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ORIGINAL ARTICLE

Exploitation of microbes for enhancing bacoside content and reduction of *Meloidogyne incognita* infestation in *Bacopa monnieri L*

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Abstract Despite the vast exploration of rhizospheric microbial wealth for crop yield enhancement, knowledge about the efficacy of microbial agents as biocontrol weapons against root-knot disease is scarce, especially in medicinal plants, viz., *Bacopa monnieri*. In the present investigation, rhizospheric microbes, viz., *Bacillus megaterium*, *Glomus intraradices*, *Trichoderma harzianum* ThU, and their combinations were evaluated for the management of *Meloidogyne incognita* (Kofoid and White) Chitwood and bacoside content enhancement in *B. monnieri* var CIM-Jagriti. A novel validated method Fourier transform near infrared was used for rapid estimation of total bacoside content. A significant reduction (2.75-fold) in root-knot indices was observed in the combined treatment of *B. megaterium* and *T. harzianum* ThU in comparison to untreated control plants. The same treatment also showed significant enhancement (1.40-fold) in total bacoside contents (plant active molecule) content using Fourier transform near-infrared (FT-NIR) method that analyses samples rapidly in an hour without solvent usage and provides ample scope for natural product studies.

Keywords Asparginase activity · FT-NIR · Indole acetic acid · Larvicidal · *Meloidogyne incognita* · Root-knot index

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Introduction

Plant growth-promoting rhizobacteria (PGPR) play significant role/s in improving the growth and yield of agricultural crops (Vacheron et al. 2013) by imparting beneficial effects on their host plants. PGPR influence the plant physiology to a greater extent through a widespread array of mechanisms, viz., N_2 fixation, increased nutrient uptake, and production of secondary metabolites, viz., IAA, enzymes, siderophores (Kloepper 2004; Ongena et al. 2007) effecting the plant, and soil health (Barriuso et al. 2008). Since these microbes are bestowed with several beneficial effects, PGPR are widely acclaimed in agricultural practices for enhancement of crop yield attributes.

Bacopa monnieri (L.) Pennell (Brahmi), a therapeutically important perennial herb used in the treatment of insomnia, insanity, depression, psychosis, epilepsy, asthma, and mental disorders (Bammidi et al. 2011), is a vulnerable host for *Meloidogyne incognita* (Kofoid and White) Chitwood (Pandey et al. 2003). Chemical nematicides are effective against these plant pests, yet their associated environmental and health hazards have restricted for their use (Food and Environment Protection act, 1985, Part III). The potential risk to nontarget organisms has stressed for the search of novel and safer alternatives for nematode management. In this regard, biological control can offer safer and cheaper alternatives, and our group is seriously involved in the characterization of such potent microbes (Pandey et al. 2011; Saikia et al. 2013). Further, with the current awareness and inclination of general masses towards natural and chemical-free products, there exists a serious need for quick and efficient estimation of

bioactive molecules, viz., bacosides, bacogenins, and hepestine (Anand et al. 2011). The study evaluates a novel Fourier transform near-infrared (FT-NIR) spectroscopy method for the quantitative and qualitative analysis of bacoside content. This method is faster than the traditionally used chromatographic methods and also does not have the problem related to sample preparation, use of solvents, etc., which are quite tedious, expensive, as well as hazardous to both environment and the human beings (Alessandrini et al. 2008).

Therefore, the present experiment was designed to envisage the influence of selected rhizospheric microbial inoculants, viz., *Bacillus megaterium* (KC978881), *T. harzianum* ThU, and *Glomus intraradices* alone and in combinations (earlier characterized for their plant beneficial traits) for their potentials against *M. incognita* and for enhancement of plant growth/yield attributes in *B. monnieri*.

Materials and methods

Plant materials

Plant runners of *B. monnieri* cv. 'CIM Jagriti' were procured from the National Germplasm Repository of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. The runners of uniform length (6–8 cm) bearing at least three nodes along with roots were used for transferring to the pots (with sterilized soil in a greenhouse under natural light conditions) as well as to the fields.

Bioinoculants and growth conditions

B. megaterium (KC978881) is regularly maintained in the Microbial Technology and Nematology Department CSIR-CIMAP, Lucknow. A single pure colony was inoculated in nutrient broth (NB, Himedia) and incubated at 28±2 °C under shaking conditions. After 48 h, the culture was centrifuged at 6,000×*g* for 5 min. The supernatant was discarded, the pellet containing cells were suspended in 0.9 % saline, and the cell density was adjusted to 1.0×10^8 CFU mL^{-1} using spectrophotometer (Spectra Max, Molecular Devices) at 610 nm.

