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RESEARCH ARTICLE

Effect of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on root-rot and wilt, growth and yield of *Coleus forskohlii*

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Root-rot and wilt caused by *Fusarium chlamydosporum* affects the cultivation of C*oleus forskohlii,* a medicinal plant grown for its roots, which contain a pharmaceutically important compound called forskolin. In this study, management of this disease under low and high inoculum levels was assessed with four arbuscular mycorrhizal (AM) fungi and a strain of *Pseudomonas fluorescens.* The AM fungus *Glomus fasciculatum* and *P. fluorescens* were the most effective treatments that reduced the severity of root-rot and wilt of *C. forskohlii* by 56–65% and 61–66%, respectively, under lower and higher levels of pathogen *F. chlamydosporum*. *G. fasciculatum* increased the dry shoot and root weight by 108–241% and 92–204%, respectively, while in plants treated with *P. fluorescens,* an increase of 97–223% and 97–172% in dry shoot and root weight, respectively, was observed. Although *P. fluorescens* was effective, it gave higher root yields only under lower inoculum level of the pathogen. *G. fasciculatum* performed equally well under both lower and higher inoculum levels. Increase in yields with both the biocontrol agents was accompanied by increase in P uptake (230–303%) and in K uptake (270–335%). The forskolin content of the roots was significantly increased (14–21%) by *G. fasciculatum*, *P. fluorescens* or *G. mosseae* under lower inoculum level of pathogen.

Keywords: *Coleus forskohlii*; biocontrol; *Fusarium chlamydosporum*; forskolin content

Coleus forskohlii Briq. (Family Labiatae), is an important medicinal plant, which grows perennially over tropical and subtropical regions of India. Its roots are the source of a labdane diterpene compound called forskolin, a potent vasodilatory, hypertensive and inotropic agent (Seamon 1984). Recently, farmers have started cultivating this crop because of its commercial potential. The plant is susceptible to root rot and wilt caused by the fungal pathogen *Fusarium chlamydosporum* (Shyla 1998), causing serious losses. Biological control involving arbuscular mycorrhizal (AM) fungi (Mohan and Verma 1996) and *Pseudomonas fluorescens* (Nautiyal 1997) is considered as a preferred disease management strategy because chemical control results in accumulation of harmful chemical residues leading to serious ecological and

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health problems. The present study was carried out to investigate the effect of four AM fungi and a strain of *P. fluorescens* in controlling root-rot and wilt of *C. forskholii.*

Pure cultures of AM fungi viz. *Glomus aggregatum* (GA), *G. fasciculatum* (GF), *G. intraradices* (GI), *G. mosseae* (GM) with an inoculum potential of 4.0 ± 1.2 infecting propagules g^{-1} and a PGPR, *Pseudomonas fluorescens* (PF 6) were obtained from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. *P. fluorescens* was multiplied in nutrient broth for 24 h at 210 rpm on a rotary shaker. The bacterial suspension was centrifuged on 10,000 rpm for 10 min. The supernatant was discarded and the pellets containing bacterial cells were suspended in 100 mM phosphate buffer. *P. fluorescens* in this suspension was 2.5×10^8 CFU mL^{-1}.

Fusarium chlamydosporum (F) was consistently isolated from infected roots and stems of the *C. forskohlii* and was multiplied on a corn meal sand medium (90 g sand and 20 g corn meal with 50 mL distilled water) for 2 weeks. Pathogen inoculum was mixed in sterile distilled water with a final concentration of 1.2×10^6 CFU mL^{-1}.

Stem cuttings of *C. forskohlii* were raised in 15×6 cm polyethylene bags containing 200 g of sterilized soil: sand: vermicompost (1:1:1/10, v/v) potting mixture. Planted cuttings were placed into agrinet shade of 60% for 50 days for rooting.

A pot experiment with four replicates adopting a completely randomized design was conducted in a glasshouse to evaluate the biocontrol potential of four AM fungi and a PGPR, *Pseudomonas fluorescens* at two pathogen inoculum levels (120 and 240 mL pot^{-1}). Two levels of pathogen alone served as controls and non-inoculated pots referred as soil only. Each pot containing 3.5 kg of sterilized soil (sandy loam, pH 6.4, 112 kg ha^{-1} available N, 9.5 kg ha^{-1} available P, 103.7 kg ha^{-1} available K and 0.44% organic carbon) was kept for 7 days after inoculation with *F. chlamydosporum* for the establishment of the pathogen. AM fungi inoculum at 10 g per pot was placed adjacent to the roots while 50-day-old *C. forskohlii* rooted cuttings were dipped in *P. fluorescens* suspension for half an hour before transplanting. N, P and K were applied at the recommended fertilizer doses (40:60:50 kg ha^{-1}) as urea (0.137 g), single super phosphate (0.586 g) and muriate of potash (0.13 g), respectively, per pot. Plant height, plant spread and number of branches were recorded at monthly interval until harvesting. Harvesting was done after 140 days of transplanting. After harvesting, rhizosphere soil samples were collected to determine microbial population of *P. fluorescens*, *F. chlamydosporum* and AM fungi spore number. Roots were collected to determine percent root colonization in AM fungi-treated plants. Nutrient uptake in root and shoot was determined (Jackson 1973) based on P and K concentration in dry matter samples.

