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# Evaluation of bacterial formulations as potential biocontrol agents against the southern root-knot nematode, *Meloidogyne incognita*

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## Abstract

**Background:** Bacterial bioagents, *Pseudomonas fluorescens*, *P. putida*, *Bacillus amyloliquefaciens*, and *B. megaterium*, have management potential against root-knot nematode (RKN), *Meloidogyne incognita*, in bottle gourd.

**Results:** Field and laboratory experiments were conducted to evaluate the effect of bacterial bioagents *Bacillus megaterium* 1% WP ( $2 \times 10^6$  CFU/g), *B. amyloliquefaciens* 1% WP ( $2 \times 10^8$  CFU/g), *Pseudomonas putida* 1% WP ( $2 \times 10^8$  CFU/g), *P. fluorescens* 1% WP ( $2 \times 10^9$  CFU/g) on egg hatching and juvenile mortality of root-knot nematode, *M. incognita*. All the bacterial species inhibited the egg hatching in *M. incognita* and caused juvenile mortality. The lowest mean egg hatching in 120 h. after treatment was observed in *P. putida* (20.9% mean egg hatching), followed by *P. fluorescens* (21.1%), *B. amyloliquefaciens* (23.7%), and *B. megaterium* (24.7%) at 4% concentration of the formulated product against (47%) egg hatching in control. The juvenile mortality was found highest (57.1% mean mortality) in *P. fluorescens* in 120 h. of exposure, followed by *P. putida* (56.13%), *B. megaterium* (54.46%), and *B. amyloliquefaciens* (53.13%) at 4% concentration against 0.46 mean juvenile mortality in control, where distilled water was used. Under field conditions, the bottle gourd seeds that were treated either with *B. amyloliquefaciens*, *B. megaterium*, *P. fluorescens*, or *P. putida* at 10 g/kg seed along with the application of neem cake (1t/ha) significantly resulted in reduced root gall index and the number of nematode juveniles in soil and roots than the non-treated control.

**Conclusion:** This study revealed that the tested bacterial bioagents, namely *B. amyloliquefaciens*, *B. megaterium*, *P. fluorescens*, and *P. putida*, showed the potential for controlling of root knot nematode (RKN) in the laboratory as well as in field conditions in bottle gourd.

**Keywords:** Biological control, Bacterial formulations, Bottle gourd, Neem cake, *Meloidogyne incognita*, *Pseudomonas*, *Bacillus*

## Background

Bottle gourd [*Lagenaria siceraria* (Mol.)] is one of the oldest and the most important cucurbitaceous vegetables grown in tropical and subtropical parts of the world (Purseglove, 1974) and considered indigenous

to Africa (Heiser, 1979). Bottle gourd is widely grown in warmer areas of India, and it is attacked by many pests and diseases including nematodes resulting in significant yield losses. Among soil pathogens, root-knot nematodes (RKN), *Meloidogyne* spp., are considered the most important nematode pests that cause great economic losses to horticultural and field crops, as they infect almost all the main crops of the world (Oka et al. 2000). The Southern root-knot nematode, *Meloidogyne incognita* (Kofoid and White 1919) Chitwood, is a major

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constraint in cucurbitaceous crop production including bottle gourd. Due to its minuscule size and farmers' lack of knowledge, the damage symptoms are confused with mineral deficiency symptoms in plants. These pests are frequently disregarded from the plant protection aspect, resulting in inconvenient agricultural losses. Plant-parasitic nematodes generally inhabit the soil and attack the underground parts of plants, so their successful and sustainable management becomes very complex (Stirling, 2014). In India, the estimated annual losses due to plant-parasitic nematodes were estimated to be about 21.3% (1.58 billion USD) (Kumar et al. 2020). Economic losses in bottle gourd due to root-knot nematodes ranged between 21 and 23% (Gowda *et. al.*, 2017). To avoid these losses, various nematicides have long been used, but due to their negative impact on the environment and their ineffectiveness after prolonged use, there are ongoing efforts to look for safer and eco-friendly control methods.

Biological control is one of the alternative management systems which includes the use of living microorganisms to suppress the population of the pest. Among the biological control agents that have been assessed against nematodes are antagonistic bacteria, nematophagous fungi, and yeasts (Forghani and Hajihassani, 2020).