T. harzianum ThU was subcultured on potato dextrose agar (PDA, Himedia) and was incubated at 28±1 °C for 96 h. After proper incubation, the mycelial mat with conidia was homogenized and suspended in 500 mL of 0.1 M phosphate buffer (K_2HPO_4; KH_2PO_4) fixing colony-forming units (CFU) mL^{-1} density at 1.2×10^6. *G. intraradices* inoculum was propagated on maize roots (*Zea mays*) for 10 weeks in a 1:1 (*v/v*) mixture of sterilized sand and soil (5 kg) of low phosphorus content (7.5 kg ha^{-1}) and subsequently left to shade-dry for 2 weeks. The inoculum potential of *G. intraradices* used in the present experiment consisted of soil containing spores (8–10 spores g^{-1}) with colonized roots of maize (Singh et al. 2012).

Nematode inoculum preparation

The inoculum for *M. incognita* was obtained from pure cultures maintained on brinjal (*Solanum melongena* L.), grown in sterilized loamy-sand soil (CSIR-CIMAP). The plants were removed with intact root systems and dipped in water for removing adhering soil and dirt particles. Mature egg masses were handpicked using forceps and sterilized with 0.5 % sodium hypochlorite, to dissolve the gelatinous matrix, and then rinsed with sterile water. The eggs obtained were incubated for 3–5 days using modified Baermann funnel method (Southey 1986) to obtain second-stage juveniles (J2). This nematode inoculum was used for in vitro (nematicidal) and bioassay experiments.

Preparation of microbial metabolites and nematicidal assay

To assess the effect of bacterial metabolite on nematicidal activity of *M. incognita*, a single colony of *B. megaterium* was cultured in 50 mL of NB media, incubated at 28±2 °C on a mechanical shaker for 48 h. The cells were harvested by centrifugation at 6,000×*g* for 10 min followed by passing through 0.22 μ Millipore filter (Millex-GV, USA) to ensure cell-free supernatant. This was designated as a standard cell-free extract of 100 % concentration which was further diluted with sterilized distilled water to 50 and 25 %, respectively, to study their effect on *M. incognita* juveniles (Niknam and Dhawan 2001). *T. harzianum* ThU was grown in 100 mL of potato dextrose broth, pH 7.0 at 4 °C on a rotary shaker (180 rpm) at 28 °C for 96 h. After proper incubation, the mycelial mat with conidia was homogenized and suspended in 500 mL of 0.1 M phosphate buffer (K_2HPO_4; KH_2PO_4), fixing colony-forming units (CFU) mL^{-1} density at 1.2×10^6.

Freshly hatched J2s (100 J2/250 μL) in distilled water were added into each well of 24-well microtitre (TCP-24T-ST, Axygen, India) plates containing different concentrations (100, 50, and 25 %) of culture filtrates (CF) of *B. megaterium* and *T. harzianum* ThU in five replicates. The plates were incubated under humidified conditions at ambient temperature for 48 h. J2s were observed under a stereo microscope (Leica S8AP0), and percentage mortality of nematodes was recorded after 72 h of exposure. The mortality of nematodes was assessed by checking the motility and confirmed by touching the juvenile with fine needle (Cayrol et al. 1989). Each treatment was replicated five times, and sterilized distilled water served as control.

Quantitative determination of protease, asparaginase, and indole acetic acid activities by *B. megaterium* and *T. harzianum* ThU

Protease, asparaginase, and indole acetic acid (IAA) production are considered as major plant beneficial activities which were quantitatively estimated. Protease activity was ascertained according to Li et al. 2012 using azocaesin (1 %) as a substrate. Briefly, the cells were grown for 24 h in a minimal media containing 1 % gelatine, harvested by centrifugation at 6,000×*g* for 10 min at 4 °C, and the supernatant was used as the crude enzyme. For determination of protease activity, crude enzyme and azocaesin (1:4) was incubated at 30 °C for 30 min; the reaction was stopped by adding 0.6 mL of TCA (10 %), and the reaction was placed for 30 min on ice. The precipitate was removed by centrifugation at 12,000×*g* at 4 °C for 10 min. The supernatant was neutralized by 1.8 N NaOH, and the absorbance was read at 420 nm.

Asparaginase activity was assessed according to Imada et al. (1973) using L-asparagine as the substrate with some modifications. The microbes were grown in modified Czapeck Dox (MCD, Himedia) broth at 28 °C for 24 h under shaking conditions. The cells were removed by centrifugation, followed by passing through 0.22 μ Millipore filter, and the filtrate was used as the crude enzyme. Crude enzyme and L-asparagine (40 mM) were mixed in equal volumes and incubated at 37 °C for 60 min. The reaction was stopped by adding TCA (1.5 %). Nessler's reagent (0.15 mL) was added to reaction mixture (0.1 mL), and the final volume was made up to 1 mL by sterile distilled water. The reaction mixture was incubated at 20 °C for 20 min, and the absorbance was recorded at 450 nm. One unit of asparaginase was quantified by the amount of enzyme catalyzed by the formation of 1 μmol ammonia per 30 min at 37 °C.

