MYCORRHIZA NEWS

The Quarterly Newsletter of Mycorrhiza Network

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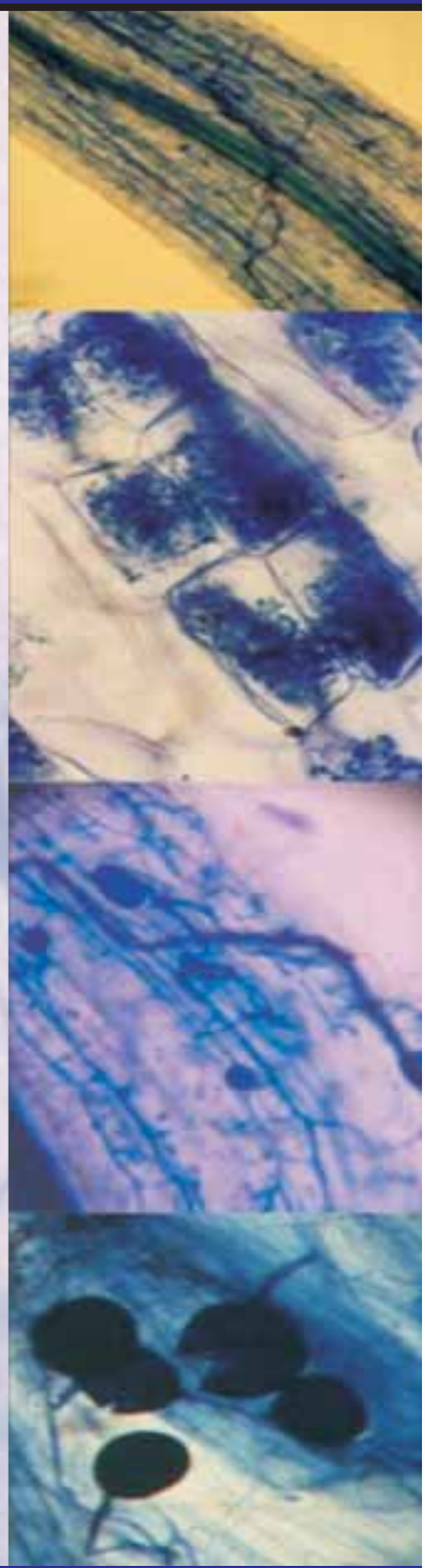

About TERI

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TERI's Mycorrhiza Network is primarily responsible for establishing the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhiza Culture Collection), and publishing the *Mycorrhiza News*. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhizologists.

Mycorrhiza News

The *Mycorrhiza News* provides a forum for dissemination of scientific information on mycorrhiza research and activities; publishes significantly important papers from eminent scientists, notes on important breakthroughs, brief accounts of new approaches and techniques, papers compiled from the RIZA database. It also provides information on forthcoming events on mycorrhiza and related subjects,lists important research references published during the quarter, and highlights the activities of the CMCC.

Contents

Research finding papers

Taxonomic series: *Glomus* from Goa

*Sharda W Khade**

15. *Glomus clavisporum* (Trappe) Almeida and Schenck

Mycologia **82**: 703–714, 1990
= *Sclerocystis microcarpus* Iqbal and Bushra
Transactions of Mycological Society of Japan
21: 57–63, 1980 (Plate 15)

Sporocarps are dark brown, globose, 285–420 µm in diameter, minutely verrucose from exposed tips of spores, which are formed radially, side by side in a single, tightly packed layer around a central plexus of tightly woven hyphae (Plate 15a). Sporocarps lack peridium. Chlamydospores are brown to dark brown in colour, clavate to sub-cylindric, 105 × 36 µm in size (Plate 15b). Spore wall is laminated, brown in colour, 12 µm thick at the apex and 3 µm thick at the sides. Spore wall is generally thickest at the apex (Plate 15b).

Distinguishing feature: Small sporocarps without the peridium and spores with thickened apex tightly packed around the central plexus.

***Distribution*:** Recorded from Valpoi in July with 16.66% frequency of occurrence. Again, recorded in April and July from Valpoi with 50% frequency of occurrence. Recorded in October from Kodar with 12.50% frequency of occurrence.

(a) (b)

Plate 15(a) Crushed sporocarp of *Glomus microcarpus* consisting of chlamydospores radially arranged around a central plexus of tightly woven hyphae (× 150)
Plate 15(b) Chlamydospores of *Glomus microcarpus* with thickened apex (× 400)

***Association*:** Found in association with *Carica papaya* plants from Western Ghats and the plateau region of Goa, India.

16. *Glomus microcarpum* Tulasne and Tulasne

Gironale Botanico Italiano **2**: 63,1845 (Plate 16)

The spores are firmly embedded in glebal hypha that are either variably swollen 10 µm wide with walls

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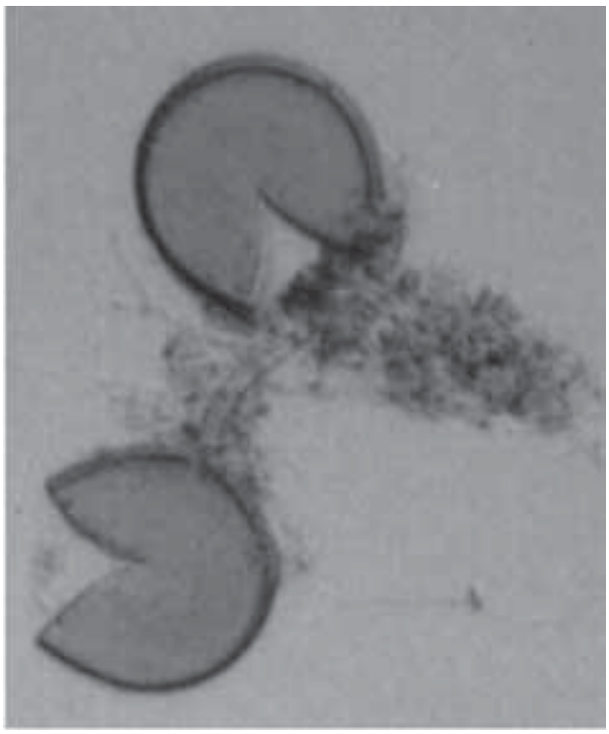
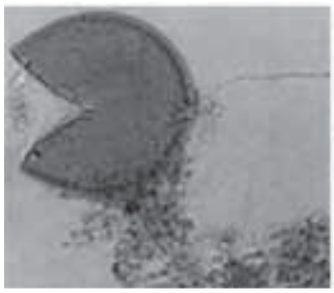
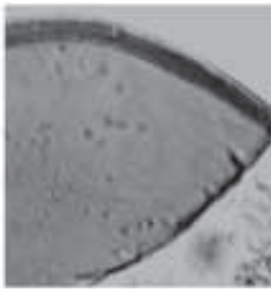


(a)



(b)



(c)

Plate 16(a) Spores of *Glomus microcarpum* embedded in glebal hypha (× 400)
Plate 16(b) A spore of *Glomus microcarpum* embedded in glebal hypha (× 400)
Plate 16(c) A portion of laminated spore wall of *Glomus microcarpum* (× 1000)

1–2 µm thick (Plates 16a and 16b). Spores are globose, 30–45 µm in diameter, and yellow to yellowish brown in colour (Plates 16a and 16b). The spore wall is smooth and composed of a single layer, 4–6 µm thick, laminated, and light yellow or yellowish brown in colour (Plate 16c). The width of the single subtending hypha at its point of attachment to the spore is 4–6 µm. Wall of the subtending hypha is 1–2 µm thick, and pore is open, partially or completely occluded by thickening of the spore wall.

