Rhizospheric biological weapons for growth enhancement and *Meloidogyne incognita* management in *Withania somnifera* cv. Poshita

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**HIGHLIGHTS**

- *Withania somnifera* roots are economically important for its withanolide content.
- *Meloidogyne incognita* is a prime concern causing serious root damage.
- Rhizospheric microbes promote plant growth.
- Some microbes possess nematicidal potentials; helpful in *M. incognita* management.

**ABSTRACT**

*Withania somnifera* L. (Family Solanaceae) is an angiospermic medicinal herb, well recognized for the immense therapeutic potentials of its roots containing several withanolides. *W. somnifera* is also a susceptible host for southern root knot nematode, *Meloidogyne incognita*. The nematode infestation in roots causes serious crop losses in terms of yield and chemo-pharmaceutical quality of this medicinal herb. In the present study, influence of five rhizospheric microbes, namely *Bacillus megaterium* (ATCC No. 14581), *Pseudomonas fluorescens* (ATCC No. 13525), *Trichoderma viride* (MTCC No. 167), *Paecilomyces lilacinus* (PDBC PL55) and *Glomus intraradices* was studied for the management of *M. incognita* in *W. somnifera* cv. Poshita under greenhouse conditions. All rhizospheric microbes, except *G. intraradices*, displayed nematicidal potentials via ovicidal and larvicidal actions in vitro and, resulted in significant improvement in plant growth parameters. The rate of nematode damage to *W. somnifera* was directly proportional to *M. incognita* (number of J2) population.

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2001), endocrine (Schliebs et al., 1997; Panda and Kar, 1998), cardio-vascular stimulatory activities (Mishra et al., 2000; Mohanty et al., 2004). Most of the therapeutic actions of W. somnifera are attributed to the presence of several steroidal lactones (withanolides, withaferins) and acyl steryl glycosides (sitosteroides VII–X) in its roots, leaves and berries (Bhattacharya et al., 1997; Kulkarni et al., 1998; Tohda et al., 2005).

W. somnifera is able to thrive in most of the soils under varied climatic conditions. In India, the plant is widely cultivated over an area of 10,780 ha in the sub-tropical and semi-temperate regions of Uttar Pradesh, Rajasthan and Andhra Pradesh (Kothari et al., 2003). However, against the annual requirement of nearly 7000 t for W. somnifera roots, India has an approximate production of 2000 t only (Patra et al., 2004). Due to increasing demand for its roots, growth promotion and resistance against diseases is a major obligation for W. somnifera cultivation. The infestation of root-knot nematode, Meloidogyne incognita (Kofoid & White) Chitwood is a major yield constraint and visible threat to W. somnifera cultivation (Sharma and Pandey, 2009; Gupta et al., 2004). M. incognita infec-
tion causes several biochemical changes such as alterations in the levels of amino acids (Sikora and Schuster, 2000), organic acids (Freire and Bridge, 1985) and reduced chlorophyll content (Ferraz et al., 1989) in the plants. Heavy infestation causes stunting, reduced tillering, yellowing, premature drying of leaf tips and margins, narrowing of leaf blades, delay in flowering, immature fruit drop, excessive root branching and reduction in the unit biomass yield of roots, leaves and their bioactive constituents (Kingland, 2001; Pandey et al., 2003).

India, the diversified land of crops and climate still relies mainly on synthetic nematicides viz. halogenated aliphatic hydrocarbons (e.g., 1,3-dichloropropene), methyl isothiocynate, oxamyl, thiona-
ril and carbofuran for nematode management. These chemical nematicides besides being costly are not so ecofriendly and poses potential risk to non-target organisms as has been emphasized under ‘Food and Environment Protection act, 1985, Part III. General-
izations on the dosage of nematicides are not possible due to their dependence upon the live standard. However, field application of aldicarb or carbofuran at 1.5 kg/ha has been found to reduce M. incognita populations effectively (Jagdale et al., 2000). Therefore, search for more benign and acceptable biological control measures is a global research priority today (Karna et al., 1996; Akhtar and Malik, 2000; Jagdale et al., 2000; Atta and Saad, 2001; Sharon et al., 2001). Rhizospheric microbes are one of the most promising groups of microorganisms for plant growth promotion, playing a crucial role in soil health and plant growth by a plethora of mech-
anisms in variety of crop plants (Siddiqui et al., 2005; Lee et al., 2011). Besides, they also serve as ideal biocontrol agents since they share the rhizosphere with their host plants, providing frontline defence for roots against pathogen attack. Among the microorgan-
isms that parasitize/reduce nematode populations by antagonistic behavior, rhizospheric microbes hold an important position where some of them have shown great potential as biocontrol agents (Akhtar et al., 2012).