IAA production was estimated according to Patten and Glick (1996) by raising the inoculants in tryptone soy broth (TSB, Himedia) medium for 24 h. To obtain cell-free crude extract, the broth-containing cells were centrifuged and the supernatant thus obtained was used as the crude enzyme. The reaction mixture was prepared by mixing the supernatant and Salkowski's reagent (74 mM $FeCl_3$ and 43 mL conc. H_2SO_4) in equal amount. The development of pink color intensity was quantified at 530 nm using a spectrophotometer. A standard curve with indole acetic acid was plotted to quantify the IAA (μg mL^{-1}) present in the culture filtrate.

Biocontrol treatments for glass house experiments

After 10 days of plant establishment, the plants were thinned to one runner per pot (17 cm×dia. 30 cm) containing 4.5 kg soil adopting a completely randomized design in set of five replicates. The bioinoculants *B. megaterium*, *G. intraradices,* and *T. harzianum* ThU, prepared according to aforementioned conditions, were used for assessing plant growth promotion potency and management of root-knot nematode in single and combined treatments. Table 1 describes the various treatments evaluated for glass house experiment. The treatments were maintained in triplicates in randomized block design. Sterilized vermicompost was added to all the treatments in equal volume, time to time to ensure proper nutrition. After 1 week of microbial inoculation, each pot was inoculated with 1,000 freshly hatched J2/pot. Plants were maintained in a greenhouse at 28°±6 °C and 13-h day length until the experiment was terminated 90 days after inoculation. Plant growth was determined by considering fresh and dry weight herb yield. Roots were rated for galling severity according to Krusberg and Nielsen (1958) on a scale of 0–4.

Field experiments

The experimental trails (during 2010 and 2011) were conducted at Research farm, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow (26.85° N, 80.92° E). The soil of the experimental plot is sandy loam in texture having alkaline reaction (pH 8.5, EC 0.40 dSm^{-1}). The available nitrogen (alkaline permanganate extractable, 168.00 kg ha^{-1}), potassium (neutral N ammonium acetate extractable, 107.00 kg ha^{-1}), and phosphorus (0.50 N $NaHCO_3$ extractable, 12.84 kg ha^{-1}) were determined according to Kiran and Patra (2003). For field experiment, carbofuran was added to soil at 0.0015 g a.i. per kilogram of soil 5 days before transplantation of runners of *B. monnieri*. Since under field conditions there is no control on the existing microbial diversity of the soil, the treatment (untreated uninoculated control) was eliminated. Other conditions were similar to glass house experiment. The runners were transplanted at a spacing of 30× 45 cm in beds using randomized complete block design with each treatment plot (12 m^2) consisting of six rows of six plants each. Proper irrigation was given at 3 to 4-day intervals. On maturation of the crop (90 days), the data for fresh herbage yield and the moisture content in herb were recorded. After harvesting (pot and field experiments), rhizospheric soil samples were collected for estimation of microbial population from each pot/plot. Nutrient uptake was determined by Jackson et al. (1973) based on N, P, and K concentration in dry matter samples.

M. incognita population in the infested roots was evaluated through reproduction factor (Rf), calculated by the formula, Rf=Pf/Pi, where Pf=final *M. incognita* population and Pi= initial *M. incognita* population (Saikia et al. 2013). The determination of disease incidence was based on the nematode population per root system, and it was expressed by the root-knot index (RKI). The RKI was determined according to Krusberg and Nielsen 1958 on a 0–4 scale.

Table 1 Details of different treatments for management of root-knot nematode *M. incognita* in *B. monnieri* (glass house experiment)

S. no.	Treatment	Details
1	Untreated uninoculated control	Without any microbial treatment and *M. incognita* involvement
2	Untreated inoculated control[a]	Without any microbial treatment but having *M. incognita* inoculation
3	Carbofuran[a]	Chemical nematicide along with *M. incognita* inoculation
4	BM[a]	*B. megaterium* and *M. incognita* inoculation
5	GI[a]	*G. intraradices* and *M. incognita* inoculation
6	TH[a]	*T. harzianum* ThU and *M. incognita* inoculation
7	BM+GI[a]	*B. megaterium*, *G. intraradices,* and *M. incognita* inoculation
8	BM+TH[a]	*B. megaterium*, *T. harzianum* ThU, and *M. incognita* inoculation

[a] Each pot was inoculated with 1,000 J2-juveniles of *M. incognita*

Sample preparation for bacoside content estimation

An in-house developed Fourier transform near-infrared (FT-NIR) method was used for the analysis of total bacoside in *B. monnieri*. For the screening of the samples, 2 mg of finely powdered *B. monnieri* plant samples was subdivided into 40 subsamples of 50 μg weight. Every subsample was subjected for FT-NIR analysis (eight times). After each analysis, the subsample was remixed before taking new readings, generating a total of 320 FT-NIR spectra for each sample.

