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ORIGINAL PAPER

Bioefficacy of novel cyanobacteria-amended formulations in suppressing damping off disease in tomato seedlings

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Abstract Biological control of plant pathogens is receiving increasing relevance, as compared to chemical methods, as they are eco-friendly, economical and indirectly improve plant quality and yield attributes. An investigation was undertaken to evaluate the potential of antagonistic cyanobacteria (*Anabaena variabilis* RPAN59 and *A. oscillarioides* RPAN69) fortified formulations for suppressing damping off disease in tomato seedlings challenged by the inoculation of a fungal consortium (*Pythium debaryanum*, *Fusarium oxysporum lycopersici*, *Fusarium moniliforme* and *Rhizoctonia solani*). Treatment with *A. variabilis* amended formulations recorded significantly higher plant growth parameters, than other treatments, including biological control (*Trichoderma* formulation) and chemical control (Thiram-Carbendazim). The *A. variabilis* amended compost-vermiculite and compost formulations exhibited 10–15 % lower disease severity and 40–50 % higher values than chemical and biological control treatments in terms of fresh weight and height of the plants. In future, in depth analyses regarding the mechanism involved in biocontrol by cyanobacteria and evaluation of these formulations under field conditions are proposed to be undertaken.

Keywords Antagonistic cyanobacteria · Damping off · Fungal consortium · Biological control · Compost formulations

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Introduction

Intensive agriculture is heavily dependent on chemicals, which has caused tremendous problems of environmental degradation and human health. In contrast, organic farming systems are exposed to problems, primarily of low yield and poor returns to investment, but are increasingly being explored as promising options. Most soil borne pathogens are difficult to control by conventional strategies such as the use of resistant host cultivars or synthetic fungicides. The lack of effective chemicals, the occurrence of fungicide resistance in pathogens, or the breakdown or circumvention of host resistance by pathogen populations are among the key factors which need urgent attention. In this context, biological control, or the use of living organisms or their products is a potential strategy for reducing the polluting effects of chemicals. Fungi and bacteria are the chief biological agents that have been studied for the control of plant pathogens, particularly soil-borne fungi. Biocontrol formulations, involving *Trichoderma*, fluorescent *Pseudomonads* have been developed and successfully used at field with a number of crops (Singh et al. 1999; Kokalis-Burelle et al. 2002). In addition, viruses, amoebae, nematodes, and arthropods have been mentioned as possible biocontrol agents (Whipps and Mcquilken 1993). Although cyanobacteria (or blue green algae), constitute the largest, most diverse and most widely distributed group of photosynthetic prokaryotes (Stanier and Cohen-Bazire 1977), and together with the eukaryotic algae, make up most of the world's biomass, they have received little attention as potential biocontrol agents of plant diseases.

Cyanobacteria are photosynthetic prokaryotes, which represent a continually renewable biomass source that can release to the environment soluble organic substances as extracellular products, including secondary metabolites (Kulik 1995; Zulpa et al. 2003). In the last few decades, they have become an attractive source of innovative classes of pharmacologically active compounds showing interesting and exciting biological activities ranging from antibiotics, immunosuppressant, anticancer, antiviral, anti-inflammatory to proteinase inhibiting agents. Frankmolle et al. (1992) reported that crude ethanolic extracts from *Anabaena laxa* Rabenh, inhibited the growth of *Aspergillus oryzae*, *Candida albicans*, *Penicillium notatum*, *Saccharomyces cerevisiae* and *Trichophyton mentagrophytes*.

Damping off disease is known to be caused by a complex of pathogenic soil inhabiting fungi *Fusarium oxysporum lycopersici*, *F. moniliforme*, *P. debaryanum* and *R. solani*, which leads to severe losses in a wide variety of crop plants including tomato (*Lycopersicon esculentum* Mill.). Composts have been recognized in recent years as disease suppressive agents, especially against soil borne pathogens. Several mechanisms have been proposed to explain the suppression of plant diseases by compost (Cronin et al. 1996), including stimulation of microbial communities antagonistic to pathogens, presence of fungicidal compounds/enzymes (de Britto Alvarez et al. 1995) and induction of systemic acquired resistance (Zhang et al. 1998). Soil inoculation of antagonistic microorganism without a suitable nutritionally adequate substrate cannot be expected to be successful (Kokalis-Burelle et al. 2002; El Hassan and Gowen 2006); hence carriers such as talc, vermiculite, peat, charcoal have been employed for developing biocontrol formulations. However, amendment of composts with pathogen antogonists is less explored.

The aim of the present study was to compare and evaluate two cyanobacteria amended compost and compost: vermiculite based formulations, as disease suppressive agents against damping off disease in tomato.

