



MYCORRHIZA NEWS

The Quarterly Newsletter of Mycorrhiza network

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Mycorrhiza News

The Mycorrhiza News provides a forum for dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers compiled from its RIZA database; 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.



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RESEARCH FINDING PAPERS

Arbuscular anti Status of the Plants Growing in the Alkaline/anti - tumor Soils of Handia, Allahabad, Uttar Pradesh

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Introduction

One of the important categories of wasteland is salt affected wasteland covering vast areas of the world, presenting a serious impediment to crop production. Plant growth and development in such soils is adversely affected either due to presence of excessive amounts of neutral soluble salts or high exchangeable sodium or both. In India approximately 7 mha land is salt affected out of which 2.5 mha lies in Indo-Gangetic plains covering the states of Uttar Pradesh, Haryana, Punjab, Delhi, and parts of Bihar. In Uttar Pradesh alone about 1.29 mha is salt affected and commonly known as 'usar' or 'reh' in local language.

In this respect, application of biological inputs in combination with some moderately salt tolerant plant species seem to be a better option for the reclamation and management of such soils. In the past few decades, it has been well established that the Arbuscular Mycorrhiza (AM) fungi enhance the ability of plants to cope with environmental stresses generally prevalent in the degraded ecosystems. Several workers have reported the presence of AM association in salt stress environments.

The objective of the present work is to investigate the distribution of AMF in the rhizosphere of different wild plants growing in the alkaline/sodic soils of Handia, Uttar Pradesh so that the adoptive and effective AMF isolates may be used in further studies to give solutions for the reclamation and utilization of such soils.

Materials and Methods

Sample Collection

A systematic survey of the alkaline/sodic sites of Handia was undertaken to assess the population and diversity of AM fungi in such soils and to study the intensity of AM association in the roots of the plants growing in vicinity.

Samples were collected during summer (April–May), rainy (August–September) and winter (December – January) seasons. During each sampling, 200g soil was collected randomly from each zone at different depths, i.e., 0–15cm, 15–30cm, and 30cm below the surface. Root samples of the plants growing in different zones were also collected for the estimation of mycorrhizal infection.

Analysis of Soil Samples

Collected soil samples were air-dried, sieved and soil extract was prepared by the method proposed by Adams *et al.* (1980). Soil pH and electrical conductivity (EC) were measured with a digital pH meter and a digital EC meter, respectively. Total organic carbon was estimated by the method given by Nelson and Sommers (1982), phosphorus by Watanabe and Olsen (1965), nitrogen by Bremner (1982), and potassium by flame photometer method (Richards, 1954). Soil characteristics of different sites are presented in table 1 (see pg 6).

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Estimation of AM Association in the Roots

Intensity of AM association in the root samples was determined by the method given by Philips and Hayman (1970). Percent root bits colonized and the percent root length colonized were expressed as the mean of three replicates.

Determination of AM Spore Population

AM spore population was determined in 50g air-dried soil (in triplicates for each sample) by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Number of spores were expressed as the mean of three replicates.

Identification of AM Fungi

AM spores were mounted in PVLG (polyvinyl-lactoglycerol) and PVLG + Melzer's reagent (1:1 v/v). Species level was identified using the synoptic keys of Trappe (1982), Schenck and Perez (1990), and INVAM species guide.

Results and Discussion

The data of analysis of all the soil samples collected from different zones of the selected site, Handia is presented in Table 1. The elemental analysis of the soil samples indicate that the soil of the site is highly alkaline. The pH of the soils ranged from 8.3 (Zone IV) to 10.2 (Zone I). However, electrical conductivity (EC) was not more than 4.0 m.mhos.cm⁻¹. EC ranged from 0.42 m.mhos.cm⁻¹ (Zone IV) to 4.06 m.mhos.cm⁻¹ (Zone I). The nutrient status of the soil was also very poor. The organic carbon and nitrogen contents were low in all the samples. Organic carbon ranged from 0.20 per cent (Zone I) to 0.48 per cent (Zone IV) and nitrogen content ranged from 2.25 per cent (Zone I) to 2.88 per cent (Zone IV).

The soils were also deficient in phosphorus and potassium content. Available phosphorus, estimated in terms of P₂O₅, ranged from 18 kg/ha (Zone I) to 40 kg/ha (Zone IV). Potassium content, estimated in terms of K₂O ranged from 125 kg/ha (Zone I) to 127 kg/ha (Zone IV).

Vegetation Composition in the Different Zones of the Selected Site

The data of vegetation composition in the different zones of the selected site, Handia, is presented in table 2 (see pg 6). A total of 19 plant species belonging to 19 genera and 10 families were identified from different zones. Least vegetation was recorded in Zone I, which increased sequentially from Zone I to Zone III. Grasses such as *Cyperus rotundus* L., *Desmostachya* sp., and *Sporobolus diander* (Retz.) P. Beauv. were the most dominant vegetation in these zones. As Zone IV was a cultivation zone, so was mainly dominated by the cultivated crops.

The largest family was Poaceae accounting for 31.7 per cent of total flora recorded. *Cyperus rotundus* L., *Desmostachya* sp., and *Sporobolus diander* (Retz.) P. Beauv. formed the pioneer vegetation in Zone I. Zone II and Zone III were dominated by the members of the family Amaranthaceae, Chenopodiaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Mimosaceae, and Poaceae. *Brassica campestris* L., *Cajanus cajan* (L.) Huth., *Oryza sativa* L. and *Triticum aestivum* L. were the most dominant crops cultivated in Zone IV.

AM association in the Roots of the Plants Growing in Different Zones of the Selected Site

Data of the AM intensity in the roots of the plants growing in different zones of the three selected sites in different seasons is presented in table 3 (see Pg 7). All the plants showed mycorrhizal association in their roots, except the members of Brassicaceae and Cyperaceae. However, the intensity of the mycorrhizal association varied with the plant species, magnitude of the stress, and with the seasonal variations. *Chenopodium album* L., member of the family Chenopodiaceae—which is otherwise reported as non-mycorrhizal—showed mycorrhizal association under stress conditions, although the intensity of the mycorrhizal infection was quite low.

The various characteristics of AM fungi such as, hyphae, vesicles, arbuscules, and intramatrical spores were observed in the roots of the plants. Maximum mycorrhizal infection was recorded in *Croton bonplandianum* Baill. and *Oryza sativa* L. while, minimum was found in *Chenopodium album* L.

In general, the intensity of AM infection showed a negative correlation with the magnitude of stress as it increased from Zone I to Zone IV. Mycorrhizal intensity ranged from 10–12 per cent in Zone I, 10–44 per cent in Zone II, 8–70 per cent in Zone III, and 13–70 per cent in Zone IV.

Maximum AM infection was recorded during winter season. However, no significant pattern of mycorrhization was traced in relation to seasonal variation. During winter season, mycorrhizal intensity ranged from 10–70 per cent, during rainy season, it ranged from 8–70 per cent, and during summer season, the intensity ranged from 10–56 per cent.

Spore Population and Diversity of AM Fungi in Different Zones of the selected Site

Data of spore population and diversity of AM fungi in different zones of the three selected sites in different seasons is presented in Table 4 (see Pg 7). Although the presence of AM fungi was recorded in all the zones at all the sites, their population and diversity varied with the magnitude of stress and with the seasonal variations.

AM spore population showed a negative correlation with the magnitude of stress as it increased from Zone I to Zone IV. Average AM spore population was 11, 18.7, 27.7, and 54 spores/50g in air-dried soil in Zone I, Zone II, Zone III, and Zone IV respectively.

A significant variation was recorded in the AM spore population in relation to the seasonal changes. Maximum spore population was recorded in the winter season and minimum in summer. During winter season the spore population ranged from 15–86 spores/50g air-dried soil, during rainy season, from 12–47 spores/50g air-dried soil, and during summer season it ranged from 6–29 spores/50g air-dried soil.

A total of 17 species belonging to two genera of AM fungi (*Glomus* and *Acaulospora*) were isolated from the different zones of the selected alkaline/sodic sites. *Glomus* was recorded as the most dominant genus with 13 species, viz., *G. caledonium*, *G. canadens*, *G. claroideum*, *G. constrictum*, *G. fasciculatum*, *G. invermaium*, *G. mosseae*, *G. occultum*, and five unidentified species named *Glomus* sp. UVK1, UVK2, UVK3, UVK4, and UVK6. It is followed by *Acaulospora* with four species, viz., *A. bireticulata*, *A. denticulata*, *A. longula*, and an unidentified species named *Acaulospora* sp. UVK1. Photographs of the identified AM fungal species are presented in Plate 1 (see pg 8).

AM fungal diversity showed a negative correlation with the magnitude of stress, as it increased from Zone I to Zone IV, however, no significant seasonal variations were recorded in diversity.

AM fungi can promote plant establishment by increasing resistance to environmental stresses, enhancing plant nutrient acquisition, and improving soil quality (Schreiner *et al.*, 1997; Jeffries and Barea, 2001).

AMF are reported to reduce the detrimental effects of soil-associated plant stresses, such as lack of nutrients, organic matter, high salinity, or high pH (Sylia and Williams, 1992; Entry *et al.* 2002). Most of the studies show that, native AM fungi grow and function better especially in stressed conditions and degraded soils as they are quite adapted to their stressed microhabitat (Caravaca *et al.*, 2003; Calvente *et al.*, 2004).

Several researchers have investigated the distribution of AM fungi in different ecological regions and their relation to soil (Aliasgharzadeh *et al.*, 2001; Wang *et al.*, 2004; Shi *et al.*, 2007). These species and isolates of AM fungi differ in their tolerance to adverse physical and chemical conditions of soil (Aliasgharzadeh *et al.*, 2001).

All the plants, except Brassicaceae and Cyperaceae, surveyed in alkaline/sodic soils of the selected site were colonized by the AM fungi but the

colonization percentages were significantly low. The results were in accordance to the earlier reports of Brown and Bledsoe (1996), Udaiyan *et al.* (1996), and Wang *et al.* (2004). Mycorrhizal intensity showed a significant correlation with the plant species and magnitude of stress. Lowest mycorrhizal intensity at Zone 1 of all the three sites may be attributed to the fact that high soil pH, low nutrients, poor plant diversity, and vegetation cover severely restrict the colonization of AM fungi (Aliasgharzadeh *et al.*, 2001; Wang *et al.*, 2004). However, AM fungi have the ability to form a zone of altered pH in the adjacent soil (Li *et al.*, 1971). Chenopod plants, which are otherwise regarded as non-mycorrhizal, are reported to be mycorrhizal under alkaline stress in the present study. Mycorrhization in chenopods under different kind of stresses, such as drought, salinity, etc., was earlier reported by Hirrel *et al.* (1978). These results may also support the conclusion of Carvalho *et al.* (2001) that the colonization by Vesicular Arbuscular Mycorrhizae (VAM) under stress conditions depend on more on host plant species than environmental stresses.

It is also reported that the AM spore population and root colonization pattern changes with seasonal variation and host plant phenology (He *et al.*, 2002; Bohrer *et al.*, 2004). Patterns of seasonal change in mycorrhizal intensity were more obvious in the annuals than in perennials. Although no significant correlation could be established in the present study between the mycorrhizal intensity and seasonal variation, however, in general mycorrhizal intensity was always higher during winter season. The highest degree of colonization of annuals in winter, i.e., during their flowering period, may have a possible explanation of increasing requirements of nutrients and water at this stage of growth (Shi *et al.*, 2007).

