MYCORRHIZA NEWS

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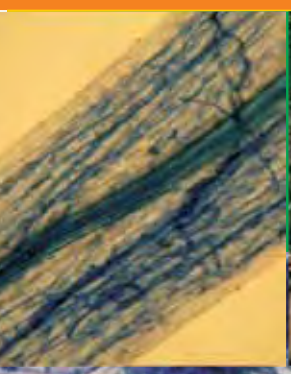

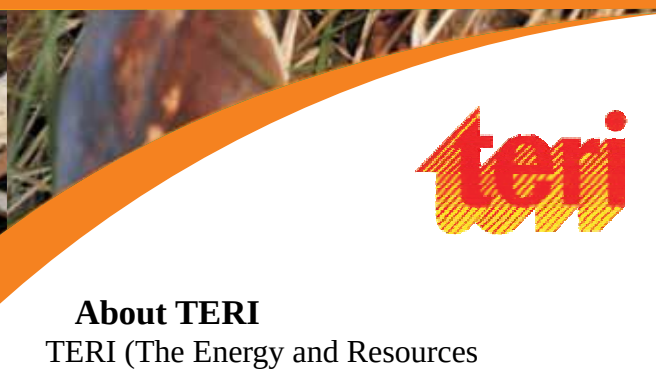
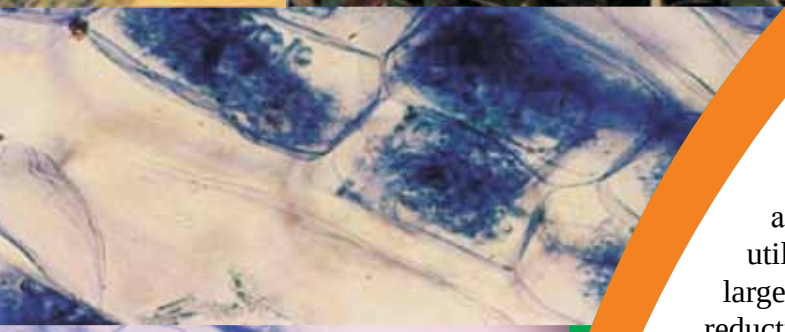
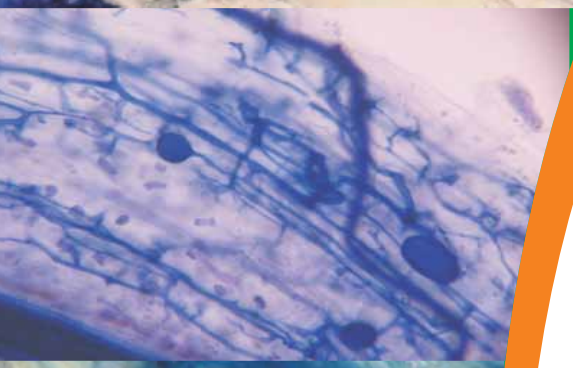

About TERI

TERI (The Energy and Resources Institute) is a not-for-profit research organization engaged in research on various aspects of energy, environment, and biotechnology, and on documentation and information dissemination. The genesis of these activities lie in TERI's firm belief that the efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

TERI's Mycorrhiza Network

Mycorrhiza Network was set up at TERI in 1988 with the objective to strengthen research, encourage cooperation, promote exchange of information and germplasm, and facilitate transfer of technology to the field through the establishment of a mycorrhiza research network. Within the Network, a Mycorrhiza Information Centre (MIC) has been set up with support from the Department of Biotechnology, Government of India, with an objective to function as a specialized centre for collection, compilation, and dissemination of information and resources on mycorrhiza; promote networking and resource sharing.

Mycorrhiza News

The *Mycorrhiza News*, a quarterly publication since 1988, provides a forum for dissemination, acquisition, interaction and communication of scientific information on mycorrhizal research activities. It publishes papers/research findings from eminent scientists covering biology, ecology, and other related aspects of mycorrhiza, including biodiversity and conservation of mycorrhizae. Among other components encompassing the newsletter include: notes on important breakthroughs; brief accounts of new approaches and techniques; research activities highlighting the Centre for Mycorrhiza Culture Collection, forthcoming events on Mycorrhiza and related events; important references of research papers published in different national and international journals.

