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Evaluation of biocontrol potential of *Arthrobotrys oligospora* against *Meloidogyne graminicola* and *Rhizoctonia solani* in Rice (*Oryza sativa* L.)

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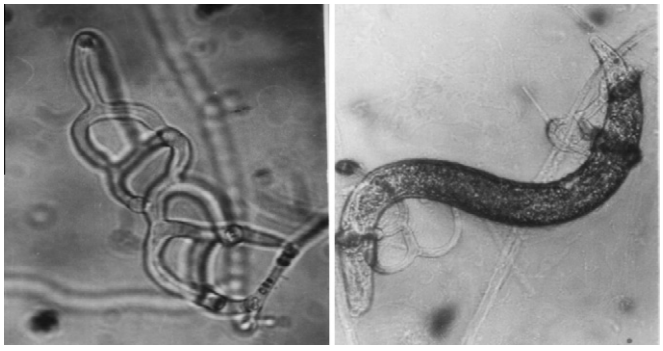
HIGHLIGHTS

- Biocontrol efficacy of *Arthrobotrys oligospora* was tested against *M. graminicola* and *R. solani.*
- *A. oligospora* was found to have potential for controlling both diseases in rice in green house and field trial.
- Ecological behavior of *A. oligospora* were tested under different conditions and found that it has better adoptability.
- We conclude that single biocontrol agent having multifarious characteristics could be a cost effective and eco-friendly.

GRAPHICAL ABSTRACT



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ABSTRACT

The nematode trapping and mycoparasitic potential of *Arthrobotrys oligospora* was tested *in vitro* against *Meloidogyne graminicola* and *Rhizoctonia solani*, respectively. Five isolates of *A. oligospora* were isolated from different locations of India. Diversity of the trapping structures is large and highly dependent on the environmental condition and nature of the fungus. In *A. oligospora*, a three-dimensional adhesive net (in response to nematode) and hyphal coils developed around the hyphae of *R. solani*. *In vitro* trap formation and predacity were tested against second-stage juveniles of *M. graminicola* (J_2) and the interactions between *A. oligospora* and *R. solani* were recorded. Under field conditions, we demonstrated the biocontrol potential of *A. oligospora* against *R. solani* causing sheath blight of rice (*Oryza sativa*) for the first time. All the isolates of *A. oligospora* parasitized and killed *M. graminicola* and *R. solani*. Application of *A. oligospora*, isolate VNS-1, in soil infested with *M. graminicola* and *R. solani* reduced the number of root knot by 57.58–62.02%, sheath blight incidence by 55.68–59.32% and lesion length by 54.91–66.66% under green house and miniplot (field) conditions. Applications of *A. oligospora* to the soil increased plant growth: shoot length by 56.4–68.8%, root length by 44.0–54.55%, fresh weight of shoot and root by 62.91–65.4% and 38.9–44.19%, respectively, as compared to the plants grown in nematode infested soil.

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1. Introduction

Root knot (caused by the nematode *Meloidogyne graminicola* Golden and Birchfield) and sheath blight (caused by *Rhizoctonia solani* Kühn) are among the most devastating and destructive diseases of upland and rain-fed lowland rice (*Oryza sativa* L.) in Asia (Ou, 1985; Prot and Matias, 1995; Sariano et al., 2000; Sariano and Reversat, 2003). In India, the disease is wide spread in rice–wheat (*Triticum aestivum* L.) cropping system with significant yield losses (Rush and Lee, 1992; Devi, 2001; Kamalawanshi et al., 2002; Kamalawanshi and Kumar, 2004). Management of these diseases is largely dependent on the integration of conventional practices with chemical control (Chin and Bhandhufalck, 1990; Damicone et al., 1993). However, chemical control using effective pesticides has various undesirable effects such as phytotoxicity to plants, soil and water pollution and unavoidable natural imbalance in the soil affecting the whole ecosystem adversely (Groth et al., 1990; Dehne and Oerke, 1998; Anand et al., 2010). Growing ecological concerns have led to intensive research on alternative methods for control of plant diseases among which biological control of phytopathogenic microorganisms has emerged as one of the most powerful approaches (Oka, 2010; Niu and Zhang, 2011).

The potential benefits of using microbial inoculants for the purpose of plant growth promotion (Niu and Zhang, 2011), biological control (Singh et al., 2007; Oka, 2010; Niu and Zhang, 2011) and induced resistance responses against the disease (Singh et al., 2002, 2003) can be achieved through modification of the rhizospheric microflora (Smith, 1995). Engineering rhizosphere through the inoculation of specific microorganisms can lead to higher levels of targeted microbial populations in the rhizosphere (Rodriguez et al., 2006). In recent years, emphasis has been given to the application of single microbial species with multipronged functions and/or binary or multiple mixtures of microbes (Bashan and de-Bashan, 2005; Meena et al., 2010).