Although encouraging success has been obtained in efforts to develop high yielding cultivars, no cultivar of M. incognita is yet available. Therefore, the management of plant parasitic nematodes on this crop relies mainly on applica-
tion of high rates of the nematicides viz. carbofuran. However, the decreasing efficacies of the chemical nematicides as well as risks associated with them have highlighted the need for a more effective and safer alternative control measures. Thus, the present study was designed to determine the effect of the rhizospheric microbes viz. Bacillus megaterium ATCC No. 14581, Pseudomonas fluorescens ATCC No. 13525, Paecilomyces lilacinus PDNC PL55, Trichoderma viride MTCC No. 167 and Glomus intraradices on the growth of W. somnifera cv. Poshita under greenhouse conditions in order to find eco-friendly and cost-effective treatments for the management of M. incognita. The nematicide carbofuran was included as a positive control since it is a popular treatment in W. somnifera cultivation.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of a high yielding cultivar of W. somnifera cv. Poshita were obtained from the National Gene Bank for Medicinal and Aromatic Plants at the Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India. The seeds were surface sterilized by soaking in 10% (v/v) sodium hypochlorite solution for 5 min, washed with distilled water and soaked for 4 h. After sterilization and soaking, healthy looking uniform sized seeds were sown in content plug plates filled with sterilized soil. Twenty-one days after sowing, healthy four leafed stage seedlings were transplanted into 70 kg soil capacity clay pots containing a mixture of auto-
claved soil (76% sand, 8% silt, 16% clay, pH 7.7) and composted farm manure in 5:1 ratio.

2.2. Culture and maintenance of biocontrol agents

B. megaterium (ATCC No. 14581), P. fluorescens (ATCC No. 13525), T. viride (MTCC No. 167), P. lilacinus (PDBC PL55) and G. intraradices (Arbuscular mycorrhizal fungi) are regularly main-
tained in the Microbial Technology and Nematology Division, CSIR-CIMAP, Lucknow. The fungal biocontrol agents (BCA) were cultured using sand maize media while the bacterial isolates were cultured in Luria broth. For multiplication, the fungal cultures were kept in a BOD incubator for 96 h at 30 ± 1 °C and the bacterial cul-
tures were placed on a rotary shaker at 28 ± 1 °C for 48 h at 200 rpm orbital shaking conditions. The fungal inoculants were mass multiplied in the previously mentioned media and after incubation, the fungal mycelial mat with conidia was homogenized and suspended in 500 mL of 0.1 M phosphate buffer (K2HPO4; KH2PO4) at 1.2 × 1010 colony forming units (CFU) mL–1. The bacterial cul-
tures, B. megaterium (ATCC No. 14581), P. fluorescens (ATCC No. 13525), as Luria broth suspensions were centrifuged at 6000g for 10 min. The supernatant was discarded and the pellet containing bacterial cells was suspended in 500 mL of 0.9% saline to a final density of 2.5 × 106 (CFU) mL–1 for B. megaterium and 1.8 × 106 (CFU) mL–1 for P. fluorescens.

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 was based on root frag-
ments colonized (70%) with G. intraradices and the sand–soil frac-
tion with AM fungus propagules (spores and mycelium) from dry maize pot culture. The roots in the pot culture were extracted from the soil, cut into 1 cm segments and thoroughly mixed with the sand–soil mix from the pot culture and stored at 5 °C until use. The inoculum potential (potential of a specific amount of inocu-

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earthen pots under greenhouse conditions. Mature egg masses from the roots were handpicked and allowed to hatch in distilled water for 48 h at 28 °C and then observed for survival under Leica stereoscopic microscope (Leica Microsystems AG, Germany). Total nematode counts in 1 mL of J2 suspensions were recorded in three independent aliquots and 1000 J2/pot were inoculated for all the treatments.