Contents

Research finding papers

Taxonomic series: *Dentiscutata from Goa*

*Sharda W Khade**

Introduction

Arbuscular mycorrhizal fungi forming spores on bulbous sporogenous cell were earlier organized in two genera of the *Gigasporaceae*: *Gigaspora* and *Scutellospora*. (Oehl, de Souza, and Sieverding 2008). Molecular analyses indicated that *Scutellospora* is polyphyletic (Oehl, de Souza, and Sieverding 2008). Therefore, *Gigasporaceae* are revised on the basis of morphological spore characters and 18S and 25S rRNA gene sequences (Oehl, de Souza, and Sieverding 2008). In light of these studies, 36 *Scutellospora* species were re-organized in three new families, including five new genera: *Scutellosporaceae (Scutellospora)*, *Racocetraceae (Racocetra, Cetraspora)*, and *Dentiscutataceae (Dentiscutata, Fuscutata, Quatunica)*. The family *Gigasporaceae* remained with a single genus *Gigaspora* (Oehl, de Souza, and Sieverding 2008). In this paper, species belonging to new genus *Dentiscutata* are reported. The key characters of the genus *Dentiscutata* are summarized below (Oehl, de Souza, and Sieverding 2008).

Etymology

From the Latin *denti* **(***culata***)** (= dentate), and *scutata* (= shielded), referring to the dentate form of the periphery of the brown germination shield.

Key characters

Sporocarps are unknown. Single spores are formed terminally on bulbous sporogenous cell with subtending hypha that arises from mycelia hyphae. Outer spore wall is three- to five-layered and continuous with the wall of the sporogenous cell. Pore between the spore and sporogenous cell is narrow and usually closed by a plug formed by the material of the outer spore wall. A hyaline middle wall and a hyaline inner wall form de novo during spore formation. The middle wall is single- to bi-layered; while the inner wall is two- to three-layered. The germination shield generally arises on the outer surface of the inner wall or beneath a thin outer layer of the inner wall. It is yellow-brown to brown in colour, generally ellipsoid (to oval), rarely reniforme to cardioforme, usually with many (8–30) large folds separating the shield into 8–30 'small compartments'. Each small compartment is generally with one circular germ tube initiation. Usually, from one or a few gti, germ tubes may arise and penetrate the middle wall and the outer wall. The periphery of the germination shield generally appears dentate in planar view. Septa is often formed in subtending hypha at some distance to the sporogenous cells. Auxiliary cells formed in hyphal mycelium are knobby without spines on the surface. Formation of typical arbuscular mycorrhizae is known but vesicle formation in roots is so far unknown (Oehl, de Souza, and Sieverding 2008).

Type species

Dentiscutata nigra (Redhead) Sieverding, de Souza, and Oehl.

Seven species were reported under the genus *Dentiscutata* by Oehl, de Souza, and Sieverding

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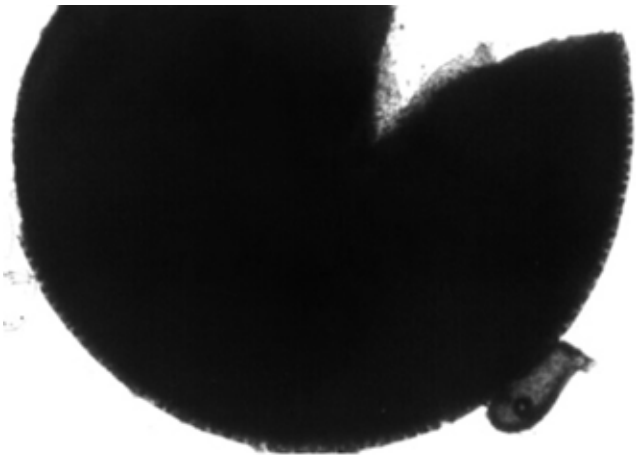
(2008). These are (1) *D. biornata* (Spain, Sieverding and Toro) Sieverding, de Souza, and Oehl; (2) *D. cerradensis* (Spain and Miranda) Sieverding, de Souza, and Oehl; (3) *D. hawaiiensis* (Koske and Gemma) Sieverding, de Souza, and Oehl; (4) *D. heterogama* (Nicolson and Gerd.) Sieverding, de Souza, and Oehl; (5) *D. nigra* (Redhead) Sieverding, de Souza, and Oehl; (6) *D. reticulata* (Koske, Mill. and Walker) Sieverding, de Souza, and Oehl; and (7) *D. scutata* (Walker and Dieder.) Sieverding, de Souza, and Oehl. The author has recently published one more new species under this genus, namely, *D. nigerita* Khade (Khade 2010). Out of the total eight species, three are reported in the present study.

