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Biocontrol potential of cyanobacterial metabolites against damping off disease caused by *Pythium aphanidermatum* in solanaceous vegetables

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An investigation was undertaken to explore the biocidal efficacy of fungicidal compound(s) produced by *Calothrix elenkenii* against damping-off disease in three vegetable crops-tomato, chilli and brinjal. Treatments included application of seeds soaked in water (control), culture filtrate and ethyl acetate extract of *Calothrix elenkenii* and Metalaxyl in potting mix inoculated with *Pythium aphanidermatum* in plastic pots. The observations taken after a period of four weeks revealed the superiority of seed treatment with ethyl acetate extracts, in terms of percent mortality and plant parameters. ANOVA revealed that the treatments, crops (tomato, chilli and brinjal) and their interactions exerted a significant influence on the parameters analyzed. Chilli recorded the highest percentage of survivors and responded best to the seed treatment with ethyl acetate extract of *Calothrix elenkenii*. Future work is being undertaken towards formulation of a biocontrol agent using *Calothrix elenkenii* and understanding the molecular basis for the biocontrol properties.

Keywords: biocontrol; *Calothrix*; cyanobacteria; damping off; *Pythium* sp.; vegetables

Introduction

Cyanobacteria (BGA) represents a valuable bioresource which has been utilised mainly as a biofertiliser in agriculture. Extensive reviews on their distribution in rice fields and their nitrogen-fixing potential exist which reveal their generic, genetic and functional diversity (Venkataraman 1972; Mandal et al. 1998; Prasanna and Nayak 2007) The less investigated aspects include the evaluation of their biochemical potential in terms of production of biologically active compounds. The significance of these organisms as producers of cyanotoxins and other novel bioactive molecules is globally recognised (Namikoshi and Rinehart 1996; Mundt et al. 2001; Kumar et al. 2005); however, their chemical potential is less explored in agriculture, especially as biocontrol agents. The extracts of cyanobacteria are known to reduce the incidence of *Botrytis cinerea* on strawberries and *Erysiphe polygoni* causing

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powdery mildew on turnips and damping off in tomato seedlings, besides reducing the growth of saprophytes, *Chaetomium globosum*, *Cunninghamella blakesleeana* and *Aspergillus oryzae*, and plant pathogens such as *Rhizoctonia solani* and *Sclerotiana sclerotium* (Kulik 1995).

Pythium species are commercially important pathogens of a variety of crops, which are ubiquitous in cultivated soils. They are considered opportunistic pathogens, as disease is more prevalent on young or weak plants. The ability of *Pythium* spp. to persist in soil for long periods, infect a wide variety of plant hosts and grow rapidly in the presence of germinating seeds provides a number of biological barriers for bio-effective controls (Ellis et al. 1999). The present investigation was undertaken to explore the biocidal efficacy of fungicidal compound(s) produced by *Calothrix elenkenii* against damping-off disease caused by *Pythium aphanidermatum* in three vegetable crops, tomato, chilli and brinjal.

Materials and methods

Organisms used in this study

Calothrix elenkenii, an isolate from rice fields of the Indian Agricultural Research Institute (IARI), was used in this investigation. This strain was observed to exhibit biocidal activity against two selected unicellular cyanobacteria and two phytopathogenic fungi (Radhakrishnan 2006). The fungal strain *Pythium aphanidermatum* was obtained from the Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi and used as the test organism.

Growth and maintenance

The cyanobacterial strain was axenised by a standard procedure employing a set of antibiotics (Kaushik 1987). The culture was routinely grown under the conditions optimised for maximum biocidal activity i.e. L:D (light:dark cycle 16–18, 5000 lux white light (50–55 μmol photons/m^2/s) and 27 $\pm$ 2° C (Radhakrishnan 2006) in nitrogen free BG-11 medium (Stanier et al. 1971). The fungal strain was grown in potato dextrose agar medium (Shadomy et al. 1985) and incubated at 30°C in a (Biochemical Oxygen Demand) BOD incubator.

