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Effect of potential bioinoculants and organic manures on root-rot and wilt, growth, yield and quality of organically grown *Coleus forskohlii* in a semiarid tropical region of Bangalore (India)

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Based on earlier results obtained in pot experiments, 2-year field experiments were conducted with five bioinoculants and neem cake under organic field conditions (with vermicompost as a nutritional supplement) to evaluate their potential to control root-rot and wilt (a complex problem involving *Fusarium chlamydosporum* and *Ralstonia solanacearum*) of the medicinal plant *Coleus forskohlii*. Plants treated with arbuscular mycorrhizal fungus (*Glomus fasciculatum*), neem cake or *Pseudomonas fluorescens* showed significantly increased plant height (15–31%), plant spread (25–33%), number of branches (63–67%) and dry root (129–200%) yields, and reduced disease incidence (47–50%) compared to controls. Increases in yields were reflected by increases in N (51–81%), P (17–76%) and K (44–74%) uptake. The forskolin content of the roots was found not to be affected by any of the bioinoculants, but forskolin yield (calculated) was increased significantly by treatment with *G. fasciculatum* (227%), neem cake (222%) or *P. fluorescens* (159%).

Keywords: *Fusarium chlamydosporum*, organic cultivation, organic fertilizers, *Ralstonia solanacearum*, root-rot/wilt

Introduction

Coleus forskohlii (family Lamiaceae) grows perennially in tropical and subtropical regions of India, Pakistan, Sri Lanka, East Africa and Brazil. Its roots are the source of a labdane diterpene compound called forskolin (also called coleonol) having the unique property of resensitizing cell receptors by activating the enzyme adenylyl cyclase, increasing the levels of cAMP (cyclic adenosine monophosphate). The *C. forskohlii* crop has economic potential as a source of forskolin for treating glaucoma, cardiac problems and certain types of cancer (Shah *et al.*, 1980). Its ethnomedicinal uses for relief of cough, eczema, skin infections, tumours and boils have been recorded (Rupp *et al.*, 1986). Because of continuous collection of roots from wild sources, this plant has been listed as endangered (Boby & Bagyaraj, 2003). Recent interest in cultivation of *C. forskohlii* and its economic potential has resulted in annual production of about 100 t from 700 ha in India (Shivkumar *et al.*, 2006).

Coleus forskohlii is susceptible to many diseases, of which root-rot and wilt are the most important, causing serious losses. Soilborne diseases are complex because of the array of organisms associated with the soil. The main pathogen associated with the disease has been identified as *Fusarium chlamydosporum* (Shivkumar *et al.*, 2006; Singh *et al.*, 2009). *Ralstonia solanacearum* has also been reported as causing vascular wilt of *C. forskohlii* (Coelho Netto & Assis, 2002). Management of diseases of medicinal plants in general, and control of soilborne plant pathogens in particular, by organic and biological methods, are being considered because chemical methods can result in accumulation of harmful residues which may lead to serious ecological and health problems (Singh *et al.*, 2009). Arbuscular mycorrhizal (AM) fungi suppressing the activity of root pathogens are well documented (Azcón-Aguilar & Barea, 1996; Whipps, 2004). *Pseudomonas fluorescens*, mainly considered as a plant growth promoting bacterium, can also suppress a wide range of plant pathogens, including *Fusarium* sp. and *R. solanacearum* (Nautiyal, 1997; Ramesh *et al.*, 2009).

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Among organic substances, neem and neem products and vermicompost, apart from being sources of plant nutrition, have also been found effective in suppressing root/soilborne pathogens (Singh *et al.*, 1980, 2011a,b; Yasir *et al.*, 2009). In pot studies, inoculation of *C. forskohlii* with *Glomus fasciculatum* and *P. fluorescens* resulted in marked reduction in disease severity and increased root yield (Singh *et al.*, 2009); and preliminary studies indicated a strong antagonistic activity of *Trichoderma viride* and *Bacillus subtilis* against *F. chlamydosporum* and *R. solanacearum*, respectively, and growth promoting potential of *Azotobacter chroococcum* in *C. forskohlii* (R. Singh and A. Kalra, unpublished data). Based on this it was deemed worthwhile to consider developing a strategy of controlling root-rot and wilt in *C. forskohlii* specifically for organic fields.

The present study is aimed at developing successful organic cultivation practices in *C. forskohlii* by minimizing disease losses through field evaluation of selected bioinoculants and neem cake in managing root-rot/wilt and improving root yields of *C. forskohlii* under organic field conditions.