Materials and methods

Organisms used in this study

A set of fungal antagonists comprising two cyanobacterial strains (*Anabaena variabilis* RPAN 59 and *A. oscillarioides* RPAN69), belonging to the germplasm of *Anabaena* isolates, from diverse agro ecologies of India (Prasanna et al. 2008) were evaluated in-depth for fungicidal activity against selected phytopathogenic fungi *Pythium debaryanum* (ITCC 95), *Rhizoctonia solani* (ITCC 4578), *Fusarium moniliforme* (ITCC 4223) and *Fusarium oxysporum lycopersici* (ITCC 4998), obtained from Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi.

Growth and maintenance

The cyanobacterial strains were axenised by standard procedures employing a set of antibiotics (Kaushik 1987), grown and maintained in nitrogen free BG 11 medium (Stanier et al. 1971), under a temperature of 27 ± 1 °C, L:D (Light: Dark cycles 16:8), white light (50–55 μmol photons m^{-2} s^{-1}). The fungal strains were grown and maintained on PDA (Potato Dextrose Agar) medium, at 28 ± 2 °C in an incubator (Commonwealth Agricultural Bureaux 1968).

Preparation of amended composts

Paddy straw compost (20 g) was prepared by the method of Gaind and Nain (2010); which was used as such and after mixing with vermiculite (1:1) as carrier. Both types of carriers (compost and compost: vermiculite) were amended with the late log phase cultures (pellets of 21 days old cultures) of selected cyanobacterial strains at 2 % inoculum rate (v/w) (on the basis of previously optimized inoculum rate; data not shown), incubated under stationary conditions in plastic bags with foam stoppers (Dukare 2010) for 10 days, maintaining 60 % water holding capacity. Four weeks old cyanobacterial cultures were selected for amendment, based on our earlier study in which the fungicidal and hydrolytic enzyme activity was highest at this stage (Prasanna et al. 2008). The chlorophyll values of the cyanobacterial cultures, used for amendment were measured using the method of MacKinney (1941) and maintained in the range of 10–20 μg ml^{-1}. Sterile water amended samples served as control.

In vitro fungicidal assay of cyanobacterial cultures

Sterile discs (Hi media Laboratories, India having no metabolite) of 5 mm diameter, were placed on the lawn of respective fungal strains. Twenty µl of filtrate of 4 weeks old cyanobacteria (Prasanna et al. 2008) or leachates from amended compost formulations were dispensed on the discs and incubated under optimal conditions. Leachates were prepared by taking 10 g compost/compost: vermiculite, suspending in 10 ml of sterile water, followed by vortexing and centrifugation (Dukare 2010). The supernatant, after passage through 0.45 μ filters was used as leachate. Nystatin and sterile water were used as positive and negative controls. Observations were taken regularly up to 72 h, on the development of inhibition zone.

Mass multiplication and preparation of fungal inoculum

Mass multiplication of the selected phytopathogenic fungi (*Pythium debaryanum*, *Rhizoctonia solani*, *Fusarium moniliforme* and *Fusarium oxysporum lycopersici*) was done on sorghum (*Sorghum bicolor*) seeds moistened with water and autoclaved thrice for 90 min on three consecutive days (Paulitz and Schroeder 2005). Flasks were inoculated with 1 week old fungal mycelium grown on PDA media and incubated at room temperature for 4 weeks, with shaking at weekly intervals. Colonized sorghum seeds were used as fungal inoculum.

Experimental set up

Nursery stage evaluation of biocontrol efficacy of the most promising cyanobacteria amended composts against soil borne pathogens of damping off disease of tomato was carried out under the controlled conditions (day/night 14/10 h; 25/18 °C) of National Phytotron Facility, IARI, New Delhi. The pots were filled with soil (500 g) and were autoclaved at 1.05 kg cm^{-2} pressure and 121 °C temperature for 1 h on three consecutive days. The potting mix comprised soil (after tyndallization to remove bacterial contaminants, if any) as the growth medium for plants in the pots and in treatments involving formulations in the ratio of 3:1; sterile soil: compost and 3:0.5:0.5 of sterile soil: compost: vermiculite in the case of blended formulation. The pots containing sterilized soil were preinoculated (TI) with fungal consortium at the rate of 5 g inoculum of each fungi per 500 g soil. The pots were kept for 1 week to establish the infection (Pandey and Dubey 1994). 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 samples from potting mixture pre inoculated with fungal consortium were analyzed for fungal spore counts at different time intervals. Uninoculated pots (TU) served as positive control.