Conclusion

Glomus spp. were the most dominant species in the alkaline/sodic soil of Indo-Gangatic plains of India and were distributed widely in the rhizosphere of most of the plant species. *G. mosseae* and *G. fasciculatum* were the most prevalent *Glomus* spp. in this kind of soil. There is a possibility that these species may improve tolerance to salt stress and may prove useful isolates for improving the overall performance of the crops under such conditions.

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Table 1: Edaphic features of the alkaline/sodic soils of different zones collected from the selected site of Handia, Uttar Pradesh

Sites/Zones	pH	EC (m.mhos.cm ⁻¹)	Organic carbon (%)	Nitrogen (%)	Phosphorus (kg/ha)	Potassium (kg/ha)
Zone I	10.2	4.06	0.20	2.25	18.0	125.0
Zone II	9.4	3.12	0.36	2.39	32.0	125.0
Zone III	8.8	1.00	0.50	2.45	34.0	130.0
Zone IV	8.3	0.42	0.48	2.88	40.0	127.0

Table 2: Vegetation composition of different zones of the alkaline/sodic soils at the selected site of Handia, Uttar Pradesh

Family	Plant/Zones	Vegetation Composition			
		Handia			
		I	II	III	IV
Amaranthaceae	<i>Amaranthus viridis</i> L	-	+	+	-
Asclepiadaceae	<i>Calotropis procera</i> (Aiton) R.Br	-	-	+	-
Brassicaceae	<i>Brassica campestris</i> L	-	-	-	+
Chenopodiaceae	<i>Chenopodium album</i> L	-	+	+	+
Convolvulaceae	<i>Evolvulus</i> sp.	-	-	+	-
Cyperaceae	<i>Cyperus rotundus</i> L.	+	+	+	+
Euphorbiaceae	<i>Croton bonplandianum</i> Baill.	-	+	+	+
Euphorbiaceae	<i>Euphorbia hirta</i> L	-	+	+	+
Fabaceae	<i>Cajanus cajan</i> (L.) Huth.	-	-	-	+
Fabaceae	<i>Indigofera</i> sp.	-	+	+	-
Fabaceae	<i>Sesbania sesban</i> (L.) Merr	-	-	+	-
Fabaceae	<i>Vigna radiata</i> (L.) Wilczek	-	-	-	+
Mimosaceae	<i>Acacia nilotica</i> (L.) Willd.	-	+	+	-
Poaceae	<i>Cynodon dactylon</i> (L.) Pres.	-	+	+	+
Poaceae	<i>Desmostachya</i> sp.	+	+	+	+
Poaceae	<i>Oryza sativa</i> L.	-	-	-	+
Poaceae	<i>Saccharum munja</i> Roxb.	-	+	+	-
Poaceae	<i>Sporobolus diander</i> (Retz.) P. Beauv.	+	+	+	-
Poaceae	<i>Triticum aestivum</i> L.	-	-	-	+

Table 3: Seasonal variation in the mycorrhizal intensity in the roots of plants growing in different zones of alkaline/sodic soils of Handia site (April - January)

Plants/Zones	MYCORRHIZATION (% ROOT BITS INFECTED)											
	Winter (Dec-Jan)				Rainy (Aug-Sep)				Summer (Apr-May)			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
<i>Acacia nilotica</i> (L.) Willd.	-	40	50	-	-	34	62	-	-	-	34	-
<i>Amaranthus viridis</i> L.	-	-	-	-	-	12	41	-	-	-	28	-
<i>Brassica campestris</i> L.	-	-	-	0	-	-	-	-	-	-	-	-
<i>Cajanus cajan</i> (L.) Huth.	-	-	-	58	-	-	-	46	-	-	-	-
<i>Calotropis procera</i> (Aiton) R. Br.	-	-	28	-	-	-	32	-	-	-	-	-
<i>Chenopodium album</i> L.	-	-	20	-	-	0	8	0	-	-	-	-
<i>Croton bonplandianum</i> Baill.	-	30	70	50	-	30	56	-	-	-	32	-
<i>Cynodon dactylon</i> (L.) Pres.	-	31	33	-	-	11	10	13	-	-	0	0
<i>Cyperus rotundus</i> L.	0	0	0	0	0	0	0	0	-	0	0	0
<i>Desmostachya</i> sp.	10	22	28	20	12	18	15	18	-	10	10	-
<i>Euphorbia hirta</i> L.	-	-	30	35	-	43	36	-	-	-	25	-
<i>Evolvulus</i> sp.	-	-	-	-	-	-	48	-	-	-	-	-
<i>Indigofera</i> sp.	-	-	-	-	-	33	29	-	-	-	-	-
<i>Oryza sativa</i> L.	-	-	-	-	-	-	-	70	-	-	-	-
<i>Saccharum munja</i> Roxb.	-	-	60	-	-	44	50	-	-	-	34	-
<i>Sesbania sesban</i> (L.) Merr.	-	-	30	-	-	-	24	-	-	-	-	-
<i>Sporobolus diander</i> (Retz.) P. Beauv.	-	-	-	-	12	26	-	-	-	10	15	-
<i>Triticum aestivum</i> L.	-	-	-	57	-	-	-	-	-	-	-	-
<i>Vigna radiata</i> (L.) Wilczek	-	-	-	-	-	-	-	-	-	-	-	56

Table 4: Seasonal variation in the population size and diversity of AM fungi in different zones of alkaline/sodic soils of Handia site (April - January)

AM Fungi/Zones	AM POPULATION SIZE/DIVERSITY (/50G SOIL)											
	Winter (Dec-Jan)				Rainy (Aug-Sep)				Summer (Apr-May)			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
<i>Acaulospora bireticulata</i> Rothwell & Trappe	-	3	3	2	-	1	1	1	-	-	-	1
<i>A. denticulata</i> Sieverding & Toro	-	-	1	-	-	-	1	-	-	-	-	-
<i>A. longula</i> Spain & Schenck	2	-	3	12	2	1	-	10	1	1	-	7
<i>Acaulospora</i> sp. UVK1	-	1	1	2	-	-	-	-	-	-	1	1
<i>G. caledonium</i> (Nicol. & Gerd.) Trappe & Gerdemann	-	-	1	2	1	-	-	-	-	-	-	-
<i>G. canadens</i> (Thaxter) Trappe & Gerdemann	-	-	6	-	-	-	2	-	1	1	-	1
<i>G. claroideum</i> Schenck & Smith	3	3	6	8	2	2	2	5	-	-	3	2
<i>G. constrictum</i> Trappe	1	3	5	9	1	-	1	4	2	-	-	2
<i>G. fasciculatum</i> (Thaxter) Gerdemann & Trappe	1	3	-	3	1	-	1	-	-	-	2	-

AM Fungi/Zones	AM POPULATION SIZE/DIVERSITY (/50G SOIL)											
	Winter (Dec-Jan)				Rainy (Aug-Sep)				Summer (Apr-May)			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
<i>G. invermaium</i> Hall	-	-	1	-	-	3	1	2	-	-	1	1
<i>G. mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe	4	3	6	12	3	4	3	11	-	2	5	5
<i>G. occultum</i> Walker (<i>Paraglomus occultum</i>)	-	-	-	3	-	1	-	1	-	1	-	-
<i>Glomus</i> sp. UVK1	2	9	2	20	-	2	4	7	-	2	3	6
<i>Glomus</i> sp. UVK2	2	5	-	5	1	2	1	3	-	-	1	1
<i>Glomus</i> sp. UVK3	-	-	-	4	1	1	1	1	-	-	2	1
<i>Glomus</i> sp. UVK4	-	-	8	-	-	-	2	2	1	1	2	-
<i>Glomus</i> sp. UVK6	-	-	-	4	-	-	-	-	1	1	-	1
	15	30	43	86	12	17	20	47	6	9	20	29

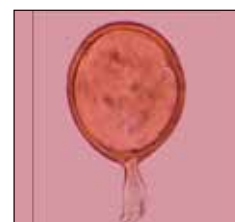
Plate 1: Dominant AM spores isolated from different zones of alkaline/sodic soils of Handia site.



Glomus mosseae



Glomus constrictum



Glomus invermaium



Glomus caledonium



Glomus canadens



Glomus claroideum



Glomus fasciculatum



Glomus occultum



Acaulospora bireticulata



Acaulospora denticulata



Acaulospora longula

Taxonomic Series: Acaulospora, Entrophospora, and Scutellospora from Odisha

Kanchan Mala Bihari and Nibha Gupta*

1. *Acaulosporalaevis*, Gardemann and Trappe. sp.

Distinguishing feature: Red brown coloured spores form sequentially on a hyaline coloured hyphae; sloughing away of the outer surface; single spiny structure emerges from the upper surface of the spore.

Variable feature: Variable size range; presence of spine.

Distribution: Recorded in Mangrove plants of Quanzhou Bay, China (in saline soil), Thar Desert, India (in semi-arid region), and Namibia. They are also recorded in association with fodder crop in Tamil Nadu.



2. *Acaulospora scrobiculata*, Trappe sp. nov.

Distinguishing feature: Presence of pyriform sporiferous saccule with pit in its lumen; evenly pitted spore surface; yellow brown coloured spores; equivalent spore size ranges.

Variable feature: Constriction within the subtending hypha.

Distribution: Recorded in the Coastal saline soil, in the semi-arid soil of West Coast of Kerala, in Western Ghats, southern India, Mangrove ecosystem, South China, agricultural soils, Sichuan province of mainland China, and also in the sand dune island of Kauai, Hawaiian.



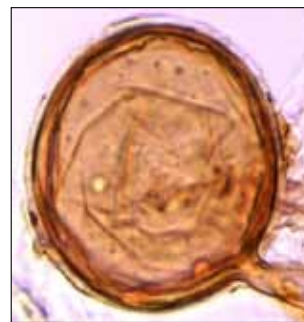
3. *Acaulospora longula*, Spain and Schenck sp. nov

Distinguishing feature: Sporocarp unknown; light yellow coloured spore; approximately same spore size range; one cicatrix present in some spore; presence of sporiferous saccule.

Variable feature: Spore singly formed; cicatrix not found.

Distribution:

Recorded in semi- natural grassland with different vegetation in Japan, in plants and rhizosphere soil of tropical plain in Tamil Nadu, India, and in forest grassland and cropland ecosystem of Tibetan Plateau, China.

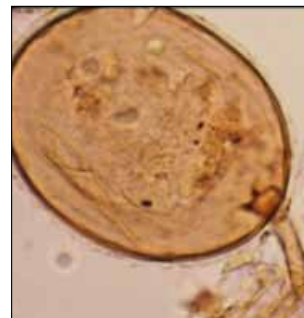


4. *Acaulospora denticulata*, Sieverding and Torn

Distinguishing feature: Sporocarp unknown; spore singly formed; yellow to dark brown coloured spore; 3–4 wall layered spores; spore contents hyaline globular; 1–2 circular projection; few spore have 1–2 hyphal attachments

Variable feature: Growing of germ tube; spore having pore.

Distribution: Recorded in coastal saline soil, west coast of Kerala, coastal sand dune, in west coast of India, in Jhum fallow and Natural forest soil of Arunachal Pradesh, India, and in hot and arid ecosystem (Semi-savannah vegetation) of Southwest China.



5. *Acaulospora foveata*, Trappe and Janos

Distinguishing feature: The spores have a pit like structure inside the lumen near the spore wall; yellow brown coloured spores formed singly; pits with round to oblong depressions; pitted spore surface.