There have been reports of rhizobacteria being effective in improving plant growth and affecting nematode growth and reproduction through different mechanisms involving the production of plant growth hormones, enhancing nitrogen-fixing ability and mineral availability in soil (Backer et al. 2018), and producing metabolites and enzymes that act directly against nematodes. As bacteria are the most abundant microorganisms in the root zone, their presence can significantly modify the rhizosphere environment and affect directly or indirectly the nematode or the host–parasite relationship. A good number of bacterial biocontrol agents has been identified for their nematicidal action on RKN and has been commercially adapted as promising sources of biopesticides (Migunova and Sasanelli, 2021). The application of *Pseudomonas fluorescens* has been found effective in reducing RKN, *M. incognita*, parameters at different concentrations when applied either as seedling root dip treatment or soil drench around the tomato plants (Sonkar et al. 2018). According to Nyodu and Das (2020), *P. fluorescens* and *Bacillus subtilis* provided comparatively better plant growth with minimum gall formation in the root system and lessened the final nematode population in tomatoes (var. Pusa Ruby). Based on these and various other past demonstrations on the effects of biocontrol agents, the present study was designed to evaluate rhizosphere bacteria, *Bacillus amyloliquefaciens*, *B. megaterium*, *P. fluorescens*, and *P. putida* against *M. incognita*, under laboratory and field conditions in bottle gourd.

## Methods

The studies were carried out in the Nematology laboratory, and Entomology farm, Department of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, H.P., India, during the year 2018–2020. Effect of different bacterial formulations on egg hatching and juvenile mortality of *M. incognita* was studied in cavity blocks under the laboratory conditions. The field evaluation of bacterial bioagents against *M. incognita* in bottle gourd cultivar Punjab Round was done in the experimental farm of Department of Entomology situated at 1262 m above mean sea level (30°51.607 ' North latitude and 77°09.951 ' East longitude).

### Bacterial formulations

The bacterial formulations, *B. megaterium* 1% WP ( $2 \times 10^6$  CFU/g), *B. amyloliquefaciens* 1% WP ( $2 \times 10^8$  CFU/g), *P. putida* 1% WP ( $2 \times 10^8$  CFU/g), *P. fluorescens* 1% WP ( $2 \times 10^9$  CFU/g), used in the study were procured from Division of Entomology and Nematology, Indian Institute of Horticultural Research (IIHR), Hessarghata Lake P.O. Bangalore 560 089, India.

### Raising of pure culture of *M. incognita*

A single egg mass of *M. incognita* was isolated from nematode culture on brinjal plants kept in a glasshouse at 23–28 °C and a minimum of 50% RH and was placed in a Petri dish filled with distilled water. Thus hatched second stage juveniles from a single egg mass were inoculated on separate brinjal seedlings grown in 500 g soil capacity pots under aseptic conditions. The seedlings were allowed to grow and were kept alive until the culture was used for testing. This nematode culture was used in additional experiments.

### Laboratory experiment

#### Extraction of *M. incognita* egg masses from infected roots

The inoculated brinjal seedlings' nematode-infected roots were carefully watered and uprooted. The roots were separated from their shoots and thoroughly washed under running water. For the extraction of egg masses, the galled roots were kept in a plastic tube filled with water. Fresh, even-sized egg masses were carefully picked with forceps and placed in Petri dishes containing distilled water for juvenile hatching.

#### Extraction of *M. incognita* juveniles from egg masses

The Petri plates containing egg masses were kept at room temperature for egg hatching. The hatched juvenile suspension was concentrated in such a way that 1 ml suspension contained 100 J2 individuals. This concentrated freshly hatched juvenile suspension was used for further studies.

### Effect of bacterial formulations on *M. incognita* egg hatching

Effects of four commercial formulations, *P. fluorescens*, *P. putida*, *B. amyloliquifaciens*, and *B. megaterium*, on *M. incognita* egg hatching were studied under laboratory conditions. Three concentrations of each formulation, i.e., 4.0, 2.0, and 1.0% (w/w basis), were evaluated for their effect on egg hatching of *M. incognita* in cavity blocks. Three different concentrations were prepared for each of the bioagents by using 100 ml sterilized distilled water. A spatula having 4gm of formulated bioagent was added in 100 ml distilled water to prepare 4% concentration and further diluted to prepare 2 and 1% concentrations. A two ml of each bacterial suspension was added to each cavity block. Uniform-sized egg masses were hand-picked carefully from the galls with the help of forceps. Five egg masses were transferred to each cavity block containing a required concentration of bioagents and were kept at room temperature. Each treatment was replicated thrice. The observations on egg hatching were recorded at 24-, 48-, 72-, 96- and 120-h intervals. After 120 h., egg masses were transferred to sterile distilled water and unhatched eggs were counted for each treatment.