Qualitative analyses of metabolite

The culture grown for three weeks was harvested by centrifugation at high speed (10–12,000 rpm) for 20 min at 15–20°C using a Sigma K 50 centrifuge. The cell-free extracts were filtered through layers of muslin cloth and pooled for extraction. The culture filtrate was extracted with ethyl acetate as solvent, using mechanical shaker for approximately 1 h. The organic phase was separated from aqueous phase and dried over anhydrous sodium sulphate. The dried extract was concentrated using a rotary evaporator. The concentrated extract was stored at 4–5°C until further analyses. Partial purification was carried out on preparatory thin layer chromatography (TLC) plates using the hexane:benzene (1:1) system and the spots were visualised using iodine vapors as visualising agent. The fraction eluted from TLC plates was tested for biocidal activity by measuring the zone of inhibition in the lawn of fungal strain.

Samples were also analysed by a HPLC (Thermo Separation Product Model Spectra System P2000) equipped with a variable wavelength UV-150 UV VIS

detector and a Rheodyne injector, connected to a Datajet reporting integrator. The stationary phase consisted of a Lichrosorb C-18 column (250 mm × 4.6 mm id) and the mobile phase was methanol: water (60:40 v/v) maintained at a flow rate of 1 ml/min with the detector wavelength set at 265 nm.

Minimum inhibitory concentration (MIC)

Standard procedures employing 96-well microplates were used for determining the MIC of the culture filtrate and extract in terms of fungicidal activity (Prasanna et al. 2006), against *Pythium aphanidermatum* in PDA medium. Positive and negative controls along with dilutions with 1–20 ppm of ethyl acetate extract and 100–1000 ppm of culture filtrate were employed. Nystatin (100 units/disc) was used as the standard antifungal compound. The presence/absence of growth at the various concentrations were observed visually and through microscopic observation of the plates for a period of five days.

Treatments and growth chamber conditions

An experiment was undertaken under controlled conditions of National Phytotron Facility, IARI, New Delhi to evaluate the fungicidal activity of the partially purified culture filtrates of *Calothrix elenkenii* on seeds/seedlings of tomato, brinjal and chilli grown in potting mix inoculated with *Pythium aphanidermatum*. 2% metalaxyl (Ridomil MZ 72 WP) was used as the recommended chemical control, both as seed treatment and soil drench.

The seeds of tomato variety *Pusa Rohini* and brinjal variety *Pusa Kranti* were obtained from the Division of Vegetable Sciences, Indian Agricultural Research Institute, New Delhi. The Chilli variety *H1* was obtained from National Seeds Corporation, Pusa Campus, IARI, New Delhi. All three varieties are known to be susceptible to damping off disease caused by *Pythium* spp. The germination percentage (as index of seed viability) of the vegetable seeds was checked.

The inoculum of *Pythium aphanidermatum* was produced on sorghum (*Sorghum bicolor*) seeds using water in equal proportion and autoclaved twice for 90 min on two consecutive days (Paulitz and Schroeder 2005). Flasks were seeded with one-week-old *Pythium aphanidermatum* mycelium grown on PDA media. Flasks were incubated at room temperature for four weeks, with shaking at weekly intervals. Colonised sorghum seeds were spread out on craft paper, dried for two days under laminar flow hood, and ground using a mixer grinder. Inoculum was passed through a 1 mm sieve and collected on a sieve of 250 μm; particles larger than 1 mm or smaller than 250 μm were not used as inoculum. Inoculum was stored at 4°C until use.

Nursery stage evaluation of biocontrol efficacy of *Calothrix* sp. culture filtrate and concentrated ethyl acetate extract against damping-off disease of vegetable crops (tomato, chilli and brinjal) was carried out with the following treatments in four replications. The potting mix consisted of vermiculite: sand (2:1) used as media for growth of plants in the pots. The pots were filled with media and were autoclaved at 15 lb/inch^2 pressure, 121°C temperature for 3 h. The *Pythium aphanidermatum* inoculum was put into the already sterilised pots filled with potting mix at the rate of 2 g inoculum per 200 g potting mix. The surface sterilised seeds of the three crops were soaked in the effective concentrations (MIC values) for 2 h in the culture

filtrates or ethyl acetate extracts or metalaxyl or water (control) and placed in the pre-inoculated potting mix. Uninoculated pots served as positive control for the respective crops.

The pots were kept for one week to establish the infection (Pandey and Dubey 1994). All treatments involved application of seeds into potting mix, pre-inoculated with *Pythium aphanidermatum* (except T1). The treatments included: T_1 - Untreated seeds (plant control); T_2 - Untreated seeds; T_3 - Metalaxyl as soil drench + untreated seeds; T_4 - Culture filtrate treated seeds; T_5 - Ethyl acetate extract treated seeds and T_6 - Metalaxyl as seed treatment.