2.4. Preparation of cell free extract from rhizospheric microbes

A single colony/1 mm plug of rhizobacterial strains was cultured in 10 mL of nutrient broth, incubated at 28 °C on a rotary shaker at 150 rpm for 48 h. The cells were harvested by centrifugation at 10,000 rpm for 10 min and the supernatant was sterilized through 0.22 μ filters. This was designated as undiluted standard cell free extract of 100% concentration that was diluted with sterilized water to 50 and 25% respectively to study their effect on M. incognita (Nickmann and Dhawan, 2001).

2.5. In vitro egg hatch (Ovicidal) test/assay

Ovicidal action of biocontrol treatments was assessed by slightly modified procedure of Su and Mulla (1998). A known number of eggs (50) were carefully transferred into each well of 24-well microtiter plate. In test wells, 500 μL of cell free culture filtrate of rhizospheric strains of 100, 50 and 25% concentrations was added. Treatment with distilled water was used as negative control. Three replicates for each treatment were maintained for the accuracy of the results. The plates were incubated under humidified condition at ambient temperature (27 °C) for 120 h. A drop of Lugol’s iodine solution was added to each well to stop further hatching, and all the unhatched eggs in each well were counted microscopically. The hatch rates were assessed by the following formula:

\[ \text{Percentage of egg mortality} = \frac{\text{no. of hatched eggs/total no. of eggs} \times 100}{\text{100}} \]

2.6. In vitro mortality test of J2 (Larvicidal assay)

Freshly hatched J2’s (50 J2/100 μL) in distilled water suspension were added into each well of 24-well microtiter plate containing 400 μL of each of the rhizospheric strains cell free filtrates of 25, 50 or 100% concentrations (three replications per treatment). The plates were then incubated under humidified conditions at ambient temperature for 48 h. J2’s populations were monitored microscopically after 12, 24 and 48 h exposure period and percentage mortality was calculated.

2.7. Biocontrol Treatments

The experiments were carried out on a complete randomized design with three replications. The various biocontrol treatments are described in Table 1. Pot experiments were carried out in winter from January to April, 2012 (mean day length 10.42–12.67 h; mean daily maximum temperature 17.9–21.8 °C; mean daily minimum temperature 6.7–12.2 °C). The bio-control agents were applied three days before M. incognita inoculation. One week after transplantation, each plant was inoculated with 1000 freshly hatched J2 per pot. In addition, the following three treatments were maintained as controls: untreated-uninoculated, -inoculated, and carbofuran at 2 kg a.i. ha⁻¹ (0.0020 g a.i./kg soil) treated-inoculated. Plants were maintained in a greenhouse at 30 ± 6 °C and 13 h day length until the experiment was terminated 90 days after inoculation. Plant growth was determined by measuring root length, shoot height, fresh and dry weight of root and shoot. Root and shoot dry weights were determined after drying in a hot air oven at 60 °C. Roots were rated for galling severity according to Krusberg and Nielsen (1958) on a scale of 0–4.

2.8. Effect of M. incognita and rhizospheric strains on W. somnifera growth

For assessing M. incognita population in the infested roots, root samples were processed by a modified maceration method (McSorley, 1999). Briefly, the roots were washed free of soil and chopped in 1 cm pieces by scissors. The chopped root were immersed in sufficient amount of water and macerated by a waring blender for 1–2 min. Water was added to this suspension and 1 mL aliquot was taken by micropipette for M. incognita counting at 400× magnifications using a compound microscope. The number of M. incognita were determined from mean of three counts was taken in each case. Sieves used in nematode extraction were U.S. standard sieve series of 100, 200 and 325 meshes with openings of 149, 74 and 44 μm, respectively.