### Effect of bacterial formulations on *M. incognita* juveniles

All the 3 concentrations (4.0, 2.0, and 1.0%) of each bacterial formulation used against *M. incognita* eggs were also evaluated for their effect on juveniles in cavity blocks under laboratory conditions. Double strength suspensions of each bacterial formulation were prepared in sterile distilled water. After adding 2 ml of each suspension, the concentrations became half i.e., 4.0, 2.0, and 1.0%. Two ml of each suspension was placed in separate cavity blocks. A concentrated 2 ml of juvenile water suspension was added to each cavity block containing required bacterial concentrations. There were 3 replications for each treatment. The cavity blocks were arranged on a laboratory table, and the data on juvenile mortality were recorded at 24-h intervals up to 120 h. of exposure. Juveniles were considered dead if they didn't move when probed with a fine needle and their body become straight. At the end of the experiment, both dead and alive juveniles were counted and percentage mortalities were calculated.

### Field experiment

#### Field evaluation of bacterial formulations against *M. incognita* in bottle gourd

As cucurbitaceous vegetables are highly prone to phytoparasitic nematodes (especially RKN), an identified nematode-sick field (Average initial nematode population was 309.1 J2s per 200 cc soil) previously planted with tomato crop in the Entomology farm of the University

was selected for this experiment. Thirty-three beds, each measuring  $2 \times 1$  m ( $2 \text{ m}^2$ ), were prepared in the selected field. Various field preparation operations were followed as per the package of practices recommended by the university. During both the years, the seeds of Punjab round variety of bottle gourd were treated with different bacterial formulations as (per the treatments) and sown in the field during the first fortnight of June (2018 and 2019). In all, 11 treatments and 3 replications were divided and randomized in the selected field. The treatments evaluated were: T1—Neem cake@1 t/ha; T2—*P. putida*@10 g/kg of seed; T3—*B. megaterium*@0 g/kg of seed; T4—*B. amyloliquifaciens*@10 g/kg of seed; T5—*P. fluorescens*@10 g/kg of seed; T6—Neem cake@1 t/ha + *P. putida*@10 g/kg of seed; T7—Neem cake@1 t/ha + *B. megaterium*@10 g/kg of seed; T8—Neem cake@1 t/ha + *B. amyloliquifaciens*@10 g/kg of seed; T9—Neem cake@1 t/ha + *P. fluorescens*@10 g/kg of seed; T10—Carbofuran@1.0 kg a.i./ha, and T11—Control (untreated check).

Standard cultural practices including irrigation, weeding, hoeing, and thinning as recommended in the university package of practices for vegetable crops were followed throughout the experiment. The experiment was terminated in the second fortnight of October when cropping ceased.

### Statistical analysis

Laboratory data obtained were subjected to angular transformation before analyzing the same by using CRD (Completely Randomized Design). The inhibition of the egg hatching was calculated using the formula:  $I (\%) = (C - T) / C \times 100$  (where  $I$ —the inhibition of the egg hatching,  $T$ —number of eggs hatched or number of juveniles in suspension in treatment,  $C$ —number of eggs hatched or number of juveniles in suspension in the control). Data obtained from the field experiment were analyzed by using RBD through OPSTAT computer program (Sheoran et al. 1998), and the treatments were compared through critical difference (CD). Analysis of variance was done as per the method suggested by Gomez and Gomez (1984).

### Soil microbial status

Soil samples were collected from plant rhizosphere and screened through 2-mm sieve. The serial dilution technique was employed for isolation and identification of viable bacteria and preparing the media for desired microflora. The autoclaved and cooled ( $45^\circ\text{C}$ ) medium was poured into sterile Petri plates. Then medium was allowed to solidify. One gram of sieved (2 mm) soil was added to 9 ml sterile water blank and shook it for 15–20 min. prepared serial dilutions  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ ,

$10^6$ ,  $10^7$ , and  $10^8$ . One milliliter of aliquots was added over cooled and solidified medium in Petri plates. Plates were rotated for uniform distribution of spores and incubated at 28 °C for 3–5 days.

## Results

### Efficacy of microbial formulations against *M. incognita* (eggs and juveniles) under laboratory conditions

#### Egg hatching

All the concentrations of bioagents, viz. *B. amyloliquifaciens*, *B. megaterium*, *P. putida*, and *P. fluorescens*, caused significant reductions in *M. incognita* egg hatching than the control. Mean percentage egg hatching was the lowest (20.9%) in *P. putida* at 4% concentration (Fig. 1). Egg hatching significantly decreased with the increase in bioagent concentrations and exposure periods from 24 to 120 h. Egg hatching remained higher at lower concentrations and exposure periods.

