

Original Research Article

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Comparative Efficiency of Biocontrol Agents in the Management of Root Borne Diseases of Coleus (*Coleus forskohlii* (Willd.) Briq.)

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ABSTRACT

A field experiment was conducted to score the comparative efficiency of biocontrol agents in the management of root borne diseases of *Coleus forskohlii* (cv.K-8). The crop has a great potential demand for forskolin (Diterpene) with wide application in treating many human diseases. However, the crop has become unpopular among farmers due to the susceptibility to many diseases of which root rot and wilt is the most serious causing loss in tuber yield and makes the crop uneconomical. The bioinoculants such as *Azotobacter chroococcum* (N fixer), *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Glomus fasciculatum* (AM Fungi) were used as potential biocontrol agents in the study. To quantify the disease severity per cent disease index (PDI) was calculated. The least disease incidence with improved plant health and maximum tuber yield was found to be in the treatment of 100% NPK with combined inoculation of all the consortia followed by plants inoculated with same consortia plus 75 % NP with 100% K. The highest disease incidence and least tuber yield were recorded in control plants with 50 % NP plus 100% K without any consortia. This indicates that economically advantageous yields with lowest disease incidence can be obtained with 75 % NP with 100% K and a saving of 25 % N can be achieved by using above consortia. This could be attributed that due to the effective use of microbial biogents with graded level of inorganic fertilizers. Hence, the present study aims at novel approaches to control diseases of *Coleus* and its economical management.

Keywords

Coleus forskohlii,
Forskolin,
Biocontrol agents,
Root borne diseases,
*Pseudomonas
fluorescens*.

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Introduction

Coleus (*Coleus forskohlii* (Willd.) Briq.) is a member of *lamiaceae* and an Indian medicinal herb. The entire plant is aromatic whether fresh or dried. The plant is grown in kitchen gardens in North Karnataka for its carrot like tubers that are used as condiment in the preparation of pickles and also used as a vegetable. This crop rose into prominence by virtue of its 'forskolin' a diterpene present in the root extracts (Shah *et al.*, 1980).

Forskolin activates almost all hormone sensitive adenylate cyclase enzymes in biological system. The crop has a great demand for forskolin which is widely used in glaucoma, cardiac problems, eczema, asthma, and hypertension and also used in the treatment of certain types of cancers. With the present annual production of about 100 tons from 700 ha in India, cultivation of *C. forskohlii* is picking up because of its economic potential. However, the crop has

not become very popular among farmers because of its susceptibility to many diseases of which root-rot and wilt is the most important, causing serious losses affecting the tuber yield. Now a day people have started growing this crop in farmsteads because of its economic potential (Vishwakarma *et al.*, 1988).

The crop is suffering from the diseases, among which root rot caused by *F. chlamydosporum* (Shyla, 1998; Boby, 2000) [*F. fusariodes* (frag. and cif.) Booth], aerial blight caused by *Rhizoctonia solani* (Shukla *et al.*, 1993) and root-knot caused by *Meloidogyne incognita* (Patel *et al.*, 1989) are the major crop production constraints. However, in the last few years, the cultivation of *Coleus* has suffered a great set back due to outbreak of bacterial wilt disease particularly in new gardens in and around Bangalore and Kolar district of Karnataka. The incidence of bacterial wilt caused by *Ralstonia solanacearum* was reported for the first time from Brazil (Netto and Assis, 2002). As it is a disease of recent origin, not much information pertaining to symptomatology, etiology, characterization of the causal organism and management of the disease are available (Vijaya Kumari, 2004).

Some fungi and bacteria are considered as potential biocontrol agents. *Trichoderma* fungi and bacteria like *B. subtilis* are of increasing interest as antagonistic soil microorganisms that control wide range of plant pathogens. Some of the important pathogens controlled by *T. harzianum* and *B. subtilis* are *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium*, *Colletotrichum*, *Pythium* spp, *Phytophthora* spp. infecting various crops.

