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Evaluating novel microbe amended composts as biocontrol agents in tomato

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ABSTRACT

An investigation was conducted to evaluate the potential of antagonistic cyanobacteria/bacterial cultures amended compost and compost tea preparations for suppressing diseases caused by plant pathogenic fungi *Fusarium oxysporum*, *Pythium debaryanum*, *Pythium aphanidermatum* and *Rhizoctonia solani* in tomato. Two types of microbe-fortified composts and the compost tea preparations, along with the recommended biological control (*Trichoderma* formulation) and chemical control (Thiram-Carbendazim), were used for inoculating the potting mixture. Comparative performance of the treatments revealed the superiority of both the composts/compost tea preparations in enhancing seed germination, seedling length and biomass in the fungi challenged treatments. The most effective control of the diseases was obtained by the composts amended with *Anabaena oscillarioides* C12 and *Bacillus subtillis* B5 and the compost tea preparations. Both treatments provided significantly better control than the other treatments in terms of reduction in disease severity, reduction of fungal load and enhancement of plant parameters. Our study reveals the efficacy of microbe-fortified composts for use in control of the studied root diseases caused by phytopathogenic fungi.

1. Introduction

Agricultural sustainability depends greatly on the development of strategies that reduce the need for costly external inputs (such as agrochemicals) and alleviate the deleterious environmental impacts often associated with the excess use of these inputs. Such an approach is embodied in the science of agroecology, which is the application of the ecological principles to the design and management of a sustainable agroecosystem. Biological control, through the use of microorganisms, offers an alternative and attractive approach for disease control, without the negative impact of chemicals. This has become an important tenet for sustainable agriculture, as biocontrol agents are easy to deliver, may activate plant resistance mechanisms like systemic/induced resistance and thereby indirectly improve plant growth and yields.

Compost based suppression of wide range of major soilborne diseases has been demonstrated in the last decade as a promising option (Hadar and Mandelbaum, 1992; Nelson and Boehm, 2002; Nakkeeran et al., 2005). Plant pathogens such as *Pythium* and *Phytophthora* spp. produce small propagules that can be suppressed through microbiostasis, which implies nutrient competition and antibiosis. Biocontrol of soilborne pathogens is known to be related to specific suppression by metabolites produced by antagonists or a specific increase in populations of selected or groups of organisms antagonistic to the pathogens (Lockwood, 1988; Liebman and Epstein, 1992).

Diverse biocontrol agents, including *Trichoderma*, *Pseudomonas* and *Pantoea* spp., are known to contribute to this effect induced by composts (Ellis et al., 1986; Hardy and Sivasithamparam, 1991; Craft and Nelson, 1996). However, such microbe mediated mechanisms of compost based suppression are generally complex and unstable as it is dependent upon maturity indices, physicochemical/biological characteristics, timing of application etc. (Temorshuizen et al., 2006). Cyanobacteria have been found to produce a wide variety of linear cyanotoxins such as aeruginosins, microginins and cyclic peptides such as anabaenopeptins, nostopeptolides and anabaenopeptides, which may not be actually toxic but have other bioactivities such as serine protease inhibition (Namikoshi and Rinehart, 1996; Golakoti et al., 2000). A large body of information on the bioactive compounds/hydrolytic or lytic enzymes produced by microbes, including cyanobacteria (Young et al., 1974; Kulik, 1995; Prasanna et al., 2008) is available. However, there are no sufficient investigations undertaken so far to exploit the efficacy of

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the combinations of composts and microbial antagonists as effective biocontrol methods.

Tomato (*Lycopersicon esculentum* Mill.) is among the world's largest vegetable crops and known as a healthy food, because of its special nutritive value and widespread production. It is one of the most important nursery-based vegetable crops cultivated for its fleshy fruits. Soilborne pathogens are considered to be the major scourge of nursery-based vegetable crops like chili, tomato and brinjal belonging to the family Solanaceae. Control measures that are commonly employed against soilborne plant pathogens include the use of resistant or tolerant cultivars, cultural practices, biological and chemical control Ferree and Madden (1986).

The present study was conducted to evaluate the potential of selected microbe-fortified composts and compost tea as biocontrol agents against soilborne plant pathogenic fungi *Fusarium oxysporum* (Schlecht. emend. Snyder & Hansen), *Pythium aphanidermatum* (Edson) Fitzp, *Pythium debaryanum* Hesse. and *Rhizoctonia solani* Kühn, which cause damping off diseases of tomato.

2. Materials and methods

2.1. Organisms used in this study

A set of fungal antagonists, comprising one cyanobacterial strain (C12, *Anabaena oscillarioides* (Bory) Bornet & Flahault) and one bacterial strain (B5, *Bacillus subtilis* Ehrenberg), which showed maximum fungicidal and hydrolytic enzyme activity in a preliminary screening under *in vitro* conditions (Dukare, 2010), were used in the present study. These isolates were obtained from the Division of Microbiology, IARI, New Delhi. The fungal strains *F. oxysporum* (ITCC4998), *P. aphanidermatum* (ITCC123), *P. debaryanum* (ITCC95) and *R. solani* (ITCC6180) were obtained from the Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi and used as test organisms.