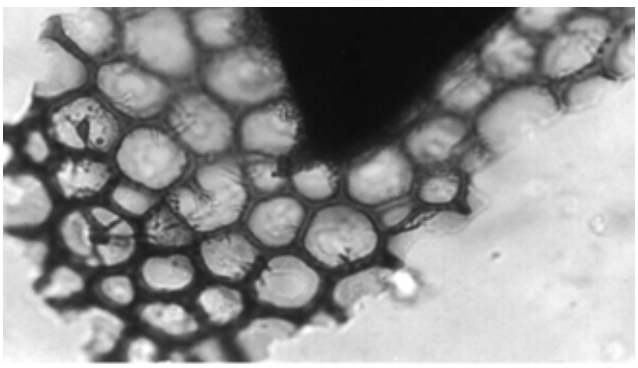
1. Dentiscutata nigra (Redhead) Sieverding, de Souza and Oehl

Mycotaxon **106**: 311–360, 2008

Spores formed singly in soil, borne laterally on a bulbous sporogenous cell, are dark-brown to black, globose, and 480–720 µm in diameter (Plate 1[a]) with an inner and outer wall. The outer wall is black, pitted with large pores, 7–10 µm in diameter, overlying smaller pores consisting of a series of coils (Plate 1[b]). The inner wall is light brown. Sporogenous cell, present on the tip of the hyphae, are brown, ellipsoidal, and measure 36–48 × 96–115 µm. These cells often produce a peg-like hypha to the spore wall. The subtending hyphae are 4–8 µm in diameter. The germination shield is oval, light-brown to brown in colour, with 90–100 × 148–160 µm diameter.



(a)



(b)

Plate 1(a) Crushed spore of *Dentiscutata nigra* with laterally attached bulbous suspensor (× 100)
Plate 1(b) Outer pitted wall in *Dentiscutata nigra* (× 1000)

Distinguishing feature Outer wall is black, pitted with large pores, and has sinuous secondary ornamentation below.

Distribution Recorded in December from Kodar with 16.66% frequency of occurrence. Recorded in January, October, and April from Kodar with 62.50 % frequency of occurrence.

Association Found in association with *Carica papaya* L. plants at plateaus of Goa, India.

2. Dentiscutata nigerita Khade

Mycosphere **1(3)**: 241–247, 2010

Azygospores are dark-brown to shining-black in colour, globose to subglobose, 300–500 × 250–650 µm in diameter (Plate 2[a]), and terminally borne on brown, elongated bulbous suspensor (Plate 2[b]) with sparsely septate hypha. Bulbous suspensor is attached at an angle to the spore. Azygospores with oily globular contents, which become opaque to buff in colour. Spore wall structure is made up of two wall groups (A and B).

Group wall A This consists of an outer wall layer (wall 1), dark-brown to black with pitted ornamentation, consisting of large pores 7–10 µm in diameter, forming a network throughout the outer circumference of the spore (Plate 2[c]). This pitted wall 1 is underlined by small porous, fine, pale-brown to transparent, membranous layer wall 2. This is followed by wall 3, which is reddish-brown to dark orange-brown consisting of sinuous interconnecting ridges that form reticulum, 1µm high, 4–8 sided meshes, 4–20 × 1–24 µm across (Plate 2[d]). Spore surface between ridges covered with conical sub-cylindrical spines, 0.5–2 µm high and 1µm apart (Plate 2[d]).