A mixture of Thiram and Carbendazim (2 g kg^{-1} each) and *Trichoderma* @ 6 g kg^{-1} were used in the powdered form as the recommended chemical and biological control treatments respectively. After 1 week of inoculation of fungi in the pots, the cyanobacteria amended and unamended compost and compost formulations, recommended chemical and biological control were put into the fungi inoculated and uninoculated pots. The seeds of tomato variety *Pusa Ruby* were obtained from the Division of Vegetable Sciences, Indian Agricultural Research Institute, New Delhi. *Pusa Ruby* variety is known to be susceptible to diseases caused by soil borne pathogens such as “damping off” by *Pythium* sp. and root/stem roots caused by *Fusarium/Rhizoctonia* spp. The seeds were surface sterilized with 0.1 % (v/v) mercuric chloride for 3 min, washed repeatedly with deionized water, and soaked in water overnight, before sowing. A fixed number of ten seeds were sown per pot and the germination percentage (as an index of seed viability) of the seeds was also checked. All treatments involved application of seeds into soil, in which six treatments having seeds into soil pre inoculated with fungal inoculum, while the other six treatments having seeds into soil were uninoculated. The main treatments included TI—fungal consortia inoculated potting mixture; TU—uninoculated potting mixture and sub treatments such as T1—chemical control (Thiram + Carbendazim); T2—biological control (*Trichoderma*—IARI formulation; Dubey et al. 2012); T3—amended compost; T4—unamended compost; T5—amended compost: vermiculite; T6—unamended compost: vermiculite. The physicochemical properties of the different carrier materials were evaluated (Table 1). The sowing of the seeds was undertaken on 18th May 2011 and harvested after 6 weeks on 3rd July, 2011. Fungal load was measured in different treatments treated mix on 18th May, after 20 days on 8th June and after 45 days of sowing on 3rd July. Disease severity was also checked daily up to 20 days of sowing.

Plant/soil parameters evaluated

Enumeration of fungal spore from potting mixture pre inoculated with fungal consortia

Samples from soil pre-inoculated with fungal consortia were analyzed for the enumeration of fungal spore count using PDA medium (amended with Triton-X 100 @ 0.1 % and L—Sorbose @ 4 g L^{-1} after autoclaving) by serial dilution techniques and plate counting method at beginning (0 day), mid (20 days) and end of the experiment (45 days). Although spores of different fungi present in the different treatments treated soil were quantified by plate counting method, the growth/presence of *Rhizoctonia solani*, which does not produce any spores, was measured on the basis of the presence/absence of growth, compared with the control plate.

Table 1 Physico chemical properties of carrier materials

Properties	Paddy straw compost	Vermiculite
pH	6.59	8.8
EC (d S m^{-1})	1.82	0.1
Organic carbon (%)	24.05	–
Nitrogen (%)	1.48	–
C/N ratio	16.22	–
Humus (%)	13.84	–
Available phosphorus (mg g^{-1})	1.40	–
WHC (%)	48.0	76.0
Bulk density (g cm^{-1})	0.94	1.0
Particle density (g cm^{-1})	61.88	138.01
Porosity (%)	36.12	70.18

–, denotes not detectable

Disease severity

Observations were recorded to measure the pre- and post emergence disease severity in terms of seeds germinated and surviving. Pre-emergence disease was expressed in terms of number of seeds germinating/seedlings exhibiting disease symptoms, out of total number of seeds sown. Post emergence disease severity was evaluated by counting the number of germinated seeds and number of healthy seedlings (not exhibiting any disease symptoms) among those germinated, by visual observations taken daily up to 20 days. The average of pre and post emergence disease severity values was computed as disease severity index and expressed in terms of %.

After 6 weeks, three plants were selected randomly from each treatment, removed carefully from the pots, washed under running water to remove adhering particles and measurements were made in terms of number of leaves, root length, shoot length, plant height and fresh weight. These seedlings were evaluated in terms of fresh weight followed by dry weight after keeping in an oven maintained at 75 °C for 24 h in paper packets. The dried seedlings were kept in a desiccator for cooling and their weight taken. These values were converted to dry weight per seedling and expressed in grams. The percent mortality values were expressed using standard methods (Ratio of the number of diseased/dead seedlings and total number of seeds sown).

Experimental design and statistical analyses

The experiment was designed as factorial randomized block design, which included both pathogen inoculated (TI) and uninoculated treatments (TU) as controls, along with different treatments of cyanobacteria amended compost and compost formulations in soil, in which the sterilized seeds were placed. Data was recorded in triplicate for the selected parameters in the tomato crop. Analysis of variance (ANOVA) was performed in accordance with treatments and selected biometrical parameters as factors to quantify and evaluate the source of variation using SPSS statistical software. Duncan's Multiple Range Test (DMRT) was employed to compare the mean performances of different strains for the specific parameters under study. The C.D. (Critical Difference) values for uninoculated (TU) and inoculated (TI) treatments are given below each column, followed by the interactions between TU and TI treatments (TU × TI), which were calculated at 5 % level of significance. The rankings are denoted by superscripts in the relevant tables and graphs, with 'a' denoting the highest rank. Standard deviation has been denoted as bars in the graphs.

Results

Preliminary studies using the cyanobacterial strains (*Anabaena variabilis* RPAN59 and *Anabaena oscillarioides* RPAN69) produced a zone of inhibition of 12–16 mm diameter (as measured by disc diffusion assay), within 3–4 days, against the four phytopathogenic fungi—*R. solani*, *F. moniliforme*, *F. oxysporum lycopersici* and *P. debaryanum*. Nystatin (100 U) was used as positive control which produced an average zone of inhibition of 20 ± 5 mm with all fungal strains tested except *F. moniliforme*. The leachates of the formulations also produced a zone of inhibition of 14–16 mm diameter within 3 days of incubation (Fig. 1).

