtilis*, $T_5$ = *M. incognita* + *P. fluorescens*, $T_6$ = *M. incognita* only and $T_7$ = Healthy control.