Yahia *et al.*, (1985) reported that *B. subtilis* inhibited the growth of *Fusarium solani*. De *et al.*, (1996) reported that use of biocontrol

agent *B. subtilis* has significantly controlled the *Fusarium oxysporum* wilt by 30-45.8 per cent. Sancher *et al.*, (1994) reported that strain UPR5C of *Pseudomonas cepacia*, inhibited the growth of *Sclerotium rolfsii*, *Rhizoctonia solani* and to a lesser extent *Fusarium solani*. Anjaiah (1998) reported that *Pseudomonas* produced Phenazine antibiotics which inhibited the mycelial growth of *F. oxysporum*, while another compound anthranilite suppressed mycelial growth of *Pythium* spp.

Ciampi *et al.*, (1996) stated that fluorescent pigment synthesized by the strain BC-8 of *P. fluorescens* was able to inhibit the growth of *P. solanacearum* by production of siderophore compound. Shubalaxmi (1999) reported that *P. fluorescens* was highly inhibitory to *Ralstonia solanacearum* followed by *B. subtilis* and *P. aeruginosa* under *in vitro* condition. Vijaya kumari (2004) studied the effect of three antagonists of which *P. fluorescens* was found most effective in inhibiting the growth of *Ralstonia solanacearum* followed by *B. subtilis*, *Lactobacillus* sp. produced the least inhibiting zone.

The lethal action of *Trichoderma viride* due to the secretion of an antibiotic substance called gliotoxins was first showed by Weindling (1932). Further, *Sclerotium rolfsii* was found susceptible to *Trichoderma lingorum* (Tode) Harz. and the antibiotic substance produced by above fungus was identified as viridine (Brain, 1951). Sychev and Shadoshinik (1982) found that *T. viridae* inhibited the growth of *Rhizoctonia solani* and *F. oxysporum*. Hartman and Fletcher (1991) reported that *T. harzianum* is most efficient to control *Fusarium* root-rot. Seed treatment with *T. viridae* and soil application of *T. viridae* and *T. harzianum* in mustard was found to reduce damping off disease by 63.7, 71.3 and 64.4 per cent respectively compared to control (Ebenezer *et al.*, 1996).

The recent concept of eco-friendly technology and sustainability is most appropriate through use of bioinoculants in combination with inorganic fertilizers meet the crop nutrient needs reduces the rate of application of chemical N fertilizers by 18-20 per cent and increasing the yield to an extent of 20-25% but also supplement various plant growth promoting substances.

Materials and Methods

Microbial bioinoculants used in the experiment and their mass multiplication

Microbial bioagents such as *B. subtilis* (9×10^8 cfu g^{-1}), *P. fluorescens* (6×10^8 cfu g^{-1}), *T. harzianum* (2×10^6 cfu g^{-1}) and *G. fasciculatum* (12500 IP g^{-1}) were used as bioagents in order to control the disease. The bioinoculants such as *A. chroococcum* (12×10^7 cfu g^{-1}) and arbuscular mycorrhizal fungus *G. fasciculatum* (12500 IP g^{-1}) used as nitrogen fixer and phosphorus mobilizer respectively. These bioinoculants were mass

produced at Biofertilizers scheme laboratory, Department of Agricultural Microbiology, UAS, Gandhi Krishi Vijnana Kendra, Bangalore.

Site Description, Experimental design and treatments

The field experiment was carried out at Sanjeevini Vatika, Division of Horticulture, University of Agricultural Sciences, GKVK, Bangalore. Microbiological investigations were done at Biofertilizer laboratory, Department of Agricultural Microbiology, UAS, GKVK, Bangalore. The experiment had 14 treatments replicated thrice was laid out in Randomized Complete Block Design (RCBD). Plant variety used: K-8 (released by IIHR), recommended fertilizer dose: 40:60:50 kg NPK ha^{-1} and recommended dose of FYM: 10 tons ha^{-1} . Forty days old healthy Coleus seedlings were transplanted on the ridges at a spacing of 45cms to main field. The treatment details as follows.