Fourier transform near-infrared (FT-NIR) absorbance spectra were recorded from 10,000 to 4,000 cm^{-1} at a resolution of 2 cm^{-1} on Antaris II analyzer fitted with InGaAs detector (Thermo Fisher Scientific, USA) in diffuse reflectance mode for each sample. Each spectrum was the average of 64 scans. Background measurements were taken before every spectral measurement. The partial least squares model for the total bacoside determination was developed using TQ Analyst. The measurements were preprocessed and analyzed using TQ Analyst Software (Thermo Fisher Scientific, USA).

Statistical analysis

Analysis of variance (ANOVA) techniques were applied for the statistical analysis of data. Duncan multiple tests applicable to RCBD were performed by ASSISTAT software version 7.6 beta. Duncan's test was performed to test the significant differences among treatments. Least significant difference (LSD) was calculated at 5 % probability level (P=0.05).

Results

Nematicidal activity

Figure 1 depicts the nematicidal potentials of *B. megaterium* and *T. harzianum* ThU (P=0.05) under in vitro conditions. The inhibition of second-stage juveniles of *M. incognita* was observed with respect to distilled water as well media controls. It was observed that *B. megaterium* possesses higher nematode mortality (75.3 %) as compared to *T. harzianum* ThU after 72 h. *G. intraradices* treatment was not included in this experiment as it cannot be cultured under in vitro conditions.

Quantitative estimation of IAA, protease, and asparaginase activities

Plant beneficial activities, viz., IAA and asparaginase were found to be higher in *T. harzianum* ThU, depicting a 36.85 μg mL^{-1} IAA and 34.77 μg mL^{-1} asparaginase activity against a 30.57- and 27.26-μg mL^{-1} production of respective activities by *B. megaterium*. The protease activity, however, was higher in *B. megaterium* (58.46 μg ml^{-1}) as compared to *T. harzianum* ThU (36.85 μg ml^{-1}) (Fig. 2).

Plant growth/yield promoting (pot and field experiments)

The growth attributes such as fresh and dry herb yield are considered important parameters for assessment of microbial activities (Tables 2 and 3). The greenhouse results reveal that all the microbial inoculants significantly enhanced plant growth (fresh and dry weights), ranging from 1.06- to 1.14-fold and 1.16- to 1.30-fold in the first harvest and continued during the second harvest. The maximum increase in the herb yield was obtained in combined microbial treatments followed by single treatments as depicted in Table 2. The bioinoculants were able to support plants in case of nutrient uptake (Fig. 3a, b). The nitrogen uptake was drastically enhanced in all the treatments, whereas maximum uptake was recorded in TH+Mi treatment (1.60-fold) followed by BM+GI+Mi (1.56-fold) as compared to untreated –Mi inoculated. The uptake of nitrogen was also enhanced (1.08–1.60-fold) in all the treatments, which clearly shows that these microbial inoculants have an unambiguous role in growth promotion of plants. ANOVA results for biomass (fresh and dry) revealed significant mean differences against controls as listed in Tables 1 and 2. Means were compared using the least significant difference (LSD) at probability level P=0.05.

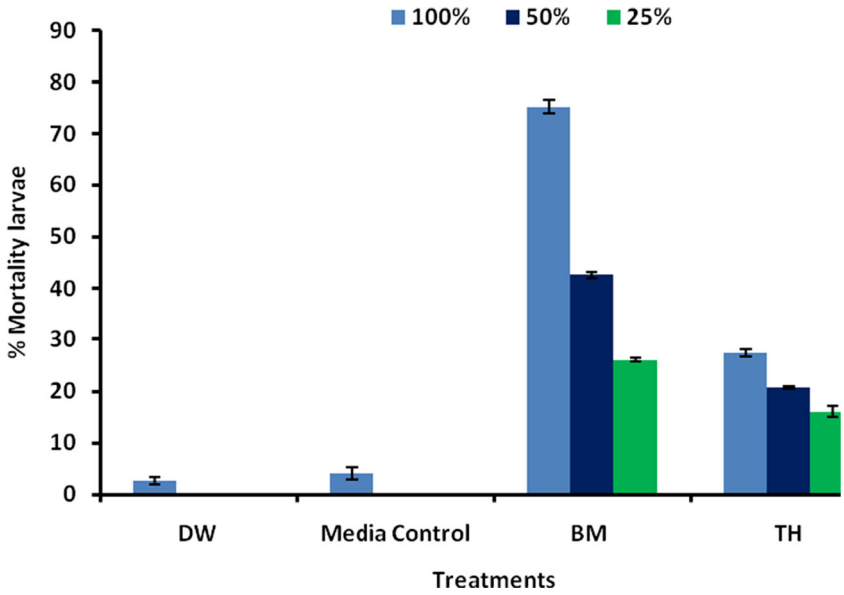

Fig. 1 In vitro nematicidal effect of different cell-free extracts of rhizospheric microbes on mortality of second-stage juveniles of *M. incognita*. Distilled water (*DW*) and media control serve as uninoculated treatments, and effect of microbial treatments is expressed in reference to these controls. Results are expressed as means of three replicates, *vertical bars* indicating standard errors of means

The same treatments were evaluated in the field conditions to evaluate the potentiality of bioagents in *B. monnieri*. The results were found to be quite similar to those of the greenhouse experiment and showed a fresh herb yield (1.14–1.55-fold) and dry herb yield (1.20–1.74-fold) increment in comparison to the uninoculated control (Table 3). The results revealed that bioinoculants have their significant role in absorption of NPK by the treated plants as compared to untreated one (Fig. 3b).