Materials and methods

Isolation of fungal and bacterial pathogens

Both the pathogens (*F. chlamydosporum* and *R. solanacearum*) were isolated from infected tissues of roots and shoots of *C. forskohlii* plants exhibiting typical root-rot and wilt symptoms grown at the CIMAP experimental farm at Bangalore, India. The identity of the fungal pathogen (*F. chlamydosporum*) was confirmed by the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute (Boby & Bagyaraj, 2003), while the virulent bacterial pathogen *R. solanacearum* was identified (white fluidal irregular colonies with pink centre) following the methods of Kelman (1954) and Vanitha *et al.* (2009). The virulent *R. solanaceraum* (isolate CIMAP-R7) and *F. chlamydosporum* (isolate CIMAP-F6) were purified and preserved at −80°C with 50% glycerol in the culture collection of CIMAP for further studies.

Preparation of bioinoculants

Glomus fasciculatum 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. fasciculatum* 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 inoculum potential (potential of a specific amount of inoculum to cause root infection under a standard set of conditions) of *G. fasciculatum* used in this study was 5·3 ± 1·3 infecting propagules g^{-1} sand–soil mixture.

Trichoderma viride (MTCC 167) was mass-multiplied on potato dextrose broth at 28°C for 7 days, after which the mycelial mat with conidia was separated from broth and homogenized and the homogenized fungal mass was suspended in 500 mL 100 mM phosphate buffer at $1{\cdot}2 \times 10^6$ colony forming units (CFU) mL^{-1} buffer.

The bacterial cultures *P. fluorescens* (ATCC 13525), *B. subtilis* (ATCC 11774), and *A. chroococcum* (MTCC 446) were multiplied in nutrient broth (*A. chroococcum* on Jensen's broth) for 36 h at 210 rpm on an incubator shaker. Bacterial suspensions were centrifuged at 5867 ***g*** for 10 min. The supernatants were discarded and the pellets containing bacterial cells were suspended in 500 mL 100 mM phosphate buffer. Suspensions contained $2{\cdot}5 \times 10^8$ CFU mL^{-1} for *P. fluorescens*, $1{\cdot}8 \times 10^8$ CFU mL^{-1} for *B. subtilis* and 2.3×10^7 CFU mL^{-1} for *A. chroococcum*.

Nursery conditions

Cuttings were raised in 15- × 6-cm polyethylene bags containing 200 g potting mixture consisting of soil, sand and vermicompost (1:1:1/10, v/v). For the neem cake treatment, the potting mixture contained soil, sand, vermicompost and neemcake (1:1:1/10:1/40, v/v). *Glomus fasciculatum* inoculum (10 g per cutting) was placed into the polybags adjacent to the cut end of stem cuttings, and for the bioinoculant treatments healthy stem cuttings were dipped in the bioinoculant suspension for 30 min before planting. Culture suspension (5 mL per bag) was also poured into the respective treatments while the polybags containing only potting mixture (soil, sand and vermicompost) served as controls. Planted cuttings were placed under 60% agrinet shade for 50 days for rooting (Singh *et al.*, 2009). The plants were then kept for 5–7 days in open conditions to harden before transplanting to the field.

Nursery observations

Five plants were randomly selected from 55-day-old nursery cuttings for observations related to plant growth (plant spread, shoot and root length) and yield parameters (root and shoot fresh and dry biomass) were recorded each year. The data presented in Table 1 are on an oven-dry basis.

Transplanting to the field

The experiment was conducted at a certified (ECOCERT) organic farm at the Central Institute of Medicinal and Aromatic Plants, Resource Center, Bangalore (India), located at 12°58′N, 77°35′E and 930 m a.s.l., where a *C. forskohlii* crop had been grown continuously for the previous 3 years and disease incidence was more than 80%. The soil of the experimental field was a red sandy loam with pH 6·4, 125 kg available N, 11·5 kg available

Table 1 Effect of bioinoculants and neem cake on growth characteristics of *Coleus forskohlii* at the nursery stage (55-day-old cuttings) prior to transplanting