Distinguishing feature: Small spores with single laminated wall.

***Distribution*:** Recorded from Kodar in April and October with 25% frequency of occurrence.

***Association*:** Found in association with *C. papaya* plants from the plateau region of Goa, India.

17. *Glomus multicaule* Gerdemann and Bakshi

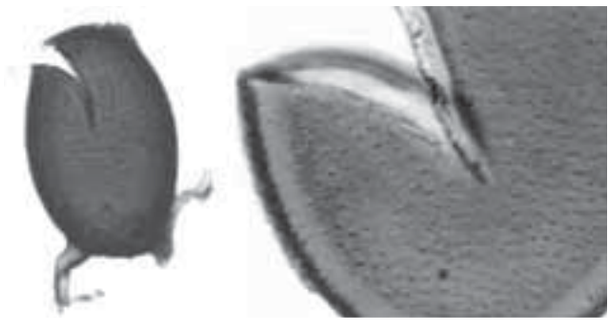
Transactions of British Mycological Society **66**: 340–343, 1976 (Plate 17)

Spores are formed singly in soil. They are ellipsoid or broadly ellipsoidal, sub-globose, 149–249 × 124 –162 µm in diameter, reddish brown to dark brown in colour with one to four hyphal attachments generally occurring at opposite ends (Plate 17a). The spore wall is 6–12 µm thick, and is thickest at the points of hyphal attachments. Rounded projections that are 1–3 µm long are regularly distributed all over the wall surface (Plate 17b).

Distinguishing feature: Rounded projections 1–3 µm long regularly distributed all over the spore surface.

***Distribution*:** Recorded from Valpoi and Collem in December with 50% frequency of occurrence.

***Association*:** Found in association with *C. papaya* plants from Western Ghats of Goa, India.



(a) (b)

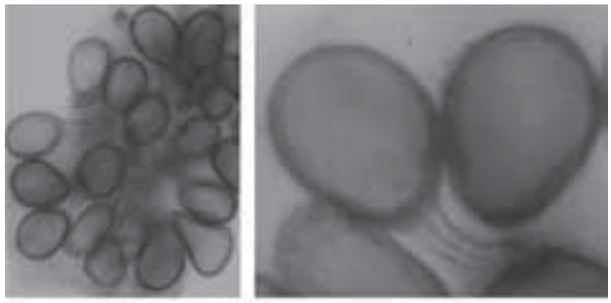
Plate 17(a) Crushed spore of *Glomus multicaule* with two hyphal attachment (× 200)
Plate 17(b) A portion of spore surface of *Glomus multicaule* covered all over with rounded projections (× 400)

18. *Glomus rubiforme* (Gerd. and Trappe), Almeida and Schenck

Mycologia **82**: 703–714, 1990.

= *Sclerocystis pachycaulis* Wu and Chen *Taiwania* **31**: 65–88, 1986 (Plate 18)

Sporocarps are yellow to yellowish brown in colour, globose to sub-globose, 170–200 × 175–250 µm in diameter. They consist of terminal chlamydospores radially arranged on a central plexus of hyphae (Plate 18a). Chlamydospores are yellow to yellowish brown, obviod to ellipsoid, sometimes irregular and 28–60 × 38–88 µm in size (Plate 18a). Chlamydospore wall is yellowish brown, 1–5 µm thick with a 1 µm thick hyaline and separable outer layer. Spore contents separated from the subtending hypha by one to two septa below the point of attachment. The attached hypha is 8–15 µm in diameter with a thick wall (up to 8 µm). Wall of attached hypha extends downwards and is usually thicker than the spore wall (Plate 18b).



(a) (b)

Plate 18(a) Crushed sporocarp of *Glomus pachycaulis* (× 200)
Plate 18(b) Spore of *Glomus pachycaulis* (× 400)

Distinguishing feature: Obviod to ellipsoid, sometimes irregular chlamydospores with wall of attached hypha extending downwards and usually thicker than the spore wall.

***Distribution*:** Recorded from Valpoi and Collem in December with 33.33% frequency of occurrence. Recorded from Valpoi in April with 25% frequency of occurrence.

***Association*:** Found in association with *C. papaya* plants from Western Ghats of Goa, India.

Reference

Almeida R T and Schenck N C. 1990. **A revision of the genus *Sclerocystis* (Glomaceae, Glomales)**. *Mycologia* **82**: 703–714

Schenck N C and Perez Y (eds). 1990. ***Manual for Identification of VA Mycorrhizal Fungi***. Gainesville, USA: INVAM, University of Florida

Influence of *Glomus fasciculatum* and bioformulations on growth parameters and yield of papaya cv. Red Lady

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Introduction

Carica papaya L. (papaya) is often considered the common man's fruit as it is one of the commercially important fruit crops of the tropics. Due to its continuous vegetative growth and flowering and fruiting habits, papaya requires high nutrients. It is estimated that the extent of nutrients removed by the whole plant at the time of harvest would be 305 kg of nitrogen (N), 103 kg of phosphorous (P), 524 kg of potassium (K), 327 kg of calcium (Ca), and 183 kg of magnesium (Mg) per hectare (ha) (Veerannah and Selvaraj 1984). In addition, the indiscriminate use of inorganic fertilizers is polluting the ecosystem. Soil microbes influence the mutability and uptake of inorganic nutrients by plants. Symbiotic endophytes and mycorrhizae are involved in the uptake of nutrient elements of vital plants. Furthermore, from the literature, it was observed that the use of bioformulations, like panchagavya, Amrit pani, microbial consortia, and Pandrapur slurry, are important alternative sources of nutrients responsible for the substantial increase in the yield of many fruit crops (Adivappar, Patil, Patil, *et al*. 2004; Manjunath 2000; Santosh, Patil, and Swamy 2004; Sukhada 1992). However, papaya has been cultivated inorganically for decades, which has resulted in the substantial decline in its productivity. Hence, the purpose of the present study was to find out the influence of the mycorrhizal fungus, *Glomus fasciculatum,* and bioformulations, namely, panchagavya, Amrit pani, and microbial consortia, on various components of vegetative growth and yield.