Total bacoside content

The FT-NIR spectra showed broad bands of overlapping absorption bands raised from harmonics and combinations of fundamental molecular vibrations. During the analysis of the secondary derivative spectra of the raw spectra obtained from the *Bacopa* samples, it was found that at three spectral regions, viz., 7,308–6,800 cm^{-1}, 8,720–8,120 cm^{-1}, and 4,546–4,420 cm^{-1}, the variations in absorbance values have significantly influenced by the total bacoside content in the plant powder as depicted in Fig. 4a and b. The total bacoside content values obtained from the model are given in Table 3. Microbial bioinoculants significantly increased the total bacoside contents (1.17- to 1.40-fold) as compared to untreated control (Fig. 4a, b).

Root-knot index and reproduction factor

The result of greenhouse experiment showed that the application of different microbes alone and in combination to soil considerably reduced the root-knot indices and *M. incognita* population on *B. monnieri*. All the treatments significantly reduced the root-knot index and *M. incognita* populations as compared to carbofuran treated and untreated-Mi inoculated

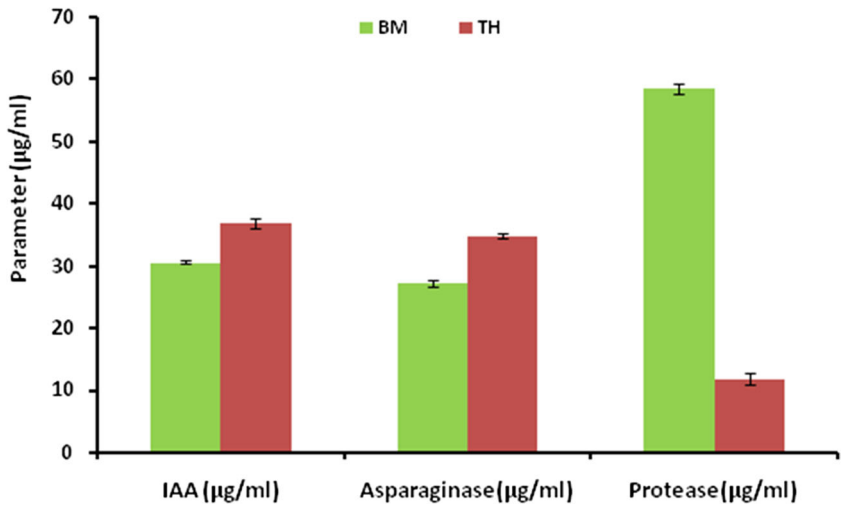

Fig. 2 Quantification of indole acetic acid, asparaginase, and protease activity in rhizospheric microbes. *BM*, *B. megaterium*; *TH*, *T. harzianum* ThU. Results are expressed as means of three replicates, *vertical bars* indicating standard errors of means

Table 2 Effect of microbial treatments on plant growth parameters of *B. monnieri* under greenhouse conditions

Treatments	First harvest		Second harvest	
	Fresh weight (g pot^{-1})	Dry weight (g pot^{-1})	Fresh weight (g pot^{-1})	Dry weight (g pot^{-1})
Untreated uninoculated control	370.6 e	68.0 d	231.3 d	41.7 c
Untreated inoculated control[a]	354.8 f	61.7 e	215.9 e	35.1 d
Carbofuran[a]	374.8 de	71.0 d	241.7 c	42.6 bc
BM[a]	405.3 a	80.0 a	257.8 a	47.0 a
GI[a]	378.7 cd	71.7 cd	243.6 bc	43.7 ab
TH[a]	385.8 bc	77.5 ab	245.0 bc	44.3 ab
BM+GI[a]	384.1 bc	77.6 ab	251.1 ab	45.4 ab
BM+TH[a]	389.7 bc	78.5 ab	253.6 a	46.2 ab
CV%	2.03	3.24	1.77	4.85
LSD (P<0.01)[**]	13.28	4.08	7.38	3.61

The averages followed by the same letter do not differ statistically between themselves

[a] Inoculated with 1,000 J2-juveniles

[**] The Duncan test at a level of 1 % of probability (ANOVA) was applied

(Fig. 5 and Table 3). It was observed that when *B. megaterium*, *T. harzianum* ThU, and *G. intraradices* applied singly, these microbes moderately reduced the root-knot index and nematode population (Fig. 5 and Table 3), whereas in combined treatments, better and significant reduction in root-knot index was observed.