Group wall B This consists of an outer (wall 4), middle (wall 5), and inner (wall 6) yellow, unit membranous walls. The germination shield is circular, yellow-to-brown, 100–110 × 150–200 µm in diameter, amorphous with wavy margin, and multi-lobed in

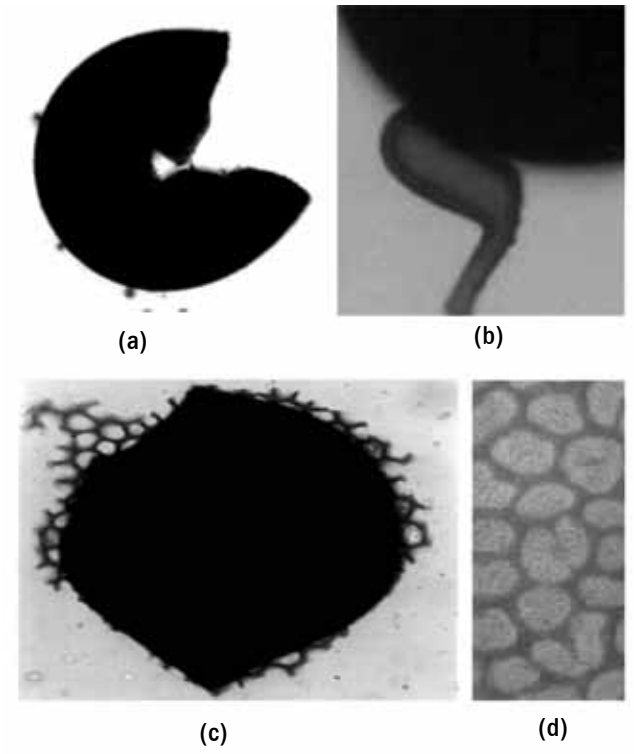


Plate 2(a) Crushed spore of *Dentiscutata nigerita* (× 100)
Plate 2(b) Elongated bulbous suspensor of *Dentiscutata nigerita* (× 300)
Plate 2(c) Portion of a spore of *Dentiscutata nigerita*, showing large porous ornamentation (× 400)
Plate 2(d) Portion of the inner spore wall of *Dentiscutata nigerita* supporting raised, straight to sinuous interconnecting ridges that form a reticulum interspersed with spines (× 1000)

appearance due to deep grooves. Bulbous suspensor, 110–125 × 55–65 µm, is attached at an angle to the spore.

Distinguishing feature *Dentiscutata nigerita* is a combination of two species: *D. nigra* (Redhead) Sieverding, de Souza, and Oehl and *D. reticulata* (Koske, Miller, and Walker) Sieverding, de Souza, and Oehl. Like *D. nigra, D. nigerita* has outer pitted black ornamentation with large pores, and like *D. reticulata,* it has reddish-brown layer consisting of straight-to-sinuous interconnecting ridges forming a reticulum with spore surface between ridges covered with polyhedral, conical or sub-cylindrical spines.
Distribution Recorded in March from Kodar with 16.66% frequency of occurrence.

Association Found in association with *Carica papaya* L. plants at plateaus of Goa, India.

3. *Dentiscutata reticulata* (Koske, Mill. and Walker) Sieverding, de Souza and Oehl

Mycotaxon **106**: 311–360, 2008

Spores were found singly in the soil, borne laterally on a bulbous sporogenous cell. Spores are dark-red to brown, globose to sub-globose, and 300–600 µm in diameter. The spore wall structure is complex, consisting of two separate groups of wall layers overlain by an alveolate reticulum. The outer wall group is three layered. The outer layer 0.5–1 µm thick, orange-brown to reddish-brown in colour. It supports raised, straight-to-sinuous interconnecting ridges that form a reticulum 0.5–1 µm high with 4–8 sided meshes measuring 2–24 × 2–30 µm across