Material and methods

The study was conducted in the split plot design with the arbuscular mycorrhizal fungus (AMF), *G. fasciculatum,* and without the AMF as the main treatment. Bioformulations and their combinations were used at seven sub-treatments: (1) panchagavya, (2) Amrit pani, (3) microbial consortia, (4) panchagavya and Amrit pani, (5) panchagavya and microbial consortia, (6) Amrit pani and microbial consortia, and (7) recommended dose of fertilizer (RDF). The experiment was replicated thrice. The papaya seeds were inoculated by placing 5 g of the AMF culture *G. fasciculatum* in a bag of 18 × 12 cm dimensions at 2 cm depth comprising 19 chlamydospores/gram of soil.

The bioformulations, namely, panchagavya, Amrit pani, and microbial consortium, were used at the rate of 3% concentration in the form of soil application except for panchagavya, which was applied both in the form of soil application and as a spray. The bioformulations and combination of these bioformulations were applied at monthly intervals to the respective plots as per the treatments. Chemical fertilizer was added to the RDF treatment at a ratio of 250:250:500 g of nitrogen, phosphorous, and potassium (NPK) per plant per year in split doses (Anonymous 2004).

Vegetative growth parameters like plant height, stem girth, number of leaves, petiole length, and yield (kg/ha) were recorded from each plant and mean values were used for statistical analysis. The data was subjected to the Fisher's method of analysis of variance, and interpretation of the data was done as given by Panse and Sukhatme (1967).

Results and discussion

The inoculation of the AMF had significantly increased the vegetative growth parameters, namely, plant height, stem girth, number of leaves, petiole length, and yield of plants as compared to non-inoculated plants (Tables 1 and 2). Plants supplied with the bioformulation combinations of panchagavya and Amrit pani recorded the maximum plant height, stem girth, number of leaves, petiole length, and yield, followed by plants supplied with panchagavya and microbial consortia as compared to other sub-treatments in the interaction effect. The AMF *G. fasciculatum* inoculated plants with panchagavya and Amrit pani recorded the highest plant height, stem girth, number of leaves, petiole length, and yield followed by the AMF *G. fasciculatum* inoculated plants along with panchagavya and microbial consortia.

The beneficial effect of the AMF *G. fasciculatum* in papaya was evident in the present investigation as all the growth parameters and yield exhibited significant improvement in the AMF inoculated plants as compared to the non-inoculated plants (Tables 1 and 2). Similar increase in vegetative growth parameters and yield were reported in papaya by Adivappar (2001). The AMF can stimulate growth and yield of

Table 1 Influence of *Glomus fasciculatum* and bioformulations on the growth parameters of papaya growth

	Plant height (cm)		Stem girth (cm)		Number of leaves		Petiole length (cm)	
Treatment	300 DAT		300 DAT		300 DAT		120 DAT	
M_1S_1	143.37		25.35		51.16		66.41	
M_1S_2	140.42		25.91		50.60		65.56	
M_1S_3	137.86		23.33		49.68		62.93	
M_1S_4	162.75		32.05		58.27		75.47	
M_1S_5	156.72		29.05		56.04		72.83	
M_1S_6	149.66		27.15		55.09		69.27	
M_1S_7	134.18		22.12		47.12		62.02	
Mean (M_1)	**146.42**		**26.42**		**52.65**		**67.78**	
M_2S_1	117.13		22.71		36.42		57.71	
M_2S_2	116.04		19.14		35.65		57.04	
M_2S_3	113.55		19.13		33.13		55.24	
M_2S_4	130.62		25.82		46.38		62.76	
M_2S_5	127.33		23.66		43.12		59.13	
M_2S_6	120.42		20.32		39.09		58.10	
M_2S_7	108.12		16.92		31.18		54.36	
Mean (M_2)	**119.03**		**21.10**		**37.85**		**57.76**	
S_1	130.25		24.03		43.79		62.06	
S_2	128.23		22.53		43.13		61.30	
S_3	125.71		21.23		41.41		59.09	
S_4	146.69		28.94		52.33		69.12	
S_5	142.03		26.36		49.58		65.98	
S_6	135.04		23.74		47.39		63.69	
S_7	121.15		19.52		39.15		58.19	
For comparing the means of	S. Em ±	CD at 5%	S. Em ±	CD at 5%	S. Em ±	CD at 5%	S. Em ±	CD at 5%
Main (M)	0.466	2.836	0.134	0.814	0.150	0.913	0.382	2.324
Sub (S)	0.493	1.438	0.202	0.589	0.420	1.226	0.442	1.291
S at same M	0.697	2.033	0.285	0.333	0.594	1.734	0.625	1.825
M at same S	0.796	2.323	0.296	0.864	0.570	1.664	0.694	2.024

DAT – days after transplanting; NS – non significant; RDF – recommended dose of fertilizer

Notes

M_1 – with *Glomus fasciculatum;* M_2– without *Glomus fasciculatum;* S_1– panchagavya; S_2– Amrit pani; S_3 – microbial consortia; S_4 – panchagavya + Amrit pani; S_5 – panchagavya + microbial consortia; S_6– Amrit pani + microbial consortia; S_7 – RDF

the host plant due to the improved association of the AM fungi and roots, resulting in better accessibility of nutrients via fungal mycotropism (Azcon and Bago 1994), growth regulators and hormones, namely, auxins, cytokinins, gibberellins, and vitamins (Craft and Miller 1974).

The improved vegetative parameters observed in the present investigation could thus be attributed to the increased *G. fasciculatum* association in terms of percent root colonization and spore count (Table 3), aided by bioformulation application, leading to

Table 2 Influence of *Glomus fasciculatum* and bioformulations on yield parameters of papaya

Treatment	Yield (kg/plant)		Yield (t/ha)	
M_1S_1	43.43		134.70	
M_1S_2	42.51		131.11	
M_1S_3	36.04		111.04	
M_1S_4	64.47		198.95	
M_1S_5	51.16		155.29	
M_1S_6	47.63		144.92	
M_1S_7	34.02		104.99	
Mean (M_1)	**45.61**		**140.14**	
M_2S_1	23.25		71.75	
M_2S_2	22.72		69.47	
M_2S_3	20.97		64.71	
M_2S_4	31.12		96.31	
M_2S_5	27.74		85.61	
M_2S_6	23.24		71.72	
M_2S_7	18.71		57.74	
Mean (M_2)	**23.97**		**73.90**	
S_1	33.34		103.23	
S_2	32.62		100.29	
S_3	28.51		87.88	
S_4	47.80		147.63	
S_5	39.45		120.45	
S_6	35.44		108.32	
S_7	26.36		81.36	
For comparing the means of	S. Em ±	CD at 5%	S. Em ±	CD at 5%
Main (M)	0.191	1.163	0.179	1.088
Sub (S)	0.336	0.979	0.266	0.776
S at same M	0.475	1.385	0.376	1.098
M at same S	0.479	1.398	0.392	1.143

DAT – days after transplanting; RDF – recommended dose of fertilizer

Notes

M_1 – with *Glomus fasciculatum;* M_2– without *Glomus fasciculatum;* S_1– panchagavya; S_2– Amrit pani; S_3 – microbial consortia; S_4 – panchagavya + Amrit pani; S_5 – panchagavya + microbial consortia; S_6– Amrit pani + microbial consortia; S_7 – RDF

increased surface area for the absorption and uptake of nutrients in the papaya plants (Tables 1, 2, 4, and 5). Besides this, *G. fasciculatum* and bioformulations are also known to release growth substances, growth regulators, hormones, and enzymes (namely acid phostase) in the rhizosphere, which might have helped in the conversion of insoluble nutrients to a soluble form. Consequently, this may have led to the increase in their availability to the plants (Table 4), resulting in the increased content of major nutrients like N, P, K, and micronutrients like iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), and cobalt (Co) (Adivappar, Patil, Patil, *et al.* 2004), thus leading to increased vegetative parameters and yield of the papaya plants.