Treatments
T ₁ -100% NPK
T ₂ -75% NP+100% K
T ₃ -50% NP+100% K
T ₄ -75% NP+100% K+ <i>Azotobacter chroococcum</i> + <i>Bacillus subtilis</i>
T ₅ -75% NP+100% K+ <i>A. c</i> + <i>B. s</i> + <i>Trichoderma harzianum</i>
T ₆ -75% NP+100% K+ <i>A. c</i> + <i>B. s</i> + <i>Pseudomonas fluorescens</i>
T ₇ -75% NP+100% K+ <i>A. c</i> + <i>B. s</i> + <i>Glomus fasciculatum</i>
T ₈ -75% NP+100% K+ <i>A. c</i> + <i>B. s</i> + <i>T. h</i> + <i>P. f</i> + <i>G. f</i>
T ₉ -50% NP+ 100% K+ <i>A. c</i> + <i>B. s</i>
T ₁₀ -50% NP+ 100% K+ <i>A. c</i> + <i>B. s</i> + <i>T. h</i>
T ₁₁ -50% NP+ 100% K+ <i>A. c</i> + <i>B. s</i> + <i>P. f</i>
T ₁₂ -50% NP+ 100% K+ <i>A. c</i> + <i>B. s</i> + <i>G. f</i>
T ₁₃ -50% NP+100% K+ <i>A.c</i> + <i>B. s</i> + <i>T. h</i> + <i>P. f</i> + <i>G. f</i>
T ₁₄ -100%NPK+ <i>A. c</i> + <i>B. s</i> + <i>T. h</i> + <i>P. f</i> + <i>G. f</i>

Fertilizer

The amount of inoculum applied to each plant was standardized by following package of practices recommended by UAS, GKVK, Bangalore. The lignite based *A. chroococcum* inoculum was applied at 5g per plant after assessing the population per gram carrier. *T. harzianum* had a population of 2×10^6 cfu g⁻¹ was applied at 2g per plant and *P. fluorescens* and *B. subtilis* which has attained a population of 10⁸ cells per ml mixed with lignite powder and applied to crop plants at 5g per plant. The air-dried AM fungal inoculum contained infected root bits of AM fungi and Chlamydo spores and applied to each plant was 10g which had a population of 12500 IP (infective propagules). The recommended dose of fertilizer (IHR recommendation) was applied in the form of urea, single super phosphate and muriate of potash respectively as per the treatments. The crop was harvested after five months of transplanting.

Major diseases of the crop

Coleus plant is highly prone to many diseases such as leaf spots, leaf blight, root rot and wilt and root knot (caused by a nematode). Of these root rot and wilt are the main diseases responsible for causing major loss of tuber yield.

Method of pathogen isolation

The disease affected tissues of shoot and tuberous roots were collected. The diseased parts was cleansed (surface sterilized) using 70% alcohol and then with distilled water. Affected parts were cut into small pieces and incisions were made to subject for serial dilution in order to isolate fungal and bacterial pathogens. They were cultivated using potato dextrose agar and nutrient agar respectively. Later pure cultures of *Fusarium chlamydo sporum* and *Ralstonia solanacearum* were obtained and preserved in cryo. The fungal pathogen was identified and confirmed (Booth, 1977). The virulent bacterial pathogen *R. solanacearum* produce pink pigmented colonies with white fluid was identified by following the methods of Kelman (1954); Vanitha *et al.*, (2009) and (Singh *et al.*, 2012).

Per cent Disease index

The crop is highly susceptible to fungal root rot and bacterial wilt caused by *F. chlamydo sporum* and *R. solanacearum* respectively. The effect of microbial bioagents on *Coleus* was determined by scoring as per cent disease index. The disease index was computed by adopting 0-4 scale to cover all broad disease symptoms (Kesavan and Chowdhury, 1977).

Scale	Symptom
0	No symptoms (healthy plant)
1	Slight drooping of leaves or vascular browning in root region and no plant mortality
2	Wilting of leaves or vascular browning extended in root region followed by no plant mortality
3	Severely wilted, withered except terminal bud
4	Dead plant

Each one was given due weightage as all the symptoms contributed for reducing the yield. Plants showing any of the above symptoms

were considered as diseased. To quantify the disease severity per cent disease index (PDI) was calculated by using the formula:

$$PDI (\%) = \frac{\text{Summation of individual scores}}{\text{Maximum grade} \times \text{Total number of plants}} \times 100$$

Microbial parameters

The initial microbial load viz., bacteria, fungi and actinomycetes in soil and at harvest of the crop is presented in Table 1. The initial soil sampling was done after land preparation and pooled by quadrant method. At harvest the treatment wise rhizosphere soil sampling was done and pooled. The microbial population was enumerated using serial dilution and plate count technique.