Two types of cyanobacterium amended compost formulations were prepared [paddy straw compost and compost: vermiculite (1:1)] and evaluated for their potential as biocontrol agents against a mixture of phytopathogenic fungi, involved in causing damping off disease in tomato. The physico-chemical characteristics of the vermiculite and paddy straw compost was evaluated and given in Table 1. Symptoms of Damping-off disease were recorded in small patches at various places in the pots. The seedlings were found to be most susceptible up to 2 weeks after emergence, and with the increasing age, the stem hardened and increased in size. Pre-emergence disease symptoms included visual observations on seeds, becoming soft, mushy, brown and decomposing, as a consequence of fungal infection. In post-emergence damping-off, the seedlings emerged, but died shortly afterwards. The affected portions (roots, hypocotyls and perhaps the crown of the plant) were pale brown, soft, water soaked, and thinner than non-affected tissue. Stunting of plants due to root rot or collar rot was also recorded. The infected stems

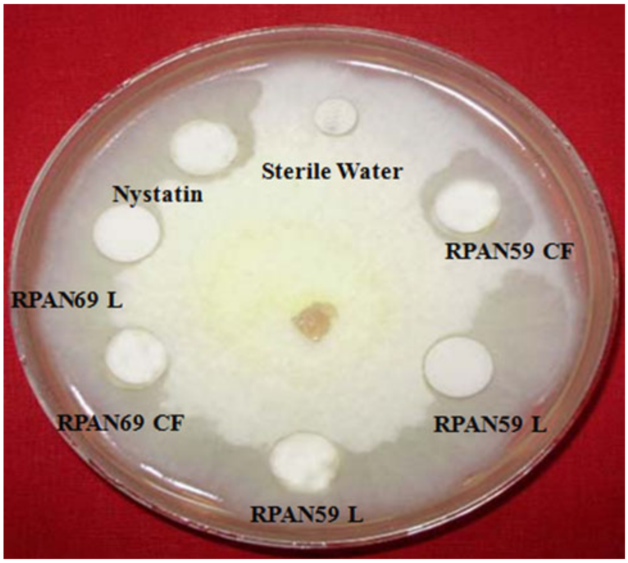

Fig. 1 Zone of inhibition produced by extracellular filtrates (CF) of *A. variabilis* (RPAN59) and *A. oscillarioides* (RPAN69) and leachates (L) from compost amended with the individual strain on the lawn of *Pythium debaryanum*. Nystatin and sterile water represent positive and negative controls

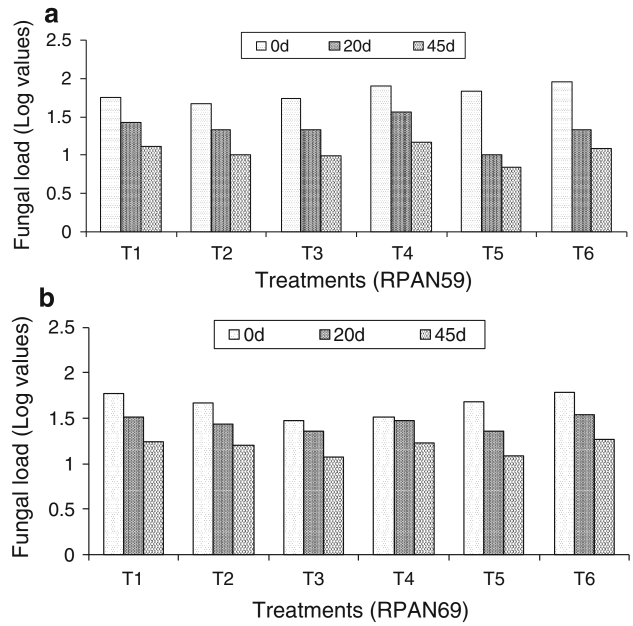

Fig. 2 Total fungal load (log values) in soil, taken from various fungi inoculated treatments involving **a** *A. variabilis* (RPAN59) and **b** *A. oscillarioides* (RPAN69) amended composts and compost formulations. Values were taken at beginning (0 d), mid stage (20 d) and end of the experiment (45 d). T1, chemical control; T2, biological control; T3, cyanobacterium (RPAN59/RPAN69) amended compost; T4, unamended compost; T5, cyanobacterium (RPAN59/RPAN69) amended compost: vermiculite; T6, unamended compost: vermiculite

collapsed when the root or lower stem became infected (Supplementary Fig. 1).