For getting the actual frequency and relative density of M. incognita in soil, 250 g soil sample from each of the replicative pots of all the treatments was collected. After mixing the soil properly, M. incognita from a composite subsample of 250 g soil were extracted by means of modified Cobb’s sieving and decanting technique followed by Baermann funnel method (Southey, 1986). The suspension was passed through nested sieves of 149, 74 and 44 μ mesh sieves. Final volume of each of the samples was made up to 50 mL and nematode numbers were counted in a 1 mL capacity counting slide under stereoscopic binocular microscope and mean of three independent counts were taken in each case. Reproduction factor (RF) was calculated by the formula, RF = Pf/Pi, where Pf = final M. incognita population and Pi = initial M. incognita population. The disease incidence was measured on the basis of nematode population per root system expressed as root knot index (Rki). The Rki was determined by counting the number of galls on the infested roots on a scale of 0–4: 0 = 0; 1 = 1–25; 2 = 26–50; 3 = 51–75; 4 = 76–100 (Krusberg and Nielsen, 1958).

2.9. Statistical analysis

Analysis of variance (ANOVA) techniques were used for the statistical analysis of data. To compare various treatments, Duncan multiple tests applicable to RCBD were performed by ASSISTAT software version 7.6 beta. Least Significant difference (LSD) was calculated at 5% probability level (P = 0.05) to compare treatment means for significance of difference between any two variables.

3. Results

3.1. Ovicidal and Larvicidal assay results

The nematidical potentials of rhizospheric microbes as evaluated by in vitro egg hatch assay for M. incognita are shown in Fig. 1. It was observed that all the rhizospheric isolates were able to reduce the hatching of M. incognita eggs. The highest ovicidal potentials were shown by P. lilacinus allowing only less than 17 eggs to hatch at 100% cell extract concentration. It was observed that the isolates were able to control egg hatch at all the tested cell extract concentrations with 100% concentration showing the maximum activity.

The effect of rhizospheric microbes on percentage egg mortality is depicted in Fig. 2. The results clearly indicate a significant inhibition (mortality) as compared to distilled water control treatment. Table 2 summarizes the larvicidal potentials of rhizospheric microbes on the J2’s at different concentrations. Hatching of M. incognita juveniles decreased with an increase in concentration of microbial strains with a maximum percentage mortality observed for P. lilacinus.
3.2. Effect of M. incognita (J2) on W. somnifera growth

M. incognita being a sedentary plant parasitic nematode causes a severe loss to the host plants and the severity increases with an increase in inoculum density as is represented in Fig. 3. Dry weight parameters provide a quick glance to the damage ratio incurred due to increased inoculum density of the parasite. The results also suggest that reduction in plant dry biomass accumulation corroborated with damage ratio due to increased inoculum density of the parasite. The rate of growth inhibition

Table 1
Experimental details (different treatments) for management of root-knot nematode M. incognita in W. somnifera cv. Poshita.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatments</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Un-control</td>
<td>Without any microbial treatment and M. incognita involvement</td>
</tr>
<tr>
<td>2</td>
<td>Un-m. control*</td>
<td>Without any microbial treatment but having M. incognita association</td>
</tr>
<tr>
<td>3</td>
<td>Carbofuran + MI</td>
<td>Chemical nematicide along with M. incognita inoculation</td>
</tr>
<tr>
<td>4</td>
<td>BM + MI</td>
<td>Bacillus megaterium and M. incognita inoculation</td>
</tr>
<tr>
<td>5</td>
<td>PF + MI</td>
<td>Pseudomonas fluorescens and M. incognita inoculation</td>
</tr>
<tr>
<td>6</td>
<td>TV + MI</td>
<td>Trichoderma viride and M. incognita inoculation</td>
</tr>
<tr>
<td>7</td>
<td>PL + MI</td>
<td>Paecilomyces lilacinus and M. incognita inoculation</td>
</tr>
<tr>
<td>8</td>
<td>GI + MI</td>
<td>Glomus intraradices and M. incognita inoculation</td>
</tr>
</tbody>
</table>

* Each pot was inoculated with 1000 J2-juveniles of M. incognita.

Fig. 1. Ovicidal effect of different cell extracts of rhizospheric microbes on M. incognita (in vitro).

Fig. 2. Effect of different cell extracts rhizospheric microbes on percentage egg mortality of M. incognita (in vitro).
Table 2
Effect of different microbial cell extracts for their larvicidal effects on J2's of *M. incognita*.