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Variable feature:

Variable size ranges

Distribution:

Recorded in Mangrove of Sundarban in Eastern India, in Hainan Island of China, Plants and rhizosphere soil of tropical plain in Tamil Nadu, India, and in association with pteridophytes in Western Ghats, Goa.



6. Entrophospora colombiana, Spain and Schenck

Distinguishing feature:

Nearly equal spore size range; granulated spore contents; germination shield like structure present in each spore at the upper surface; new spore growing from the inside of mature spore at the attachment site.



Variable feature: Germination shield like structure.

Distribution: Recorded in fly ash pond and mine site of Neyveli lignite corporation in Tamil Nadu, India, in association with physic nut in Thailand, and also recorded in different soil management system in Rolim de Moura, North region of Brazil.

7. Entrophospora infrequens, (Hall) Ames and Schneider

Distinguishing feature:

Same spore shape; 2–4 small to large hyphal scars; dark brown coloured shield like structure present at the upper surface of the spore.



Variable feature:

Vacuolated spine absent; variable spore sizes.

Distribution: Recorded in Coastal saline soil, west coast of Kerala, in tea gardens of Chittagong, Bangladesh, and in soil samples from different locations of Finland.

8. Gigaspora gigantea, Endogone gigantea, Nicol and Gerd

Distinguishing feature: Sporogenous cell present in the large spore.

Distribution: Recorded in coastal sand dune, west coast of India, in fly ash pond and mine site in Tamil Nadu, India, and also recorded in different soil in Ahmednagar, India.

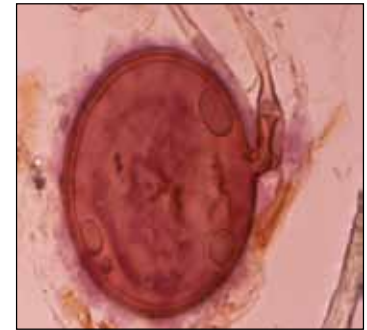


9. Scutellospora minuta, (Ferrer and Herrera) Walker and Sanders

Distinguishing feature:

Spore contents reticulate; presence of 1.9 µm pore; germination shield like structure from which the germ tube originates.

Variable feature: Germination shield from which germ tube arises.



Distribution: Recorded in rhizosphere and non-rhizosphere soil growing tomato in Nasik, Maharashtra.

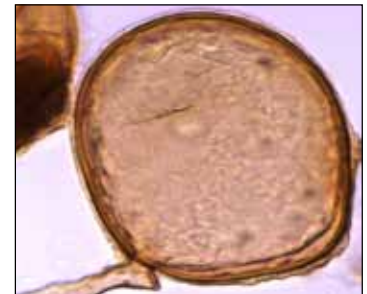
10. Scutellospora pellucida, Gigaspora pellucida, Nicolson and Schenck sp. nov.

Distinguishing feature: Germ tube extending outwards through germination shield like structure; elongated spores with brownish coloured base; some spores are attached to each other.

Variable feature: Variable spore size range.

Distribution:

Recorded in Mangrove of Sundarban, Eastern India, in stressed soil of Bailadila iron ore sites in Bastar region, M P, in Natural and cultivated vegetation of Argentina, and in



association with angiosperms present in the Western Ghats of Goa.

11. *Scutellospora erythropha*, *Gigaspora erythropha* Koske and Walker sp. nov.

Distinguishing

feature: Spore formed singly or in clusters; 4–5 wall layered spore; two small ring structures are observed within the germination shield plate; new spore formed from the spore mother cell at the neck of hyphal attachment; bulbous projection in interior spore surface.



Variable feature: Variable spore size ranges.

Distribution: Recorded in coastal sand dune in west coast of India.

12. *Scutellospora verrucosa*, (Koske and Walker) Walker and Sanders

Distinguishing feature: Pale yellow coloured spore; rounded warty structure form at the base; germ tube arises from a germination shield like structure; 2–3 cicatrix with germination shield structure; germination orb can be seen; recurved septation at subtending hypha.



Variable feature: Variable spore size.

Distribution: Recorded in coastal sand dune vegetation of Goa, in aquatic and marshy plant species in Goa, India, and also recorded in association with sedge, Tibetan Plateau.

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Growth in *Vigna mungo* as influenced by *Glomus fasciculatum*, *Pseudomonas fluorescens* Os25, and *Trichoderma viride*

Sujatha N and Ammani K*

Results and Discussion

Total Nitrogen Content of *Vigna Mungo*

The total nitrogen content of *V. mungo* increased significantly with treatments of the biocontrols and mycorrhiza. The ANOVA results are documented in Table 4.

While in the uninoculated control of *V. mungo* the nitrogen content was 61.07 ± 0.11 $\mu\text{g/g}$ dry weight, with *P. fluorescens* Os25, *G. fasciculatum*, and *T. viride* inoculated plants the nitrogen content was 155.99 ± 0.76 $\mu\text{g/g}$ dry weight, 120.87 ± 0.52 $\mu\text{g/g}$ dry weight, and 118.85 ± 0.75 $\mu\text{g/g}$ dry weight, respectively.

The efficiency of *P. fluorescens* Os25 individually and in the presence of *G. fasciculatum* and *T. viride* to improve the total nitrogen content was studied. When

compared to the nitrogen content of plants treated only with *P. fluorescens* Os25 (155.99 ± 0.76 $\mu\text{g/g}$ dry weight), the nitrogen content in dual inoculation of *P. fluorescens* Os25 + *G. fasciculatum* treated plants (181.55 ± 0.25 $\mu\text{g/g}$ dry weight) was found to be increased by 16.38 per cent, and with dual inoculation of *P. fluorescens* Os25 + *T. viride* the nitrogen content (177.56 ± 0.39 $\mu\text{g/g}$ dry weight) showed an increase by 13.82 per cent. The nitrogen content in *V. mungo* plants (172.70 ± 0.32 $\mu\text{g/g}$ dry weight) treated with dual inoculation of *G. fasciculatum* and *T. viride* showed an approximated increase of 42.88 per cent, compared to those treated only with *G. fasciculatum* (120.87 ± 0.52 $\mu\text{g/g}$ dry weight).. These results suggest the efficiency of *P. fluorescens* Os25, *G. fasciculatum*, and *T. viride* in increasing the nitrogen content of the plant.

Table 4. Total nitrogen content of *Vigna mungo* at 35 DAI

Treatment	Root N ($\mu\text{g/g}$ dry weight)	Shoot N ($\mu\text{g/g}$ dry weight)	Total ($\mu\text{g/g}$ dry weight)
Uninoculated seeds	15.03 \pm 0.06 ^a	46.04 \pm 0.05 ^d	61.07 \pm 0.11 ^c
<i>P. fluorescens</i> Os25	48.72 \pm 0.52 ^{ad}	107.27 \pm 0.34 ^d	155.99 \pm 0.76 ^c
<i>G. fasciculatum</i>	40.74 \pm 0.54 ^{edfg}	80.13 \pm 0.11 ^d	120.87 \pm 0.52 ^c
<i>T. viride</i>	39.36 \pm 0.34 ^{ac}	79.49 \pm 0.41 ^d	118.85 \pm 0.75 ^c
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i>	51.30 \pm 0.26 ^{bc}	130.15 \pm 0.01 ^d	181.55 \pm 0.26 ^c
<i>P. fluorescens</i> Os25 + <i>T. viride</i>	50.11 \pm 0.09 ^b	127.45 \pm 0.49 ^{ded}	177.56 \pm 0.39 ^c
<i>T. viride</i> + <i>G. fasciculatum</i>	47.09 \pm 0.06 ^b	125.61 \pm 0.38 ^{df}	172.70 \pm 0.32 ^{cad}
<i>F. oxysporum</i>	7.11 \pm 0.09 ^b	15.52 \pm 0.42 ^{gf}	22.83 \pm 0.53 ^{ad}
<i>R. bataticola</i>	9.16 \pm 0.01 ^a	16.35 \pm 0.30 ^{abc}	25.51 \pm 0.32 ^{dc}
<i>P. fluorescens</i> Os25 + <i>F. oxysporum</i>	10.76 \pm 1.19 ^a	29.01 \pm 0.16 ^{ac}	39.78 \pm 1.34 ^{cd}
<i>P. fluorescens</i> Os25 + <i>R. bataticola</i>	13.06 \pm 1.01 ^a	30.5 \pm 0.30 ^{acc}	43.56 \pm 0.72 ^{gf}
<i>G. fasciculatum</i> + <i>F. oxysporum</i>	9.49 \pm 0.29 ^a	21.75 \pm 0.10 ^{cb}	31.24 \pm 0.36 ^{fr}
<i>G. fasciculatum</i> + <i>R. bataticola</i>	9.82 \pm 0.03 ^a	23.73 \pm 0.02 ^b	33.54 \pm 0.05 ^{gf}
<i>T. viride</i> + <i>F. oxysporum</i>	9.79 \pm 0.06 ^a	21.72 \pm 0.01 ^c	31.51 \pm 0.08 ^{gh}
<i>T. viride</i> + <i>R. bataticola</i>	9.84 \pm 0.13 ^a	21.14 \pm 0.25 ^{de}	30.59 \pm 0.29 ^{gh}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i> + <i>F. oxysporum</i>	25.14 \pm 0.02 ^a	60.12 \pm 0.01 ^{de}	85.26 \pm 0.01 ^{ghr}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i> + <i>R. bataticola</i>	26.82 \pm 0.55 ^a	61.09 \pm 0.07 ^{fgh}	87.91 \pm 0.52 ^{fr}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i>	59.07 \pm 0.08 ^a	149.48 \pm 0.47 ^{fgh}	208.55 \pm 0.43 ^{fg}
LSD	1.56	0.34	0.45

\pm Standard deviation

$p \leq 0.05$

Values suffixed with different letters on the same column indicate significant differences

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Saudibet *et al.* (2002) reported that the nitrate uptake by the roots of spring wheat is stimulated by *Azospirillum brasilense* strain. Based on the observations he concluded that the shoot:root ratio and the plant nitrate and nitrogen contents were higher in the inoculated plants. Sophie and Bruno (2004) proposed that PGPR could induce an increase in nitrate uptake rate, leading to stimulated shoot growth rate driven by the improved nitrogen nutrition.

It is believed that the AM symbiosis relieves plant from phosphorus stress which in turn benefits the N₂-fixing nitrogenase system of the other symbiont, resulting in enhanced fixation levels, and improved nitrogen status of the plant. This promotes plant growth and functioning, thus benefiting mycorrhizal development (Fraga-Beddiar and Le Tacon, 1990; Bethlenfalvai, 1992).

The nitrogen content was significantly lower in plants grown in phytopathogen amended soils as evidenced in table 4. The highest total nitrogen content was recorded when the plants in phytopathogen amended soils were treated with *P. fluorescens* Os25 or *G. fasciculatum*, or *T. viride*. In the presence of phytopathogens, the maximum nitrogen content was observed in plants inoculated with *P. fluorescens* Os25. These inoculated plants were grown in *R. bataticola* amended soils and found to have 70.75 per cent higher total nitrogen content (43.56 ± 0.72 µg/g dry weight) compared to the untreated plants grown in *R. bataticola* amended soils. Similarly, in plants treated with *G. fasciculatum* and *T. viride* and grown in *R. bataticola* amended soils, the total nitrogen content was found to increase by 31.47 per cent, and 19.91 per cent respectively, when compared to the untreated plants grown in *R. bataticola* amended soils.