In the present study, the population of fungal colonies in treatments involving RPAN59 amended compost application revealed an almost tenfold reduction in the treated soil (from log 1.957 to log 0.845 and log 1.782 to log 1.066) at mid (20 days) and end (45 days) of the experiment respectively (Fig. 2a). T5 (RPAN59 amended compost: vermiculite) recorded lowest values at mid (20 days) and end (45 days) stage of sampling. Application of RPAN69 amended compost and compost: vermiculite in fungi inoculated soil, was the most superior and almost similar in reducing fungal load (Fig. 2b).

The pot experiments with tomato inoculated with fungal consortium, amended with RPAN69 compost showed a significant increase in terms of number of leaves as well as length and weight of the plant (Figs. 3 and 4). The treatment involving RPAN59 amended formulations recorded the highest values, which was significantly greater than the other treatments, such as unamended compost: vermiculite and compost. The amended compost: vermiculite and compost recorded the highest values of plant growth parameters, even in the fungal consortia challenged treatments (Table 2; Fig. 3). RPAN69 amended compost treatment resulted in a threefold increase in fresh weight of plant over control, as compared to chemical and biological control, which recorded the lowest yields (Table 3; Fig. 4). A significant effect of treatment (TU × TI) was observed and RPAN69 amended compost in TI, recorded significantly higher values of plant parameters, as compared to TU treatments. On the other hand, RPAN59 amended compost: vermiculite formulation was superior, even in the fungal challenged treatments and recorded significantly higher values for plant growth parameters.

In terms of mortality, lowest value of 43.34 % was recorded in treatment involving application of compost and vermiculite amended with *A. variabilis* (RPAN59) culture, followed by treatments in which the seeds had been treated with *A. variabilis* (RPAN59) amended compost. In treatments involving RPAN69, lowest % mortality was recorded in amended compost treated plants (Fig. 5).

Disease severity was recorded in terms of pre and post-emergence stages which revealed a 20 and 3 % reduction in pre-emergence values in the treatments receiving compost and compost: vermiculite formulations, over biological and chemical control respectively, especially those involving RPAN59 amendment (Table 4).

Discussion

The challenge to produce fruits, vegetables and cereals which can fulfil the demands of the consumers, not only in terms of yields, but also in terms of safe and improved quality has triggered research into environment-friendly

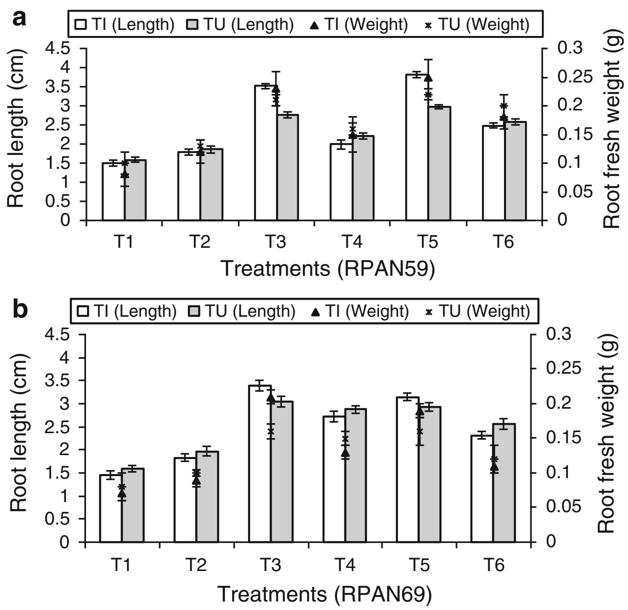

Fig. 3 Influence of **a** *A. variabilis* (RPAN59) and **b** *A. oscillarioides* (RPAN69) amended compost (s) and compost formulations on root length and root fresh weight of fungi inoculated and uninoculated tomato seedlings. *Vertical error bars* indicate the standard deviation among the three replications. T1, chemical control; T2, biological control; T3, cyanobacterium (RPAN59/RPAN69) amended compost; T4, unamended compost; T5, cyanobacterium (RPAN59/RPAN69) amended compost: vermiculite; T6, unamended compost: vermiculite