Statistical analysis

The data collected in this study was subjected to statistical analysis suitable to RCBD. Duncan's multiple range test (DMRT) was done to separate the treatment means (Little and Hills, 1978).

Results and Discussion

Microbial parameters

General microflora initial and at harvest

Initial bacterial, fungal and actinomycetes population was 18×10^6 cfu g⁻¹, 14.75×10^6 cfu g⁻¹ and 10×10^4 cfu g⁻¹ soil respectively (Table 1). At harvest, the maximum bacterial population was found in the rhizosphere soil of the treatment (T₁₄) which had *A. chroococcum*, *B. subtilis*, *T. harzianum*, *P. fluorescens* and *G. fasciculatum* with 100 % NPK (41.0×10^6 cfu g⁻¹ soil) which was statistically on par with the treatment (T₈) inoculated with same microbial consortia with 75 % NP plus full dose of K (40.0×10^6 cfu g⁻¹ of soil). The lowest bacterial population was found in the rhizosphere soils of uninoculated plot (T₃) treated only with 50 % NP plus full dose K (15.0×10^6 cfu g⁻¹ of soil). The similar trend was found with respect to fungal and actinomycetes population in treatment (T₁₄) (31.90×10^3 cfu

g⁻¹ soil) and (26.90×10^4 cfu g⁻¹ soil) which was statistically on par with the treatment (T₈) (30.0×10^3 cfu g⁻¹ of soil) and (27.0×10^4 cfu g⁻¹ of soil) respectively. The lowest fungal and actinomycetes population was found in the rhizosphere soils of uninoculated plot (T₃) treated only with 50 % NP plus full dose K (13.0×10^3 cfu g⁻¹ of soil) and (10.76×10^4 cfu g⁻¹ of soil) respectively (Table 1). Treatments differed significantly with respect to bacterial, fungal and actinomycetes population. The microbial analysis of soil after harvest of the crop has showed improvement in microbial population in soil due to biofertilizers application compared to initial population.

The above results are in accordance with the observations made earlier by Srihari and Sreenivasa (1995) who recorded higher microbial population in the rhizosphere of Chilli when inoculated with *Glomus macrocarpum* and *Bacillus polymyxa* as compared to uninoculated control plants. The results of this study has clearly showed that combined inoculation of all the consortia supplemented with full recommended NPK levels is more advantageous to crop growth and in obtaining maximum profitable yield.

Management of diseases

Water stagnation in coleus fields may lead to severe infections of *Fusarium* and *Ralstonia*, therefore water stagnation in the standing crop should be avoided.

Control of Fusarial/bacterial diseases by microbial bioagents

Per cent disease index

The number of infected plants kept increasing from the date of planting to harvest in all the treatments. But the least number of affected plants at harvest was found in the treatment of

A. chroococcum, *B. subtilis*, *T. harzianum*, *P. fluorescens* and *G. fasciculatum* supplemented with full recommended NPK (47.03%). The next best treatment was 75% NP with full dose of K with same microbial consortium (48.20%). The highest number of plants infected was found in uninoculated plants which received no microbial bioagents and supplemented only 50 % NP with full dose of K (84.63%) (Table 2).

Arbuscular mycorrhizal (AM) fungi suppressing the activity of root pathogens are well documented (Mohan and Verma, 1996). *P. fluorescens* mainly considered as a PGPR and can suppress a wide range of plant pathogens including *Fusarium* (Nautiyal, 1997; Johanson *et al.*, 2003).

Some reports clearly indicated that the root-rot/wilt of *C. forskohlii* could be significantly reduced by the application of bio-agents like *T. viride*, *P. fluorescens* and AM fungus like *G. fasciculatum* and *G. mosesae* (Boby and Bagyaraj, 2003; Singh *et al.*, 2009). Paramasivan *et al.*, (2007) reported that the use of bioinoculants like *T. viride* and *P. fluorescens* reduced the disease incidence by 20-21%. Boby and Bagyaraj (2003) and Singh *et al.*, (2009) reported that inoculation of bio-inoculants (*T. viride*, *G. fasciculatum*, *G. mosesae* and *P. fluorescens*) significantly increased the forskolin content of the roots.