Severity of root-rot disease was measured on a 0–4 scale of Kesavan and Chowdhary (1977) where 0 = no symptoms, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 = > 75% root tubers were affected by rot. Five root tubers were examined from each replicate. Based on the scoring of root disease symptoms of each treatment, the percentage disease index (PDI) was calculated as follows:

$$\text{PDI} = (\text{Sum of numerical grading recorded}/\text{Number of roots observed} \times \text{highest numerical rating}) \times 100$$

Forskolin in the *C. forkohlii* roots was estimated by HPLC (Shimadzu LC10ATVP) as suggested by Schaunberg and Khan (2003). Wet sieving and

decanting method (Gerdemann and Nicholson 1963) was used for isolation and estimation of AM fungal spores and Phillips and Hayman (1970) method was used for estimation of percent root colonization by mycorrhizal fungi. *Pseudomonas* population in the root zone soil was determined using King's B medium (King, Ward, and Raney 1954). *Fusarium* population was estimated by using PCNB peptone medium (Nash and Snyder 1962). The data were analyzed statistically by analysis of variance method (ANOVA). Significant differences among treatments were based on the *F*-test in ANOVA. Treatment means were compared using least significant difference (LSD) at $P < 0.05$ (Snedecor and Cochran 1989). There were two trials conducted within the 2-year period. All the data are presented as standard error of mean (SEM) and standard error of mean difference (SED). The results and discussion are based on the average of the trials during the 2-year period.

The plant growth parameters data, presented in Table 1, showed that *F. chlamydosporum* at higher level caused a significant decrease in the plant height of *C. forskohlii* compared to lower level of pathogen or non-inoculated soil. At both levels of pathogen inoculum, *G. fasciculatum* or *P. fluorescens* significantly increased the plant height, plant spread and number of branches compared to pathogen alone. Increase in plant height of *C. forskohlii* due to inoculation with *G. mosseae* or *P. fluorescens* are in agreement with the findings of Boby and Bagyaraj (2003).

Higher level of pathogen significantly increased disease severity by 33.5% compared to lower level of pathogen alone. AM fungi (except *G. aggregatum*) or *P. fluorescens* at both pathogen levels significantly reduced the percentage disease but *P. fluorescens* was most effective at lower level of pathogen, whereas *G. fasciculatum* was most effective at higher level of pathogen (Figure 1A).

There was a significant reduction (46%) in dry shoot weight between higher and lower pathogen level alone. At higher inoculum level of *F. chlamydosporum*, a significant reduction (44%) was observed in dry shoot weight compared to non-inoculated soil. At lower pathogen inoculum level, only *P. fluorescens* or *G. fasciculatum* produced significantly higher dry shoot yield compared to the lower level of pathogen alone. All AM fungi or *P. fluorescens* increased shoot yield at higher pathogen level compared to pathogen alone. *G. fasciculatum* or *P. fluorescens* showed a significant increase in dry shoot yield at both pathogen levels compared to non-inoculated soil (Figure 1B).

There was a significant reduction (41–43%) in dry root yield at higher pathogen level compared to lower pathogen level or non-inoculated soil. At higher pathogen level, significantly higher dry root yield was obtained in *G. fasciculatum* (16.9 g plant^{-1}) or *P. fluorescens* (13.9 g plant^{-1}) treated pots followed by *G. mosseae* (10.2 g plant^{-1}) compared to the higher level of pathogen alone, whereas at lower pathogen level *P. fluorescens* or *G. fasciculatum* equally performed well and produced significantly higher dry root yield compared to the lower level of pathogen alone. Plants treated with *G. fasciculatum* or *P. fluorescens* also yielded significantly higher dry root yield at both levels of pathogen compared to non-inoculated soil (Figure 1C).

At lower pathogen level, treatments of *G. fasciculatum*, *G. mosseae* or *P. fluorescens* significantly increased the forskolin content by 14–21% compared to lower level of pathogen alone (Table 2). This is in agreement with the findings of earlier study (Boby and Bagyaraj 2003) which reported increase in forskolin concentration in the roots of *C. forskohlii* due to application of bio-inoculants.