Treatment	Shoot length (cm)	Root length (cm)	Plant spread (cm)	Dry shoot weight (g/plant)	Dry root weight (g/plant)
TV	17·6bc	10·8ab	13·2a	0·81a	0·044bc
BS	15·8ab	11·6b	15·0a	0·75a	0·017a
AZ	19·6bc	12·0b	16·4ab	1·31b	0·059c
GF	20·4bc	13·4b	20·8c	1·05b	0·069c
PF	19·0bc	12·4b	18·6b	0·98ab	0·063c
NC	20·6c	12·4b	20·2c	1·19b	0·078c
VC (Control)	13·8a	9·20a	14·4a	0·77a	0·013a
LSD [$P < 0·05$]	3·33	2·27	2·89	0·27	0·022

VC: vermicompost; TV: *Trichoderma viride*; BS: *Bacillus subtilis*; AZ: *Azotobacter chroococcum*; GF: *Glomus fasciculatum*; PF: *Pseudomonas fluorescens*; NC: neem cake.

Data are averages of two trials (10 plants per treatment) during the 2-year period; values in vertical columns followed by different letters are significantly different at $P \leq 0·05$ by ANOVA (LSD) test.

P, 123·7 kg available K ha^{-1} and 0·44% organic carbon. The treatments were imposed in plots (1·8 m wide × 3·6 m long) with three replications arranged in a randomized block design in naturally infected soil. Each plot was separated by 50-cm flat ridges (guard row) to mitigate the effects of adjoining plots. The initial soil samples (200 g) were collected with a soil auger (0–15 cm) from five points in each replicated treatment. The soil samples were pooled, mixed and subsamples used to record the initial microbial population (beneficial and pathogenic) prior to transplanting and application of bioinoculants. The soil showed uniform infestation of root-rot-complex pathogens ($6·0–6·6 \times 10^4$ *F. chlamydosporum* CFU and $3·6–3·9 \times 10^6$ *R. solanacearum* CFU g^{-1} soil) throughout the field. The rooted cuttings (55-day-old nursery cuttings) of *C. forskohlii* were planted on both edges of ridges at a spacing of 60 × 45 cm (row spacing was 60 cm, with 45 cm between plants within each row and each ridge separated by a 20-cm channel used for irrigation). There were four rows per plot. Planting dates for the rooted cuttings were 20 June 2008 and 22 June 2009 and the same plots with the same treatments were maintained for the 2 years. Vermicompost was used as an organic nutrient supplement to support plant growth in all treatments; controls received vermicompost alone. The N requirement of *C. forskohlii* (40 kg ha^{-1}) was fulfilled in bioinoculant-treated plots by the application of vermicompost applied at the rate of 4 t ha^{-1}; whereas in neem cake-treated plots, one quarter of the N requirement (10 kg ha^{-1}) was met by application of neem cake (500 kg ha^{-1}) (Parker India Group, Chennai, India) and the rest of the N requirement (30 kg ha^{-1}) by the application of vermicompost (3 t ha^{-1}). Neem cake contained 1·98% N, 0·88% P and 0·92% K. The vermicompost used in the experiment was produced from a mixture of distillation waste (plant-spent de-oiled herb) of lemongrass, patchouli and geranium in a vermicomposting unit using *Eudrilius eugineae*, an epigeic species of earthworm, at a certified organic farm. The vermicompost contained 1·01% N, 0·45% P and 0·61% K. Transplanting was done into planting holes with a depth of 10–12 cm and a diameter of 8–10 cm by placing intact balls of earth (soil mixture) without polythene covers. Each bed contained 32 plants (only 12 plants were considered for the various observations) in seven treatments: *T. viride* (TV), *B. subtilis* (BS), *A. chroococcum* (AZ), *G. fasciculatum* (GF), *P. fluorescens* (PF), neem cake (NC) and vermicompost control (VC).

Plant growth observations and wilt in field conditions

Five randomly tagged plants out of 12 plants from each plot were considered for growth parameters (plant height, plant spread and number of branches). Plant growth parameters such as plant height (measured from soil surface to the growing tip of the plant), number of branches and plant spread were recorded at the time of harvesting. Percentage wilt incidence (PWI; yellowing and drooping of leaves, browning of vascular tissues of stem) was assessed in the field with plants of each replicated plot before harvest; PWI = (number of wilted plants/total number of plants) × 100.