Discussion

There is growing interest for the exploitation of microbial bioinoculants for the management of plant diseases and enhancement of plant growth/yield. In this regard, rhizospheric microbes may offer more environment friendly alternatives than chemical pesticides. The present study showed that under in vitro conditions, *B. megaterium* and *T. harzianum* ThU increased the mortality of *M. incognita* juveniles as compared to media and sterilized water control. These findings are in agreement of other researchers showing lethal effect of several microbes on *M. incognita* (Kiewnick and Sikora 2006; Hashem et al. 2008). Plant growth/yield promotion by rhizospheric microbes has been demonstrated in the present experiment. Plant beneficial activities, viz., IAA, asparaginase, protease, etc. are considerably important for enhanced biomass yield. Microbial bioinoculants in the present experimentation played a vital role in enhancing plant's growth/bacoside contents by stimulating nutrient uptake from the soil.

In the present experiment, different microbial consortia for the diminution of *M. incognita* infestation were employed in order to discover a biological alternative against the chemical

Table 3 Effect of microbial treatments on plant growth parameters, total bacoside content, and disease reduction in *B. monnieri* under field conditions

Treatment	Fresh weight (tons ha^{-1})	Dry weight (tons ha^{-1})	Total bacoside content (%)	Root-knot index (RKI)	Disease reduction (%)
Untreated control	7.80 d	1.71 d	0.52 d	3.66 a	0.00 e
Carbofuran	8.37 cd	1.95 cd	0.64 bc	2.00 b	45.35 c
BM	11.28 a	2.71 ab	0.73 a	1.33 b	63.66 a
GI	8.87 c	2.06 bc	0.61 c	2.33 b	36.34 d
TH	9.40 bc	2.46 ab	0.65 bc	2.33 b	36.34 d
BM+GI	10.23 b	2.52 ab	0.66 b	1.66 b	54.64 b
BM+TH	12.09 a	2.98 a	0.67 b	1.33 b	63.66 a
CV%	0.98	0.62	4.52	26.45	3.31
LSD (P<0.01)[**]	5.89	15.54	0.05	0.93	2.47

The averages followed by the same letter do not differ statistically between themselves