These results clearly suggest the efficiency of *Glomus fasciculatum* and *Trichoderma viride* besides *P. fluorescens* Os25 in the biocontrol of phytopathogens such as *Fusarium* and *Rhizoctonia*, thereby increasing the plant nitrogen content. Filion *et al.* (1999) suggested that the AM mycelium alter the microbial environment as non-favourable, hence detrimental to pathogens. In addition, *T. viride* controls phytopathogens by competing for both rhizosphere colonization and nutrients.

Total Phosphorus Content of *Vigna mungo*

In the uninoculated control of *Vigna mungo*, the phosphorus content was 400.03 ± 0.02 . The phosphorus content increased with treatments using biocontrols. The total phosphorus content was highest in plants inoculated with *P. fluorescens* Os25 (900.66 ± 0.21 µg/g dry weight). Plants which received *G. fasciculatum* and *T. viride* treatments recorded total phosphorus content of 810.77 ± 0.59 µg/g dry weight and 780.32 ± 0.07 µg/g dry weight, respectively. These results indicate the efficiency of *P. fluorescens* Os25, *G. fasciculatum* and *T. viride* in increasing the phosphorus content of the plants with *P. fluorescens* Os25 being the most efficient.

According to the present study, total phosphorous content in the dual inoculation of *P. fluorescens* Os25 + *G. fasciculatum* treated plants (997.16 ± 11.82 µg/g dry weight) was highest followed by *P. fluorescens* Os25 and *T. viride* co-inoculation (850.85 ± 0.02 µg/g dry weight). The co-inoculation of *T. viride* with *G. fasciculatum* recorded the lowest phosphorus content of 736.94 ± 32.17 µg/g dry weight.

These results clearly suggest that the co-inoculation of the plants with other biological agents enhanced the growth of the plants. This could be attributed to the synergistic effect of the biological agents.

The total phosphorus content in *V. mungo* was significantly lower in phytopathogen amended soils. In *F. oxysporum* and *R. bataticola* amended soil, the phosphorus content decreased by 62.59 per cent and 60.41 per cent, respectively when compared to the control.

Amendments with biocontrols and phytopathogens showed a marked difference in total phosphorus content of the plants when compared to individual biocontrol treatments or single phytopathogen treatments. The ANOVA results are documented in Table 5. In *R. bataticola* amended soils, the total phosphorus content of the plants treated with *P. fluorescens* Os25 showed an increase by 15.29 per cent when compared with the plants treated with *G. fasciculatum*, and by 12.95 per cent when compared with *T. viride* treated plants.

Table 5. Total phosphorus content of *Vigna mungo* at 35 DAI

Treatment	Root P (µ/g dry weight)	Shoot P (µ/g dry weight)	Total (µ/g dry weight)
Uninoculated seeds	100.13±0.03 ^a	300.00±0.00 ^b	400.03±0.02 ^a
<i>P. fluorescens</i> Os25	400.62±0.18 ^a	500.02±0.01 ^b	900.66±0.21 ^b
<i>G. fasciculatum</i>	350.83±0.57 ^a	460.14±0.03 ^b	810.77±0.59 ^b
<i>T. viride</i>	340.00±0.00 ^a	440.48±0.25 ^c	780.32±0.07 ^b
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i>	406.73±11.64 ^{ad}	590.43±0.2 ^{cb}	997.16±11.82 ^b

Treatment	Root P (μg dry weight)	Shoot P (μg dry weight)	Total (μg dry weight)
<i>P. fluorescens</i> Os25 + <i>T. viride</i>	360.12 \pm 0.02 ^{fg}	490.72 \pm 0.005 ^{bc}	850.85 \pm 0.02 ^b
<i>T. viride</i> + <i>G. fasciculatum</i>	300.14 \pm 0.02 ^{gh}	436.80 \pm 32.15 ^{bc}	736.94 \pm 32.17 ^b
<i>F. oxysporum</i>	50.55 \pm 0.33 ^a	99.14 \pm 0.02 ^b	149.69 \pm 0.35 ^b
<i>R. bataticola</i>	57.14 \pm 0.02 ^{gh}	101.25 \pm 0.08 ^a	158.39 \pm 0.09 ^b
<i>P. fluorescens</i> Os25 + <i>F. oxysporum</i>	70.15 \pm 0.02 ^{gh}	120.44 \pm 0.06 ^{dfg}	190.59 \pm 0.05 ^c
<i>P. fluorescens</i> Os25 + <i>R. bataticola</i>	79.34 \pm 0.11 ^{de}	130.29 \pm 0.05 ^{gh}	209.63 \pm 0.13 ^c
<i>G. fasciculatum</i> + <i>F. oxysporum</i>	60.41 \pm 0.30 ^{ed}	110.26 \pm 0.16 ^{gh}	170.67 \pm 0.45 ^c
<i>G. fasciculatum</i> + <i>R. bataticola</i>	65.26 \pm 0.03 ^{efgh}	116.56 \pm 0.29 ^{ef}	181.82 \pm 0.28 ^c
<i>T. viride</i> + <i>F. oxysporum</i>	63.23 \pm 0.02 ^a	107.66 \pm 0.14 ^{ef}	170.98 \pm 0.03 ^{dfg}
<i>T. viride</i> + <i>R. bataticola</i>	67.24 \pm 0.04 ^a	118.36 \pm 0.32 ^{fgh}	185.60 \pm 0.36 ^{efg}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i> + <i>F. oxysporum</i>	89.22 \pm 0.05 ^a	135.06 \pm 0.09 ^{fgh}	224.28 \pm 0.09 ^{efgh}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i> + <i>R. bataticola</i>	90.15 \pm 0.02 ^a	140.32 \pm 0.37 ^{adef}	230.47 \pm 0.39 ^{efgh}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i>	420.34 \pm 0.32 ^a	595.09 \pm 0.09 ^{adc}	1015.43 \pm 0.23 ^{gh}
LSD	2.34	1.98	3.45

\pm Standard deviation

$p \leq 0.05$

Values suffixed with different letters on the same column indicate significant differences

Similarly, Akkopru and Demir (2005) reported that the dual inoculation with *Glomus intraradices* and *P. fluorescens* strains reduced disease severity in the pathosystem of *Fusarium oxysporum* f. sp. *lycopersici* and tomato by 8.6–58.6 per cent. Indra and Subbiah (2003) found that the incidence of root rot in black gram caused by *Macrophomina phaseolina* was significantly reduced by 50 per cent when treated with *Trichoderma* spp.

The improvement in the nitrogen content of the plants treated with biocontrols in pathogen amended soils of *V. mungo* can be attributed to the chitinase production by the biocontrols. Similarly, Benhamou *et al.* (1996) reported that chitinase and glucanase activities significantly increased in the treated palms when the antagonists *Trichoderma* and *P. fluorescens* were applied either alone or in combination.

Mycorrhizal fungi are also known to enhance plant resistance to soil-borne pathogens. Several direct mechanisms are involved including production of antibiotic substances by the mycorrhizal fungi and the associated plant roots (Sylvia and Sinclair, 1983; Tsantrizos *et al.*, 1991), mechanical exclusion of the pathogens by the mantle of the ectomycorrhizae (Duchesne, 1994), and competition between mycorrhizal fungi and other biotrophic pathogens for the root cortex and its nutrient resources (Graham, 2001).

The most important result from the present research was the enhanced biocontrol performance

in triple inoculation of *P. fluorescens* Os25, *G. fasciculatum*, and *T. viride* in phytopathogen amended soils.

Percent Colonization of *G. fasciculatum* in *Vigna mungo* Roots

The colonization by *G. fasciculatum* in *V. mungo* roots is represented in Table 6. The percentage of hyphal colonization and vesicular colonization by *G. fasciculatum* in *V. mungo* plants was calculated. The efficiency of *Glomus fasciculatum* individually and in the presence of *P. fluorescens* Os25 in colonizing the plants was studied. In *V. mungo*, the percentage of hyphal and vesicular colonization by *G. fasciculatum* was 51.17 ± 1.75 and 31.16 ± 2.75 , respectively. In the plants treated with *P. fluorescens* Os25, the percentage of hyphal colonization (62.76 ± 2.75) of *G. fasciculatum* was increased by 22.64 per cent and vesicular colonization (42.71 ± 1.86) by 37.06 per cent when compared to the colonization by *G. fasciculatum* in biocontrol untreated plants. Maximum vesicular colonization of 65.16 ± 2.61 was observed in triple inoculation of the plants. It showed an increase by 51.34 per cent when compared to the *G. fasciculatum* colonization of biocontrol untreated plants.

There was a decrease in AM infection in phytopathogen amended soils. In the plants grown in *F. oxysporum* amended soils, the hyphal colonization by *G. fasciculatum* decreased by 43.33 per cent and in *R. bataticola* amended soils, the vesicular colonization

by *G. fasciculatum* decreased by 57.77 per cent when compared to *G. fasciculatum* colonization of the plants grown in phytopathogen non-amended soils.

In the plants grown in *F. oxysporum* amended soils and co-inoculated with *P. fluorescens* Os25 and *T. viride* the hyphal colonization by *G. fasciculatum* was 50.17 ± 1.61 , an increase by 73 per cent when compared to the colonization in biocontrol untreated plants grown in pathogen amended soils. In the

plants grown in *R. bataticola* amended soils and co-inoculated with *P. fluorescens* Os25 and *T. viride*, the vesicular colonization by *G. fasciculatum* was 27.31 ± 2.61 , while the colonization by *G. fasciculatum* in biocontrol untreated plants grown in the pathogen amended soils was 13.16 ± 3.16 . The biocontrol treatments improved the percentage of *Glomus* colonization in phytopathogen amended soils. The ANOVA results are documented in Table 6.

Table 6. Per cent colonization of *G. fasciculatum* in roots of *Vigna mungo*

Treatment	% Hyphal Infection	% Vesicular Infection
<i>G. fasciculatum</i>	51.17±1.75 ^a	31.16±2.75 ^a
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i>	62.76±2.75 ^{dfg}	42.7±1.86 ^b
<i>T. viride</i> + <i>G. fasciculatum</i>	52.31±2.16 ^{fg}	30.21±1.91 ^{cdf}
<i>G. fasciculatum</i> + <i>F. oxysporum</i>	29±1.15 ^{de}	15.71±2.16 ^{df}
<i>G. fasciculatum</i> + <i>R. bataticola</i>	28.16±2.75 ^{sd}	13.16±3.16 ^{df}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i> + <i>F. oxysporum</i>	50.17±1.61 ^{dfg}	27.16±1.7 ^{fgh}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i> + <i>R. bataticola</i>	51.11±2.61 ^{fgh}	27.31±2.61 ^{fg}
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i>	65.16±2.61 ^{fgh}	47.16±2.16 ^a
LSD	0.34	0.09

± Standard deviation

p ≤ 0.05

Values suffixed with different letters on the same column indicate significant differences

Although the rhizosphere appears to be too complex to allow manipulation, specific bacteria can be applied to seeds or roots causing alteration in the composition of the rhizosphere. Such manipulations have important and exciting implications. In addition to the manipulation to discourage disease-causing organisms, it could be used to promote the activity of beneficial ones such as mycorrhizal fungi, actinorhizae, *Rhizobium* spp., and associative nitrogen fixers. Thus, the focus of attention has now shifted from plant-microbe interactions to plant-microbe-microbe interactions.

Some investigations have shown that biological activities are markedly enhanced in two or three member associations of organisms (Tilak *et al.*, 1982; Varma and Mathur, 1989; Barea *et al.*, 1998). Such syntropic associations are of ecological importance with implied agricultural significance. In the present study, the colonization by *G. fasciculatum* was enhanced in the plants treated with biocontrols suggesting the positive effect of the biocontrols on *G. fasciculatum*.