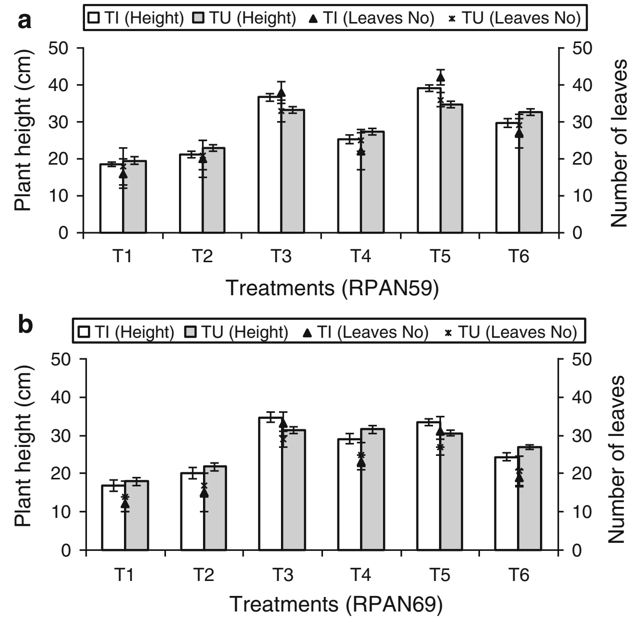

Fig. 4 Plant height and number of leaves of fungi inoculated and uninoculated tomato seedlings as influenced by **a** *A. variabilis* (RPAN59) and **b** *A. oscillarioides* (RPAN69) amended compost and compost formulations. *Vertical error bars* indicate the standard deviation among the three replications. T1, chemical control; T2, biological control; T3, cyanobacterium (RPAN59/RPAN69) amended compost; T4, unamended compost; T5, cyanobacterium (RPAN59/RPAN69) amended compost: vermiculite; T6, unamended compost: vermiculite

options. Biological control offers an attractive alternative without the negative impact of chemical control. A number of cyanobacteria and eukaryotic algae produce various biologically active compounds which have ecological roles as allelochemicals, and exhibit immense utility in the commercial development of algicides, herbicides and insecticides (Kulik 1995; Prasanna et al. 2008; Prasanna et al. 2010a, b). Among cyanobacteria, *Nostoc muscorum* is known to be effective against "damping off" disease caused by fungi (de Caire et al. 1990) and several *Anabaena* and *Calothrix* strains exhibit fungicidal activity against species of *Pythium*, *Fusarium* and *Rhizoctonia* (Moon et al. 1992; Prasanna et al. 2008; Radhakrishnan et al. 2009; Manjunath et al. 2010). Our earlier studies have shown that several cyanobacteria—including *Calothrix* sp. and *Anabaena* strains exhibit fungicidal activity which could be correlated with the production of hydrolytic enzymes and the homologues for chitosanase and endoglucanase have been detected in several *Anabaena* strains (Prasanna et al. 2008; Prasanna et al. 2010a; Gupta et al. 2010, 2011). The production of hydrolytic enzymes and their role in biocontrol has been reported earlier for bacterial strains (Singh et al. 1999; Grover et al. 2009; Dukare et al. 2011).

The cyanobacterial strains used in this study (*Anabaena variabilis* RPAN59 and *Anabaena oscillarioides* RPAN69) had shown significant inhibition against the four phytopathogenic fungi *R. solani*, *F. moniliforme*, *F. oxysporum lycopersici* and *P. debaryanum* (Prasanna et al. 2008). In an earlier investigation, assays were developed for evaluating the fungicidal activity and the role of hydrolytic enzymes in these ubiquitous photosynthetic prokaryotes (Chaudhary et al. 2010).

Compost based suppression of a wide range of major soil borne diseases has been demonstrated in the last decade as a promising option (Nakkeeran et al. 2008). Organic amendments, including composts are widely used in agriculture, especially in horticultural crops to improve soil structure which allows for better water transmission, and enhance biochemical and microbial activity leading to improved disease resistance of plants. Besides the suppression of the spread of disease at nurseries and field, composts can also provide nutrients and organic matter, thereby eliminating or reducing the need for fertilizer or the use of expensive peat mixes. As composts are derived from agricultural wastes/residual materials and by-products, they are generally free from xenobiotics and heavy metal contamination and can be considered as suitable candidates for exploitation as carriers for biocontrol agents. It is recognized that inoculation with bacterial/fungal antagonists may not be always successful because of the

Table 2 Influence of *A. variabilis* (RPAN59) amended compost formulations on selected plant growth parameters of tomato seedlings

Treatments	Plant fresh weight (g)		Shoot fresh weight (g)		Plant height (cm)		Shoot length (cm)	
	TI	TU	TI	TU	TI	TU	TI	TU
T1—chemical control	1.04[f]	1.180[f]	0.660[e]	1.080[f]	18.500[f]	19.490[e]	17.000[f]	17.900[e]
T2—biological control	1.37[e]	1.590[e]	1.250[d]	1.460[e]	21.190[e]	22.960[d]	19.400[e]	21.100[d]
T3—RPAN59 amended compost	3.557[b]	2.630[b]	3.327[b]	2.420[b]	36.720[b]	33.297[b]	33.200[b]	30.533[b]
T4—unamended compost	1.620[d]	1.880[d]	1.470[d]	1.720[d]	25.223[d]	27.410[c]	23.233[d]	25.200[c]
T5—RPAN59 amended compost: vermiculite	4.130[a]	2.900[a]	3.880[a]	2.680[a]	39.043[a]	34.737[a]	35.233[a]	35.767[a]
T6—unamended compost: vermiculite	2.120[c]	2.440[c]	1.940[c]	2.240[c]	29.747[c]	32.590[b]	27.267[c]	30.000[b]
C.D.	0.077	0.099	0.357	0.096	1.488	1.116	1.488	1.108
C.D. (TI × TU)	0.326		0.360		1.601		1.404	