**The Duncan test at a level of 1 % of probability (ANOVA) was applied

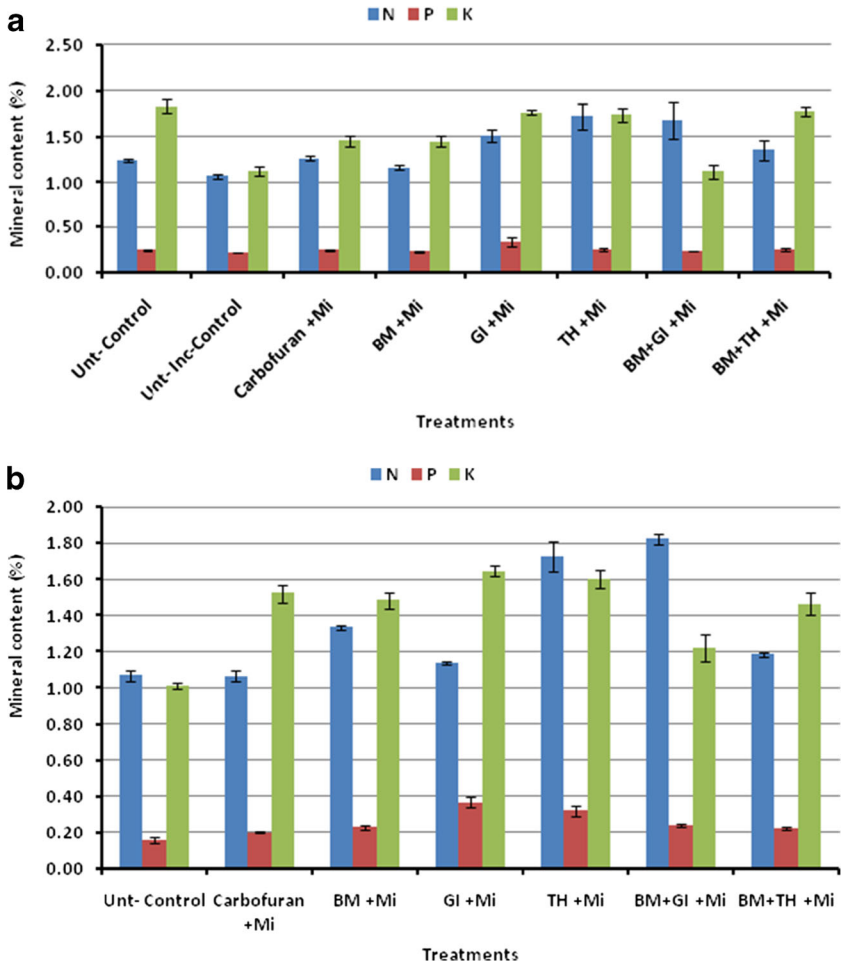

Fig. 3 a Effect of microbial treatments on mineral uptake (NPK) of *B. monnieri* under green house. *Unt-Control*, untreated uninoculated control; *Unt-Inc-Control*, untreated inoculated control; *Carbofuran+ Mi*, chemical control with *M. incognita* inoculation; *BM+ Mi*, *B. megaterium* with *M. incognita* inoculation; *GI+ Mi*, *G. intraradices* with *M. incognita* inoculation; *TH+ Mi*, *T. harzianum* ThU with *M. incognita* inoculation; *BM+ GI+ Mi*, *B. megaterium* and *G. intraradices* with *M. incognita* inoculation; and *BM+ TH+ Mi*, *B. megaterium* and *T. harzianum* ThU and with *M. incognita* inoculation. Results are expressed as means of three replicates and *vertical bars* indicate standard errors of the means. **b** Effect of microbial treatments on mineral uptake (NPK) of *B. monnieri* under field condition. *Unt-Control*, untreated uninoculated control; *Carbofuran+ Mi*, chemical control with *M. incognita* inoculation; *BM+ Mi*, *B. megaterium* with *M. incognita* inoculation; *GI+ Mi*, *G. intraradices* with *M. incognita* inoculation; *TH+ Mi*, *T. harzianum* ThU with *M. incognita* inoculation; *BM+ GI+ Mi*, *B. megaterium* and *G. intraradices* with *M. incognita* inoculation; and *BM+ TH+ Mi*, *B. megaterium* and *T. harzianum* ThU and with *M. incognita* inoculation. Results are expressed as means of three replicates, and *vertical bars* indicate standard errors of the means

nematicides. It was observed that *B. megaterium* alone and in combination with *T. harzianum* ThU and *G. intraradices* reduced root-knot index and reproduction of *M. incognita* and enhanced the fresh and dry weight of *B. monnieri*.

The application of near-infrared (NIR) measurements for medicinal and aromatic plants (MAPs) have emerged as a powerful technique towards quantitative analysis of bioactive molecules present in plant tissues (Ajayakumar et al. 2012). The results reveal that microbial association not only enhances the growth parameters in *B. monnieri* but also significantly modulates bacoside content. The observations explained appreciable correlations between functional rhizospheric microbes and growth-promoting traits, viz., fresh weight, N, P, and K uptake, and bacoside yield as major constituents. The results suggest that total bacosides content was found to be 1.40-fold, higher in comparison to control that is strongly supported by the positive correlation among enhancement of yield attributes such as fresh biomass/dry herb yield and managing the root - not nematode depicted to bands of FT-NIR.

The macronutrients nitrogen and phosphorus are the major plant nutrients responsible for plant health, influencing vegetative and reproductive phase of the plant growth, respectively. In the present study, nitrogen content in the biomass of the inoculated plants was much higher in all the treatments. The phosphorus and potassium uptake results were quite contrasting to N_2 uptake as the phosphorus content does not deviate very much with different treatments but have significant difference in comparison to control.

The present experimentation clearly indicates that in both green house and field conditions, the selected strain