Harvesting

Harvesting was done 140 days after transplanting each year. Plants were manually uprooted with care not to damage the tubers. Fresh and dry root and shoot weights were recorded from each replicated net plot. Samples (shoot or root) were taken randomly, mixed, and then a subsample of 200 g fresh shoot was dried in a hot-air oven at 80°C for 24 h, while fresh root samples (200 g) were chopped into 1- to 2-cm pieces and shade-dried for 5–7 days before determining their moisture content and nutrient concentrations. From the moisture content and biomass yields, dry matter yields were calculated. Powdered dry roots (passed through a 0·2-mm sieve) were used to measure nutrient uptake and forskolin production. At the time of harvesting, rhizospheric soil samples (200 g) were collected in the same manner as for the initial soil sampling to determine microbial populations (both beneficial and pathogenic) from each bed. Disease severity was measured after harvesting, taking into account the tubers harvested from each replicated net plot. Nutrient concentration (NPK) in dried roots and

shoots was determined as per Jackson (1973). The total uptake by plants was determined by considering nutrient concentration and total dry matter yield.

Percentage disease index (PDI)

Disease severity was measured on a 0–4 scale where 0 = healthy roots (no symptoms) and 4 = >75% root tubers affected by rot (blackening, oozing and putrefaction of roots and plant death). Based on the scoring of root-rot severity of plants in all three replicates of each treatment, the percentage disease index (PDI) was calculated as: PDI = (sum of numerical gradings recorded/number of roots observed × highest numerical rating) × 100.

Forskolin estimation

Forskolin content in *C. forkohlii* roots was estimated by HPLC. Powdered dried roots (1 g) were weighed and transferred into a conical flask (25 mL) to which 10 mL acetonitrile was added. The samples were sonicated four times for 15 min each. The combined extractions were concentrated and diluted to the final volume of 10 mL with acetonitrile. Samples were filtered (0·45-μm nylon filter membrane) and 25 μL injected into a Shimadzu LC10ATVP HPLC equipped with a Phenomenex Luna 5 μm, C 18 (2) 100 Å; 250 mm × 4·6 ID; operation parameters: oven temperature 30°C, mobile phase acetonitrile:water with flow rate of 1 mL min^{-1}. Standards preparation, calibration and gradient elution was done as per Schaneberg & Khan (2003). Theoretical forskolin yield was calculated based on percentage forskolin content and dry root yield.

Microbial population estimation

To assess AM fungi colonization, fine roots from host plants were cut into 5-mm sections, cleared with 10% KOH and stained with 0·05% trypan blue. The percentage of root length colonized by mycorrhizal fungi was calculated as reported by McGonigle *et al.* (1990). Positive counts for mycorrhizal colonization included the presence of aseptate hyphae/vesicles/arbuscules. A wet sieving and decanting method was used for isolation and estimation of AM fungal spores. *Trichoderma*, *Pseudomonas*, *Bacillus* and *Azotobacter* populations in the root-zone soil were determined by a serial dilution technique with 0·85% saline solution using *Trichoderma*-selective medium (Papavizas & Lumsden, 1982), King's B medium (King *et al.*, 1954), *Bacillus* isolation and cultivation medium (Claus & Berkeley, 1986) and Jensen's medium (Jensen & Petersen, 1954), respectively. The density of pathogen populations (*F. chlamydosporum* and *R. solanacearum*) were estimated using PCNB peptone (Nash & Snyder, 1962) and 2,3,5-triphenyl tetrazolium chloride (TTC/TZC) semiselective medium (Kelman, 1954), respectively.

Statistical analysis

For all parameters, data from the two trials (years), with seven treatments with three replicates each, were analysed statistically by analysis of variance (ANOVA) for a randomized complete block design. The experimental data of the two trials had similar variance values, so the data were combined for all further analyses. Significant differences among treatments were based on the *F*-test in ANOVA and treatment means were compared using the least significant difference (LSD) at $P \leq 0{\cdot}05$ (Snedecor & Cochran, 1989). The data on percentage root colonization by AM fungi was analysed using arcsine square transformed values. The standard error (SE) of the mean in vertical bar charts was computed with SigmaPlot 10 software.

Results

Effect of bioinoculants and neem cake on growth characteristics and shoot and root biomass in the nursery

Significantly greater shoot length was observed in all bioinoculant treatments, except *B. subtilis*, compared with the control; the greatest increase (49%) was observed with neem cake, followed by *G. fasciculatum* (48%) (Table 1). Root length was significantly increased relative to the control in all treatments except *T. viride*, the increase ranging from 26% with *B. subtilis* to 46% with *G. fasciculatum*. A significant increase in plant spread was also observed with *G. fasciculatum* (45%), neem cake (40%) and *P. fluorescens* (29%). Dry shoot weights were significantly higher in plants treated with *A. chroococcum* (70%), neem cake (55%) and *G. fasciculatum* (36%) than in controls. Similarly, dry root weight was also significantly higher than in controls in all treatments except *B. subtilis*; the greatest increase was observed with neem cake (500%), followed by *G. fasciculatum* (431%) and *P. fluorescens* (385%) (Table 1).