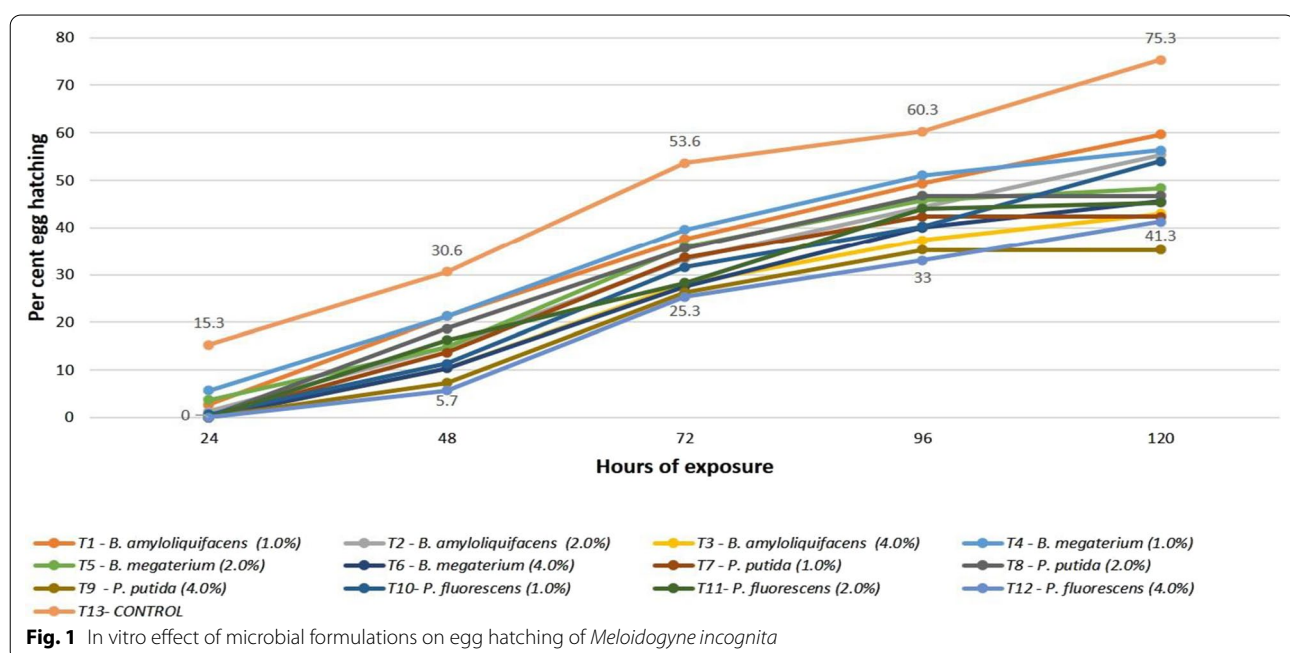
#### Juvenile mortality

All the concentrations of bioagents, viz. *B. amyloliquifaciens*, *B. megaterium*, *P. putida*, and *P. fluorescens*, caused significant mortalities of *M. incognita* second-stage juveniles than the control. Maximum mean juvenile mortality, i.e., 57.1%, was recorded in *P. fluorescens* at 4% concentration, followed by *P. putida*, *B. megaterium*, and *B. amyloliquifaciens* (Fig. 2). Juvenile mortality was significantly increased with the increase in exposure period from 24 to 120 h. Interaction of concentrations and exposure periods was found significant. The highest mortality

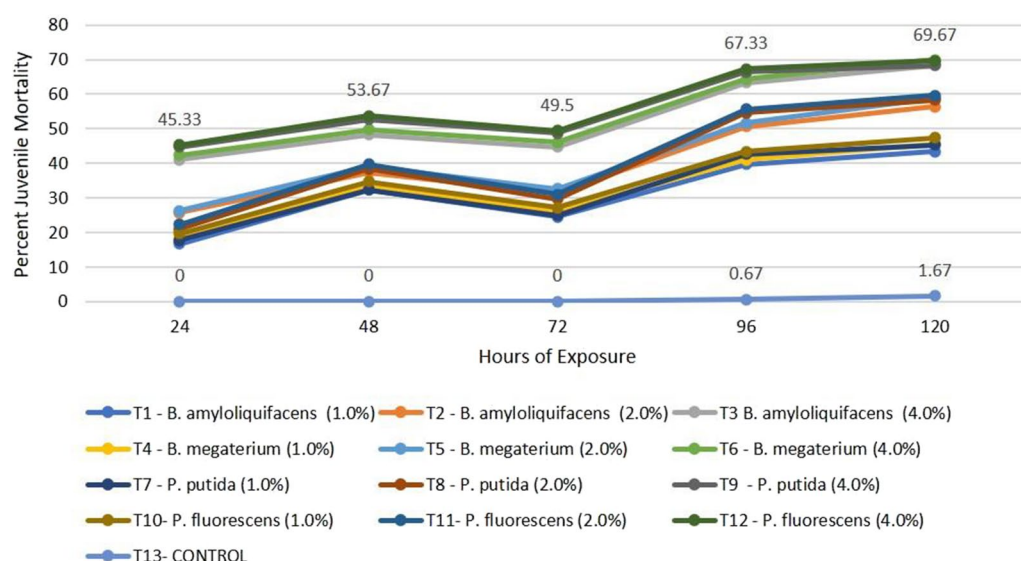
was recorded in *P. fluorescens* at 4% concentration at 120 h. of exposure period, i.e., 69.67%. Juvenile mortality remained lower at lower concentrations and exposure periods.