Table 3 Influence of *Glomus fasciculatum* and bioformulations on the AM fungi spore count in the soil and per cent root colonization in the papaya root

Treatment	Spore count		Root colonization (%)	
M_1S_1	493.68		92.50	
M_1S_2	473.60		88.75	
M_1S_3	517.73		87.27	
M_1S_4	669.33		96.75	
M_1S_5	632.51		94.33	
M_1S_6	587.68		93.66	
M_1S_7	132.78		48.96	
Mean (M_1)	**501.05**		**86.03**	
M_2S_1	105.50		34.12	
M_2S_2	103.00		35.66	
M_2S_3	115.50		33.47	
M_2S_4	123.50		36.76	
M_2S_5	117.54		35.82	
M_2S_6	97.96		34.73	
M_2S_7	61.05		18.20	
Mean (M_2)	**103.43**		**32.39**	
S_1	299.59		63.31	
S_2	288.33		61.21	
S_3	316.61		60.37	
S_4	396.41		66.76	
S_5	375.03		65.08	
S_6	342.82		64.20	
S_7	96.91		33.58	
For comparing the means of	S. Em ±	CD at 5%	S. Em ±	CD at 5%
Main (M)	0.154	0.939	0.174	1.056
Sub (S)	0.464	1.354	0.541	1.580
S at same M	0.656	1.915	0.766	2.235
M at same S	0.627	1.830	0.730	2.130

DAT – days after transplanting; RDF – recommended dose of fertilizer

Notes

M_1 – with *Glomus fasciculatum;* M_2– without *Glomus fasciculatum;* S_1– panchagavya; S_2– Amrit pani; S_3 – microbial consortia; S_4 – panchagavya + Amrit pani; S_5 – panchagavya + microbial consortia; S_6– Amrit pani + microbial consortia; S_7 – RDF

Table 4 Influence of *Glomus fasciculatum* and bioformulations on the NPK content of papaya (petiole)

Treatment	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
M_1S_1	1.00		0.39		3.81	
M_1S_2	0.99		0.37		3.79	
M_1S_3	0.98		0.35		3.53	
M_1S_4	1.39		0.48		4.07	
M_1S_5	1.27		0.43		3.88	
M_1S_6	1.07		0.40		3.78	
M_1S_7	0.98		0.27		3.65	
Mean (M_1)	**1.10**		**0.38**		**3.79**	
M_2S_1	0.83		0.27		3.32	
M_2S_2	0.81		0.27		3.17	
M_2S_3	0.78		0.25		3.24	
M_2S_4	0.85		0.29		3.52	
M_2S_5	0.84		0.28		3.44	
M_2S_6	0.84		0.28		3.33	
M_2S_7	0.83		0.26		3.28	
Mean (M_2)	**0.83**		**0.27**		**3.33**	
S_1	0.92		0.33		3.57	
S_2	0.90		0.32		3.48	
S_3	0.88		0.30		3.38	
S_4	1.12		0.38		3.80	
S_5	1.06		0.36		3.66	
S_6	0.95		0.34		3.56	
S_7	0.91		0.27		3.46	
For comparing the means of	S. Em ±	CD at 5%	S. Em ±	CD at 5%	S. Em ±	CD at 5%
Main (M)	0.017	0.103	0.004	0.025	0.022	0.133
Sub (S)	0.034	0.098	0.007	0.019	0.036	0.106
S at same M	0.048	0.139	0.009	0.027	0.051	NS
M at same S	0.047	0.138	0.010	0.028	0.052	NS

DAT – days after transplanting; NS – non significant; RDF – recommended dose of fertilizer

Notes

M_1 – with *Glomus fasciculatum;* M_2– without *Glomus fasciculatum;* S_1– panchagavya; S_2– Amrit pani; S_3 – microbial consortia; S_4 – panchagavya + Amrit pani; S_5 – panchagavya + microbial consortia; S_6– Amrit pani + microbial consortia; S_7 – RDF

Table 5 Influence of *Glomus fasciculatum* and bioformulations on the soil NPK content of the papaya field

	Soil NPK content (kg/ha)					
Treatment	N		P		K	
M_1S_1	205.30		39.13		73.09	
M_1S_2	205.42		41.07		70.30	
M_1S_3	183.63		40.20		68.38	
M_1S_4	147.62		38.31		69.92	
M_1S_5	165.07		47.41		71.46	
M_1S_6	173.45		39.59		67.14	
M_1S_7	132.66		83.61		86.47	
Mean (M_1)	**173.31**		**47.05**		**72.39**	
M_2S_1	220.98		54.37		75.41	
M_2S_2	210.42		54.91		80.76	
M_2S_3	203.39		52.78		74.13	
M_2S_4	198.21		57.52		74.00	
M_2S_5	216.30		50.53		72.39	
M_2S_6	209.80		54.44		70.62	
M_2S_7	293.54		87.68		91.00	
Mean (M_2)	**221.81**		**58.89**		**76.90**	
S_1	213.16		46.75		74.25	
S_2	207.92		47.99		75.53	
S_3	193.51		46.49		71.26	
S_4	172.92		47.92		71.96	
S_5	190.69		48.97		71.93	
S_6	191.63		47.02		68.88	
S_7	213.10		85.65		88.73	
For comparing the means of	S. Em ±	CD at 5%	S. Em ±	CD at 5%	S. Em ±	CD at 5%
Main (M)	3.566	21.698	0.916	5.573	0.333	2.026
Sub (S)	6.038	17.623	1.936	5.650	1.546	4.513
S at same M	8.538	24.923	2.738	NS	2.187	6.382
M at same S	8.672	25.313	2.695	NS	2.052	5.988

DAT – days after transplanting; NS – non significant; RDF – recommended dose of fertilizer

Notes
M_1 – with *Glomus fasciculatum;* M_2– without *Glomus fasciculatum;* S_1– panchagavya; S_2– Amrit pani; S_3 – microbial consortia; S_4 – panchagavya + Amrit pani; S_5 – panchagavya + microbial consortia; S_6– Amrit pani + microbial consortia; S_7 – RDF

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Effect of mulches on the efficiency of microbial inoculants and growth of *Ailanthus excelsa* in calcareous mined spoil*