Effect of bioinoculants and neem cake on growth parameters at harvesting in field conditions

Plants treated with *G. fasciculatum*, neem cake or *P. fluorescens* showed significant increases in plant height (31%, 27% and 15%, respectively) compared to the control. Plant spread was significantly increased in treatments with *G. fasciculatum* (33%), *P. fluorescens* (27%), *B. subtilis* (26%) and neem cake (25%). Plants inoculated with *G. fasciculatum*, *P. fluorescens* and neem cake showed significant increases in number of branches (67%, 65% and 63%, respectively) (Table 2).

Effect of bioinoculants and neem cake on dry root and shoot yields and forskolin yield of *C. forskohlii*

Plants inoculated with *G. fasciculatum* and neem cake performed equally well, showing significant increases in dry root yield of 193% and 200%, respectively, and

Table 2 Effect of bioinoculants and neem cake on growth and yield parameters of *Coleus forskohlii* at harvesting in field conditions

Treatment	Plant height (cm)	Plant spread (cm)	Number of branches	Dry shoot yield (t ha^{-1})	Dry root yield (t ha^{-1})	Forskolin yield (kg ha^{-1})
TV	41·7ab	43·7ab	20·3a	1·34a	0·18a	1·1a
BS	40·0ab	46·7b	19·3a	1·36a	0·17a	1·02a
AZ	40·2ab	41·3ab	18·3a	1·49a	0·22a	1·32ab
GF	49·6c	49·3b	28·3b	2·58b	0·41c	2·71c
PF	43·6b	47·1b	28·0b	2·01a	0·32bc	2·15bc
NC	48·2c	46·3b	27·7b	2·64b	0·42c	2·67c
VC (Control)	38·0a	37·1a	17·0a	1·33a	0·14a	0·83a
LSD [$P < 0·05$]	4·1	7·0	6·8	0·8	0·1	0·84

TV: *Trichoderma viride*; BS: *Bacillus subtilis*; AZ: *Azotobacter chroococcum*; GF: *Glomus fasciculatum*; PF: *Pseudomonas fluorescens*; NC: neem cake; VC: vermicompost.
Data are averages of two trials (six replicates for each treatment) during the 2-year period; values in vertical columns followed by different letters are significantly different at $P \leq 0·05$ by ANOVA (LSD) test.

increases in dry shoot yield of 94% and 98.5%, respectively, compared with controls. *Pseudomonas fluorescens* was also effective in improving root yield (by 129% compared to non-inoculated plants). Forskolin concentration was not affected by any of the bioinoculant or organic manure treatments, but as a result of higher root yields, the total forskolin yield (calculated) was significantly higher in plants treated with *G. fasciculatum* (227%), neem cake (222%) or *P. fluorescens* (159%) than in controls (Table 2).

Effect of bioinoculants and neem cake on incidence and severity of root-rot/wilt in field conditions

All the bioinoculants (except *T. viride* and *B. subtilis*) significantly reduced (18–43%) percentage wilt incidence compared with the control, the greatest reduction being observed with *G. fasciculatum* (43%) and neem cake (43%), followed by *P. fluorescens* (36%) (Fig. 1a). Apart from reducing the incidence of the disease, the above treatments, i.e. neem cake, *G. fasciculatum* and *P. fluorescens* significantly reduced the severity of the disease as indicated by percentage disease index, compared with controls (Fig. 1b). The highest reduction in disease index was observed in plants treated with neem cake (50%), followed by *G. fasciculatum* (48·4%) and *P. fluorescens* (47·3%), but these treatments were not significantly different from each other (Fig. 1b).