Table 1. Effect of bio-inoculants on growth parameters.

Treatments	Plant height (cm)[1]	Plant spread (cm)[1]	Number of branches[1]
GA+120F	57.21[bc]	39.17 [bc]	9.13 [b]
GF+120F	78.25 [d]	49.75 [c]	13.38 [c]
GI+120F	46.96 [ab]	36.71 [bc]	8.00 [ab]
GM+120F	64.50 [cd]	37.00 [bc]	9.00 [b]
PF6+120F	81.88 [d]	56.00 [c]	12.25 [c]
120F	50.50 [b]	28.38 [ab]	6.75 [ab]
GA+240F	42.88 [ab]	31.63 [ab]	8.50 [ab]
GF+240F	76.00 [d]	39.75 [bc]	10.75 [bc]
GI+240F	48.38 [ab]	34.46 [ab]	7.13 [ab]
GM+240F	58.38 [b]	33.13 [ab]	7.88 [ab]
PF6+240F	76.00 [d]	43.50 [bc]	9.75 [bc]
240F	37.25 [a]	21.50 [a]	4.75 [a]
Soil only	50.42 [b]	30.04 [ab]	7.50 [ab]
SED	5.5640	6.3542	1.9221

SED, Standard error of mean difference.
[1]Average of the trials during the 2-year period; values in vertical columns followed by different superscripted letters are significantly different at $P < 0.05$ by ANOVA (LSD test); legend as in Figure 1.

Application of AM fungi or *P. fluorescens* significantly improved the uptake of P and K at both levels of the pathogen with marked increase in *G. fasciculatum* or *P. fluorescens* treated pots (Table 2). AM fungi or *P. fluorescens* significantly reduced the rhizosphere population of *F. chlamydosporum* at both levels of pathogen. Maximum reduction of the pathogen population in the rhizosphere was observed with *G. fasciculatum* followed by *P. fluorescens* under lower and higher pathogen levels (data not shown). On the other hand, the population of *P. fluorescens* remained at appreciable levels ($1.2+0.1 \times 10^6$ CFU g^{-1} soil) even at the time of harvest and was not affected due to levels of pathogen inoculum. There was no significant difference in mycorrhizal spores count among treated AM fungi. Significantly higher root colonization (66% at higher and 71% at lower pathogen level) was observed in the case of *G. fasciculatum*-treated plants at both levels of pathogen. Even in the presence of higher inoculum level of *F. chlamydosporum*, disease reduction and plant growth parameters were considerably improved by inoculation with *P. fluorescens* or *G. fasciculatum.* Earlier Boby and Bagyaraj (2003) have reported satisfactory control of root-rot and wilt and improvement in growth in *C. forskohlii* by inoculation with *Trichoderma viride* and *G. mosseae.* AM fungi are known to increase the resistance of plants to soil-borne pathogens (Prashanthi, Kulkarni, Srenivasa, and Kulkarni 1997; Bagyaraj 2006) by modification of cell wall, production of antimicrobial compound and altered rhizosphere microflora (Sampangi and Bagyaraj 1989). The disease suppressive effects of *P. fluorescens* are also well established (Weller 1988) and may result from production of antibiotics, siderophores, hydrocyanic acid, salicylic acid and competition for nutrients (Sharma 2006). Enhanced uptake of phosphorus and potassium due to AM fungal inoculation, observed in the present study, is in conformity with the observation made by several earlier workers (Sieverding and Toro 1988; Boby and Bagyaraj 2003). The percent mycorrhizal colonization by different AM fungi in the presence of