Root rot and wilt disease

Initially, symptoms such as yellowing and wilting of leaves is observed eventually roots turn brown to black color show oozing, putrefaction and decaying of roots (Singh *et al.*, 2011). The fungal pathogen causing the disease has been identified as *Fusarium chlamyosporum* (Shyla, 1998; Singh *et al.*, 2009). *Fusarium solani* causing root-rot of *C. forskohlii* has also been reported by Bhattacharya and Bhattacharya (2008).

Wilt as reported to be caused by *Ralstonia solanacearum* affects vascular tissues in *Coleus barbatius* (Coelho and Assis, 2001; Chandrashekara and Prasannakumar, 2010). Kamalakannan *et al.*, (2006) reported *Macrophomina phaseolina* has also been isolated from the disease complex. Yellowing and drooping of the leaves, blackening of the stem, rotting of the roots and basal stem and peeling of stem bark and root epidermis is commonly observed.

Effect of microbial bioagents on Tuber yield and Forskolin yield per hectare

The maximum tuber yield (t ha⁻¹) and forskolin (kg ha⁻¹) yield were recorded in the treatment combination (T₁₄) inoculated with *A. c.*, *B. s.*, *T. h.*, *P. f.* and *G. f.* with 100 % NPK (13.49, 20.37) followed by treatment (T₈) inoculated with same microbial consortia along with 75 % NP plus full dose of K (12.87, 18.93) which was statistically on par with the treatment (T₇) having the combined inoculation of *A. c.*, *B. s.* and *G. f.* (12.76, 17.03). The control treatment supplemented with 50% NP plus full dose K showed the lowest tuber yield and forskolin content (8.05, 6.29) (Table 3).

The higher yields attributed to the maximal disease suppression of combined effect of microbial bioagents and influence of inorganic fertilizers. Similar results were obtained in *Mentha arvensis* by Singh *et al.*, (1988) and Kumarvel (2003) in *Artemesia annua*.

In vitro study of microbial bioagents against *Ralstonia solanacearum* and *Fusarium chlamyosporum*

In vitro screening for inhibition of *Ralstonia solanacearum* and *Fusarium chlamyosporum* by *P. fluorescens*, *B. subtilis* and *T. harzianum* was tested under laboratory

condition and the results are presented in Table 4. Among the three antagonists tested *P. fluorescens* showed maximum inhibition over control (69.38 %) against *Ralstonia solanacearum*. The lowest was recorded in *B. subtilis* which showed only 14.33% inhibition over control. It has been also reported earlier that *Pseudomonas fluorescens* antagonistic to the *Fusarium roseum* and *Pythium* under *in vitro* conditions (Dahiya *et al.*, 1988). The earlier studies also showed that *P. fluorescens* is capable of suppressing wide range of pathogens like *Fusarium oxysporum* f.sp. *dianthi* in carnation (Dujiff *et al.*, 1995), *Gaeumannomyces tritici* causing take all disease in wheat (Weller, 1983) and *F. oxysporum* f. sp. *ciceris* causing wilt in chick pea (Dileep Kumar and Dube, 1992). *Pseudomonas* is also known to produce chitinases, hydrogen cyanide and proteases (Woeng *et al.*, 1998) and also phenazine antibiotics (Anjaiah *et al.*, 1998) which are inhibitory to root pathogens.

Similarly, *T. harzianum* (49.00 %) proved to be highly antagonistic against *F. chlamydosporum* over control. The other two *P. fluorescens* (37.00 %) and *B. subtilis* (33.00 %) were shown less effective against *F. chlamydosporum*.

Arbuscular mycorrhizal per cent root colonization and spore number in rhizosphere soil

Plants treated with *A. chroococcum*, *B. subtilis*, *T. harzianum*, *P. fluorescens* and *G. fasciculatum* with full recommended NPK (T₁₄) showed maximum root colonization and spore count (per 50 g of soil) (81.81% and 114.02 respectively) which was statistically on par with the treatment (T₈) of 75% NP plus

100 %K with *A. chroococcum*, *B. subtilis*, *T. harzianum*, *P. fluorescens* and *G. fasciculatum* (80.25% and 112.31 respectively). Whilst the lowest per cent root colonization and spore count was observed in uninoculated treatment (T₃) which had only 50% NP with full dose of K (25.46% and 53.83 respectively) (Table 5).