Similarly, Bhowmik and Singh (2004) found that PGPR, including *Pseudomonas fluorescens*, *P. striata*, *Azotobacter chroococcum*, and *Azospirillum* sp. enhanced the root colonization by *Glomus mosseae*. Biologically active substances such as amino acids, plant hormones, vitamins, other organic compounds, and volatile substances produced by soil microorganisms can also stimulate the growth rates of AM fungi (Azcon-Aguilar and Barea, 1995; Barea, 2000).

Pseudomonas spp. strain F113 capable of synthesizing the antibiotic 2,4-diacetylphloroglucinol, did not exhibit antifungal activity against *Glomus mosseae*, but was able to stimulate mycelial development from *G. mosseae* spores and the overall processes involved in the formation of AM association in tomato growing soil (Barea *et al.*, 1998).

Thus, the study proved that *P. fluorescens* enhances the colonization of *G. fasciculatum*.

Seedling Vigour Index

The seedling vigour index which indicates the growth efficiency of the plant was much pronounced in the tripartite association of *V. mungo* seedlings with

P. fluorescens Os25, *Trichoderma viride*, and *Glomus fasciculatum*. *P. fluorescens* Os25 proved to be a highly efficient strain with a seedling vigour index of 6163 in the treated seedlings and 4764 in the control. Higher seedling vigour index was observed in *G. fasciculatum* treated seedlings (5611) when compared to *T. viride*

treated seedlings (5576). In *V. mungo* seedlings inoculated with both *P. fluorescens* Os25 and *G. fasciculatum*, higher seedling vigour index amounting to 46.11 per cent increase over control and 5.34 per cent increase over *G. fasciculatum* + *T. viride* treatment was observed (Figure 2 and Table 7).

Figure 2. Seedling Vigour Index

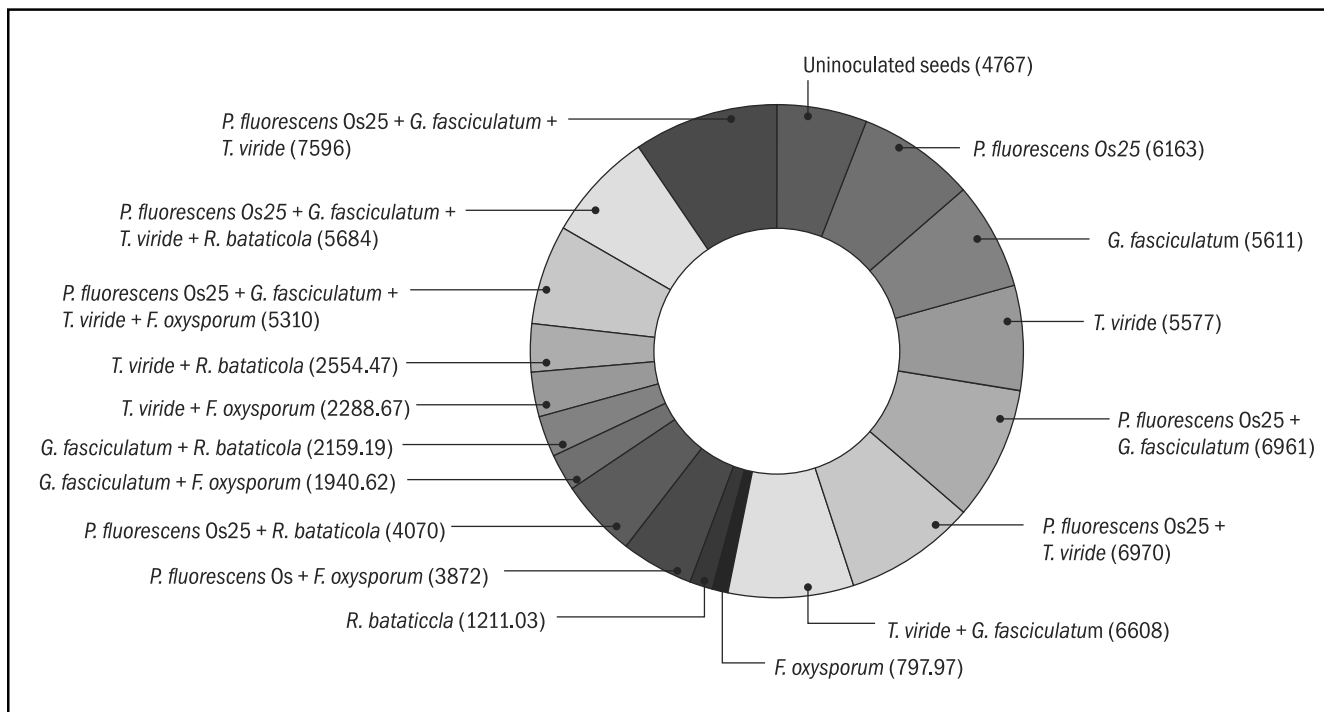


Table 7. Seedling Vigour Index

Treatments	Seedling Vigour Index	Treatments	Seedling Vigour Index
Uninoculated seeds	4764	<i>P. fluorescens</i> Os25 + <i>R. bataticola</i>	4070
<i>P. fluorescens</i> Os25	6163	<i>G. fasciculatum</i> + <i>F. oxysporum</i>	1940.62
<i>G. fasciculatum</i>	5611	<i>G. fasciculatum</i> + <i>R. bataticola</i>	2169.19
<i>T. viride</i>	5576	<i>T. viride</i> + <i>F. oxysporum</i>	2288.67
<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i>	6961	<i>T. viride</i> + <i>R. bataticola</i>	2554.47
<i>P. fluorescens</i> Os25 + <i>T. viride</i>	6970	<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i> + <i>F. oxysporum</i>	5310
<i>T. viride</i> + <i>G. fasciculatum</i>	6608	<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i> + <i>R. bataticola</i>	5684
<i>F. oxysporum</i>	797.97	<i>P. fluorescens</i> Os25 + <i>G. fasciculatum</i> + <i>T. viride</i>	7596
<i>R. bataticola</i>	1211.03		
<i>P. fluorescens</i> Os25 + <i>F. oxysporum</i>	3872		

When the *V. mungo* seedlings were treated with *F. oxysporum* and *R. bataticola*, the seedling vigour index reduced to 83.25 per cent and 74.58 per cent respectively, when compared to the control. When the seedlings were co-inoculated with the biocontrols in phytopathogen amended soils, the seedling vigour index increased considerably. In *F. oxysporum* amended soils the seedling vigour index of the plants treated with *P. fluorescens* Os25 was 3872 while with *T. viride* inoculated plants it was 2288.67. An increase of 69.18 per cent in seedling vigour index was observed.

Production of secondary metabolites such as siderophores is yet another mechanism by which *P. fluorescens* Os25 acts as a biocontrol agent, thereby increasing the seedling vigour. Fluorescent siderophores, which have a very high affinity for ferric iron, are secreted during growth under low iron conditions. The resulting ferric-siderophore complex is unavailable to other organisms, but the producing strain can utilize this complex via a very specific receptor in its outer cell membrane (Buyer and Leong 1986.). This way, fluorescent *Pseudomonas* strains may restrict the growth of deleterious bacteria and fungi at the plant root.

In the seedlings with tripartite association of *P. fluorescens* Os25, *G. fasciculatum* and *T. viride* and grown in *F. oxysporum* amended soils, the seedling vigour index was found to be 5310, i.e., an increase by 7.68 per cent when compared to the control.

Use of environmentally friendly biological control agents and mycorrhiza can more effectively control the soil-borne phytopathogens. The emanating fungal mycelium improves soil aggregation and thus modifies soil structure in comparison with rhizosphere soil (Schreiner and Bethlenfalvay, 1995). They considerably alter root exudation and are therefore expected to influence rhizosphere populations as well (Hayman, 1983). In the same way, changes in the population of pathogen antagonists in the mycorrhizosphere could indirectly explain many reported protective effects of the mycorrhizal symbiosis against plant diseases.

Biocontrol is the final result of different mechanisms acting synergistically to achieve disease control. Biocontrol results either from competition for nutrients and space or as a result of the ability of biocontrol agents to produce and resist metabolites or modify the rhizosphere, so that pathogens cannot grow.

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Medicinal Value of Edible Ectomycorrhizal Fungi: Potential Example of Sustainable Resource Utilization

S Krishna Sundari*

Introduction

Natural products represent a rich source of biologically active and highly diverse compounds with recognized potential in drug discovery and development. Plants have been a major focus of investigation for novel biomolecules of natural origin. In recent years, pharmaceutical companies are devoting a lot of research time and expense in developing these natural products to produce more affordable and cost effective remedies. However, due to over exploitation of this rich biodiversity and imbalance in depletion of natural resources from wilderness *vs.* restoration efforts of medicinal plants in natural ecosystems, some plant species have become threatened or even endangered. As a result, sustainable usage of such natural plant resources is currently questioned by ecologists.

Since ages, mushrooms are used for their medicinal properties. Many species of mushrooms have been used in folk medicine for many years particularly in eastern countries including Japan, China, and tribal lands of India. Mushroom derived compounds are used in the treatment of sexual impotence and dysfunction, high cholesterol levels, high blood pressure, neurological disorders, cancer therapy, and many other health problems (Talpur *et al.*, 2002; Paterson, 2006; Sullivan *et al.* 2006; Preuss *et al.*, 2010). Ferreira *et al.* (2010) made an extensive review on bioactive compounds from mushrooms and also highlighted their antitumor and immune-modulating properties. Some advantages of using mushrooms over plants as sources of bioactive compounds are that the fruiting body can be produced in much less time and the mycelium can be rapidly produced (*in vitro*) in liquid culture which can be manipulated to produce optimal quantities of active products using bioreactors. Nevertheless, these medicinal mushrooms are not necessarily edible and hence are to be used as prescribed by experienced practitioners only.

Ectomycorrhizae — the higher basidiomycetous fungi are universally recognized as important symbiotic fungi and form an integral part of the forest ecosystems. They are, economically, one of the most important groups of fungi forming a symbiotic relationship with tree species and are commonly found

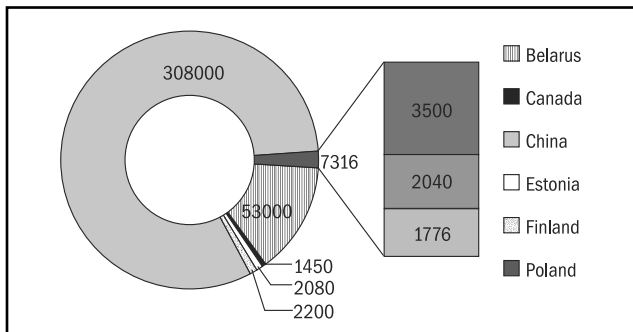
in temperate as well as alpine forests. These symbiotic relationships with the roots of the living tree host are an important part of ecosystem sustenance assisting in nutrient cycling and balance. Ectomycorrhizal fungi form fruiting structures called mushrooms. Some of these ectomycorrhizal mushrooms are edible, hence are cultivated as part of sustainable social forestry management. The edible ectomycorrhizae (EEM) are long recognized as a source of food and nutrition. However, consumption of EEM is limited to those areas/locations of wilderness from where their fruiting bodies can be harvested. A symbiotic relationship between EEM and host tree species is essentially required to develop these mushrooms. Recent literature reports the existence of medicinal properties in these ectomycorrhizal fungi. Extracting bioactive compounds from EEM fulfills the criteria of sustainability, since their growth and multiplication in wilderness would mean maintaining their host symbionts, thus promoting reforestation/afforestation. Also, they can be cultured using artificial cultivation techniques, causing no damage to ecosystem and with compliance to industrial standards.