<table>
<thead>
<tr>
<th>Treatments/Incubation time</th>
<th>25%</th>
<th>50%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 h</td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Control</td>
<td>50.00 ± 0.0 (100)</td>
<td>49.00 ± 0.3 (100)</td>
<td>48.33 ± 0.3 (100)</td>
</tr>
<tr>
<td><em>B. megaterium</em></td>
<td>41.67 ± 0.33 (83.33)</td>
<td>39.00 ± 0.58 (79.59)</td>
<td>35.67 ± 0.33 (73.79)</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>39 ± 0.58 (78)</td>
<td>36.33 ± 0.88 (74.15)</td>
<td>31.33 ± 0.88 (64.83)</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>35.67 ± 0.88 (71.33)</td>
<td>31.00 ± 0.58 (63.27)</td>
<td>26.67 ± 0.88 (55.17)</td>
</tr>
<tr>
<td><em>P. lilacinus</em></td>
<td>33.00 ± 0.58 (66)</td>
<td>26.33 ± 0.33 (53.74)</td>
<td>20.00 ± 0.58 (41.38)</td>
</tr>
</tbody>
</table>

J2-juveniles (mean values) with ± standard error (SE) are indicated. Values in parentheses indicate the percentage mortality against distilled water control.
was also found to be in accordance with population density of J2’s in the roots. The results emphasize that the rate of damage is directly proportional to the number of J2.

The multiplication of M. incognita inside the host roots as assessed by counting the number of galls, measured as RKI gave a good estimation of disease caused by M. incognita. The expression of M. incognita multiplication was based on the method given by Krusberg and Nielson (1958). RKI data also supports the damage due to M. incognita at higher densities. Treatments with P. fluorescens exhibited RKI of 1.7 (less than 50 galls), comparable to carbofuran treatment (Fig. 4). However, treatments consisting of B. megaterium, T. viride and P. lilacinus were more effective in controlling M. incognita population having an RKI of 1.3 (less than 25 galls). The plants having G. intraradices treatment showed a little higher RKI value (2.0; 50 galls/plant), yet it was much less than untreated- M. incognita inoculated control, RKI = 2.7 (more than 50 galls).

In terms of the reproduction factor (Rf) treatment involving T. viride as the biocontrol agent was found most effective resulting in least final population count of the parasite (Pf = 2620). P. lilacinus treatment, on the other hand, resulted in a maximum Rf of 3.89 that was still much less than in the untreated-inoculated control (Fig. 5). Except for the treatment having P. lilacinus, all other microbial treatments resulted in a lower Rf value as compared to carbofuran treatment (Rf = 2.653) that clearly indicated their better efficacy in controlling M. incognita populations in W. somnifera roots.

The results clearly show that in the treatments of rhizospheric bacteria, Rf was similar to that of chemical nematicide, carbofuran but in combined treatments, Rf was drastically reduced proving the efficacy of microbial agents.

3.3. Growth parameters results

The overall plant growth inhibition data due to nematode population build up in infected plants is summarized in Fig. 6. The nematode significantly reduced plant growth (22.7 cm, shoot length and 11.3 cm, root length) in comparison to control plants (34 cm, shoot length and 12.3 cm, root length), leaf surface area (9.3 mm) against 12.3 mm of control plants besides other growth parameters like
plant canopy, number of flowers and fruits etc. (Fig. 6). ANOVA results for biomass depicted by root and shoot fresh and dry weight in *W. somnifera* cv. Poshita revealed significant mean differences against control (Table 3). The tests performed included Fisher’s least significant difference (LSD) at $P = 0.05$ and Duncan’s test for comparing control with treatment means (Zar, 1999). CV% value