These results have clearly showed that Arbuscular Mycorrhizal population has proliferated in soil by synergistic interaction with other introduced microbial bioagents in the rhizosphere soil. These findings uphold the views of Praveen Kumar (2003) reported such increased population of VA Mycorrhiza in the rhizosphere soils of Gherkin due to introduced microorganisms and Govind Rao *et al.*, (1989) found 100% root colonization in *Mentha arvensis*.

Benefit: Cost ratio

The plants receiving 100% NPK with *A. chroococcum*, *B. subtilis*, *T. harzianum*, *P. fluorescens* and *G. fasciculatum* recorded maximum Benefit: cost ratio of 1.76:1 which is due to highest tuber yield in turn resulting in increased net returns (91,471 rupees per hectare). However the combined inoculation of above microorganisms with 100% NPK was found economically viable in obtaining maximum yields which were statistically on par with the treatment of 75% NP plus full dose of K with same microbial consortium which show a net saving of 25% NP chemical fertilizer application with maximum yield. These results are in conformity with the findings of Mahantesh (2002) who recorded maximum benefit: cost ratio in onion due to *Azospirillum* inoculation.

Table.1 General microbial population of soil initial and at harvest of Coleus

Treatments	General microbial population (Cfu g ⁻¹ soil)					
	Initial			At harvest		
	Bacteria (No.x 10 ⁶)	Fungi (No. x 10 ³)	Actinomycetes (No. x 10 ⁴)	Bacteria (No.x 10 ⁶)	Fungi (No. x 10 ³)	Actinomycetes (No. x 10 ⁴)
T ₁	18.00	14.75	10.00	18.20	15.50	12.05
T ₂				16.00	15.53	13.25
T ₃				15.00	13.00	10.76
T ₄				36.70	20.33	20.00
T ₅				25.90	28.10	24.70
T ₆				36.70	21.03	17.73
T ₇				33.25	20.50	20.23
T ₈				40.00	30.00	27.00
T ₉				29.70	25.80	26.93
T ₁₀				29.50	27.90	22.50
T ₁₁				36.50	23.10	20.23
T ₁₂				25.70	22.95	18.00
T ₁₃				37.20	28.25	17.00
T ₁₄				41.00	31.90	26.90
SEm ±				0.239	0.323	0.146
CD (0.05)				0.695	0.940	0.425

Notations as in Table 1

Notations: *A. c* =Azotobacter chroococcum *B. s* = Bacillus subtilis *T. h* = Trichoderma harzianum;
P. f =Pseudomonas fluorescens *G. f* = Glomus fasciculatum DAP = Days after planting
 NS =Non significant * =Significant at 0.05

Table.2 Effect of microbial bioagents on per cent disease index at harvest in Coleus

Treatments	Per cent Disease index at harvest
T ₁	62.02
T ₂	63.87
T ₃	84.63
T ₄	59.00
T ₅	56.37
T ₆	49.31
T ₇	49.90
T ₈	48.20
T ₉	58.71
T ₁₀	57.81
T ₁₁	53.82
T ₁₂	54.07
T ₁₃	52.29
T ₁₄	47.03
SEm ±	1.60
CD (0.05)	4.8

Table.3 Influence of microbial bioagents on forskolin content and dry weight of *Coleus* tubers per hectare

Treatments	Fresh weight of tubers (t ha ⁻¹)	Forskolin yield (Kg ha ⁻¹)
T ₁	11.69	13.03
T ₂	8.69	8.18
T ₃	8.05	6.29
T ₄	12.01	15.18
T ₅	12.71	16.66
T ₆	12.17	15.55
T ₇	12.76	17.03
T ₈	12.87	18.93
T ₉	10.02	9.99
T ₁₀	10.77	11.85
T ₁₁	10.11	10.79
T ₁₂	11.07	12.22
T ₁₃	11.97	14.44
T ₁₄	13.49	20.37
SEm ±	0.53	0.41
CD (0.05)	1.60	0.12

Table.4 In vitro effect of bioagents on *Ralstonia solanacearum* (wilt pathogen) and *Fusarium chlamydosporum* (root rot pathogen)