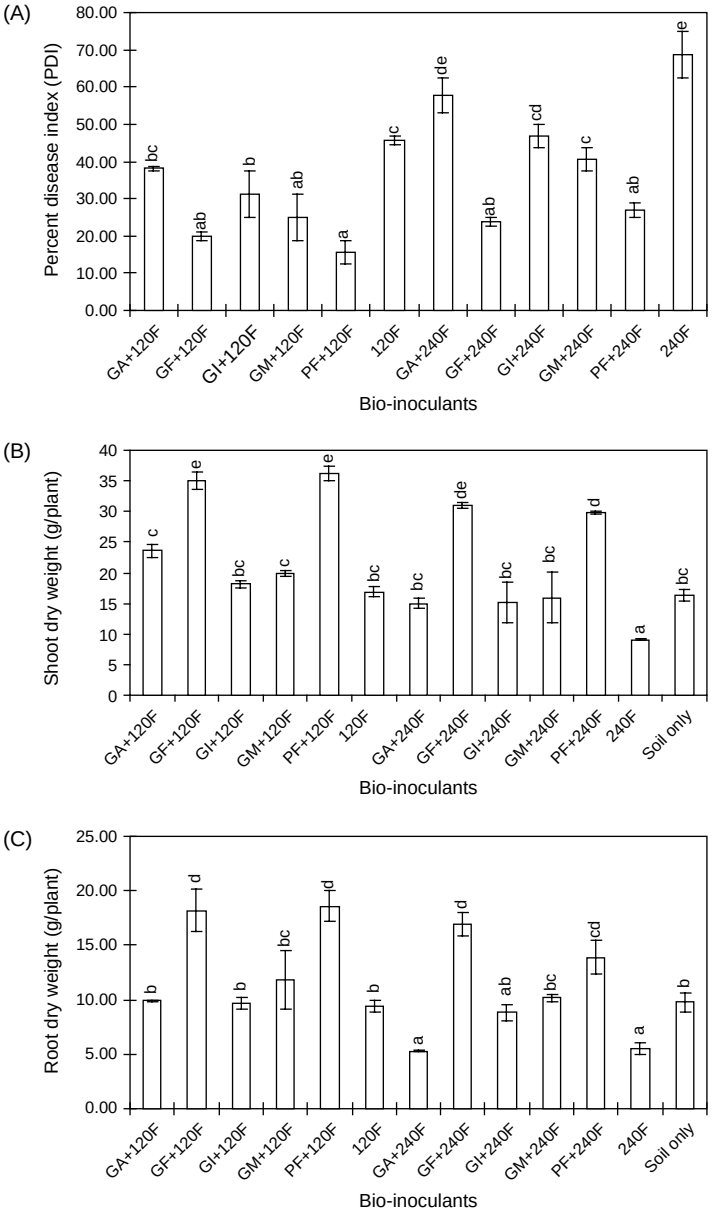

Figure 1. Effect of bio-inoculants on Percent Disease Index (PDI) and yields of *C. forskohlii*. (A) Mean PDI, (B) Mean shoot dry yield, (C) Mean root dry yield. Error bars are presented as standard error of mean (SEM). Different letters above the error bars show significant difference at $P < 0.05$ by ANOVA (LSD test). GA, *Glomus aggregatum*; GF, *Glomus fasciculatum*; GI, *Glomus intraradices*; GM, *Glomus mosseae*; PF6, *Pseudomonas fluorescens*; 120 F, 120 mL suspension of *Fusarium chlamydosporum*; 240 F, 240 mL suspension of *Fusarium chlamydosporum*.

Table 2. Effect of bio-inoculants on P and K uptake forskolin content.

Treatments	P uptake (mg/plant)[1]	K uptake (mg/plant)[1]	Forskolin content (mg/100 g dry roots)[1]
GA+120F	66.50 ^{cd}	495.00 ^{de}	890.00 ^{ab}
GF+120F	77.50 ^{d}	600.00 ^{f}	1010.00 ^{c}
GI+120F	59.50 ^{cd}	385.00 ^{c}	795.00 ^{a}
GM+120F	59.00 ^{c}	390.00 ^{c}	920.00 ^{bc}
PF6+120F	81.00 ^{d}	555.00 ^{ef}	975.00 ^{bc}
120F	23.50 ^{ab}	150.00 ^{ab}	795.00 ^{a}
GA+240F	36.50 ^{b}	195.00 ^{b}	790.00 ^{a}
GF+240F	71.50 ^{c}	500.00 ^{de}	835.00 ^{ab}
GI+240F	50.00 ^{bc}	270.00 ^{b}	820.00 ^{ab}
GM+240F	59.50 ^{cd}	390.00 ^{c}	835.00 ^{ab}
PF6+240F	72.50 ^{cd}	495.00 ^{de}	820.00 ^{ab}
240F	18.00 ^{a}	115.00 ^{a}	820.00 ^{ab}
Soil only	30.50 ^{ab}	140.00 ^{ab}	895.00 ^{ab}
SED	8.448	36.576	47.871

SED, Standard error of mean difference.
[1]Average of the trials during the 2-year period; values in vertical columns followed by different superscripted letters are significantly different at $P < 0.05$ by ANOVA (LSD test); legend as in Figure 1.

F. chlamydosporum suggests that *G. fasciculatum* is a better colonizer of *C. forskohlii*, thereby resulting in better symbiotic response and suppression of the pathogen. Also, higher population of *P. fluorescens* might have contributed to the higher levels of protection from the pathogen *F. chlamydosporum*. The results of the present study clearly demonstrate the potential of *G. fasciculatum* or *P. fluorescens* as biocontrol agents in suppressing the wilt and root-rot severity of *C. forskohlii* caused by *F. chlamydosporum* even at higher inoculum level and may play an important role in minimizing chemical inputs and improving yields in *C. forskohlii*.

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