### Efficacy of microbial formulations against *M. incognita* under field conditions

Lower nematode populations, galling indices, and higher fruit yields were recorded in treatments, where bottle gourd seeds were treated with microbial formulations than the control. The application of neem cake increased the efficacy of microbial bioagents resulting in further reductions in nematode population, galling index, and increase in yield. The pooled data of 2018 and 2019 revealed significant reductions in soil and root populations of RKN in bottle gourd in treatment T9, where bottle gourd seeds were treated with *P. fluorescens* along with field application of neem cake@1 t/ha, which was followed by treatments involving *P. putida* and *B. megaterium* for seed treatment along with neem cake. The neem cake application though resulted in a lower RKN population in soil and roots, it was found statistically at par with control. The neem cake application though resulted in a lower root-knot nematode population in soil and roots, it was found statistically at par with control. The treatments with microbial formulations increased the fruit yield of bottle gourd from 3.61 to 21.46%, with the maximum increase in treatment involving seed treatment with *P. fluorescens* along with field application of neem cake, followed by treatment involving seed treatment with *B.*







**Fig. 2** In vitro effect of microbial formulations on second stage juveniles of *Meloidogyne incognita*

*megaterium* along with neem cake and *P. putida* along with neem cake.

### Final nematode population

This study revealed that during both years, bottle gourd plots with bacterial treatments resulted in lower soil populations of *M. incognita* J<sub>2</sub> (Table 1; Fig. 3). The final soil population of RKN juveniles ranged from 124.5 to 215.0 J<sub>2</sub> per 200 gm of soil against 332.5 juveniles per 200 gm of soil in untreated check. The seed treatment with *P.*

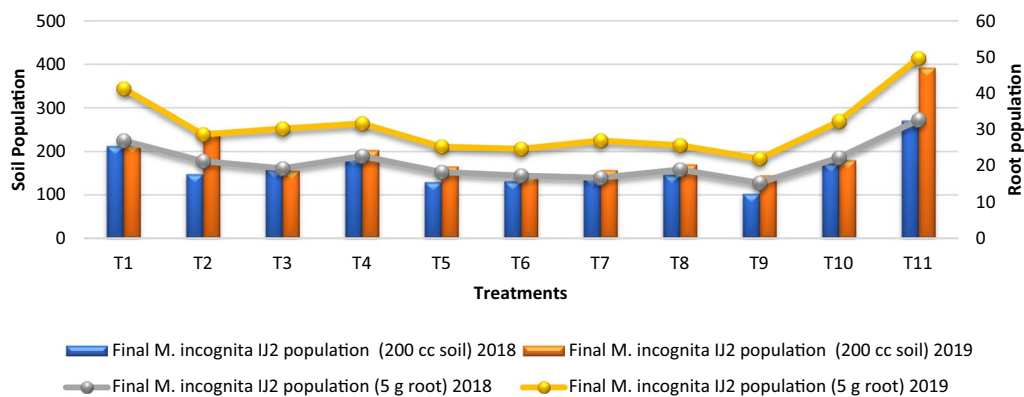
*fluorescens* and neem cake (T<sub>9</sub>) harbored the lowest RKN soil population of, i.e., 124.5 juveniles per 200 cc soil which was also found at par with J<sub>2</sub> population in other treated plots.

The mean J<sub>2</sub> population in T<sub>1</sub> (Neem cake@1t/ha) and T<sub>2</sub> (seed treatment with *P. putida*@10 g/kg of seed) also showed statistical similarity with J<sub>2</sub> population in untreated plots. All the treatments, except T<sub>1</sub> (Neem cake@1t/ha), resulted in significant lower *M. incognita* J<sub>2</sub> populations in bottle gourd roots than the untreated

**Table 1** Efficacy of different microbial formulations against root-knot nematode (average initial nematode population = 309.1 J<sub>2</sub>s per 200 cc soil) in bottle gourd (Pooled for 2018–2019)