Experimental observations

The percentage mortality was calculated by dividing the number of surviving seedlings by the total number of seeds sown. Five plants were selected randomly in each treatment and the number of leaves per plant counted. The average length of root, shoot, height and fresh weight of five seedlings were taken. The same five seedlings selected for fresh weight measurement were kept in an oven maintained at 75°C for 24 h in paper packets and dried seedlings were kept in a desiccator for cooling. These values were converted to dry weight per seedling and expressed in grams.

Experimental design and statistical analyses

The experiment was designed as a completely randomised design, which included both pathogen-inoculated and non-inoculated (T1) control in vermiculite–sand potting mix in which the treated seeds were placed. The experiment was undertaken twice to ascertain the results. Data was recorded in quadruplicate for the parameters in the various crops. Analysis of variance was performed with treatment and crop as factors to quantify and evaluate the source of variation. CD values were calculated at 1% level of significance. In the graphs, Standard deviation values have been denoted as error bars in the Figures.

Results

The cell-free culture filtrates of *Calothrix elenkenii* from a 21 day old culture was employed as it exhibited maximum inhibition zone of 20 mm when tested against *Pythium aphanidermatum*, the causal organism of damping-off disease of vegetable crops. Among the different solvent systems used for the extraction of polar and non-polar compounds, ethyl acetate was found to be the most potent and used for further work. Ethyl acetate extract of *Calothrix elenkenii* was observed to be active against the fungi tested. The MIC values using the micro-dilution method indicated that 850 ppm of culture filtrate and 16.6 ppm of the extract was inhibitory towards *Pythium aphanidermatum*. The broth dilution assay for determining MIC of the ethyl acetate extract against *Pythium aphanidermatum* also provided similar results. The extracted toxin was partially purified using preparatory TLC. The band showing antifungal activity was extracted and subjected to further characterisation. HPLC analyses of the TLC purified extract revealed a single peak indicating presence of a single compound with a retention time of 3.19 min.

In vitro *evaluation of biocontrol potential under controlled conditions*

An experiment was undertaken in pots (4 cm in diameter, with 200 g sterilised potting mix) under the controlled conditions of the National Phytotron Facility, IARI for a period of four weeks to evaluate the biocidal effect of the culture filtrates and ethyl acetate extracts of *Calothrix elenkenii* on *Pythium aphanidermatum* challenged seeds/seedlings of three vegetable crops, namely tomato (var *Pusa Rohini*), brinjal (*var Pusa Kranti*) and chilli (var *H1*). All the varieties used in this experiment were sensitive to the damping-off disease caused by the pathogen. The vigour of the seeds was determined by placing 50 seeds (in three replicates) in seedling agar. Tomato seeds germinated after 6 d, while brinjal and chilli seeds germinated after 7 and 10 d, respectively. All three crops exhibited a germination percentage of 95%. The pots were irrigated with Hoagland's solution frequently to maintain the optimum moisture in the potting mix. After four weeks, the seedlings were 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, fresh weight, dry weight, plant height and percent mortality.

Effect on tomato (*Lycopersicon esculentum Mill.*)

In terms of mortality, the lowest value of 17.5% was recorded in treatment (T5) involving application of ethyl acetate extract followed by treatments in which the seeds had been treated with metalaxyl and potting mix drenched with metalaxyl (Figure 1). Water soaked seeds recorded a mortality percentage of 38.75% (T1), while untreated seeds challenged with *Pythium aphanidermatum* (T2) recorded the highest mortality percentage of 64.2%. The number of leaves ranged from three to seven in all the treatments and statistical analyses revealed the significance of the treatment at the 1% level of probability. Treatment T5, involving application of ethyl acetate extract, gave the highest values of 7 followed by T3 in which the potting mix was drenched with metalaxyl (2%). The fresh weight and dry weight values of the seedlings grown in the presence of ethyl acetate extract were twofold higher than those observed in T6 treatment (seeds treated with metalaxyl) and significantly higher than the values recorded in T4 seeds grown in metalaxyl drenched potting mix (Figure 1). In terms of root length, T5 treatment recorded the highest values of 7.525 cm, which was significantly higher than the values recorded in all other treatments. A similar trend was observed in the observations recorded for shoot length and significant differences were observed among the treatments (Table 1).