2.2. Growth and maintenance

The cyanobacterial strain was axenized by a standard procedure employing a set of antibiotics (Kaushik, 1987). The culture was routinely grown under the conditions optimized for maximum biocidal activity i.e. L: D light: dark cycle 16–18, 5000 lux white light (50–55 mmol photons/m^2/s) and 27–28 °C in nitrogen free BG-11 medium (Stanier et al., 1971). The bacterial strains were grown and maintained in nutrient broth. The fungal strain was grown in potato dextrose agar medium (CAB, 1968) and incubated at 28 °C.

2.3. Preparation of microbe amended composts

Paddy straw compost (the composition is given as Supplement Table 1) prepared as described by Gaind and Lata (2010) was used for preparing the fortified biocontrol formulations. Based on previously optimized inoculum rate (1% v/w), two types of compost mixture (I and II) were prepared under stationary conditions in plastic bags with foam stoppers (Dukare, 2010). Compost mixture I was prepared by adding one cyanobacterial (C12) and one bacterial strain (B5) to paddy straw compost, while compost mixture II was prepared by amendment of paddy straw compost with the bacterial strain alone. A compost mixture amended with cyanobacterial strain alone was also prepared, but it did not exhibit significant fungicidal/hydrolytic enzyme activity as compared to the other compost mixtures and therefore was not used for testing with crop (Dukare, 2010). Both the amended compost mixtures were incubated for 10 days. Compost tea (5% w/v) was prepared from both the compost mixtures (I and II) by vigorous stirring in stoppered flasks using a shaker (250 rpm) followed by standing for 7–8 days and filtering through 100 micron mesh sieve (Scheuerell et al., 2005).

2.4. Mass multiplication and preparation of fungal inoculum

The selected phytopathogenic fungi (*F. oxysporum, P. aphanidermatum, P. debaryanum* and *R. solani*) were multiplied using sorghum seeds [*Sorghum bicolor* (L.) Moench]. The inoculum of the fungi was produced on sorghum seeds moistened with water (1:1) and autoclaved twice for 90 min on two consecutive days (Paulitz and Schroeder, 2005). Flasks containing sorghum seeds were inoculated with one week-old fungal culture grown on potato dextrose agar (PDA) media. Flasks were incubated at room temperature for four weeks and shacked once a week. Colonized sorghum seeds were used as fungal inoculums at a rate of 15 g in 500 g of potting mix.

2.5. Treatments and growth chamber conditions

The seed of tomato variety Pusa Ruby was obtained from the Division of Vegetable Sciences, Indian Agricultural Research Institute, New Delhi. Pusa Ruby is known to be susceptible to damping off diseases caused by soilborne pathogens such as *Pythium* spp. and root/stem rot caused by *Fusarium/Rhizoctonia* spp.

The disease control experiment was conducted under the controlled conditions in the National Phytotron Facility, IARI, New Delhi to evaluate the biocontrol potential of the microbe amended compost mixtures and compost tea on tomato plants (cv. Pusa Ruby) grown in potting mixture pre-inoculated (T1) with the fungal consortium comprising the species of *Fusarium*, *Pythium* and *Rhizoctonia* as described above. The potting mix consisted of 2 vermiculite: 1 sand (The composition and characteristics are given as Supplementary Table 2) used for the growth of plants in the pots (about 15 cm in diameter) and in treatments of compost mix I and II. It was comprised of potting mix: fortified compost in the ratio of 3:1. The pots were filled with potting mix and autoclaved at 1.055 kg/m^2 pressure and 121 °C for 1 h for 3 consecutive days. Thiram and carbendazim at a rate of 2 g kg^{-1} were used as the recommended chemical control treatment as soil drench and *Trichoderma* at a rate of 20 beads kg^{-1} as a recommended biological control practice (Pandey and Dubey, 1994) as positive controls. In order to maintain sufficient humidity required for efficient disease establishment and development of fungal mycelia, the pots were placed in a polythene covered enclosure.

The surface sterilized seeds of tomato which had been soaked in water overnight were used for sowing. Treatments (Table 1) involved application of seeds into potting mix, pre-inoculated with

Table 1
Details of treatments included in pot experiment under green house conditions of the National Phytotron Facility.

Treatments	Fungal plant pathogen consortium inoculated (T1)	Non-inoculated (T2)
Chemical control (Thiram + Carbendazim as soil drench)	T1A	T2A
Biological control (*Trichoderma* as soil drench)	T1B	T2B
Potting mix + non-amended paddy straw compost	T1C	T2C
Potting mix + Compost mix I (*Anabaena* sp. C12 + *Bacillus* sp.B5)	T1D	T2D
Potting mix + Compost mix II (*Bacillus* spp. B5)	T1E	T2E
Potting mix + Compost tea I (*Anabaena* sp. C12 + *Bacillus* spp. B5)	T1F	T2F
Potting mix + Compost tea II (*Bacillus* sp. B5)	T1G	T2G