Treatments	Inhibition zone (mm)
<i>Trichoderma harzianum</i> + <i>Ralstonia solanacearum</i>	3.8
<i>Pseudomonas fluorescens</i> + <i>Ralstonia solanacearum</i>	6.9
<i>Bacillus subtilis</i> + <i>Ralstonia solanacearum</i>	1.4
<i>Ralstonia solanacearum</i> (Control)	0.0
<i>Trichoderma harzianum</i> + <i>Fusarium chlamydosporum</i>	4.9
<i>Pseudomonas fluorescens</i> + <i>Fusarium chlamydosporum</i>	3.7
<i>Bacillus subtilis</i> + <i>Fusarium chlamydosporum</i>	3.3
<i>Fusarium chlamydosporum</i> (Control)	0.0

Table.5 Mycorrhizal root colonization in *Coleus*

Treatments	Per cent root colonization	Spore count per 50 g soil
T ₁	30.00	64.76
T ₂	28.99	63.95
T ₃	25.46	53.83
T ₄	37.84	66.00
T ₅	39.44	66.23
T ₆	40.17	68.39
T ₇	74.66	70.50
T ₈	80.25	112.31
T ₉	30.65	65.00
T ₁₀	35.03	65.08
T ₁₁	38.62	68.37
T ₁₂	66.00	69.04
T ₁₃	68.18	72.33
T ₁₄	81.81	114.02
SEm ±	0.94	1.17
CD (0.05)	2.71	3.41

Notations as in Table 1

Table.6 Economics of Coleus cultivation using bioinoculants

<i>Treatments</i>	Cost of fertilizers (Rs ha ⁻¹)	Cost of biofertilizers (Rs ha ⁻¹)	Total cost of cultivation (Rs ha ⁻¹)	Gross income (Rs ha ⁻¹)	Net income (Rs ha ⁻¹)	Benefit: cost ratio (per ha)
T ₁	2162	-	52249	118500	66251	2.6:1
T ₂	1719	-	51806	82500	30694	0.59:1
T ₃	1276	-	51363	73500	22137	0.43:1
T ₄	1719	800	52606	130500	77094	1.46:1
T ₅	1719	1200	53006	136500	82294	1.54:1
T ₆	1719	1200	53006	132500	78294	1.47:1
T ₇	1719	920	52726	139500	83014	1.60:1
T ₈	1719	1720	53526	142000	85914	1.59:1
T ₉	1276	800	52163	100500	47537	0.91:1
T ₁₀	1276	1200	52563	112300	58537	1.11:1
T ₁₁	1276	1200	52563	106300	52537	0.99:1
T ₁₂	1276	920	52563	114500	60475	1.15:1
T ₁₃	1276	1720	53083	126500	70857	1.32:1
T ₁₄	2162	1720	53969	148000	91471	1.67:1

Notations as in Table 1

Similar findings were also reported by Maheshwari *et al.*, (2000) who recorded maximum B:C ratio (3.7) in Isabgol inoculated with *Azotobacter* and phosphate solubilizing bacteria (Table 6).

Labour expenses for different operations were calculated as Rs. 250/- per day for both men and women labourers. Gross income was calculated by multiplying the Forskolin yield with the price prevailing in the market and expressed as total income per hectare. Net income was calculated by deducting all the cost of cultivation from gross income. Cost benefit ratio was calculated by dividing the net income by cost of cultivation.

In conclusion, the results clearly reveal that microbial bioagents with graded different levels of nitrogen, phosphorus and potash application showed beneficial effect on pathogenic suppression in Coleus. The similar effect is reflected in tuber yield and forskolin

content wherever maximum disease suppression is observed. Maximal disease suppression was found in the treatment of 100 % NPK with consortia of bioagents and that of lowest in the tubers of uninoculated control plants. Maximum tuber yield can be obtained in plants treated with 100 % NPK along with microbial consortia.

The next best superior yield was in the treatment of 75 % NP plus full dose of K with microbial consortia which were found statistically significant over 50 % NP plus 100 % K alone. This clearly shows that economically advantageous yields can be obtained with 75 % NP with full dose of K and a saving of 25 % N can be achieved by using the above microbial consortia. The use of microbial bioagents a cost effective and ecofriendly approach to control phytopathogenic fungi and bacteria. It can be concluded that microbial bioagents can be used to control fungal root rot and bacterial

wilt as the causative agents are found to be susceptible.

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