Effect on chilli (*Capsicum annum* L.)

The mortality percentage was highest in untreated *Pythium* challenged seeds, followed by treatment involving water soaked uninoculated seeds (Figure 2). The lowest values were recorded in metalaxyl treated seeds, followed by treatment involving seed treatment with ethyl acetate extract of *Calothrix elenkenii*. The mean values in terms of number of leaves ranged from 2.5 to 7.0 with the highest values being recorded in treatment T5 (seeds treated with ethyl acetate extract of *Calothrix elenkenii*) followed by T6 and T4. Fresh weight and dry weight of the seedlings (Figure 2) was also significantly higher in ethyl acetate extract treated seedlings (treatment T5) as compared to other treatments in which *Pythium*

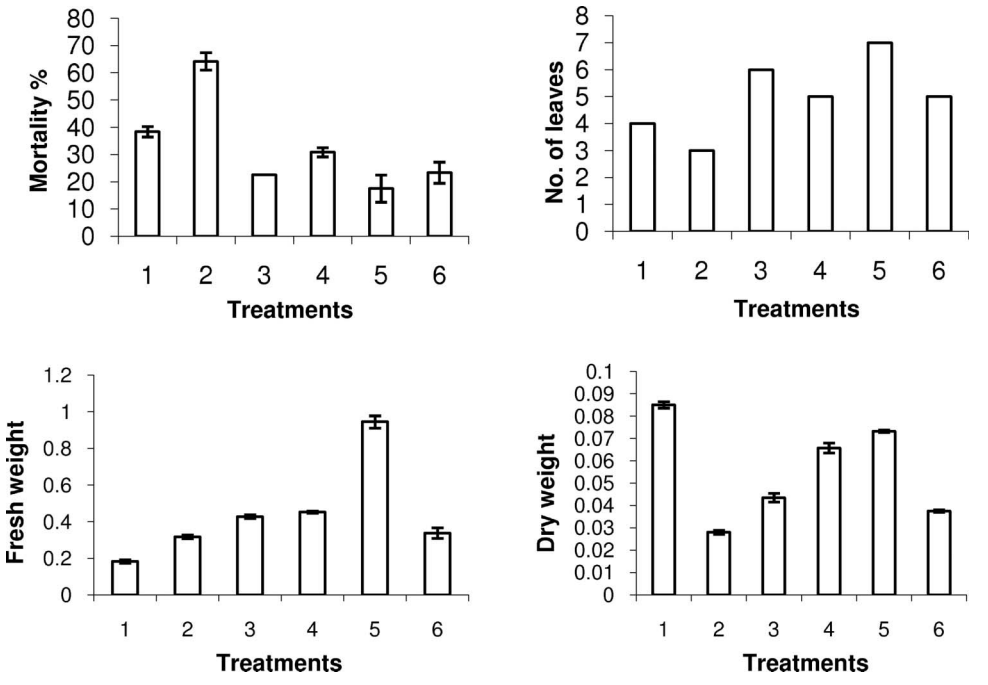

Figure 1. Influence of culture filtrate/ethyl acetate extract of *Calothrix elenkenii* on selected biometrical parameters of tomato (*Lycopersicon esculentum* Mill.) seedlings challenged with *Pythium aphanidermatum* and evaluated after four weeks of incubation. Treatments included 1 - Untreated seeds; 2 - Untreated seeds (Control without *Pythium* inoculation); 3 - Metalaxyl as soil drench + untreated seeds; 4 - Culture filtrate treated seeds; 5 - Ethyl acetate extract treated seeds and 3 - Metalaxyl as soil drench + untreated seeds and 6 - Metalaxyl as seed treatment. All treatments involved application of seeds into potting mix, pre-inoculated with *Pythium aphanidermatum* (except T_1).

Table 1. Comparative evaluation of influence of different treatments on root and shoot length of seedlings of tomato, chilli and brinjal.