Treatment	Final <i>M. incognita</i> J <sub>2</sub> population		Root gall index	Yield (t/ha)	% Increase in yield over control
	200 cc soil	5 g root			
T1—Neem cake@1 t/ha	215.0 (14.60) <sup>a</sup>	34.2(5.82)	2.83	14.39	3.61
T2— <i>P. putida</i> @10 g/kg of seed	198.3 (13.62)	25.0(5.00)	2.41	15.23	8.93
T3— <i>B. megaterium</i> @10 g/kg of seed	157.3 (12.54)	24.8(4.98)	2.31	16.12	13.96
T4— <i>B. amyloquifaciens</i> @10 g/kg of seed	195.0 (13.95)	27.2(5.20)	2.53	15.50	10.51
T5— <i>P. fluorescens</i> @10 g/kg of seed	148.2 (12.14)	21.8(4.65)	1.98	16.05	13.58
T6—Neem cake@1 t/ha + <i>P. putida</i> @10 g/kg of seed	130.0 (11.73)	21.0 (4.56)	1.83	17.33	19.97
T7—Neem cake@1 t/ha + <i>B. megaterium</i> @10 g/kg of seed	148.8 (12.15)	21.8(4.66)	2.03	17.56	21.01
T8—Neem cake@1 t/ha + <i>B. amyloquifaciens</i> @10 g/kg of seed	158.7 (12.47)	22.3 (4.73)	2.24	16.51	15.99
T9—Neem cake@1 t/ha + <i>P. fluorescens</i> @10 g/kg of seed	124.5(11.14)	18.7(4.31)	1.68	17.66	21.46
T10—Carbofuran@1.0 kg a.i./ha	177.0(13.29)	27.3(5.23)	2.30	17.08	18.79
T11—control (untreated check)	332.5 (17.87)	41.2 (6.41)	3.42	13.87	—
CD ( <i>P</i> = 0.05)	3.59	0.70	0.76	2.61	—

<sup>a</sup> Figures in the parentheses are square root transformed values



**Fig. 3** Trend of root knot nematode population in roots and soil during 2018 and 2019

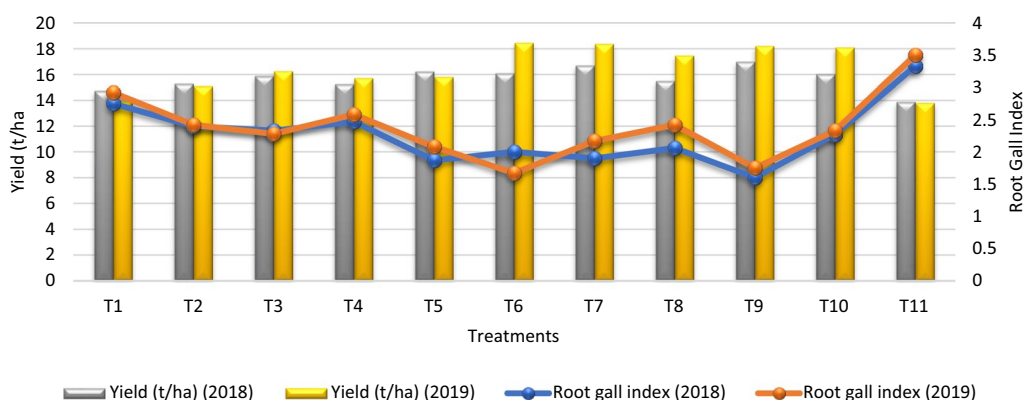
check (Table 1). Minimum root population, i.e., 18.7 J<sub>2</sub> per 5 g of the root, was recorded in T<sub>9</sub> (combined use of neem cake and seed treatment with *P. fluorescens*), which was also found at par with root population in other treatments except for T<sub>1</sub> (Neem cake alone). Minimum total *M. incognita* J<sub>2</sub> population (124.5 and 18.7 J<sub>2</sub>s in soil and root, respectively) was also recorded in T<sub>9</sub> i.e. 143.2 *M. incognita* J<sub>2</sub> per 200 cc of soil, followed by T<sub>6</sub> (T<sub>1</sub> + T<sub>2</sub>), T<sub>7</sub> (T<sub>1</sub> + T<sub>3</sub>), T<sub>3</sub> (seed treatment with *B. megaterium*@10 g/kg of seed), T<sub>5</sub> (seed treatment with *P. fluorescens*@10 g/kg of seed), T<sub>8</sub> (T<sub>1</sub> + T<sub>4</sub>), T<sub>4</sub> (seed treatment with *B. amyloliquefaciens*@10 g/kg of seed), T<sub>2</sub> (seed treatment with *P. putida*@10 g/kg of seed), and T<sub>1</sub> (Neem cake 1t/ha) against 204.3 nematodes in carbofuran (T<sub>10</sub>) and 373.5 nematodes in untreated check. The results showed that plots with the combined use of bacterial formulations with neem cake harbored a lower *M. incognita* J<sub>2</sub> population than the plots where bacterial formulations were applied without neem cake.

### Root gall index

The treatments with bacterial formulations significantly resulted in a lower galling index in bottle gourd than the control (Table 1; Fig. 4). Minimum gall index (1.68) was recorded in T<sub>9</sub> (seed treatment with *P. fluorescens*@10 g/kg seed and neem cake at 1t/ha combination was used), which was also found statistically similar to treatments with microbial formulations and treated check, but was found superior to alone application of neem cake (T<sub>1</sub>).