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT. TI, Fungal consortia inoculated potting mixture; TU, uninoculated potting mixture; C.D. denoted the critical difference among the means of different treatments for each parameter within TI and TU and their interactions is given as (TI × TU)

Table 3 Influence of *A. oscillarioides* (RPAN69) amended compost formulations on selected plant growth parameters of tomato seedlings

Treatments	Plant fresh weight (g)		Shoot fresh weight (g)		Shoot length (cm)		Plant dry weight (g)	
	TI	TU	TI	TU	TI	TU	TI	TU
T1—chemical control	0.960[f]	1.190[d]	0.890[f]	1.110[d]	15.333[e]	16.333[d]	0.360[f]	0.390[f]
T2—biological control	1.330[e]	1.550[c]	1.240[e]	1.447[c]	18.333[d]	19.803[c]	0.540[e]	0.650[e]
T3—RPAN69 amended compost	3.850[a]	2.963[a]	3.640[a]	2.803[a]	31.333[a]	28.333[a]	1.447[a]	1.243[a]
T4—unamended compost	2.597[c]	2.710[a]	2.467[c]	2.560[a]	26.333[b]	28.647[a]	0.953[c]	1.050[c]
T5—RPAN69 amended compost: vermiculite	3.357[b]	2.840[a]	3.167[b]	2.680[a]	30.333[a]	27.653[a]	1.340[b]	1.147[b]
T6—unamended compost: vermiculite	1.860[d]	2.260[b]	1.750[d]	2.140[b]	22.080[c]	24.347[b]	0.730[d]	0.890[d]
C.D.	0.072	0.229	0.066	0.232	2.064	1.111	0.055	0.058
C. D. (TI × TU)	0.254		0.243		1.435		0.080	

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT. TI, fungal consortia inoculated potting mixture; TU, uninoculated potting mixture; C.D. denoted the critical difference among the means of different treatments for each parameter within TI and TU and their interactions is given as (TI × TU)

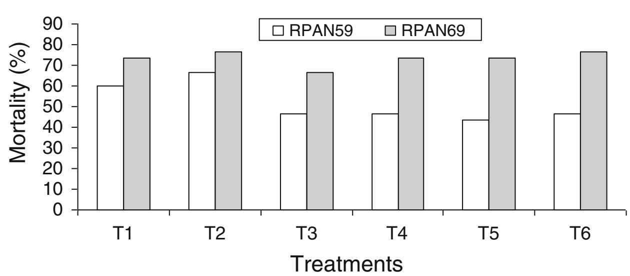

Fig. 5 Effect of different treatments on mortality of tomato plants challenged with fungal consortia. T1, chemical control; T2, biological control; T3, cyanobacterium (RPAN59/RPAN69) amended compost; T4, unamended compost; T5, cyanobacterium (RPAN59/RPAN69) amended compost: vermiculite; T6, unamended compost: vermiculite

competition with native flora/fauna for nutrients and availability of optimal moisture/temperature conditions. A combination of a biological antagonist with suitable organic substrate, can obviate to a great extent, such challenges in the soil environment. Also, a number of microbes, including *Pseudomonas* and *Pantoea* spp. present, are known to contribute to this effect induced by composts (Craft and Nelson 1996). The 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). Several mechanisms have been proposed to explain the suppression of plant diseases by compost; including beneficial chemical components in compost, stimulation of microbial communities antagonistic to pathogens (Chen et al. 1987; Dukare et al. 2011), and induction of systemic acquired resistance have been implicated in the suppression of plant disease (Zhang et al. 1998). However, very few initiatives have been undertaken especially to exploit combinations of composts and microbial antagonists to develop effective biocontrol formulations.

Compost is commonly used as a fertilizer and soil amendment, and vermiculite is known for its utility as a conditioner and matrix in potting mixes. Vermiculite is additionally an inexpensive carrier which is widely

Table 4 Influence of different treatments on disease indices of fungal pathogen challenged tomato seedlings

Treatments	*A. variabilis* (RPAN59)			*A. oscillarioides* (RPAN69)		
	Pre emergence	Post emergence	Disease severity	Pre emergence	Post emergence	Disease severity
T1—chemical control	36.67	36.83	36.75	63.34	27.27	45.30
T2—biological control	53.34	28.56	40.95	63.34	36.36	49.85
T3—amended compost	33.34	19.99	26.66	46.67	37.50	42.08
T4—unamended compost	36.67	15.79	26.23	60	33.35	46.67
T5—amended compost: vermiculite	33.34	15.0	24.17	56.67	38.47	47.57
T6—unamended compost: vermiculite	33.34	19.99	26.66	63.34	36.36	49.85

Pre emergence disease (%) = (total number of seeds sown − number of germinated seedlings) × 100/total number of seeds sown

Post emergence disease (%) = (number of germinated seedlings − number of surviving seedlings not exhibiting any disease symptoms) × 100/number of germinated seedlings

Disease severity (%) = average of pre and post emergence disease severity

available and possesses multilamellate structure and excellent moisture holding and anti-crusting properties. The combination of an organic material with an inorganic matrix can provide an ideal physical, chemical and biological composition and the plant growth promoting effect of our formulations, and the slow release of nutrients from compost, or indirectly through the positive effect on the cation exchange capacity, can lead to effective biofertilization and biocontrol (Bashan 1998).