Nutrient uptake and rhizospheric bioinoculant species populations

The data on nutrient uptake are presented in Table 3. N uptake improved significantly in plants treated with neem cake (81%), *G. fasciculatum* (55%), *P. fluorescens* (51%) and *Azotobacter* (44%). Uptake of P and K were also higher in the *G. fasciculatum* (69% and 73%, respectively) and neem cake (76% and 74%, respectively) treatments when compared with the controls. Higher uptake of nutrients resulted in an increase in root and shoot

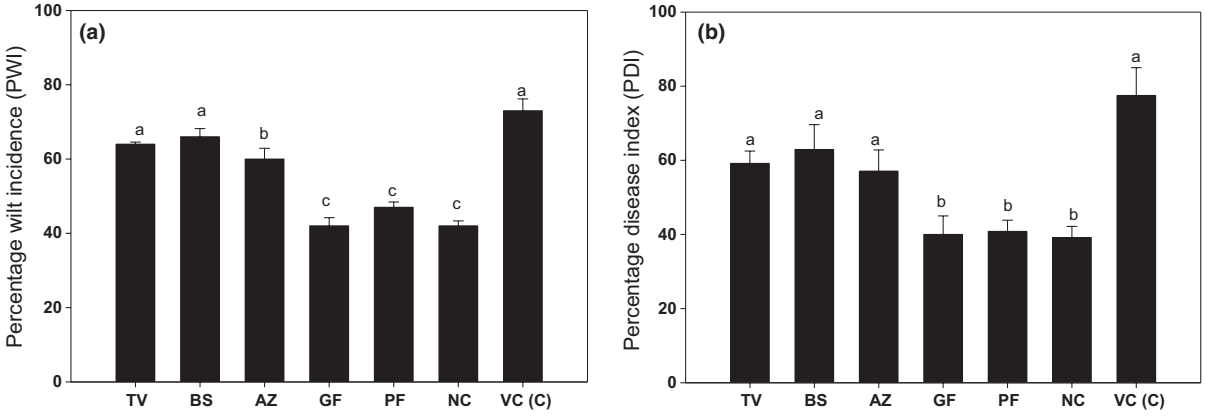

Figure 1 Effect of bioinoculants and neem cake on mean (six replicates for each treatment during 2-year period) (a) percentage wilt incidence (PWI) and (b) percentage disease incidence (PDI) of *Coleus forskohlii* caused by a complex of *Fusarium chlamydosporum* and *Ralstonia solanacearum*. TV, *Trichoderma viride*; BS, *Bacillus subtilis*; AZ, *Azotobacter chroococcum*; GF, *Glomus fasciculatum*; PF, *Pseudomonas fluorescens*; NC, neem cake; VC (C), vermicompost control. Error bars represent standard error of mean (SEM). Different letters above error bars show significant difference at 95% confidence level ($P \leq 0·05$) by ANOVA (LSD test).

Table 3 Effect of bioinoculants and neem cake on nutrient uptake by *Coleus forskohlii* under field conditions

	Shoot uptake (kg ha^{-1})			Root uptake (kg ha^{-1})			Total uptake (kg ha^{-1})		
Treatment	N	P	K	N	P	K	N	P	K
TV	18·62a	4·89ab	22·31a	0·89a	0·39a	2·44a	19·51a	5·28a	24·75a
BS	19·35ab	4·62a	21·96a	1·04a	0·44ab	2·70a	20·39ab	5·06a	24·66a
AZ	26·97ab	4·82ab	24·62a	1·00a	0·55ab	3·16ab	27·97b	5·37a	27·78ab
GF	28·03b	7·49b	35·68b	1·99b	0·94b	6·05b	30·02b	8·43b	41·73b
PF	27·78b	5·10ab	30·06a	1·41ab	0·73b	4·76b	29·19b	5·83ab	34·82b
NC	32·78b	7·94b	36·11b	2·35b	0·83b	5·90b	35·13b	8·77b	42·01b
VC (Control)	18·6a	4·62a	21·86a	0·80a	0·36a	2·27a	19·40a	4·98a	24·13a
LSD [$P < 0·05$]	8·4	2·8	9·1	0·7	0·3	2·0	8·2	2·8	9·2

TV: *Trichoderma viride*; BS: *Bacillus subtilis*; AZ: *Azotobacter chroococcum*; GF: *Glomus fasciculatum*; PF: *Pseudomonas fluorescens*; NC: neem cake; VC: vermicompost.

Data are averages of two trials (six replicates for each treatment) during the 2-year period; values in vertical columns followed by different letters are significantly different at $P \leq 0·05$ by ANOVA (LSD) test.

yields; the effects were prominent in the case of *G. fasciculatum*, *P. fluorescens* and neem cake.