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**Table 3**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot fresh wt. (g)</th>
<th>Root fresh wt. (g)</th>
<th>Shoot dry wt. (g)</th>
<th>Root dry wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-control</td>
<td>31.33d</td>
<td>5.87c</td>
<td>5.37b</td>
<td>2.17d</td>
</tr>
<tr>
<td>Un-in. control*</td>
<td>18.33e</td>
<td>4.23d</td>
<td>3.67c</td>
<td>1.13e</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>34.6b</td>
<td>7.03b</td>
<td>5.72b</td>
<td>3.01b</td>
</tr>
<tr>
<td>BM + MI*</td>
<td>38.67b</td>
<td>7.77b</td>
<td>5.82b</td>
<td>2.71b,c</td>
</tr>
<tr>
<td>PF + MI*</td>
<td>42.67a</td>
<td>9.93a</td>
<td>8.50a</td>
<td>3.90a</td>
</tr>
<tr>
<td>PL + MI*</td>
<td>37.33b,c</td>
<td>7.47b</td>
<td>5.33b</td>
<td>2.92b</td>
</tr>
<tr>
<td>GI + MI*</td>
<td>35.33c</td>
<td>7.67b</td>
<td>5.47b</td>
<td>2.67b,c</td>
</tr>
<tr>
<td>CV%</td>
<td>4.77</td>
<td>7.46</td>
<td>5.17</td>
<td>7.62</td>
</tr>
<tr>
<td>LSD</td>
<td>2.85</td>
<td>0.92</td>
<td>0.51</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* Each treatment was inoculated with 1000 J2-juveniles of *M. incognita*.

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*Fig. 5.* Effect of microbes on Reproduction factor (RF) of *M. incognita* in *W. somnifera*.

*Fig. 6.* Effect of rhizospheric microbes on plant growth parameters in *W. somnifera*. 

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for fresh weight (shoot) was 4.77 while it was 5.17 for shoot dry weight among various treatments. LSD value of 0.05 for percentage root weight, both fresh (0.92) and dry (0.35), indicated a highly significant result for various treatments. The Duncan test at 5% probability was applied to the treatments. Mean values for treatments followed by the same letter do not differ statistically between themselves. All the treatments were significantly effective in growth promotion The ANOVA results for fresh and dry shoot and root weight are represented in Table 4. The results significantly correlate the biocontrol agents, effective as growth promoting also.

The application of the various treatments significantly increased plant growth parameters of *W. somnifera* cv. Poshita and suppressed nematode soil population and root gall index when compared to non-treated inoculated plants. The treatments, *T. viride*, *B. megaterium* and *P. fluorescens* significantly increased plant growth, and were found to be effective for growth promotion. The results show that microbials agents completely counteract the negative impact of the nematode and reduce nematode population and root knot index, and were equally effective to carbofuran treatment.

### 4. Discussion

Buhrer (1938) first reported root knot nematodes (*Meloidogyne sp.*) on *Mentha arvensis*. The intricate association of plant parasitic nematodes causes significant damage to almost all the medicinal plants studied till date (Saikia et al., 2012). The magnitude of crop damage due to nematode–plant interactions has established *M. incognita* as the most important nematode pests limiting production, with infestations reported on *Mentha arvensis*, *Ocimum sanctum*, *Papaver somniferum*, *Bacopa monnieri*, *Withania somnifera* and *Artemesia annua* (Pandey et al., 2009; Sikora et al., 2003). Gradual declines characterized by unthrifty growth and yellowing of leaves due to reduced the uptake of nutrients such as P, K, Zn, Mn and Cu are the prominent symptoms of *M. incognita* (Ferraz et al., 1988). The complete eradication of *M. incognita* from agricultural soils is nearly impossible due to the polyphagous nature of this pest. Inhibiting egg hatching in *M. incognita* is helpful in reducing its populations in soil and roots (Meyer et al., 2004). The root knot infested plants are stunted with smaller and chlorotic leaves, fewer rootlets and root-hairs and the roots bear small knobs/galls. There are several earlier reports indicating an inverse relationship between nematode population and total foliage yield (Rhoades, 1984).

*W. somnifera* is economically important for its bioactive phyto-molecules present in its roots, which are a major source of numerous alkaloids (*sominiferine*, *somnine*, *withanine*, *tropane*, isopelletierine, *cuscohygrine*, *anaferine*, *anahygrine*, *visamine*, etc.) and withanolides, a group of naturally occurring oxygenated ergostane-type steroids. *W. somnifera* is reported to be a highly susceptible host for *M. incognita* race 2 (Pandey et al., 2009). It has been demonstrated that application of carbofuran or aldicarb at 5 kg a.i./ha three times, every 3 months, results in increased growth and vigor of seedlings in both primary and secondary nurseries (Koshy, 1987; Ali, 1986, 1987; Eapen, 1995). Since these nematocides do not kill but only inactivate nematodes, repeated use is necessary to ensure good yield. However, the potential side effects associated with these synthetic treatments restricts their enormous usage. The use of antagonistic microorganisms can be considered suitable for nematode management programs, but still need advanced field testing (Pandey et al., 2003).