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Introduction

Application of different mulches in degraded soil is helpful for the establishment, growth, and survival of plants. Due to moisture loss, limestone mined spoils become dry and compact, which prevents the establishment and growth of plants. Moisture in the soil can be retained by the use of different mulches available in the plantation site. Various organic mulches such as straw, hay, and crop residues have been applied on mine spoil (Schivley 1979). Micro-climate of the rhizosphere can be modified through the application of suitable mulches on drastically disturbed areas (Graves and Carpenter 1979). Mulches also protect the soil by reducing the impact of raindrops and water run-off. Mulching treatments have been reported to be effective in lowering the soil temperature in the rhizosphere. Moreover, organic mulches, after decomposition, can improve the fertility status of the spoils and help in the growth and development of plants in disturbed sites. Application of different mulches improves moisture content of infertile soil and plays an important role in microbial efficiency through increased multiplication, particularly of arbuscular mycorrhizal fungi (AMF) in the soil. The present study was conducted to investigate the effect of different mulches on the influence of bio-inoculants in the growth of *Ailanthus excelsa* in the soil of mined out spoil. After successful experiments in the nursery, the technology was transferred to the field.

Materials and methods

For the study of nutrients content in the soil, the physico-chemical properties and concentration of trace elements in limestone mined out spoils and soil samples of overburden materials were collected from dumps of different age groups, including 10 years, 5 years, and 0 year old (fresh spoil) dumps. The soil samples were carefully collected in polyethylene bags and their openings were tied with rubber bands.

Estimation of trace elements (Ni, Pb, Mn, Zn, Fe, Cu, and Co) in the limestone mines spoil was done using the diethylene triamine pentaacetic acid (DTPA) extraction method prescribed by Lindsay and Norvell (1978). The physico-chemical analysis of the soil was made using the method given by Black (1965). To conduct nursery experiments, composite spoil material was collected in bulk in n ylon sacs and imported to the laboratory. Spoil material was manually ground to suitable size and sterilized twice a day in an autoclave at 121 °C and 15 lbs for 40 minutes, every alternate day. This sterilized spoil material was used for experimentation. Different mulches, namely, stone mulch, bark mulch, leaf litter mulch, and coir mulch (coconut peelings), either singly or in combination, were used. Seeds of *A. excelsa* were collected from the campus of the Tropical Forest Research Institute, Jabalpur, from a single tree and surface sterilized with 1% sodium hypochlorite solution for two minutes for removal of surface infectants. Healthy seeds were sown in the sterilized calcareous soil filled in earthen pots. Regular watering was done using sterilized water. After sprouting of seeds, the culture of AMF and *Pseudomonas fluorescens* bacteria were inoculated in the root zone of seedlings. The AMF and *P. fluorescens* were isolated from the limestone mined spoil and multiplied in bulk. The seedlings were first treated with the AMF consortium and *P. fluorescens*. After microbial treatments, the plants were supplemented with different mulches. The broth culture of *P. fluorescens* was developed in King's B medium. The AMF culture was prepared in the trap crop *Zea mays* and placed in the vicinity of the roots of the seedlings. Stone pieces were collected from the spoil material, and after steam sterilization, pieces of suitable sizes were placed around the collar of seedlings in such a manner that the entire exposed soil was completely covered. For the purpose of leaf litter mulch, dried leaves of *Butea monosperma* were collected, cut, and crushed into small pieces. Sterilized leaves were placed around the

* This work is a part of a PhD thesis approved by the Forest Research Institute University, Dehradun, India

seedlings. Coconut peelings were then collected and cut into small pieces. After steam sterilization, these pieces were put around the seedlings as coir mulching treatment. The dried bark of *B. monosperma* was cut into pieces; after steam sterilization, it was applied as mulch. For the purpose of bark and leaf litter mulch amendment, the bark and leaves of *B. monosperma* were cut into pieces and applied as mulch. All the mulching materials were sterilized in an autoclave at 121 °C and 15 lbs pressure for one hour. Two controls were maintained; control I was supplemented with AM and *P. fluorescens* without any mulching treatment and control II contained spoil soil without any microbial and mulching treatment.

Results and discussion

From Tables 1a and 1b, it is evident that the spoils were deficient in essential nutrients, alkaline in reaction, highly calcareous, and poor in organic carbon contents. The soil characteristics of different aged dumps indicated the status of the soil, which shows the unsuitability of the soil for plant growth. The concentration of different trace elements in the spoils of different age groups is presented in Table 2. The concentration of Ni, Pb, Fe, and Cu decreased with the age of the spoils, while the concentration of Mn, Zn, and Co increased. All such variations may be due to the interaction between plant roots of growing vegetation and microbial succession occurring in the mined spoils. Increased levels of Cd in Zn mines were reported by several researchers (Figge, Hetrick, and Wilson 1995). Jastrow, Zimmerman, Dvorak and Hinchman (1981) observed a variation in the concentration of different trace elements in coal mine spoil and reported maximum concentration of Zn among all the heavy metals. A high level of Zn was also reported by Stucky, Bauer, and Lindsey (1980) in acidic mine spoils. Heavy metals may have a beneficial effect on living organisms as micronutrients (Mortvedt, Giordano, and Lindsay 1972). However, when high concentration levels are taken up by plants, their toxic effects have been observed, especially on taller plants, including forest trees, (Bazzaz, Carlson, and Rolfe 1974) as well as on soil micro-organisms (Jackson and Watson 1977). Toxic levels of concentration of heavy metals in mine spoil are important indicators for the establishment and further growth, survival, and development of plants on these areas. However, any such eventuality was not noticed in this study.

Table 1a Nutrient status (NPK) of limestone mined spoils of different age group dumps

	Available nutrient (kg/ha)		
Age of spoil (year)	N	P	K
10	64.78[a]	14.92[a]	239.7[a]
5	62.12[b]	4.96[b]	188.0[b]
0 (fresh spoil)	32.80[c]	3.46[c]	173.9[c]
CD (0.05)	3.0968	1.0202	7.9767
SE ±	1.3429	0.44242	3.4591

kg/ha – kilogram per hectare; N – nitrogen; P – phosphorus; K – potassium

Note Different alphabets in the same column indicate significant difference between the treatments at a 5% rejection level.