Mushroom Production and Cultivation of EEM

Mushroom production is a fairly established trade and according to FAO (Boa, 2004), China is a leading producer of mushrooms (Figure 1). Currently, India stands 54 in the world ranking and is not a major producer/exporter of any of the mushroom varieties. It may be due to the low per capita consumption of mushrooms in India (25 g/year), as compared to USA (> 3.8 kg/year). Currently three varieties of mushrooms are cultivated in India, viz., the white button mushroom (*Agaricus bisporus*), the paddy straw mushroom (*Volvarelliella volvacea*), and the oyster mushroom (*Pleurotus sajor-caju*). However, there has been a steady increase in the consumption of exotic mushrooms in India alongwith the regular button mushrooms (Harsh and Joshi, 2008). There are no large scale concentrated efforts to grow EEM for trade, though EEM fruit bodies are sold in local markets involving native collectors from tribal lands (Bhaben *et al.*, 2011).

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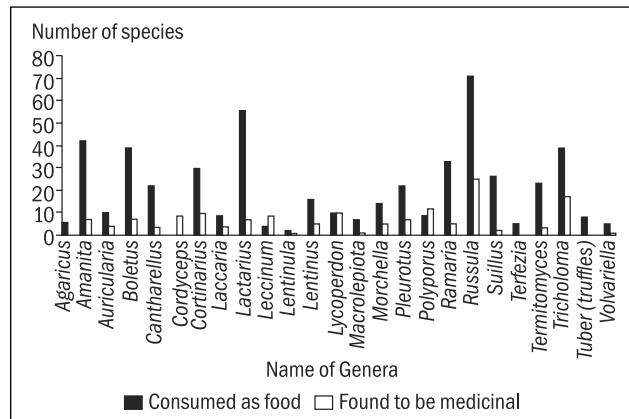
Figure 1: Country wise production of wild edible mushrooms (amount in tonnes)



The edible mycorrhizal mushrooms include some of the world's most expensive foods and have a global market of millions of US dollars. Some examples of the edible ectomycorrhizal mushrooms are *Cantharellus* sp., *Amanita* sp., *Termitomyces* sp., *Lactarius* sp., Truffles, *Tricholoma matsutake*, *Russula* sp., *Boletus edulis*, etc. Among these the most expensive and sought after varieties are *Tuber melanosporum* (Périgord black truffle), *Tuber magnatum* (Italian white truffle), *Tricholoma matsutake* (matsutake), *Boletus edulis* (The King boletus), *Cantharellus cibarius* (chanterelle), and *Amanita caesarea* (Caesar's mushroom). Despite this, few have been cultivated with any degree of success, owing to various complexities involved in cultivating EEM, such as vulnerability to contamination, host plant dependency, and a lack of understanding of each mushroom's trophic relationships and other requirements including biotic, edaphic, and climatic.

Amongst the 25 most important edible mushroom producing genera, 22 of them also have representative members of medicinal mushrooms (Boa, 2004). However, the proportion of medicinal mushrooms reported is far less in number as compared to the number of edible mushroom species (Figure 2). Two genera, *Russula* and *Lactarius* have maximum number of edible mushroom species followed by *Amanita*, *Boletus*, and *Tricholoma*. Incidentally, all these genera live symbiotically with plants and are ectomycorrhizal in nature. In fact, 13 (viz., *Amanita*, *Boletus*, *Cantharellus*, *Cortinarius*, *Laccaria*, *Lactarius*, *Leccinum*, *Ramaria*, *Russula*, *Suillus*, *Terfezia*, *Tricholoma*, and *Tuber*) out of 25 edible mushroom producing genera are ectomycorrhizal in nature (Figure 2). An in-depth research on important medicinal properties of these EEM would not only open interesting avenues of research with easily culturable and extractable fungal metabolites for industrial purposes, but also would increase their economic importance by encouraging farming and consumption of these EEM fungi. Growing EEM in native habitat would automatically mean establishing and maintaining tree host species, thus encouraging joint social forestry initiatives.

Figure 2: Edible mushroom producing genera with reported medicinal properties



Medicinal Properties of EEM Forming Fungi

Natural bioactive compounds with medicinal properties can be a valuable source of nutraceuticals or therapeutics. Studies on medicinal properties of mushrooms have shown marked health promoting benefits such as immunomodulation, anti-atherosclerotic, anti-inflammatory, analgesic, anti-tumorous, chemopreventive, chemoprotective, sleep promoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, hypoglycemic, anti-fibrotic, hepatoprotective, anti-diabetic, anti-androgenic, anti-angiogenic, anti-herpetic, anti-oxidative and radical scavenging, anti-aging, estrogenic, anti-ulcer, and many other exceptional nutritional and medicinal properties (Zhan *et al.*, 2010; Wani *et al.*, 2010). The medicinal properties of mushrooms are generally attributed to their ability to produce bioactive molecules such as polysaccharides, tri-terpenoids, low molecular weight proteins, glycoproteins, and immunomodulating compounds (Thakur and Singh, 2013). Higher Basidiomycetes contain long-chained large-molecular weight polysaccharides which when present in specific configurations or linkages (beta 1-3 glucan and beta 1-6 glucan) have strong effects on the immune system of humans (Wasser, 2002; Quang *et al.*, 2006). Mushrooms also contain a variety of antioxidant enzymes such as superoxide dismutase, peroxidases, and other complex secondary metabolites such as phenolic compounds, polyketides, flavonoids, triterpenoids, and steroids which are specific to each mushroom and have important role in balancing the internal reactive oxygen species (ROS) concentrations.

Research on the medicinal properties of EEM is fairly recent and is focused mainly on their anti-oxidative potential or their anti-microbial properties. There are few reports about their immune-protective and antitumour properties also. Most of the studies

however, were not exclusively on EEM, but were either on edible mushrooms in general or on medicinal mushrooms like *Ganoderma* sp. In this manuscript the work on medicinal properties of EEM is extracted from all such publications and presented under 3 subheads:

- Anti-oxidative potential of EEM
- Anti-microbial nature of EEM
- Other medicinal properties of EEM

Anti-oxidative Potential of EEM

Oxidative stress is one of the common triggers for various pathological conditions including cancer, hyper tension, Alzheimers, hypercholesterol, obesity, type II diabetes, etc. More and more efforts are being made to identify natural sources of antioxidants that can alleviate conditions of oxidative stress and maintain intracellular balance of reactive oxygen species (ROS). Many bioactive compounds have shown to possess anti-oxidative activity, including phenols, flavonoids, ascorbic acid, etc., along with anti-oxidative enzymes such as catalase, peroxidases and superoxide dismutase. Asatiani *et al.* (2007) have worked with submerged mycelial cultures of basidiomycetous mushrooms from different ecological niches and studied their free radical scavenging activity as a means to understand their anti-oxidative potential. Amongst the isolates they have tested, only three isolates were ectomycorrhizal (EM) in nature and only one is edible EM. Highest free-radical scavenging activity was observed in *Ganoderma lucidum*, the known medicinal mushroom. The authors have reported that free-radical scavenging activity depends upon the solvent used for extraction (water, ethanol) and showed that hot water extracts had better activity over organic extracts. Keyhani *et al.* (2009) have quantified the key intracellular anti-oxidative stress enzymes—superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), from *Pleurotus ostreatus* (edible, non-mycorrhizal). From the crude extract of *P. ostreatus*, the authors have reported up to 7.4 units SOD, 11.7 units CAT, and 0.037 units POX per mg protein respectively. Further, Keyhanf and Keyhani (2012) have evaluated the EEM *Cantharellus cibarius* for its key intracellular anti-oxidative stress enzymes—superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX). Similarly, Yang *et al.* (2011) worked with ethanolic extracts of isolates from the genus *Phellinus* and showed their antioxidant activity, inhibitory effects on the growth of human tumour cells, and the capacity to protect PC12 cells against H₂O₂ induced oxidative damage. The strongest antioxidant activity was found in the extracts of *Ph. baumii* PB-10. The IC₅₀ values for superoxide

radical and hydrogen peroxide scavenging activity of *Ph. baumii* PB-10 was recorded to be 3.76 µg/mL and 4.24 µg/mL, respectively. They have attributed the ROS scavenging activity to the presence of flavonoids in the fungal extracts/isolated fractions. One group from Kolkata (Pal *et al.*, 2010) have worked with the edible but non-mycorrhizal mushroom from the genus *Pleurotus* and have reported higher anti-oxidative activity in the hot water extract of the mushroom as compared to cold water or methanolic extracts. They have found flavonoids, lycopene, and beta carotene in the hot water extracts. The results were purely based on *in vitro* biochemical assays such as oxygen radical and NO scavenging assays, carotene assay, etc., and no *in vivo* tests for oxidative stress induced cellular damage/protection have performed.

Sundari *et al.* (2013) studied the edible ectomycorrhizal isolates of *Lactarius* and *Boletus* sp. and reported anti-oxidative properties of edible ectomycorrhizal fungi *Lactarius deliciosus* and *Boletus edulis*. They also tested the ability of EEM derived bioactive components to offer protection against oxidative stress associated cellular damage in *Saccharomyces* yeast. The anti-oxidative potential of ectomycorrhizal fungi was also compared with *Ganoderma lucidum* (medicinal mushroom) and *Agaricus bisporus* (non-mycorrhizal mushroom). Phenols, flavonoids, and polysaccharides were the non-enzymatic antioxidants observed in EEM, while superoxide dismutase (SOD), catalase and, peroxidases were the anti-oxidative enzymes quantified from mycelia extracts of EEM. The results have revealed better anti-oxidative potential in *Boletus* sp. as they showed highest SOD activity (1.2 units/mg protein), phenolic content (8.1mg of gallic acid equivalent (GAEs) per gm dry weight), and flavonoid concentrations (41.7 mg quercetin equivalent per gram of dry weight). *Saccharomyces cerevisiae* was subjected to oxidative stress (0–100mM acetic acid) and the cellular damage was recorded by studying DNA fragmentation and cell viability. Pre-treatment with *Boletus* spp. extracts gave enhanced cell protection. At a concentration of 17mg/ml there was a 39 per cent increase in cell viability. The presence of catalase enzyme also enhances the anti-oxidative activity of the mycorrhizal mycelial extracts. The DNA fragmentation assay showed protection against DNA damage in pre-treated yeast cells. The study showed that edible ectomycorrhizal fungal metabolites can provide protection against oxidative damage in *S. cerevisiae*. Reis *et al.* (2011) studied two non-edible EM isolates, *Paxillus involutus* and *Pisolithus arhizus* for their antioxidant properties and for their ability to produce tocopherols (*in vivo*-fruiting bodies and *in vitro*-mycelia).

While the antioxidant properties were studied in terms of DPPH radical-scavenging activity, reducing power, and inhibition of carotene bleaching, concentration of tocopherols was determined by high performance liquid chromatography (HPLC) coupled to a fluorescence detector. Authors have reported highest antioxidant properties in fruiting bodies of EM. They have also reported scavenging effects on free radicals (EC₅₀ = 0.61 and 0.56 mg/ml) and inhibition of lipid peroxidation capacity (EC₅₀ = 0.40 and 0.24 mg/ml for *Paxillus involutus* and *Pisolithus arhizus*, respectively). They noted higher levels of total tocopherols in mycelia of *Pisolithus arhizus* (154.39 µg/g dry weight).