Rhizospheric microorganisms prove to be a potential source of novel natural products for exploitation in agriculture and are recognized as a promising group in terms of diversity and pharmaceutical potential (Masadeh et al., 2004). The reduction of inoculum density or disease producing activities of a pathogen by one or more microorganisms, termed as biocontrol is achieved by competition, hyperparasitism, induced resistance etc. Mycoparasitism and production of volatile and non-volatile antibiotics are important mechanisms operating in the case of *Trichoderma sp.* At CIMAP, earlier our group has identified a strain of *T. harzianum* that has been used to produce a disease-free, healthy nursery with a reduction in root-knot nematode infection and improving the growth and yields of *M. arvensis* (Kalra et al., 1996). With the growing public concern regarding environmental pollution and the increase in the costs of pesticides, efforts to develop non-chemical disease management strategies are indicated.

The pot experiment results demonstrated the nematocidal potential of the biocontrol agents tested, especially *P. fluorescens* and *B. megaterium*, which resulted in increased plant growth and yield of *W. somnifera*. Several mechanisms may be involved in the suppression of nematode soil populations and enhancement of plant growth and yield. Microbial extracts often contain pheno-lic compounds, organic acids and secondary metabolites (Spencer et al., 2003) which have been demonstrated to have nematicidal activity (Shaukat et al., 2004). This study revealed a drastic reduction in hatching of eggs by microbial inoculants with a significant reduction in RJK and increase in total weight of the plant. Bacteria destroy nematodes continuously in virtually all soils because of their constant association with nematodes in the rhizosphere. Although a large number of bacteria have shown antagonistic effects against nematodes but the most important genera include *Rhizobium* (*R. leguminosarum*), *Bradyrhizobium japonicum*, *Mesorhizobium sp.*, *Azorhizobium sp.*, *Pseudomonas* (*P. fluorescens* and *P. aeruginosa*) and *Bacillus* (*B. subtilis*). Similarly, Wescott and Kluepfel (1993) had reported that *Bacillus* and *Pseudomonas sp.* inhibit egg hatching by the production of exotoxic compounds as a result of cellular metabolism, which can also affect nematode juveniles. This antagonistic effect against *M. incognita* is due to the permeability changes of juveniles cuticle which is characterized by its selective permeability and this effect is more pronounced with molting inside eggs.

In this investigation, the addition of bio-control organisms improved plant growth and further suppressed *M. incognita* population as compared to the chemical nematicides, carbofuran. Reducing the hatching of *M. incognita* eggs will help in delaying the population build-up of *M. incognita* which will help in the efficient and harmless control of this pathogen. The possible mechanism for nematode population reduction might be related to the microbial ability to envelop or bind the root surface with

### Table 4

ANOVA results for root and shoot fresh and dry weight in *W. somnifera* cv. Poshita.

<table>
<thead>
<tr>
<th>Degree of freedom (df)</th>
<th>Mean sum of squares (ss)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot fresh weight (g)</td>
</tr>
<tr>
<td>Treatments</td>
<td>7</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
</tr>
<tr>
<td>F-value</td>
<td>-</td>
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<td>P-values</td>
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* Significant at a level of 1% probability.
carbohydrate lectin, thereby interfering with normal host recogni-
tion by the nematode. The use of rhizospheric microbes for the
management of nematode populations is apparently effective
and environmentally friendly compared to synthetic nematicides.
The pot experiment was a preliminary test revealing the rhizospheric
microbes as potential tools for nematode management in the near
future.

5. Conclusions

Rhizospheric microbes are a poorly investigated group of micro-
organisms that represent an abundant and dependable source of
bioactive and chemically novel compounds with potential for
exploitation in a wide variety of medical, agricultural, and indus-
trial arenas. The mechanisms through which these microorganisms
exist and respond to their surroundings must be better understood.
The results presented in this study show that rhizospheric mi-
crobes play an important role for protecting plants against diseases
besides enhancing their growth parameters.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in
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