Inter- or intra-specific interactions within fungi or with nematodes are part of the microbiological complexities in the rhizospheric environment leading to beneficial results. Although the nematode trapping fungus, *Arthrobotrys oligospora* Drechsler has not been studied intensively as biocontrol agent, it has a variety of trapping structures with functional nematode trapping devices (Barron, 1977; Singh et al., 2007; Khan et al., 2011; Simon and Anamika, 2011). The parasitism of *R. solani* by *A. oligospora* was studied previously (Persson et al., 1985; Olsson and Persson, 1994) but these studies remained confined to the *in vitro* conditions. Currently, no evidence exists on the biological control of *R. solani* by *A. oligospora* in field conditions. In order to explore the biocontrol efficacy of *A. oligospora* against *M. graminicola* and *R. solani*, the present investigation was carried out to assess (i) the potential of *A. oligospora* in *in vitro* to parasitize and kill *M. graminicola* and *R. solani*, (ii) the *in vitro* growth pattern of *A. oligospora*, and (iii) the efficacy of *A. oligospora* against sheath blight and root-knot diseases of rice.

2. Materials and methods

2.1. Media, fungicide and growth conditions

Potato dextrose agar (PDA) medium (pH 5.8) from HiMedia, India, was used for stock culturing of *R. solani* BHU-1. Corn meal agar (CMA) medium (pH 7.0) was prepared with 20 g each of corn infusion and agar per liter and used in a ratio of 1:10 and 1:20 for evaluating trap formation and parasitism of *M. graminicola* by the nematophagous fungi *in vitro*. Under specific conditions, acidic or alkaline pH of the medium was maintained using sterilized tartaric acid (10%) and NaOH (1 N), respectively. The fungicide and nematicide (Tilt 25 EC and carbofuran, respectively) were obtained from Syngenta Pvt. Ltd., India. All experiments were performed at 25 ± 2 °C unless otherwise stated.

2.2. Isolation, purification and maintenance of fungal isolates

Isolates of *A. oligospora* was obtained from rhizospheric soil samples collected at different locations in India: Banaras Hindu University (BHU), Varanasi (isolate VNS-1 and 2), Ghazipur (isolate VNS-3), Mau (isolate VNS-4) and Azamgarh (isolate VNS-5) in 2007–2008. Isolation of *A. oligospora* was performed as per the method of Duddington (1955) with slight modifications (Bandyopadhyay and Singh, 2000). Monoconidial cultures of all the isolates were prepared (Singh et al., 2004) and identified with the help of microscopic identification key (Drechsler, 1937; Cook and Godfrey, 1964; Haard, 1968; Barron, 1977). Cultures of each isolate were maintained on CMA medium by regular sub-culturing at 15 day intervals. Cultures were preserved and stored in the depository of Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi, India.

R. solani BHU-1 was isolated from infected rice plants growing at the Agricultural Farm, BHU, Varanasi, India, in 2008–2009. Infected parts were cut into small pieces, surface sterilized by 0.1% $HgCl_2$ followed by washing three times with sterile double distilled water and inoculated separately on potato dextrose agar and selective medium (Ko and Hora, 1971) for the isolation of *R. solani*. Inoculated cultures were further purified by single hyphal tip culture method. *R. solani* isolates were identified and characterized on the basis of microscopy, somatic interaction, anatomy of hyphal septal pore and cellular nuclear number and nature of genetic relationship (Anderson, 1982; MacNish et al., 1993a,b). *R. solani* AG-IIA (tester isolate, ITCC-4110) was used to confirm the identity of the fungus (Ogoshi, 1987; Vilgalys and Cubeta, 1994). Pathogenicity tests were carried out with susceptible rice cultivar MTU 7029 grown in plastic pots (15 cm diameter) in green house. *R. solani* was grown on barley (*Hordium vulgare* L.) seeds as per the methods described by Naito et al. (1993). At the time of application, the population of *R. solani* was 2.05×10^4 cfu g^{-1}. Mass culture of *R. solani* was spread evenly after 20 days of sowing on the surface of each pot (at 10 g/kg soil, 2.05×10^4 cfu g^{-1}) having 60% soil moisture content. After 21 days of inoculation of *R. solani,* the percentage of diseased seedlings per pot and lesion length (cm) on sheath was recorded to prove pathogenicity.

2.3. Radial growth and trap formation

The radial growth of selected isolates of *A. oligospora* was observed for eight days on CMA medium at 25 ± 2 °C. Effect of different compositions of corn meal agar: CMA, CMA 1:5, CMA 1:10, CMA 1:20, and water agar (1.5%) on trap formation were tested in the presence of *M. graminicola* (100 J_2). Petri dishes containing different composition of CMA and water agar were inoculated with *A. oligospora* VNS-1 and incubated at 25 ± 2 °C. Number of traps produced was recorded at 4, 8, 12, 24, 48, 60, and 72 h from 10 microscopic fields (1.6 mm^2) at 10× magnification from the center, middle and periphery of the fungal colony and average number of traps was calculated. The experiments were conducted three times in five replications.

2.3.1. Nematode population

Trap formation by *A. oligospora* VNS-1 against *M. graminicola* was studied as per the methods of den Belder and Jansen (1994). Freshly collected second-stage juveniles (975 J_2) from rice root galls were washed (five times) with sterile distilled water containing 50 ppm streptomycin. A drop of water containing 20, 40, 60, and 100 J_2 of *M. graminicola* was inoculated into Petri dishes containing *A. oligospora* VNS-1 in CMA medium (1:10; pH 7)