Treatment	Root length (cm)			Shoot length (cm)		
	Chilli	Brinjal	Tomato	Chilli	Brinjal	Tomato
T1[a]	3.750	4.950	3.300	7.05	6.300	7.200
T2	1.737	2.425	2.150	5.30	4.025	6.100
T3	3.610	5.775	4.275	12.375	8.450	8.625
T4	3.135	5.550	5.225	13.365	8.075	8.375
T5	3.917	8.675	7.525	14.935	8.975	9.125
T6	3.740	5.350	3.400	12.775	8.000	8.650
C D @1%	0.406	0.181	0.231	0.406	0.170	0.181
Crops × treatment		0.072			0.068	

[a]Except T_1, all treatments, involved application of seeds into potting mix, pre-inoculated with *Pythium aphanidermatum*. Details of treatments as given for figures.

challenged seedlings were evaluated. In terms of root length (Table 1) T5, T6 and T1 recorded statistically *at par* values while shoot length was significantly higher in treatment T5.

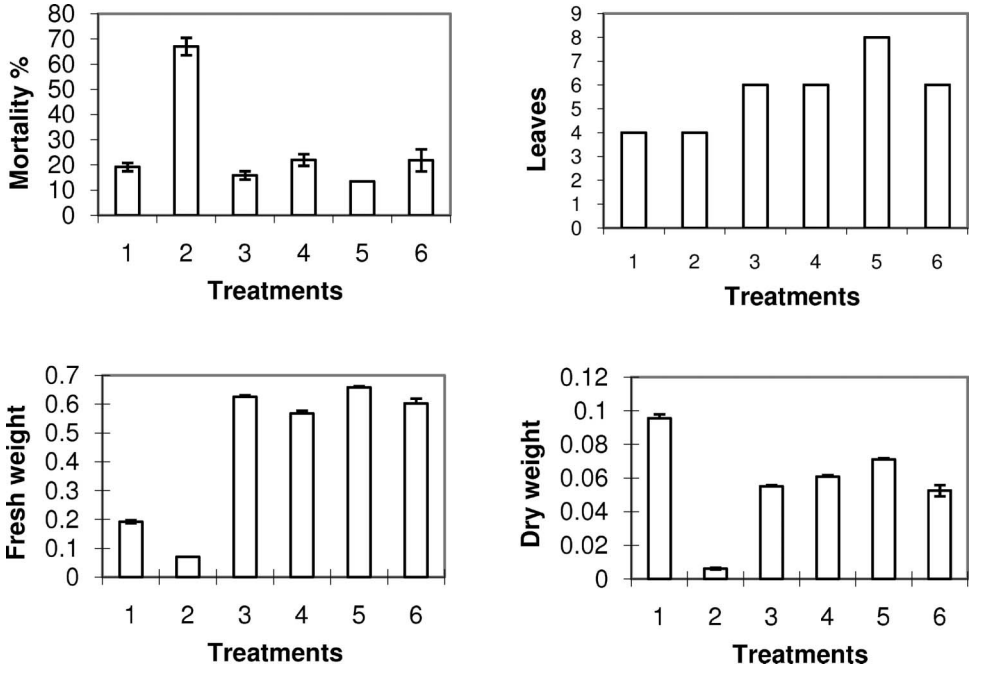

Figure 2. Influence of culture filtrate/ethyl acetate extract of *Calothrix elenkenii* on selected biometrical parameters of chilli (*Capsicum annum* L.) seedlings challenged with *Pythium aphanidermatum* and evaluated after four weeks of incubation (treatments as given for Figure 1).

Effect on brinjal (*Solanum melangena* L.)

The application of ethyl acetate extract as seed treatment (T5) recorded the lowest mortality (13.3%), followed closely by treatment (T3) involving potting mix drenched with metalaxyl (15.87%). Untreated *Pythium* challenged seedlings (T2) recorded 64.2% mortality (Figure 3). Among the six treatments, T5 (seed treatment with ethyl acetate extract of *Calothrix elenkenii*) recorded the highest number of leaves (eight) as compared to 4.0 in untreated seedlings. Six leaves were recorded in all the other treatments. A severe (9–10 fold) enhancement in fresh weight was recorded in all the treatments except T1. The highest values were recorded in T5 followed by T3 and T6. A similar trend was observed in dry weight values recorded in all the treatments (Figure 3). A fourfold increase in root length was recorded in T5 treatment compared to T2 (untreated). In terms of shoot length, the highest values of 8.975 cm were recorded in T5, followed by 8.45 cm in T3 and 8.045 cm, 8.0 cm in T4 and T6 respectively (Table 1). Compared to treatment involving water soaked seeds (T1), treatment involving the application of metalaxyl as potting mix drench/seed treatment (T3, T6) and filtrate/ethyl acetate extracts (T4, T5), in general, recorded higher values for all the parameters analysed.