Table 1b Physico-chemical properties of limestone mined spoils of different aged dumps

	Age of spoil (year)		
Property	10	5	0
Organic matter (%)	0.22	0.20	0.10
Organic carbon (%)	0.13	0.11	0.05
$CaCO_3$ equivalence (%)	7.3	9.2	18.8
Exchangeable Mg^{+2} (me/100 g)	11.8	10.0	9.4
Exchangeable Ca^{+2} (me/100 g)	27.8	26.4	37.6
Ph	8.2	8.4	8.5
EC (mmhos/cm)	0.12	0.15	0.19

me – mili equivalent; mmhos – milimhos; cm – centimetre

Table 2 Status of trace elements in limestone mined spoils

	Concentration of trace elements (ppm)						
Age of spoil (year)	Ni	Pb	Mn	Zn	Fe	Cu	Co
10	0.028	0.36	0.75	0.41	0.43	0.21	0.24
5	0.11	0.57	0.41	0.24	0.48	0.38	0.17
0	0.13	1.3	0.17	0.20	0.57	0.45	0.02
CD (0.05)	0.082363	0.037019	0.0065932	0.0087675	0.018891	0.0087367	0.029970
SE ±	0.029665	0.013333	0.0023747	0.0031578	0.0068041	0.0031467	0.010794

ppm – parts per million; Ni – nickel; Pb – lead; Mn – manganese; Zn – zinc; Fe – Iron; Cu – copper; Co – cobalt

Table 3 reveals that the seedlings supplemented with stone mulch showed the best growth performance, followed by the leaf litter mulching supplements. Seedlings amended with coir mulch and bark mulch showed good growth over both the controls. Control I (AMF and *P. fluorescens*) also improved the growth of seedlings in comparison to control II (spoil soil only). Spore population of the AMF increased significantly over control I. Maximum spore population was recorded in the mulching treatment with stone mulch, followed by leaf litter mulch. Seedlings supplemented with coir mulch and bark mulch also attained a good number of AM spores in comparison to control I. Root colonization was maximum in seedlings supplemented with stone mulch (58.33%), followed by leaf litter mulch (54%) and coir mulch (53%). Seedlings amended with bark mulch also gained good AM root colonization (49.33%) in control I (43%). Seedlings supplemented with bark and leaf litter mulch could not gain significant root colonization (44.33%) in comparison to control I. In a soil condition like limestone mined spoils, which has low soil nutrient levels with poor nutrient cycling capacity, inoculation of beneficial microbes and application of suitable mulches will be helpful in the establishment of plants in nutrient poor mined spoils. Sarles and Emanuel (1977) demonstrated the effect of tree bark, sawmill waste, and other composted organic residues on the establishment of different plants on disturbed sites, which helped in the growth and development of vegetation and reduction in water loss. Moisture loss is prevalent on such wastelands, which could be minimized by using different mulches based on the availability and requirement of the site to be treated. Schivley (1979) reported favourable effects of fly ash, sewage sludge, and organic waste as mulches on mined lands. The application of sawdust and green manure as mulch has increased productivity of some vegetable crops on mine spoils (Morse 1979). Since calcareous spoils restrict microbial activity, release of nutrients was slow. Johnson and McGraw (1988) reported significant increase in plant biomass, AM spore density, and root colonization by applying paper mill sludge as mulching treatment. Mulching was found to be effective in lowering soil temperature in the root zone as well as in conserving soil moisture and checking evaporation (Chandra and Jamaluddin 2004). Wieder, Carrel, Rapp, *et al.* (1983) reported an increase in vegetable productivity in strip mine spoil through application of sawdust and green manure. A number of researchers have recommended AMF inoculation for successful afforestation of mined wastes (Khan 1981; Loree and Williams 1987). Gaur and Rana (1990) recommended dual inoculation of phosphorus solubilizing bacteria and AMF. Mulching may alter the surface micro-climate and help conserve soil moisture during seedling establishment. Mulching amendments assist in seedling establishment by improving moisture regime, moderating surface temperature, decreasing soil erosion, and improving soil fertility. Mulching would have several effects on the physical conditions of the soil, which may have a positive influence on the microbial activities in the soil. This study reports that mulching was found to be significantly effective in increasing the root and shoot length, collar diameter, and biomass over controls (without mulches [control I]; spoil soil only [control II]). Among the different mulches, stone mulch was found to be most effective on the growth and biomass of *A. excelsa* when applied in combination with AMF and *P. fluorescens*. The synergistic effect of

Table 3 Effect of different mulches on effectiveness of AMF and *Pseudomonas fluorescens* on growth of *Ailanthus excelsa* in mined spoil soil

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Number of AMF spores/100 g soil	Root colonization (%)
Spoil soil + AMF + PF +stone mulch	20.97^{d}	7.22^{c}	3.20^{d}	377.30^{e}	58.33^{e}
Spoil soil + AMF + PF + leaf litter mulch	19.30^{cd}	6.68^{bc}	2.92^{d}	356.00^{d}	54.00^{d}
Spoil soil + AMF + PF coir mulch (coconut peelings)	17.65^{c}	6.00^{ab}	2.50^{c}	342.30^{c}	53.00^{cd}
Spoil soil + AMF + PF + bark mulch	17.37^{c}	5.73^{ab}	1.52^{b}	339.30^{c}	49.33^{c}
Spoil soil + AMF + PF + bark + leaf litter mulch	14.43^{b}	5.10^{a}	1.33^{ab}	320.00^{b}	44.33^{b}
Spoil soil + AMF + PF (control I)	14.50^{b}	6.00^{ab}	1.32^{ab}	313.70^{b}	43.00^{b}
Spoil soil only (control II)	11.87^{a}	5.02^{a}	1.12^{a}	0.00^{a}	0.00^{a}
CD (0.05)	2.5155	1.0977	0.39407	9.8534	3.9111
SE ±	1.2317	0.53748	0.19296	4.5233	1.7951

PF – *Pseudomonas fluorescens*; AMF – arbuscular mycorrhizal fungi

Note Same letters in the same column indicate that the treatments are significantly not different from each other at a 5% rejection level.

the inoculation of AMF and *P. fluorescens* facilitated the availability of nutrients to the plants, and application of stone mulch supplemented water retention and better moisture condition in the rhizosphere. Stone mulch also helped reduce thermal stresses on the soil surface. The mulching treatment increased the AMF population in the rhizosphere of the treated plants. Thus, suitable mulches may also contribute to population multiplication of beneficial microflora in degraded soils. In this study, the application of stone mulch was found to be most effective in the growth and development of the test plant species *A. excelsa* in the mined out spoil. Hence, the inoculation of AMF and *P. fluorescens* bacteria, along with stone mulch, is recommended for establishment and improved growth of plants on limestone mined out spoils. The study also suggests that the inoculation of microbial consortium essentially consists of AMF and phosphorus solubilizing bacteria while taking up large-scale afforestation on limestone mined out areas. The study concludes that the synergistic action of mulches and microbial inoculants has a significant effect on the growth and establishment of plants, particularly in degraded lands.

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Varietal affinity of Tea (*Camellia chinensis*) plants for arbuscular mycorrhizal associations

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Introduction

Mycorrhizal fungi are reported to be beneficial for several host plants in nutrient mobilizations and uptake, inducing tolerance to drought, soil salinity or alkalinity, and resistance to pathogens (Feng Gu, Bai Dengsha, Yang Maoqui, *et al.* 1999; Karaki and Raddad 1998; McGoningle, Miller, and Jaung 1999; Bryla and Duniway 1998; Tholkappian, Sivasrovanan, and Sunafanam 2000). Tea plants are also reported to have mycorrhizal association (Karthikeyan, Muthukumar, and Udaiyan 2005; Majstrick 1974; Otieno 1995; Rajagopal and Ramarathinan 1997; Singh, Pandey, Chaurasia, *et al.* 2008). Application of a large amount of inorganic and organic fertilizers to the tea soils also affects the microbial population and their role in nutrient cycling. Furthermore, a few laboratories are engaged in analysing different aspects of microbial activity of tea soils (Hayatsu 1993; Kanazawa and Kunito 1996; Pandey and Palni 1997; 2004). A study on the mycorrhizal association of tea plants grown in Bhuyanpirth tea estate of Orissa was carried out and the observations made are presented in subsequent sections.