### Crop yield

During both years, the treatments with bacterial formulations along with neem cake application resulted in higher fruit yield than the control (Table 1; Fig. 4). Maximum fruit yield (17.66 t/ha) was recorded in T<sub>9</sub>, i.e., combination of seed treatment with *P. fluorescens*@10 g/kg of seed and application of neem cake at 1t/ha, followed by the treatments T<sub>7</sub>, T<sub>6</sub>, T<sub>8</sub>, T<sub>3</sub>, T<sub>5</sub>, T<sub>4</sub>, T<sub>2</sub>, and T<sub>1</sub>, which were statistically comparable to treated check (T<sub>11</sub>). However, the differences in yield among treatments



**Fig. 4** Trend of root galling and yield attained during 2018 and 2019

were found nonsignificant. The microbial formulations resulted in 8.93 to 21.46% increase in bottle gourd fruit than the control.

### Soil bacterial count

Application of bacterial formulations and neem cake increased the total bacterial count in the soil (Fig. 5). The highest soil bacterial count (i.e.,  $128.0 \times 10^6$  per g of soil) was recorded in T6, in which seed treatment with *P. putida* was combined with neem cake, which was also at par with T7 ( $119.66 \times 10^6$  per g of soil) and followed by T8 ( $112.66 \times 10^6$  per g of soil). The soil bacterial count in T2 was found statistically at par with the treatments T3, T7, T8, and T9, while bacterial soil population in T3 was also at statistical similarity with T4 and T9. The lowest soil bacterial count ( $61 \times 10^6$  per g of soil) was recorded in T10 (Carbofuran 3G), which was lower than the untreated check (T11).

### Discussions

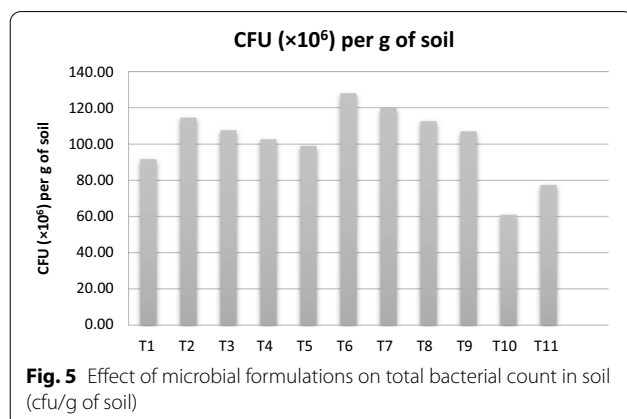
A good number of bacterial biocontrol agents was identified for their nematicidal action on RKN and becomes a promising source of biopesticides. Among the biological control agents, antagonistic bacteria, nematophagous fungi, and yeasts are promising agents against nematodes (Jayakumar, 2019). Migunova and Sasanelli (2021) presented a detailed insight about antagonistic interactions of various potential bacterial species, including the species used under the present study against plant-parasitic nematodes. The present investigations referring to bio-efficacy of various bacterial formulations (*P. fluorescens*, *P. putida*, *Bacillus amyloliquefaciens*, and *B. megaterium*) against RKN were more or less in agreement with the findings of Mane and Mhase, 2017, who reported inhibition of egg hatching and juvenile mortality of *M. incognita*.

The egg hatch inhibition and juvenile mortality of RKN might be due to nematicidal effects of volatile bacterial metabolites. Liu et al. (2013) suggested that release of an unknown compound, plantazolicin, which is structurally similar to telomerase inhibitor telomestatin, might be responsible for the nematicidal activity of *B. amyloliquefaciens*.

In unison with the present results, various studies have reported reductions in nematode populations with the application of microbial formulations under field conditions. Minimum *M. incognita* root population and galling were reported in tomato plants treated with *P. fluorescens*, which could be due to the production of phytohormones that indirectly increased the overall root growth (Noureldeen et al. 2021). Radwan et al. (2012) also reported the inhibition of *M. incognita* by *P. putida* and *B. megaterium* in both soil and roots with reduced galling in tomatoes. Reduction in final nematode population and root gall index through the application of neem leaves was recorded in bottle gourd (Singh and Patel, 2015). According to Siddique et al. (2001), the plant growth-promoting rhizobacteria reduced galling and egg masses on the roots of tomatoes caused by RKN, resulting in enhanced production. The inference of Weller et al. (2002) also suggested that some species of *Pseudomonas* bacteria were aggressive colonizers of plant rhizosphere with a broad-spectrum antagonistic activity against plant pathogens like nematodes.