The microbial populations (CFU g^{-1} soil) of *Trichoderma*, *Bacillus*, *Azotobacter* and *Pseudomonas* were 1×10^3, $1·1 \times 10^4$, $4·5 \times 10^3$ and $2·2 \times 10^4$, respectively, before transplanting. Apart from increasing percentage AM root colonization and number of mycorrhizal spores in the rhizosphere, *G. fasciculatum* treatment also increased populations of other bioinoculants in the root-zone soil compared with controls (Table 4). Higher numbers of mycorrhizal spores and percentage root colonization were also observed in the neem cake treatment (Table 4). In general, the population of a particular bioinoculant was increased in the rhizosphere of the plants inoculated with the same organism (Table 4).

Efficacy of bioinoculants in plant pathogen suppression

In our earlier studies (A. Kalra, unpublished data), *T. viride, P. fluorescens* and neem cake showed greater inhibition against *F. chlamydosporum* and *R. solanaceraum* both under plate assay (by measuring inhibition zone) and in nursery conditions (by estimating reduction in root rot severity and population of both the pathogens).

Bioinoculant- and neem-cake-treated plants reduced *F. chlamydosporum* populations at harvesting, the reduction being greatest with *G. fasciculatum* (55%), followed by *P. fluorescens* (54%) and neem cake (54%). The *R. solanacearum* population was also significantly reduced by *G. fasciculatum* (42·6%), neem cake (42·3%), *P. fluorescens* (41·1%) and *B. subtilis* (33·4%) (Table 5).

Discussion

The results clearly indicate that efficient bioinoculants (*G. fasciculatum* and *P. fluorescens*) and neem cake significantly improved plant growth parameters of *C. forskohlii*, both in nursery and field conditions, a finding also supported by Earanna *et al.* (1999) and Gill & Singh (2002). Generally, the objective of nursery inoculation is not to achieve a growth response, but rather to establish the symbiosis with the plant so that it can be effectively transferred to the field (Sylvia, 1989).

Table 4 Mean populations of bioinoculant species in the root-zone soil of *Coleus forskohlii* at harvesting

	Root-zone bioinoculant species population					
Treatment	*Trichoderma* (×10^3 g^{-1} soil)	*Bacillus* (×10^3 g^{-1} soil)	*Azotobacter* (×10^3 g^{-1} soil)	*Pseudomonas* (×10^3 g^{-1} soil)	Mycorrhizal spores/50 g soil	Percentage root colonization
TV	1·9c	60b	8bc	98c	102b	30bc
BS	1·9c	90d	9c	99c	94ab	27ab
AZ	1·8c	80cd	10c	95c	101b	31bc
GF	1·7bc	88d	10c	100c	240c	68d
PF	1·8c	95d	9c	110d	101b	30bc
NC	1·9c	40a	7b	88bc	102b	34c
VC (Control)	1·2a	33a	5a	34a	88a	25a
LSD [$P < 0·05$]	0·27	10·8	1·67	9·6	11·5	3·18

TV: *Trichoderma viride*; BS: *Bacillus subtilis*; AZ: *Azotobacter chroococcum*; GF: *Glomus fasciculatum*; PF: *Pseudomonas fluorescens*; NC: neem cake; VC: vermicompost.

Data are averages of two trials (six replicates for each treatment) during the 2-year period; values in vertical columns followed by different letters are significantly different at $P \leq 0·05$ by ANOVA (LSD) test.

Table 5 Effect of bioinoculants and neem cake on mean rhizospheric population of *Fusarium chlamydosporum* and *Ralstonia solanaceraum* of *Coleus forskohlii* at harvesting

	Rhizospheric population	
Treatment	*Fusarium* ($\times 10^4$ g^{-1} soil)	*Ralstonia* ($\times 10^6$ g^{-1} soil)
TV	3·67a	3·33a
BS	3·67a	2·33b
AZ	3·65a	3·33a
GF	2·26b	2·01b
PF	2·30b	2·06b
NC	2·30b	2·02b
VC (Control)	5·00a	3·50a
LSD [$P < 0·05$]	1·78	1·06

TV: *Trichoderma viride*; BS: *Bacillus subtilis*; AZ: *Azotobacter chroococcum*; GF: *Glomus fasciculatum*; PF: *Pseudomonas fluorescens*; NC: neem cake; VC: vermicompost.
Data are averages of two trials (six replicates for each treatment) during the 2-year period; values in vertical columns followed by different letters are significantly different at $P \leq 0·05$ by ANOVA (LSD) test.