Kumari *et al.* (2011) studied three species of EEM mushrooms namely *C. friesii*, *C. sub cibarius*, and *C. cinereus* collected from North-Western Himalayan region of India and exhibited their anti-oxidative potential. They termed these EEM as easily accessible sources of natural antioxidant. Kosanic *et al.* (2012) studied two ectomycorrhizal mushrooms *Boletus aestivalis*, *Boletus edulis* of which the later forms edible mushrooms, for their *in-vitro* antioxidant and antimicrobial activities. They have estimated the free radical scavenging activity and reducing power of the acetonic and methanolic extracts of the mushrooms along with the total phenolic content and flavonoids in the extracts to prove their bioactive properties. Dama *et al.* (2010) showed the impact of storage temperature and length of storage on potential of anti-oxidative enzymes. They studied the isozyme profile of anti-oxidative enzymes from mushrooms and reported that the activity of enzymes particularly superoxide dismutase and peroxidase are function of storage temperature and time. Kuka and Cakste (2011) detected and quantified phenolic compounds such as gallic acid, caffeic acid, catechin, epicatechin, and rutin from two strains of *Boletus edulis*. They also determined carotene and lycopene in them and further proved the ROS scavenging activity and anti-oxidative properties in them.

Anti-microbial Nature of EEM

The antimicrobial properties of phenolic extracts of Portuguese wild edible mushroom species (*Lactarius deliciosus*, *Sarcodon imbricatus*, and *Tricholoma portentosum*) were investigated by Barros *et al.* (2007). They calculated the minimal inhibitory concentrations (MICs) of total phenol and flavonoid extracts of mushrooms against gram positive bacteria (*Bacillus cereus* and *B. subtilis*) and fungi *Candida albicans* and *Cryptococcus neoformans*. Authors reported differential inhibition of fungal members and better antibacterial activity over antifungal properties, though *E. coli* showed resistance to mushroom extracts. *In vitro* studies were conducted by Ameri *et al.* (2011) to

investigate the anti-staphylococcal activity of crude extracts of two strains of *Pisolithus albus* collected from Pune, India. Though these isolates are ectomycorrhizal in nature, their fruiting bodies are not edible. The authors tested the antimicrobial activity against thirty clinical isolates of methicillin resistant *Staphylococcus aureus* and reported strong activity by ethyl acetate and methanol extracts. The MIC was shown in a range of 0.62 to 1.2 mg/ml.

Ramesh and Pattar (2010) studied the methanolic extracts of six edible mushrooms from Western Ghat region of India, of which two, viz., *Cantharellus cibarius* and the *Ramaria* sp. are EEMs. The major bioactive components found in extracts of these isolates are phenols (range 3.20 to 6.25 mg/mL), flavonoids, and ascorbic acid. The authors reported a higher concentration of these substances in four edible but non-mycorrhizal mushrooms and hence better antioxidative properties in them. The EEM tested by these scientists showed resistance to both gram positive and gram negative bacteria (*S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*). Liu *et al.* (2013) demonstrated the anti-oxidative and antibacterial properties of *Ramaria* sp. Alves *et al.* (2012) published a detailed review on antimicrobial activity of basidiomycetous mushrooms. They reported low molecular weight compounds mainly secondary metabolites, such as sesquiterpenes and other terpenes, steroids, anthraquinones, benzoic acid derivatives, quinolones, etc., and also primary metabolites such as oxalic acid in basidiomycetous mushrooms. High molecular weight compounds reported by them included mainly peptides and proteins. They included references of edible ectomycorrhizal mushrooms such as *Lactarius deliciosus*, *Boletus edulis*, *Cantherillus* sp., *Ramaria* sp., etc., in their study.

Other Medicinal Properties of EEM

Apart from antioxidative and antimicrobial properties, basidiomycetous mushrooms are also studied for their medicinal properties, viz., antitumour and immunoprotective/immune modulating properties. *Boletus* mushrooms are valuable food source, low in calories, lipids and high in vegetable proteins, minerals and vitamins (Kuka and Cakste, 2011). A study of the amino acid composition has showed *Boletus edulis* to have the highest total amino acid content of all the mushrooms tested. The major amino acids isolated were glutamine, alanine, glycine, serine, and proline. *B. edulis* was reported to show antioxidative, anti-microbial, and even antitumour properties. Barranco *et al.* (2010) studied 10 basidiomycetous mushrooms from the Mexican territory for their antioxidant, immune-modulating, cytotoxic, and antimicrobial properties. Antioxidant activity was

determined by dichlorodihydrofluorescein diacetate (DCFDA) assay and cytotoxicity was estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay in Chang liver cells. Of the ten isolates studied by the group, three were EEM (*Suillus luteus*, *Lentinus lepideus*, and *Suillus lakei*) and have shown strong antioxidant activity (20–80 per cent) which was attributed to the presence of alkaloids, tannins, and other carbohydrates. *Geastrum* sp. (some members are EEM) reported to have curative properties for eye infections and asthma. Dore *et al.* (2007) studied the tissues of this mushroom and found that the glucan-rich extract of *G. Saccatum* has antioxidant as well as anti-inflammatory properties mediated principally due to inhibition of nitric oxide synthase (NOS) and cyclooxygenase (COX) activities. Zhao *et al.* (2003) reported the presence of an antitumour lectin (AAL) consisting of two identical subunits of 15.8 kDa isolated from the fruiting bodies of the edible mushroom *Agrocybe aegerita*. Authors reported that AAL have strong inhibition effect on the growth of human tumour cell lines HeLa, SW480, SGC-7901, MGC80-3, BGC-823, HL-60, and mouse sarcoma S-180 through analysis by Hoechst 33258 staining, flow cytometry, and TUNEL (terminal transferase deoxytidyl uridine end labelling) analysis of slides of tumour tissues excised from BALB/c mice. They also demonstrated the apoptosis-induction activity of the lectin. Lee *et al.* (2004) characterized novel antihypertensive ACE (Angiotensin I converting enzyme) inhibitory peptides from edible mushrooms belonging to the genus *Tricholoma* and *Pholiota*. They reported higher I_{50} value from water extracts of *T. giganteum* and *P. adiposa* fruiting bodies.

Anand and Chowdhry (2013) analysed the bioactive compounds with antitumour potential in some of the new wild varieties of mushroom of Rajouri Dist. of Jammu and Kashmir region. They observed both low-molecular-weight compounds, viz., quinones, cerebrosides, isoflavones, catechols, etc., and high-molecular-weight compounds, viz., homo and heteroglucans, glycans, glycoproteins, etc., in mushrooms. They reported that the polysaccharide isolated from mushrooms have potent antitumour activity against sarcoma 180, mammary adenocarcinoma 755, leukemia L-1210, and against a host of other tumours. Ke *et al.* (2003), have extracted crude polysaccharide-protein complex from *Lactarius deliciosus* fruit bodies and proposed a possible role of these extracts in immune modulatory functions

Conclusion

Over exploiting natural ecosystems for mass harvest of medicinal plants is placing a huge stress on biodiversity

reserves and is even leading to slow disappearance of these valuable biodiversity reserves. Hence, there is an urgent need to look for natural resources with important medicinal/industrial applications that would not compromise the ecosystem functions and would also not spoil the rich biodiversity in nature. Exploring EEM would be a very sustainable option because on one hand they would provide important and safe biomolecules and on the other hand, promoting their growth in natural surroundings will automatically lead to conserving their natural ecosystem, i.e., the forests. The available literature on medicinal properties of EEM is limited and fairly recent. Particularly, in Indian context, scientific research on the medicinal properties of native EEM is practically scarce. Hence, the indigenous EEM represent a hugely untapped source of medicinally important bioactive compounds. While their edibility would ensure that EEM fruiting bodies can be more readily incorporated into the food habits of people as natural functional foods, their amenability to *in vitro* culture would facilitate mass culture of these organisms in fermenters and production of active metabolites throughout the year in a scientifically controlled environment ensuring product purity. The medicinal properties of indigenous EEM have numerous social and economic benefits. It hugely supports biodiversity conservation and socio-forestry initiatives. These properties make EEM a best example of value addition and of sustainable utilization of nature's sources for biotechnological application benefiting mankind.

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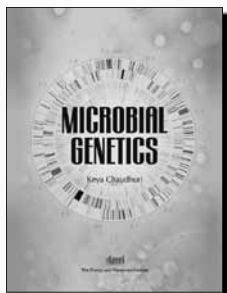
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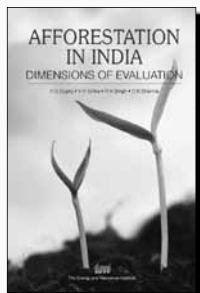


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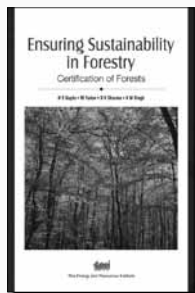
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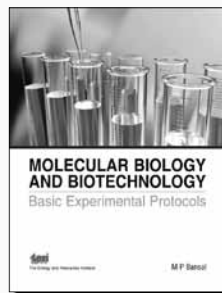
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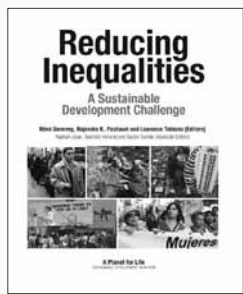
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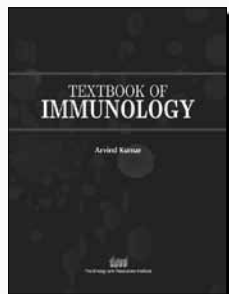
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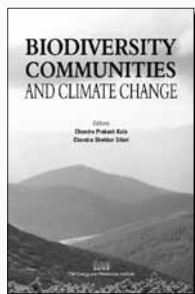
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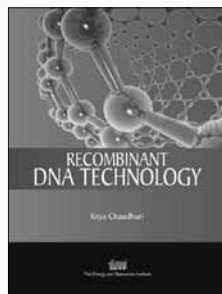
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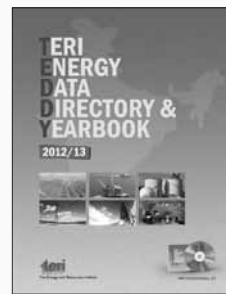
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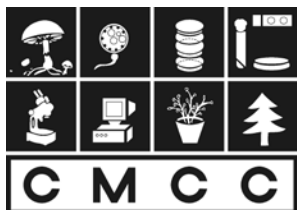


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CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Glimpses of CMCC's Varied Collection in India

Chaitali Bhattacharya* and Alok Adholeya#

A good culture collection bank is one which houses wide range of diversity among the collected species. In Centre for Mycorrhizal Culture Collection, the diversity augmentation is fashioned through collections and isolations made from varied bio-diverse regions around the globe. In this edition we would provide glimpses of our collections made from different Indian agro-climatic or biodiversity zones. There is always a significant relationship between land form and its corresponding climatic conditions. This relationship leads to evolution of diverse soil type and natural vegetation of a particular locale. Mycorrhizal biodiversity is the degree of variation that exists within a given species due to its varied place of existence. India exhibits a variety of landscapes and climatic conditions, reflected in the evolution

of different soil types and its corresponding flora. We try to screen out the mycorrhizal biodiversity by doing isolation from these diverse soil and climatic zones. It has been observed that among terrestrial biodiversity there is always a latitudinal gradient in species diversity, implying that the tropical region has greater biodiversity than the poles. India has some of the world's most bio-diverse regions and also has wide range of ecozones which include desert, high mountains, highlands, tropical and temperate forests, swamplands, plains, grasslands, and areas surrounding rivers. It hosts three biodiversity hotspots: the Western Ghats, the Himalayas, and the Indo-Burma region. According to the National Biodiversity Authority there are 22 Agro-biodiversity Hotspots of India :