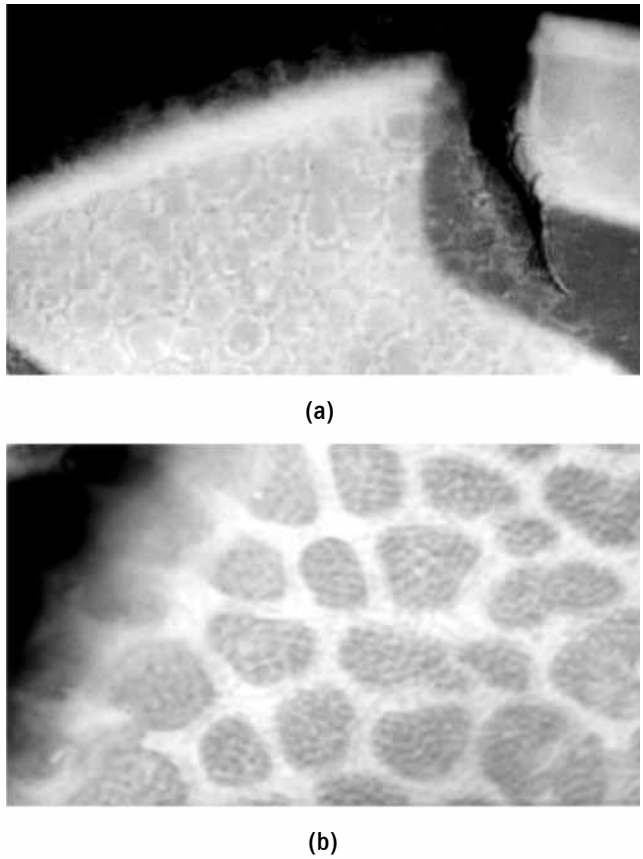


Plate 3(a) Reticulate ridges on outer spore wall supporting a detachable alveolate reticulum (× 1000)
Plate 3(b) Reticulate ridges supporting a detachable alveolate reticulum on the inner side of the outer spore wall (× 1000)
Note Spore surface between ridges is covered with spines in Plates 3(a) and 3(b)

(Plate 3[a]). Spore surface between ridges is covered with polyhedral, conical or sub-cylindrical spines or narrow, straight, curved or angular ridges 0.5–1 µm high and <1 µm apart (Plate 3[b]). The middle layer

is hyaline to pale yellow, and 5–11 µm thick. The inner layer is hyaline and 0.3–0.7 µm thick. Reticulate ridges on outer wall support a detachable alveolate reticulum 0.5–2 µm wide and 2–6 µm high. The inner wall group is tri-layered, totalling 3 µm in thickness. Sporogenous cell is formed at the tip of thick walled, sparsely septate or coenocytic pale-brown hyphae. The sporogenous cell measure 45–60 × 90–100 µm, with peg-like protrusions extending 10–20 µm towards the spore wall. The germination shield is pale-brown to brown, subglobose, irregular, and measure 100–165 µm in diameter.

Distinguishing feature The spore wall structure is complex, overlain by an alveolate reticulum. Reticulate ridges on outer and inner walls support a detachable alveolate reticulum. The spore surface between ridges is covered with polyhedral, conical or sub-cylindrical spines or narrow, straight, curved or angular ridges.

Distribution Recorded in December from Collem, Old Goa, and Colva with 50% frequency of occurrence; in January and April from Valpoi with 75% frequency of occurrence; in January, April, July, and October from Old Goa with 62.5% frequency of occurrence; in June, July, August, September, and October from Old Goa with 100% frequency of occurrence.

Association Found in association with *Carica papaya* L. plants at Western Ghats, and plateaus and coastal areas of Goa, India.