Discussion

Cyanobacteria compose most of the world's biomass, as they occur in fresh/marine water and terrestrial (soil) habitats, hence, are suitable candidates for exploitation as

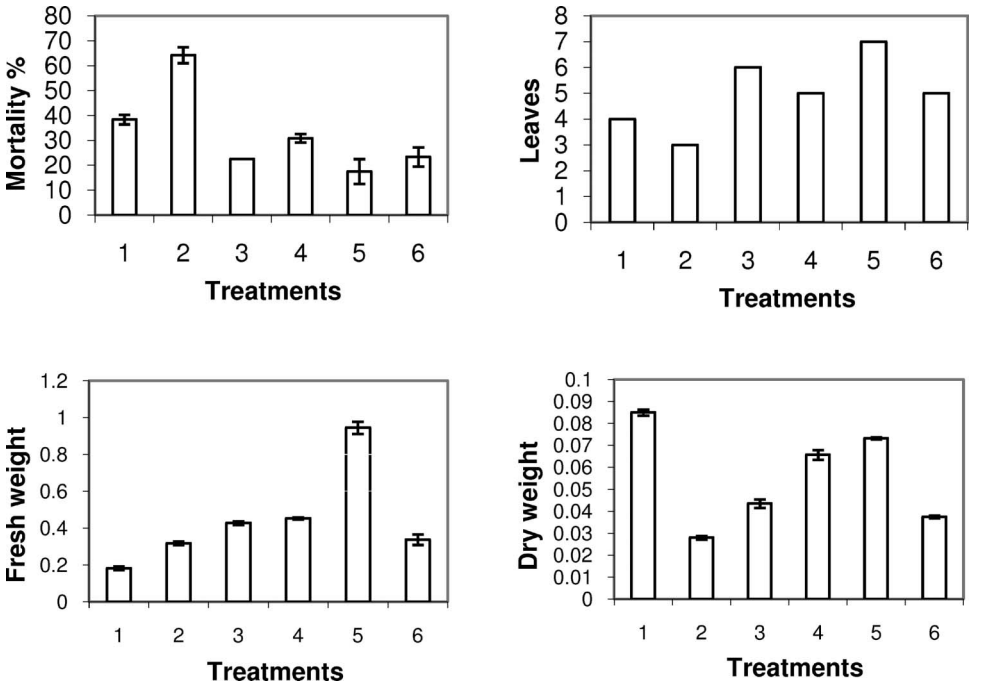

Figure 3. Influence of culture filtrate/ethyl acetate extract of *Calothrix elenkenii* on selected biometrical parameters of brinjal (*Solanum melongena* L.) seedlings challenged with *Pythium aphanidermatum* and evaluated after four weeks of incubation (treatments as given for Figure 1).

biocontrol agents of plant pathogenic fungi. However, despite the availability of a large body of information on the bioactive compounds produced by these microorganisms, very few initiatives have been undertaken to exploit them as biocontrol agents, especially against phytopathogenic fungi, which cause severe damage to important crops.

Pythium is a mycoparasite, which affects seedlings of various crops, especially vegetables and grasses, at both the pre- and post-emergence stage (Van West et al. 2003). The seedling blight and "damping off" disease caused by *Pythium* sp. leads to severe effects on roots, wilting of seedlings and is readily recognised as small spots/patches, but microscopic examination of root tissues is essential to detect its presence. Damping off disease is caused mainly by *Rhizoctonia* and *Pythium* species that live in soil. Although more than 40 damping off organisms attack vegetables, *Pythium* is the most common fungus attacking and germinating seeds and causing pre-emergence and post-emergence damping off disease. *Pythium aphanidermatum* is a cosmopolitan Oomycetous fungus belonging to the order Perenosporales with a wide host range. It is an aggressive species of *Pythium* causing damping off, root and stem rots and blights of grasses and fruits. It is a cause of much economic concern on most annuals (e.g. vegetables), beets, cucurbits and grasses and often considered one of the water molds because it survives and grows best in wet soils, especially under greenhouse conditions (in which humidity and warm temperature are prevalent). As it infects seeds, juvenile tissue, lower stems, fruit rot and roots, plants are most vulnerable to infection during the germination and juvenile stages. Despite the

availability of a number of sanitation measures and chemical formulations for application on seed or soil, the widespread nature of this fungal pathogen makes it a serious problem in several diseases in vegetable crops (Evans and Bhatt 1977). Considering the losses incurred, and the problems related to residues of chemicals, especially in vegetables, a need exists for development of biocontrol agents. Also, the aggressive nature of *Pythium aphanidermatum* requires directed biocontrol measures especially for vegetables such as tomato, brinjal and chilli, which are important crops, especially in the Indian subcontinent.