Tuberous roots are the main economic part of *C. forskohlii*. *Glomus fasciculatum*, *P. fluorescens* and neem cake produced significantly higher dry root yields. Earlier reports indicating the usefulness of bioinoculants in improving growth and yield support these results (Earanna *et al.*, 1999; Singh *et al.*, 2009). Higher root yields might also be caused by the effectiveness of these bioinoculants and neem cake in controlling plant pathogens (Singh *et al.*, 1980, 2011a,b; Govindachari, 1992) and in providing nutrition to the plants. Bioinoculants and neem cake also increased forskolin yield, which is supported by the results of Boby & Bagyaraj (2003).

Percentage disease index was reduced (by approximately 50%) by bioinoculants *G. fascuculatum* and *P. fluorescens* and by neem cake. Similar trends, i.e. reduction of severity of root pathogens, were observed by Earanna *et al.* (1999) and Ramesh *et al.* (2009). Mycorrhizal fungi are known to colonize feeder roots and thereby compete with root pathogens for space, and increase the resistance of the plant to pathogens by modification of cell walls, production of antimicrobial compounds and altered rhizosphere microflora inimical to the pathogens (Azcón-Aguilar & Barea, 1996; Boby & Bagyaraj, 2003; Shivkumar *et al.*, 2006). Reduction of wilt incidence and root-rot by neem cake and an AM fungus (*G. fasciculatum*) was reflected in terms of increase in root and shoot yield of *C. forskohlii*. Vermicompost, used as an organic supplement and rich in beneficial microbes (Gopal *et al.*, 2009) known for disease suppression (Yasir *et al.*, 2009), also reduced the incidence and severity of disease. Other studies have also shown that vermicompost supports the growth of beneficial microbes (Kalra *et al.*, 2010; Singh *et al.*, 2011a,b). Fluorescent pseudomonads have been widely tested against bacterial and fungal pathogens because of their competition for limited carbon sources in the rhizosphere and their antagonism, mainly attributed to the production of secondary metabolites such as antibiotics (Vanitha *et al.*, 2009).

Bioinoculants *G. fasciculatum*, *P. fluorescens* and *A. chroococcum* and neem cake significantly influenced nutrient uptake (NPK). The plants inoculated with *G. fasciculatum* showed highest K uptake. Smith & Read (2008) reported higher nutrient uptake by plants colonized by AM fungi. Hyphae of mycorrhizal fungi have the potential to increase the absorbing surface area of the root for nutrients (Rousseau *et al.*, 1994). *Pseudomonas fluorescens* is well documented for its plant growth promotion activities and inhibition of plant pathogens (Ramesh *et al.*, 2009). Neem cake, rich in nutritive value in terms of N, P and K, may have helped the plants via increased nutrient availability.

As well as increasing percentage AM root colonization and number of AM spores, the populations of other bioinoculants were also increased in plants treated with *G. fasciculatum*. AM colonization might influence the species composition of the soil microbial community by increasing the populations of beneficial microbes (Krishnaraj & Sreenivasa, 1992). Bansal & Mukerji (1994) demonstrated a positive correlation between AM fungi and the rhizospheric bacterial population. The present study, however, also indicated the negative effects of *B. subtilis* on populations and colonization by *G. fasciculatum*. This must be taken into consideration when forming microbial-based consortia involving AM fungi and *B. subtilis*.

Trichoderma viride and *P. fluorescens* showed inhibitory effects against *F. chlamydosporum*, a finding supported by Saharan *et al.* (2008). It is also well documented that *G. fasciculatum* and *P. fluorescens* are potent inhibitors of pathogens such as *Fusarium* and *Ralstonia* (Shivkumar *et al.*, 2006; Ramesh *et al.*, 2009; Singh *et al.*, 2009). According to Cook & Sequeira (1991), *R. solanacearum* is a poor competitor outside the plant system, so there is good potential for its biological control. In the present study, as well as bioinoculants (*G. fasciculatum* and *P. fluorescens*), neem cake also reduced pathogenic populations of *R. solanacearum* and *F. chlamydosporum*. However, further studies of the combined effects of *G. fasciculatum*/*P. fluorescens* and neem cake are needed.

The present study clearly indicated that root-rot and wilt complex of *C. forskohlii* could be reduced by soil amendments such as neem cake and vermicompost, and bioinoculants such as *G. fasciculatum* and *P. fluorescens*. This management approach will be particularly useful under organic farming conditions, especially for medicinal plants, where the use of chemicals is restricted because of health and residue considerations.

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