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Seasonality of arbuscular mycorrhizal symbiosis in the rhizosphere of *Rauwolfia serpentina* and *Tylophora asthamatica*

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Introduction

The dynamics and ecology of soil micro-organisms vary with soil characteristics, plant species, and climate (Lorgio, Julio, and Peper 1999). Environmental factors that affect and alter soil microbial assemblage structure and functions are complex. Environmental variables such as soil pH, nutrient availability, hydrologic conditions of soil, and soil temperature can influence the distribution and activity of soil micro-organisms (Anand, Ma, Okonski, *et al*. 2003; Entry, Rygiewicz, Watrud, *et al*. 2002). Diversity and functioning of arbuscular mycorrhizal (AM) fungi communities have traditionally been based on root colonization estimates and AM spore count. Apart from this, spore population dynamics is also regulated by various biotic and abiotic factors, nutrient limitation, stress, and so on. Germination rate and efficiency of these fungi depend upon the composition of AM fungi species with change of season (Shreshtha, Shreshta, Khadge, *et al*. 2007; Mandyam and Jumpponen 2008).

Despite the importance of AM fungi in the physiology and nutrition of plants, as well as in shaping plant communities (Van der Heijden, Klironomos, Ursic, *et al*. 1998; Smith, Nicholas, and Smith 1979), factors affecting the presence, diversity, spore density, and root colonization by AM fungi in soil are not well understood. This is due to the fact that the causes of correlation of soil and plant factors with AM fungi have not been established yet. Moreover, AM fungi are able to associate with a variety of host plants but the fungal sporulation rates are host dependent (Lugo and Cabello 2002). There is variation in vesicular–arbuscular mycorrhizal (VAM) colonization with change in season. Mycorrhizal colonization varies within and between years (Li, Yang, and Zhao 2005) as well as among plant species (Ruotsalainen, Vare, and Vestberg 2002). Despite the broad range of seasonal and temporal variations, several studies have reported similar patterns of AM seasonality across habitats (Lekberg, Koide, and Twomlow 2008). The establishment of plants under field conditions is influenced by seasonal effects, depending upon the efficiency of the indigenous VAM fungi. Information on seasonal variation of spore count and root colonization of VAM fungi is useful for timely inoculation of suitable species. Timely inoculation is of utmost importance as the seedlings suffer shock treatment when transferred from nursery to field, thus increasing the mortality rate.

Keeping this in view, the present study investigated natural VAM fungal spore population dynamics in the rhizosphere soil and its colonization in *Rauwolfia serpentina* and *Tylophora asthamatica* as a function of seasonal variation.

Materials and methods

Survey and site description

The plants studied were grown in pots in polyhouse conditions. Kurukshetra is a small district in Haryana, India. It lies at 29°58' N latitude and 76°5' E longitude and is about 250 m above sea level. The climate of this area is tropical monsoonal type. The three seasons in a year are (1) summer (March to May), which is hot and dry, (2) rainy season (June to September), which is warm and wet, and (3) winter season (October to February), which is cool and dry. The annual rainfall is 500–1500 mm, which precipitation occuring mostly during the monsoon period (June to September). The mean maximum temperature varied from 21.7°C (December) to 37.5°C (May), while the mean minimum temperature varied from 4.7° (December) to 25.8°C (May) during the sampling year.

Sampling

Roots and soil samples from the rhizosphere of the plants under study were collected at the end of each month. Five different plants of each type were randomly selected for the study. About 10 g of the rhizospheric soil, along with fine roots of 2 mm diameter, were collected from 15–30 cm deep soil from each plant and taken in polythene bags. The samples were then mixed together to form a composite sample and stored at 5°C for further studies. Three replicates were taken for further analysis from each composite sample.

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Estimation of VAM root colonization

Mycorrhizal root colonization in all the plants was studied by rapid clearing and staining technique (Phillips and Hayman 1970).

Estimation of VAM spore population

The VAM spores were isolated from soil samples using wet sieving and decanting technique (Gerdemann and Nicolson 1963). Quantification was done by gridline intersect method (Adholeya and Gaur 1994).

Identification of VAM fungal spores

Intact VAM spores were examined and identified by following the manuals of Schenck and Perez (1987); Morton and Benny (1990); and Mukerji (1996).

Moisture content, pH variation, and temperature of soil

Soil moisture content was determined every month. About 25 g of soil was weighed, dried at 105°C, cooled, and weighed again. The loss of weight on drying was noted and the moisture percentage was calculated by the following formula.

$$\text{Moisture (\%)} = \frac{\textit{loss of weight on drying}}{\textit{Initial sample weight}} \times 100$$

The pH variation was also recorded for the samples studied by mixing soil and distilled water (1:2) using a digital pH meter.