Screening programs undertaken at the CCUBGA, IARI in the last few years, on identifying cyanobacterial strains producing biocidal compounds (with emphasis on fungicidal activity), revealed the potential of several strains. Among them, the culture filtrates of two strains *Calothrix elenkenii* and *Anabaena* sp. exhibited algicidal activity and fungicidal activity (Radhakrishnan 2006). The culture filtrates and partially purified compounds produced by *Calothrix* sp. brought about significant inhibition of the growth of *Pythium debaryanum* and *P. aphanidermatum* (Radhakrishnan 2006). In India, no published work is available, to our knowledge, using cyanobacteria or their metabolites as biocontrol agents. The present investigation therefore was undertaken to evaluate the efficacy of the fungicidal compound(s) produced by *Calothrix* sp. against damping off disease caused by *Pythium aphanidermatum* in selected vegetables (brinjal, tomato and chilli).

The extracted metabolite was partially purified using preparatory TLC. The band showing antifungal activity was extracted and subjected to further characterisation by HPLC, wherein a single peak was observed. As a prelude to the development of a biocontrol agent, nursery stage evaluation of the fungicidal efficacy of the culture filtrate and ethyl acetate extract was undertaken along with requisite controls, including recommended chemical control measures (metalaxyl 2% as soil drench and seed treatment). The observations of the experiment at National Phytotron Facility, IARI which were taken after four weeks (i.e. after seeds were placed) revealed very interesting results. The tomato seedlings examined revealed significant visible differences in the vigour of the seedlings. In terms of root and shoot length, treatment T5 involving ethyl acetate extract treated seeds performed the best. Lower mortality was also observed in this treatment. Fresh weight and dry weight was also significantly higher in T5 treatment. The successful production of tomato is known to be much dependent upon the health of the nursery developed, which in turn is severely affected by damping off and other soil-borne diseases which can lead to 30–60% loss at pre- and post-emergence stages of the crop. Many strategies are available to control these diseases; however, the major components of integrated control (IPM) involve the use of chemical drenches (2% metalaxyl, methyl bromide) or seed treatment with Thiram, Captan, Ceresan, Agrosan G.N@ 3 g/kg of seeds. Some of the biocontrol strategies include the use of isolates of *Pseudomonas cepacia* or *Gliocladium virens* or *Bacillus cepacia* (Mao et al. 1998) which in combination provide better control and significant enhancement of plant fresh weight. It was observed that the method of application is of great significance i.e. seed treatment or soil drench. In our investigation, the application of culture filtrate/ethyl acetate extract/metalaxyl as seed treatment exhibited a significant effect on reducing the severity of the disease and significantly higher values for all the parameters were recorded. In terms of fresh weight/dry weight, percent mortality, number of leaves, shoot/root length, significant differences were observed in these treatments,

as compared to application of metalaxyl as soil drench. Kulik (1995) concluded that seed treatment is the most cost effective and easier approach for many crops and root drenches should be applied to high value transplanted crops.