The combination of neem cake with bacterial treatments further enhanced the soil bacterial count. The increase in soil microbial count with the application of nematode antagonistic bacteria also reported by other workers. Mane and Mhase (2017) observed that treatments with *P. fluorescens* resulted in  $1 \times 10^9$  cfu/cc of soil. Qiao et al. (2017) obtained similar results in case of *Bacillus* spp., when applied to the tomato rhizosphere. Holajjer et al. (2018) identified DAPG-producing isolates of *P. putida* as an antagonist to *M. arenaria* and caused significant high mortality after an exposure period of 72 h at 100% concentration and recorded maximum reduction (51.30%) in root galling with the combination of seed treatment and soil application of *P. putida* DAPG1.

The results of the present study inferred that bacterial bioagents *B. amyloliquefaciens*, *B. megaterium*, *P. fluorescens*, and *P. putida* had potential for the control of RKN in the laboratory as well as under field conditions in bottle gourd, i.e., resulted in inhibition of egg hatching and juvenile mortality of *M. incognita*. The application of neem cake further increased the efficacy of microbial agents. The seed treatment with *P. putida* and neem cake harbored the lowest RKN population of, i.e., 144.3 juveniles per 200 cc soil. The treatments with bacterial formulations along with neem cake application resulted in



higher fruit yield (17.66 t/ha). Abd El-Aal et al. (2021) recommended the use of organic modifications with productive rhizobacterial strains to enhance the effect of the bacteria against *M. incognita*. Drenching with *B. subtilis* @10 ml/liter at transplanting resulted about 19.2, 58.1, and 43.1% improvement in shoot height, dry weight of shoot and root, respectively, whereas 62.2 and 62.3% reductions were recorded in galls and egg masses (Dash et al. 2015). Reduction in root galling in tomato due to treatment of *B. pumilis* isolate ZHA90 under greenhouse conditions was reported by Cetintas et al. (2018). Sreegyathri et al. (2018) reported a higher fruit yield and reduction in root gall index in bitter melon with *Pseudomonas* (Pf 1) and *Bacillus* (PG 12) isolates at Coimbatore, Tamil Nadu. Nyodu and Das (2020) also reported minimum galls and egg masses per root system and final nematode population and maximum plant growth parameters in tomato (var. Pusa Ruby) with *P. fluorescens* @20 g/kg of seed.

In extension to Terefe et al. (2009), not many studies determine the role of colony-forming units of bacterial concentrations to be applied for the control of plant parasitic nematodes. However, Aballay et al. (2021) contradicts the previous statements indicating that higher cfu formulations do not enhance the degree of nematode control, emphasizing on the use of lower concentrations in integrated management modules. On the other hand, the lowest bacterial counts obtained from carbofuran treated soil may be attributed to the harmful effect of the chemical (Carbofuran 3G) on bacteria.

## Conclusions

All the bioagents, viz. *B. amyloquefaciens*, *B. megaterium*, *P. fluorescens*, and *P. putida*, inhibited egg hatching and caused juvenile mortality of *M. incognita* under laboratory conditions, whereas, under field conditions, all the bioagents caused a reduction in root and soil population, root gall index, and found to enhance the fruit yield in bottle gourd. These formulations outperformed in the presence of neem-based soil amendments against *M. incognita* in bottle gourd providing an insight into the pathogenic relationship between the biocontrol agents and the pathogen. The present study proposed long-term research to improve the pathogenic potential of biocontrol agents against *M. incognita*.

## Abbreviations

CFU: Colony-forming unit; DAPG: 2,4 Diacetylphloroglucinol; IIHR: Indian Institute of Horticultural Research; J<sub>2</sub>: *M. incognita* second-stage juveniles; NHB: National Horticulture Board; RKN: Root-knot nematode; WP: Wettable powder.

## Acknowledgements

We would like to acknowledge Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India-173230, for supporting this research. We are also grateful to Professor and Head, Department of Entomology, Dr. YSPUHF, Nauni, (H.P.), India, for providing necessary facilities during experiments. The authors are thankful to the Project Coordinator (AICRP, Nematodes) for financial assistance and technical guidance for carrying out these studies and IIHR Bangalore for supplying the biocontrol agents.

## Authors' contributions

MS designed the experiment and conducted the field experiments. PR conducted the laboratory experiments along with field experiments, analyzed the data, and drafted the manuscript with inputs from all authors. HP, MS, and MS collaborated closely with PR in the whole process and writing of the manuscript. All authors read and approved the final manuscript.

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## Funding

AICRP, Nematodes (HCR-193-07).

## Availability of data and materials

All data generated or analyzed during this study are included in this article.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

We, the authors do not have any competing interests.

Received: 27 December 2021 Accepted: 17 March 2022

Published online: 28 March 2022

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