Materials and methods

The study was carried out on tea plants grown in the Bhuyanpirh tea estate belonging to M/S Orissa Tea Plantation Ltd situated in Tarmakanta, which is about 48 km away from the Keonjhar district of Orissa, India. The plantation is situated in an area which was once covered by dry mixed deciduous sal forests on an elevation of more than 600 m. The soil was red clay-loam and poor in nutrient content.

The whole tea plantation was divided into different sectors under two blocks. The variation among the blocks was only due to the difference in fertilizer treatment. The rate of fertilizer applied in blocks 1 and 2 were urea 151.91 and 126.51 kg/ha, muriate of potash (MOP) 84.70 and 74.63 kg/ha, and Single super phosphate (SSP) 187.50 and 125.15 kg/ha, respectively. In each sector, 12 000 plants/ha of different varieties of tea were present. All plants were irrigated with the help of sprinklers. The whole plantation area was shaded by plants such as *Albizzia odoratissima*, *Leucaena leucocephala*, *Albizzia lebbek*, and *Acacia auriculiformis*. The plants of different tea varieties were of 15–17 years old and rarely treated with pesticides and weedicides. Samples were collected randomly in triplicates from various tea varieties grown in different sectors under the two blocks. Roots were collected, along with their rhizosphere soil (up to a depth of 25 cm). Care was taken during root collection so as not to mix other plants' roots with the test samples.

The percentage mycorrhizal infection of the tea roots was estimated according to the method of Phillips and Hayman (1970). Root samples were cleaned with 10% KOH and autoclaved for 15–20 minutes at 15 p/i; the autoclaved root samples were treated with 6N HCl for 5 minutes. The cleared roots were then stained with 0.05% cotton blue. The percentage infection was calculated by the function of number of root bits infected to the number of root bits observed. Besides percentage colonization, the number of vesicles of the roots were observed and recorded.

Wet sieving and decanting method was carried out to assess the spore population in the rhizosphere soil (Gerdemann and Nicolson 1963). About 100 g of representative soil samples of each location (in triplicate) was suspended in sufficient quantity of water and stirred thoroughly. The resulting soil suspension was sieved through meshes of sizes 400, 300, 200, and 100 um and placed one below the other in the same order. The residues, after sieving, were filtered (Whatman no. 1) and observed under stereomicroscope for spore count (Meiji, Japan). The soil pH was measured by Mettler Toledo MA 235 pH/Ion Analyzer.

Results

Mycorrhization was observed in the tea varieties studied. The maximum colonization was observed in the variety Tenali (16.80%) which was followed by TV 18 (13.42%.) (Table 1). Other varieties such as Betzan, Nandadevi, and TV 1 have shown low percentage of colonization. The total number of vesicles/colony also varied considerably. The roots of the variety Tenali were distributed with highest number of vesicles (95.44), while other tea varieties were observed with less number of vesicles in their AM colonized roots. Accordingly, the vesicles number/cm of infected roots was found to be equally distributed at a range of two

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to four. The total number of AM spores present in the soils was also determined and the maximum spores were obtained from the rhizosphere of TV 18 (786.44 spores/100 g soil) followed by Nandadevi (769.33 spores/100 g of soil) in block 1.

The tea variety Betzan and TV 18 growing in Block 2 was also found to be mycorrhizal. The Betzan variety of Block 2 had comparatively higher percentage of colonization, whereas TV 18 had more or less similar per cent colonization in both blocks (Table 2). The number of vesicles in these varieties was also higher as compared to that of Block 1. The number of vesicles/cm root was also observed to increase in Block 2. The soil pH varied from 4.8 to 5.02 and the spore/100 g of rhizosphere soil was observed to be more or less the same (Table 2).

Statistical analysis showed no significant differences in the colonization of different varieties obtained from Blocks 1 and 2. The varietal differences for the total number of vesicles, vesicles/cm infected roots and number of spores were found to be significant. The Betzan and TV 18 grown in Block 2 showed significant variation with respect to the rhizospheric spore population. Significant intra-varietal differences in vesicle number/root, vesicles number/cm root, and spore population was observed with Betzan and TV 18 grown in the two different blocks.

Discussion

Arbuscular mycorrhizal fungi (AMF) have been reported from different geographical regions with a very wide range of host plants (Sadadou and Hargas 2000). The occurrence of mycorrhization in the tea varieties in the survey supports the universal occurrence of AMF and also confirms the findings of Wang-Shousheng, He Shoulin, Wang DeJun, *et al.* (1997) and Otieno (1995).

The contribution of soil to supply nutrient to plants increased with rhizosphere acidification. Uptake kinetics were also affected by rhizosphere acidification (Gillespie and Pope 1991). Since acidic soils limit the establishment, growth, and persistence of plants in acidic soils particularly during the early stages, AMF will likely contribute in solving this problem (Staley and Voigt 2000). Tea plants were not only observed as mycorrhizal, but a significant difference in their mycorrhization was also noted among the five different varieties. Mycorrhizal association is a triangular relationship between mycorrhiza, soil, and plants. It may affect the mycorrhization in quantity as well as quality. The occurrence of more percentage colonization and more number of vesicles per root gives rise to host preferability.

Chlamydospore formation in the rhizosphere soil by AMF decreases with increase in the soil pH (Sidhu and Behl 1997). In Block 2, the presence of more number of spores with slightly low pH also confirms this finding. The variability in density of AM spores in the rhizosphere soil of tea variety may also be due to the presence of shade tree species. Variation among the Betzan and TV 18 growing in different blocks with respect to their mycorrhization pattern and quantity may also be due to the fertilizer treatment, as root infection rate decreases with increasing application of fertilizer (Zhi 1995).