Table 1: Agro-biodiversity Hotspots of India

S.No.	Hotspot Region	Areas Covered
1	Cold Desert	Western Himalyas covering Ladakh and Kargil. Upper reaches of Lahuai-Spiti districts of Himachal Pradesh
2	Western Himalayan	Districts of Srinagar, Anantnag, Udhampur, Riasi, Kathna in Jammu and Kashmir, all the districts of Himachal Pradesh except the cold arid region, and all the districts of Uttarakhand
3	Eastern Himalayan	All the districts of Arunachal Pradesh, Sikkim, and Darjeeling district of West Bengal
4	Brahmaputra Valley	Dhubri, Kokrajhar, Bongaigaon, Barpeta, Nalbari, Goalpara, Kamrup, Golaghat, Darrang, Morigaon, Nagaon, Sonitpur, Jorhat, Lakhimpur, Sibsagar, Dibrugarh, Dhemaji, and Tinsukia
5	Khasia-Jaintia-Garo Hills	All the seven districts of Meghalaya, i.e., East Garo Hills, West Garo Hills, South Garo Hills, East Khasi Hills, West Khasi Hills, Jaintia Hills, and Ri-Bhoi
6	North-Eastern Hills	All the districts of Manipur, Mizoram, Nagaland, Tripura, and the adjoining Cachar and North Cachar districts of Asom
7	Arid Western	Sikar, Nagaur, Pali, Hanumangarh, Ganganagar, Jalore, Sirohi, Jodhpur, parts of Jaisalmer and Bikaner, Udaipur, Dungarpur, Churu, and Jhunjhunu districts of Rajasthan
8	Malwa Plateau and Central Highlands	Malwa plateau, Central highlands, Mewar plateau, and semi-arid south-eastern Rajasthan. Shadel, Raisen, Bhopal, Sehore, Shajapur, Indore, Ujjain, Mandasaur, Rajgarh, Hoshangabad, Narsinghpur, Jabalpur, Mandla, and Umari districts
9	Kathiawar	Ahemadabad, Surendranagar, Jamnagar, Rajkot, Porbandar, Junagadh, Amreli, Bhavnagar, Bharuch, Surat, Navsari, Valsad, Banaskantha, and Anand districts of Gujarat
10	Bundelkhand	Districts of Jhansi, Banda, Chitrakoot, Hamirpur, Jalaun, and Lalitpur in Uttar Pradesh and Damoha, Datia, Panna, Sagar, Tikamagarh, and Chattarpur in Madhya Pradesh

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S.No.	Hotspot Region	Areas Covered
11	Upper Gangetic Plains	Districts of Hardoi, Sitapur, Barabanki, Lucknow, Unnao, Rae Bareilly, Kanpur, Kannuj in Central Uttar Pradesh, and the districts of Maharajganj, Sidharatnagar, Kushinagar, Deoria, Sant Kabir Nagar, Gorakhpur, Basti in North-eastern Uttar Pradesh
12	Lower Gangetic Plains	Districts of Paschim Champaran, Purbi Champaran, Gopalganj, Siwan, Sitamarhi, Muzaffarpur, Saran, Buxar, Bhojpur, Patna, Rohatas, Jahanabad, Vaishali, Samastipur, Darbhanga, Madhubani, Sheohar in North Bihar
13	Gangetic Delta	Broadly includes the deltaic 24-Parganas districts, and the districts of Hoogly, Howrah, Nadia, Bardhaman, Birbhum, and Murshidabad
14	Chotanagpur	Districts of Singhbhum, Gumla, Ranchi, Lohardaga, Palamau and Hazaribagh and Santhal Pargana in Jharkhand, and Mayurbhanj district in Odisha
15	Bastar	Districts of Bastar, Bilaspur, Durg, Jashpur, Kabirdham, Kanker, Kirba, Korla, Mahasamund, Kondaigaon, and Rajnangoan of Chattisgarh
16	Koraput	Districts of Malkangiri, Sonabeda, Jeypore, Koraput, Nabrangpur, Kalahandi, Bolangir, Rayagada in Odisha, and districts of north eastern Andhra Pradesh, i.e., Srikakulam, Vijanagaram, and Vizagapatnam
17	Southern Eastern Ghats	Districts of Chittoor, Ananthapur, Cuddapah, and Kurnool in Andhra Pradesh
18	Kaveri	Districts of Chengalput, South Arcot, North Arcot, Thiuannamalai, Tiruchirapalli, Pudukottai, Thiruarur, Vellore, Kanchipuram, Dharmapuri, Salem, Namakkal, Karur, and Dindigal
19	Deccan	Districts of Jalna, Hingoli, Parbhani, Beed, Nanded, Latur, Osmanabad, Solapur, Sangli, Gondia, Gadchiroli in Maharashtra, districts of Adilabad, Karimnagar, Warangal, and Khamman in Andhra Pradesh, and districts of Bidar and Gulbarga in Karnataka
20	Konkan	Coastal districts of Thane, Raigad, Ratnagiri, Sindhudurg and part of Sahyadri districts of Pune, Satara and Kolhapur districts of Maharashtra, all the districts of Goa and Uttar Kannada district of Karnataka
21	Malabar	Districts of Kasargod, Kannur, Wayanad, Kozikode, Malappuram, Palakkad, Thrissur, Idukki, Ernakulam, Alappuzha, Kollam, Kottayam, Pathanamthitta, and Thiruvananthapuram in Kerala, Udhamandalam (Nilgiri) and Kanyakumari districts of Tamil Nadu, and districts of Dakshin Kannada, Kodagu and Udipi in Karnataka
22	Islands	Andaman and Nicobar Islands and Lakshadweep

Source: <http://nbaindia.org/content/500/55//biodiversityrelatedi.html>

Depending on heat index and moisture supply, there are four climatic types in India:

- **Arid zone** — Here rainfall is below 30 cm and the distribution of rainfall is extremely uncertain even in the rainy season. Region covered is Western Rajasthan.
- **Semi-arid zone** — This is the dry farming region of the country. Region and state covered are Northern and Eastern Rajasthan, Gujarat, Marathwada, Mysore, Rayalaseema, Punjab, Delhi, and Western Uttar Pradesh.
- **Sub-humid** — This climatic complex consists of temperate sub-tropical conditions. Region and state covered are Central Uttar Pradesh, Western and Central Madhya Pradesh, Vidarbha, Eastern Uttar Pradesh, Bihar, Sub-mountain tracts of Uttar Pradesh, Himachal Pradesh, West Bengal, and Nilgiri.
- **Humid** — This represents conditions of tropical crop cultivation which mostly cover hot and humid conditions. Region and states covered are Konkan, Kerala, Coastal Chennai, Asom, Odisha, West Bengal, Eastern Madhya Pradesh, and Coastal Andhra Pradesh.

Our constant endeavour is to explore the rich biodiversity of India and to create a valuable germplasm bank for Mycorrhiza. Our stocks originate from diverse habitat that includes: trap cultures created from valuable rich natural consortia and many other parts of India.

Table 2: Germplasm isolates collected from different states

S.No.	Collections	Isolates
1	Natural Consortium	
2	Punjab	35 Isolates
3	Uttranchal-Mukteshwar	27 Isolates
4	Tamil Nadu	28 Isolates
5	Jaisalmer	13 Isolates
6	Rajasthan	13 Isolates
7	Odisha	12 Isolates
8	Meghalaya-Shillong	6 Isolates
9	Maharashtra	2 Isolates
10	Leh	4 Isolates
11	Goa	2 Isolates
12	Haryana	35 Isolates

S.No.	Collections	Isolates
13	Chattisgarh	10 Isolate
14	Asom	21 Isolates
15	Andhra Pradesh	69 Isolates
16	Madhya Pradesh, Panchmarhi	25 Isolates
17	Uttar Pradesh	15 Isolates

Our bank is housing almost 350 plus accessions (trap cultures) from which there is a constant search of

new pure isolates. This entire process of collection and isolation is time compelling, labour demanding, and space subjugating. There is a constant formation of isolates followed by their morpho-taxonomic and molecular characterization. We have under mentioned accessions (pure cultures/monosporals) isolated from different parts of India which are readily available for our researches. India being a large country, a lot of zonal characterization is yet to be done.

Table 3: Bank of Mycorrhizal Culture

S.No.	Bank Code	Culture Code	Culture
1	CMCC/AM-1102	Andhra Pradesh	<i>Rhizophagus irregularis</i>
2	CMCC/AM-1103	Rajasthan	<i>Glomus</i> sp.
3	CMCC/AM-1104	Madhya Pradesh	<i>Rhizophagus irregularis</i>
4	CMCC/AM - 1201	Natural consortia	<i>Glomus etunicatum</i>
5	CMCC/AM - 1202	Rajasthan	<i>Glomus etunicatum</i>
6	CMCC/AM - 1203	Madhya Pradesh	<i>Glomus etunicatum</i>
7	CMCC/AM - 1204	Uttaranchal	<i>Glomus etunicatum</i>
8	CMCC/AM - 1205	Andhra Pradesh	<i>Glomus etunicatum</i>
9	CMCC/AM - 1206	Madhya Pradesh	<i>Glomus etunicatum</i>
10	CMCC/AM - 1207	Natural consortia	<i>Glomus etunicatum</i>
11	CMCC/AM - 1208	Rajasthan	<i>Glomus etunicatum</i>
12	CMCC/AM - 1209	Natural consortia	<i>Glomus claroideum</i>
13	CMCC/AM - 1210	Natural consortia	<i>Glomus claroideum</i>
14	CMCC/AM - 1301	Rajasthan	<i>Glomus hoi</i>
15	CMCC/AM - 1302	Madhya Pradesh	<i>Glomus hoi</i>
16	CMCC/AM - 1303	Natural consortia	<i>Glomus hoi</i>
17	CMCC/AM - 1401	Natural consortia	<i>Funneliformis mosseae</i>
18	CMCC/AM - 1402	Uttaranchal	<i>Funneliformis mosseae</i>
19	CMCC/AM - 1403	Rajasthan	<i>Funneliformis mosseae</i>
20	CMCC/AM - 1408	Uttaranchal	<i>Funneliformis mosseae</i>
21	CMCC/AM - 1408	Natural consortia	<i>Funneliformis mosseae</i>
22	CMCC/AM - 1408	Haryana	Uncultured <i>Funneliformis</i>
23	CMCC/AM - 1501	Natural consortia	<i>Glomus coronatum</i>
24	CMCC/AM - 1601	Natural consortia	Unculturable <i>Glomus</i> spp.
25	CMCC/AM - 1701	Rajasthan	<i>Paraglomus majewskii</i>
26	CMCC/AM - 1801	Natural consortia	<i>Diversispora spurca</i>
27	CMCC/AM - 1802	Rajasthan	<i>Diversispora spurca</i>

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:

- *Applied Soil Ecology*
- *Crop Protection*
- *Environmental and Experimental Botany*
- *Fungal Biology*
- *Journal of Theoretical Biology*
- *Pedosphere*
- *Scientia Horticulturae*
- *Soil Biology and Biochemistry*

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