Another important vegetable crop which is prone to a number of diseases and pests is brinjal, which is more popular in the Indian sub-continent. In our investigation, percentage mortality was drastically reduced through application of chemical and biocontrol treatments and values were statistically at par with the untreated, uninfected treatment (positive control). However, a significant enhancement in fresh weight and number of leaves was recorded through the application of culture filtrate/ethyl acetate extract of *Calothrix elenkenii* treated seeds. Published literature revealed that soil solarisation of nursery beds or seed treatment followed by soil application using bacterial and fungal antagonists recorded a lower incidence of damping off in a solanaceous vegetable nursery (Rahman et al. 2003; Konkanthimath and Ramesh 2004). In our investigation, the application of ethyl acetate extract as seed treatment recorded the lowest disease incidence (measured as percentage mortality) besides significant enhancement in seedling vigour. Compared to tomato and brinjal, chilli is not as susceptible to diseases in general. However, the economic losses incurred as a result of fungal diseases can be immense. Commercial cultivation of chilli is mainly for use in value addition or as green peppers, wherein the use of chemicals for disease control is not advisable, in view of residue problems. Hence, biocontrol of pathogens assumes significance. In our investigation, the root length of chilli seedlings grown from seeds treated with ethyl acetate was statistically at par with those of metalaxyl treatments (T3 and T6). However, the shoot length of seedlings from treatments T4 and T5 were significantly higher, indicative of a better response of the biocontrol treatment. Percent mortality was, however, lowest in T6 treatment (metalaxyl used as a seed treatment) but interestingly, values recorded in T5 treatment were much higher than the recommended practice (T4) i.e. metalaxyl as soil drench. In our study, ANOVA revealed that all the treatments and crops analysed exhibited a significant influence on the parameters, as well as the interaction of the crop and treatments (Supplementary Table 1). The overall effect of the different treatments when evaluated cropwise revealed significant differences in root and shoot length of the seedling belonging crops. Chilli and brinjal seeds treated with ethyl acetate extract recorded significantly higher values in terms of root length while chilli seeds recorded statistically at par values with metalaxyl treated seeds/application of metalaxyl as potting mix drench. However, in terms of shoot length, T5 treatment recorded significantly higher values as compared to all other treatments. ANOVA revealed that the crop x treatment interaction (Supplementary Table 1) was also significant. A comparative evaluation of percent survivors, as a result of the treatments employed, revealed that metalaxyl treated seeds of chilli recorded the highest percentage of survivors followed by chilli seeds treated with ethyl acetate extract and seeds receiving metalaxyl as soil drench (Supplementary Figure 1). However, brinjal and tomato seedlings raised from ethyl acetate extract treated seeds recorded the highest percentage of survivors. Crop-wise analyses revealed that chilli seedlings recorded the lowest percentage of survivors in T2, indicative of its relative hardiness, followed by tomato and brinjal, which exhibited higher sensitivity to *Pythium* infection. The plant height of seedlings was significantly influenced by the treatments. Tomato crop recorded higher values with T5

treatment, while in chilli, T5 treatment recorded a maximum height with statistically at par values in T4 and T6 treatments. In brinjal, plant height was also maximum in T5 treatment. Crop-wise analyses revealed that chilli recorded the maximum height of seedlings i.e. 17.263 cm, followed by brinjal and tomato. Interestingly, all these values were recorded in the T5 treatment. Brinjal seedlings recorded lowest values of 6.45 cm, which is threefold lower than the highest values recorded (Supplementary Figure 2). The superior performance of T5 treatment, even over T1 (positive control i.e. water soaked seeds grown in *Pythium*-free potting mix), reveals the potency of the extract, which on the one hand is inhibitory towards *Pythium aphanidermatum*, but is stimulatory towards the growth of the seedlings of tomato, chillies and brinjal. The highest values in terms of number of leaves, root length, fresh weight and dry weight were recorded in brinjal crop. But, in terms of mortality percentage, chilli was observed to be most hardy, which also recorded the highest values for overall plant height as well as length of shoot. The effect of different treatments over all the crops when analysed showed that the treatment T5 outperformed in terms of all the parameters analysed. Also, significantly higher values were recorded compared to all other treatments in terms of length of root and shoot, number of leaves, fresh weight of seedlings, even higher than the control in which water soaked uninoculated seeds were grown in *Pythium*-free potting mix. Additionally, our investigation also provides a PGP (plant-growth promoting) role of the extract/filtrate and enhances its utility in agriculture.

The use of plant growth promoting rhizobacteria for biocontrol of plant diseases is currently one of the major areas of research in biology. Future research needs to be focused towards identifying the biochemical and molecular mechanisms involved in the growth stimulation of plants (i.e., tomato, brinjal, chilli, in this case) and inhibition of *Pythium aphanidermatum* by the metabolite. Such biocontrol agents, which provide multiple benefits, may provide useful pointers to improving afforestation practices and establishment of plants in diverse inhospitable/barren habitats, besides their promise as multifaceted bioinoculants in organic farming practices popular in present day agriculture.

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