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

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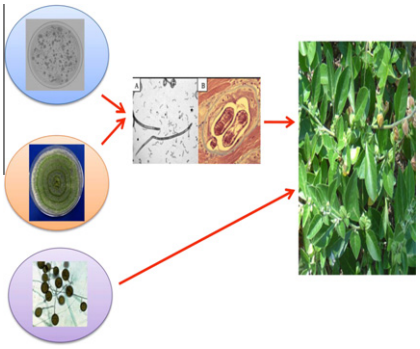
HIGHLIGHTS

- *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.

GRAPHICAL ABSTRACT

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



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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.

1. Introduction

Withania somnifera L. commonly known as Ashwagandha or Indian ginseng is a valuable medicinal herb from family Solanaceae. The plant has a long documented history of usage as "Rasayana" drug in Ayurveda with strong anti-inflammatory (Rasool et al., 2000), antioxidant (Panda and Kar, 1997), immunomodulatory (Rai et al., 2003; Archana and Namasivayam, 1999), anti-cancerous (Devi et al., 1992; Mohan et al., 2004), anti-stress and adaptogenic (Bhattacharya et al., 2002) properties. Besides, the plant is also known to possess rejuvenating action for treating several neurodegenerative (Bhattacharya et al., 1987; Naidu et al., 2003; Ahmad et al., 2005; Dhuley, 2001; Chaudhary et al., 2003; Jain et al.,

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2001), endocrine (Schliebs et al., 1997; Panda and Kar, 1998), cardiovascular 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 (sitoindosides 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* infection 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, thionazin and carbofuran for nematode management. These chemical nematicides besides being costly are not so ecofreindly and poses potential risk to non-target organisms as has been emphasized under 'Food and Environment Protection act, 1985, Part III. Generalizations 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 (Kalra 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 mechanisms 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 microorganisms 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 *W. somnifera* resistant to *M. incognita* is yet available. Therefore, the management of plant parasitic nematodes on this crop relies mainly on application 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* PDBC 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 7.0 kg soil capacity clay pots containing a mixture of autoclaved 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 maintained 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 cultures 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 (K_2HPO_4; KH_2PO_4) at 1.2×10^6 colony forming units (CFU) mL^{-1}. The bacterial cultures, *B. megaterium* (ATCC No. 14581), *P. fluorescens* (ATCC No. 13525), as luria broth suspensions were centrifuged at 6000*g* 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 2.5×10^8 (CFU) mL^{-1} for *B. megaterium* and 1.8×10^8 (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 fragments colonized (70%) with *G. intraradices* and the sand–soil fraction 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 inoculums potential (potential of a specific amount of inoculum to cause root infection under a standard set of conditions) of *G. intraradices* used in this study was 8.9 ± 1.3 infecting propagules g^{-1} sand–soil mixture. Bacterial agents were inoculated as 10 mL of 10^8 CFU/pot and fungal isolates as 10 mL of 10^6 CFU/pot. Inoculums of arbuscular mycorrhizal fungi, *G. intraradices* used in the present experiment consisted of soil containing spores (8–10 spores g^{-1}) and colonized roots of maize (*Zea mays* L.).

2.3. Nematode isolation

The population of *M. incognita* was maintained on brinjal (*Solanum melongena* L.) grown in sterilized loamy-sand soil in