Tomato is one of the most important vegetables grown and consumed worldwide due to its high nutritive value, especially as a source of vitamins A and C. Damping off and related soil borne diseases are a serious problem as they cause high seedling mortality in nurseries and fields. The lack of effective soil fungicide treatments for this disease has stimulated the search for suitable environmental friendly alternatives. In our study, compost/compost: vermiculite preparations containing cyanobacteria were evaluated for their disease suppressiveness against fungal consortia challenged tomato. The symptoms of infection, as recorded in control treatments and to a lesser extent in treatments receiving cyanobacterial formulations included reduced seed germination, poor or weak seedlings showing discolorations. Such symptoms of infection are usually not lethal, but plant growth and yield are reduced due to impairment of nutrient uptake from the soil by the plant. A consistent positive correlation between disease suppression and decrease of pathogen populations was recorded in our study, which indicates that population decline may be one of the mechanisms underlying the biological control using microbe based amendments.

Two classes of biological control mechanisms known as general and specific suppression have been described for compost-amended substrates. The mechanisms involved, are based on competition, antibiosis, hyper parasitism, and induction of systemic acquired resistance in the host plant (Hoitink et al. 2001). One of the beneficial properties of compost is the microbially induced suppression of soil borne plant pathogens and disease (Hoitink et al. 1987). Chen et al. (1987) reported that suppressive composts possess a higher microbial activity than conducive ones. They suggested that a high microbial activity, as in our case, combined with production of hydrolytic enzymes, can cause depletion in essential nutrients for the survival and multiplication of the pathogen, thus preventing infection of the host. It can also be surmised that the antagonistic cyanobacteria may also activate certain disease resistance systems in plants.

Our study also highlights that compost can play an important role in improving plant growth, which may be an indirect effect of the plants being less prone to infection by pathogens. Both RPAN59 amended vermiculite: compost formulations and compost showed effective control over *R. solani*, *F. moniliforme*, *F. oxysporum lycopersici* and *P. debaryanum* while RPAN69 amended compost performed better, followed by vermiculite: compost formulation in terms of biocontrol. In the present investigation, the plant growth attributes were the highest in fungi challenged cyanobacteria (RPAN59/RPAN69) amended formulations treated plants, as the severity of disease was also suppressed up to a great extent in these fungi challenged plants by our cyanobacterial formulations. This can be attributed to the production of secondary metabolites, plant defense mechanisms would take precedence, as has also been recorded by other researchers (Chen et al. 2000; Singh et al. 2011). In the other treatments, the higher plant growth in fungi unchallenged (TU) plants, can be as a result of channeling of nutrients/metabolic products towards growth and plant vigour vis a vis fungi challenged plants. In the present investigation, although all the treatments had almost similar mortality rates, the lowest % mortality was recorded in treatments involving application of

A. variabilis (RPAN59) amended vermiculite: compost and RPAN69 amended compost treated plants. Although, there was a decrease in disease severity in all treatments, involving chemical, biological or composts, formulations with *A. variabilis* RPAN59 showed a very drastic reduction, and lowest post emergence disease severity of 15 % was recorded in T5 (amended compost: vermiculite). For both strains, amended compost and compost: vermiculite were the top two ranked treatments in terms of all parameters in fungal inoculated treatments which revealed the promise of the cyanobacterial strains in disease suppressiveness. Overall, disease severity indices ranged from 24 to 26 % in *A. variabilis* RPAN59 amended formulations and 46–49 % in the case of *A. oscillarioides* RPAN69 formulations. *A. variabilis* amended composts performed better in terms of reduction in disease severity and enhancement in plant growth attributes. Chemical and biological control also reduced the disease severity but the efficacy of antagonists in the microbe amended composts was more promising, as it also brought about plant growth promotion. The effect of amended composts/formulations was more pronounced at post emergence stage over the chemical and biological controls. Disease severity at both pre and post emergence stages and overall (33, 15.0, 24.17 % respectively), was lowest in RPAN59 amended compost formulations. Such composts need to be evaluated at field level in "sick plots" for validation of biocontrol potential.

The highlight of this study is the observation that cyanobacterium amended formulations brought about a significant reduction in disease severity, besides increase in the plant growth of phytopathogenic fungi challenged tomato seedlings. Such novel formulations can be a successful strategy for sustainable and environment-friendly agriculture. Further research into plant defense enzymes is in progress to understand the "cross talk" between the fungal pathogens, plants and cyanobacterial metabolites and identify target molecules.

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