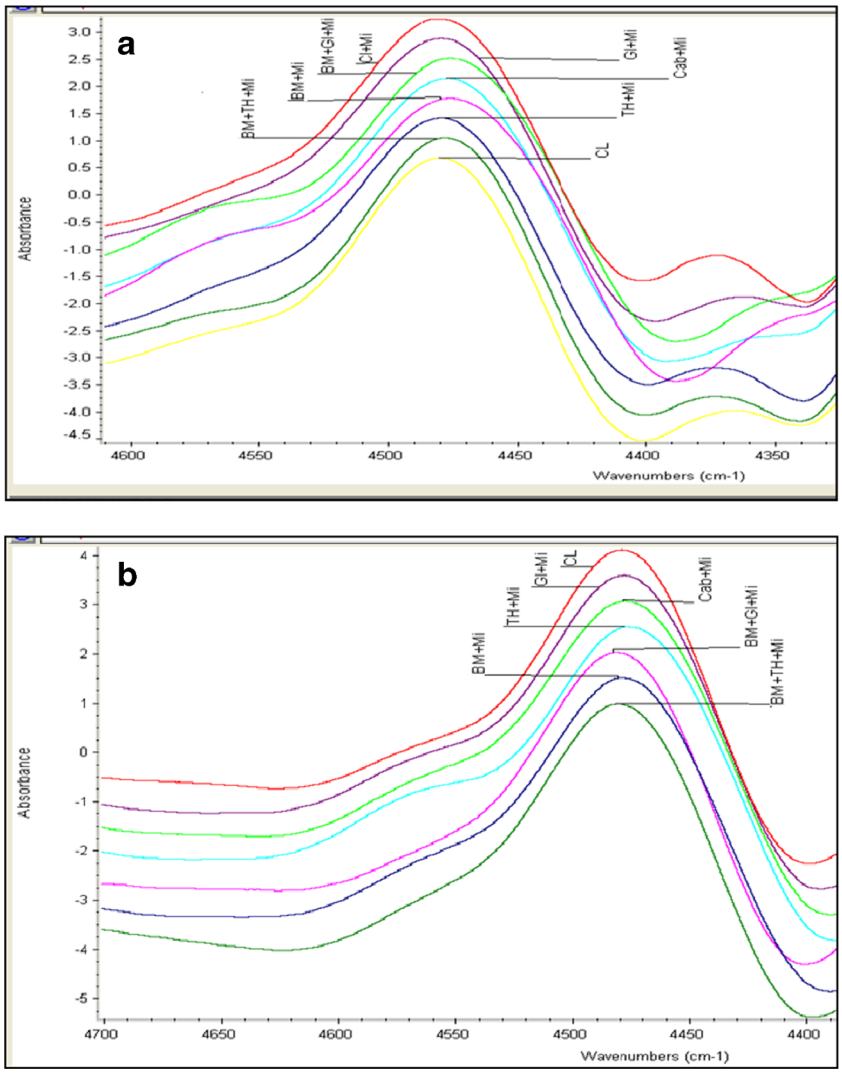

Fig. 4 **a** The pattern of FT-NIR spectra analysis of total bacoside content under green house. *CL*, untreated uninoculated control; *CL+Mi*, untreated inoculated control; *Cab+Mi*, chemical control carbofuran with *M. incognita* inoculation; *BM+Mi*, *B. megaterium* with *M. incognita* inoculation; *GI+Mi*, *G. intraradices* with *M. incognita* inoculation; *TH+Mi*, *T. harzianum* ThU with *M. incognita* inoculation; *BM+GI+Mi*, *B. megaterium* and *G. intraradices* with *M. incognita* inoculation; and *BM+TH+Mi*, *B. megaterium* and *T. harzianum* ThU with *M. incognita* inoculation. **b** The pattern of FT-NIR spectra analysis of total bacoside content under field condition. *CL*, untreated uninoculated control; *Cab+Mi*, chemical control carbofuran with *M. incognita* inoculation; *BM+Mi*, *B. megaterium* with *M. incognita* inoculation; *GI+Mi*, *G. intraradices* with *M. incognita* inoculation; *TH+Mi*, *T. harzianum* ThU with *M. incognita* inoculation; *BM+GI+Mi*, *B. megaterium* and *G. intraradices* with *M. incognita* inoculation; and *BM+TH+Mi*, *B. megaterium* and *T. harzianum* ThU with *M. incognita* inoculation

B. megaterium reduces the *M. incognita* infestation, which was achieved by the competition, hyperparatism, production of lytic enzymes, secondary metabolites, and induces systemic resistance, etc. The extracellular protease from *Bacillus* sp. plays an important in the diminution of nematodes in the soil (Barriuso et al. 2008). The mechanism of an enzyme L-asparaginase plays a major role in nitrogen metabolism and ammonia liberate from the hydrolysis of L-asparagine and is

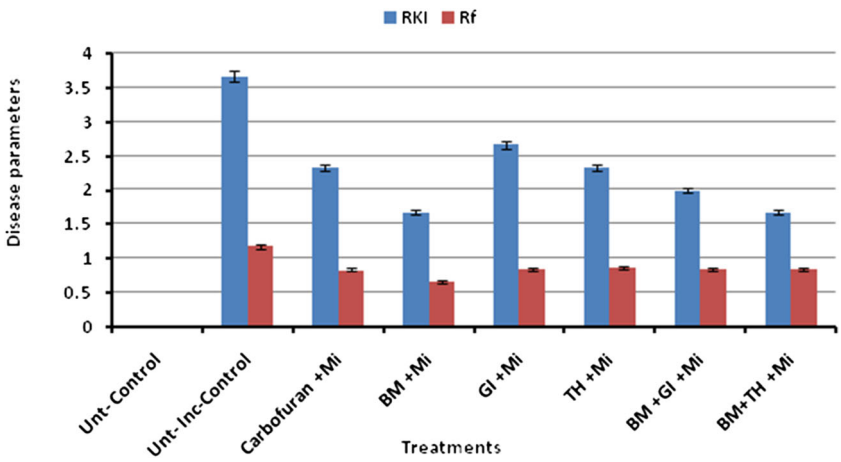

Fig. 5 Effect of microbial treatments on root-knot index (*RKI*) and reproduction factor (*RF*) in *B. monnieri* under green house. Results are expressed as means of three replicates, and *vertical bars* indicate standard errors of the means

necessary for the protein synthesis. Positive correlation of the combined treatment of *B. megaterium* with *T. harzianum* ThU has been suggested as one of the important criteria of biocontrol in the present study.

With this experiment, we conclude that consortia of native rhizospheric bacteria with *Trichoderma* are able to manage root-knot disease effectively and even performed better than chemical nematicides as they colonize root and have direct interaction making impact on plant health and nematode management. The above study will be a leading technology for development of sustainable practices towards nematode management and maintaining plant and soil health.

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Conflict of interest The authors declare that there exists no potential conflict of interest among them.

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