It may be concluded that the mycorrhizal fungi were able to tolerate acidic pH and could develop healthily. Their occurrence within the host plants also depends upon the type of host and its associates. The colonization percentage and the number of vesicles are important considerations that account for the efficiency of mycorrhizal fungi. In the present study, it has been observed that low fertilizer level encourages

Table 1 Parameters on AM colonization in different tea varieties growing in Block 1

Tea variety	% colonization	No. of root vesicles/root	Vesicle/cm root	pH of soil	No. of spores/100 g soil
Betzan	8.00 ± 2.29	19.55 ± 8.00	2.11 ± 0.26	5.02 ± 0.04	685.08 ± 65.95
Nandadevi	8.33 ± 1.46	34.33 ± 18.23	4.77 ± 1.44	4.90 ± 0.08	769.33 ± 68.96
Tenali	16.80 ± 3.64	95.44 ± 40.81	4.66 ± 0.86	5.01 ± 0.06	615.22 ± 83.70
TV 1	6.71 ± 0.52	20.66 ± 2.88	3.77 ± 0.43	4.94 ± 0.07	633.55 ± 73.72
TV 18	13.42 ± 2.04	33.11 ± 10.24	3.00 ± 0.62	4.87 ± 0.04	786.44 ± 64.01

AM – arbuscular mycorrhizal

Table 2 Parameters on AM colonization in different tea varieties growing in Block 2

Tea variety	% colonization	No. of root vesicles/root	Vesicle/cm root	pH of soil	No. of spores/ 100 g soil
Betzan	24.38 ± 5.52	145.88 ± 39.94[a]	4.66 ± 0.72[a]	4.99 ± 0.05	475.11 ± 74.91
TV 18	11.75 ± 2.80	119.33 ± 17.38[b]	6.33 ± 0.88[b]	5.16 ± 0.04	724.11 ± 95.85

AM – arbuscular mycorrhizal

[a] Significant at 5% level in comparison to block wise; [b] Significant at 0.1% level in comparison to block wise

formation of more vesicles as compared to high fertilizer level.

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Economical benefits of applying mycorrhizal inoculum in the commercial cultivation of banana in the fields of Kerala

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Introduction

India is largely an agrarian country, but agricultural production is directly affected by factors like rapidly degrading land, water stress, and climatic variations, all of which are prevalent in the subcontinent. In agricultural and horticultural crop production, application of large amounts of fertilizers and pesticides is accepted and is a normal practice (Smith and Read 1997). This has helped improve the production, but there is growing concern over the adverse effects of the use of chemicals on soil productivity and the state of the environment (Premsekhar and Rajashree 2009). Prolonged use of chemicals has degraded the soil structure and further use of fertilizers in agriculture would only make it a non-profitable venture.

The *State of the Environment Report, 2009* (MoEF 2009) has identified the intensive need to link agricultural practices to the environment by developing sustainable solutions for food security as 146.82 million hectares (Mha) out of the total geographical area of 306 Mha has been identified as degraded land.

Mycorrhiza, the only known fungal system categorized as a biofertilizer, helps plant roots tap soil nutrients that are otherwise beyond their reach. For plants, this means better uptake of nutrients, offering stress tolerance, enriching soil, increasing plant health, and decreasing dependence on chemical fertilizers. As the soil flora and fauna are also adversely affected by the overuse of chemical inputs, it becomes important that the population of mycorrhizae in the rhizosphere be artificially increased by its direct application into the fields. This may be done by several methods, including seed coating, inoculation of seedlings by the root dip method, band application with powder form of the inoculum, and placing the inoculum in the planting pits or trenches with close contact to the propagation material.

Mycorrhiza's resource saving potential

Arbuscular mycorrhizal fungi (AMF) is associated with increased uptake of phosphorus for enhancing drought tolerance of the host plant (Bethlen Falvay, Brown, Ames, *et al.* 1998). In addition to phosphorus nutrition, mycorrhiza symbiosis assists host plants to use nitrogen forms that are unavailable to non-mycorrhizal plants, thereby contributing to plant growth and nutrition (Subramanian, Santhanakrishnan, and Balasubramanian 2006). Crop yield increase (Khaliq and Sanders 2000) and increased growth responses with reduced fertilizer levels (Carpio, Davies, and Arnold 2005) have been reported in plants inoculated with mycorrhizae. It has also been reported that mycorrhizal plants may have tolerance to the disease (Smith and Read 1997) due to the resistance imparted by increased nutrient uptake. Inferences from these reports allow us to consider that mycorrhizae can bring in economic benefits in commercial crop production by decreasing the resources required, namely, chemical fertilizers and plant protection chemicals.

Selection of site and cultivation

The field trials were set up in the Thrissur district of Kerala. The soils in Kerala are acidic and hence result in phosphorus fixation, making it nutrient deficient for plants. The trials were organized in three plots, with 250 plants per plot. The crop selected for the study was banana, as it is a high value crop of the state that requires heavy doses of fertilizers and plant protection chemicals for good yields.

For planting, pits of 25–50 cm^3 were taken depending on the size of the rhizomes or suckers (planting material), which are five months old. TERI commercial product mycorrhiza (200 propagules/g) was applied at the rate of 10 g per pit. Rhizomes (with short roots emerging from it) were placed in the pit and earthed up. The fertilizer application dose consisted of 415 g urea, 575 g Raj Phos, and 600 g

muriate of potash (MOP) per plant in five split doses. In the field trials, it was decided that 125 plants per field were to be treated with mycorrhizae products, following 75% recommended fertilizer levels for nitrogen and phosphorus fertilizers, while keeping the levels of MOP unchanged. Another 125 plants were kept as control (without mycorrhizal inoculum and following 100% of the recommended fertilizer dose). Dates of flowering, fruiting, and harvest were recorded in order to see signs of earliness. Apart from the growth, response and yield attributes were also recorded.

Results

Earliness

The crop flowered a week earlier than the non-inoculated plants. Other subsequent stages of development were also ahead by seven days. This facilitated early harvest, thus giving farmers the advantage of an early crop.

Increase in yield

An increase of 8% in yield on an average was observed. The number of fruits per hand and number of hands per bunch showed an increase of 16% and 5%, respectively, as compared to that of the non-inoculated plants.

Reduction in applied fertilizer levels

The plants showed good growth compared to non-inoculated plants, with 75% of the recommended fertilizer of nitrogen and phosphorus.

Reduced incidence of rhizome rot

The plots were affected with Sigatoka leaf spot disease. The inoculated and non-inoculated plants were affected alike. However, the incidence of rhizome rot was seen only in the non-inoculated plants.

Perceived economical benefits

With the local market rates of urea, Raj Phos, and MOP at ₹5/kg, ₹6/kg, and ₹5.5/kg, respectively, the per plant cost of the fertilizer came to ₹9 in the case of full dose fertilizer application. With 75% of the recommended fertilizer and the additional cost incurred per plant for the inoculum, the new cost per plant is ₹7. The cost reduced by 22% accompanied by an 8% increase in the yield. Moreover, the earliness in the market will also help the farmers get early revenues.

Environmental care

The reduced application of chemicals would prevent land from degradation and could be put to use for cultivation. Gradually, chemicals could be completely given up, thus turning it into organic agriculture.

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Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals:

- *Agriculture, Ecosystems, and Environment*
- *Applied Soil Ecology*
- *Forest Ecology and Management*
- *Fungal Biology*
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- *Journal of Hazardous Materials*
- *Mycorrhiza*
- *Plant Physiology and Biochemistry*
- *Saudi Journal of Biological Sciences*
- *Science of The Total Environment*
- *Soil Biology and Biochemistry*

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the author for correspondence, marked with an asterisk)
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