The temperature of the soil was recorded by a soil thermometer every morning of the study period.

Statistical analysis

The data was analysed statistically using Pearson correlation method.

Results and discussion

Rhizosphere is the most active part of soil surrounding plant roots. It harbours different types of micro-organisms. It is one of the hot spots where soil biota aggregate and resources are more abundant with faster mass and energy transformation (Coleman and Crossley 1996). Many important aspects of plant–soil interactions are mediated by rhizospheric processes (Fu and Cheng 2002). Thus, the rhizosphere can be considered as a dynamic environment determined by reciprocal interaction between soil, plants, and microflora associated with roots. The present study was undertaken to assess the rate of VAM colonization, arbuscule and vesicle formation in the roots, and endomycorrhizal spore population in the rhizosphere of *R. serpentina* and *T. asthamatica*. There was much variation in the above factors during the different months of the year-long study. The distribution of AM fungal species has been related to soil pH, temperature, and moisture content of the soil.

Table 1 gives the seasonal variation of mycorrhizal association in *R. serpentine* (Figures 1[a] and 1[b]). It is clear from the table that spore morphotypes increased steadily from August (114.6 ± 1.24) and reached its maximum value in October (172.3 ± 2.05). The spore count then declined steadily and reached its minimum in July (9.7 ± 2.05). Thus, this plant showed maximum abundance of spores in the rainy season followed by the winter season. The maximum abundance of spores was recorded in the summer season.

Mycorrhizal colonization was on the higher side, from August (90.3 ± 0.89) to November (100 ± 0), and the lowest percentage colonization was recorded for the month of January (42.3 ± 1.69). Thus, VAM root colonization was maximum in the beginning of the rainy season and minimum in the winter season. Correlation of VAM spore count and percentage root colonization with moisture content was significant at 0.01 level. Negative correlation was observed with temperature.

Table 2 gives the seasonal variation of mycorrhizal association in *T. asthamatica* (Figures 2[a] and 2[b]). It is clear from the table that the density of VAM spores was maximum in October (161.3 ± 1.69) but the maximity ranged from July (114.7 ± 1.24) onwards. The minimum spore count was found in the months of May (28.7 ± 2.86) and June (25.7 ± 1.69). Similar to *R. serpentine*, the maximum endospore population was recorded in the rainy season followed by the winter season. The minimum spore count was recorded in the summer season. The intensity of root colonization was maximum in October (100 ± 0) and November (100 ± 0) and minimum in December (16.7 ± 4.71) and January (24.2 ± 4.23). Thus, root colonization was maximum in late summer and minimum in winter. Correlation between spore count and root colonization was found to be significant at the 0.05 level.

From the results, it is clear that AM fungal root colonization and spore density have seasonal dynamics. The growth of the host, spore density, and root colonization are functions of plant/fungus combinations (Sanders and Fitter 1992). The different range of spore density and root colonization are due to a wide range of hosts and seasonal changes. Sporulation rates of AM fungi have been found to be host dependent. This is probably one of the reasons that the plants under study have shown different results when compared.

It is well known that fungus colonization is influenced by soil moisture (Ghosh, Biswas,

Bhattacharya, *et al.* 2007). Root colonization and spore density reached their maximum values in summer or rainy season in *R. serpentina* and *T. asthamatica*. This may be due to high metabolic activity of plants and soil moisture in these seasons. The observations of seasonal changes and their relationship to root colonization, soil moisture, and host phenology were similar to the results of other studies. Apple, Thee, Smith-Longozo, *et al.* (2005) investigated seasonal dynamics of mycorrhizal colonization in response to precipitation in the Mojave desert and reported highest colonization in rainy season and lowest during summer drought periods. Cruz, Ishii, Matsumoto, *et al.* (2004) reported 80% AM infection in summer and decreased colonization in October in bahia grass. This is in corroboration to the present investigation, where AM infection increased from 90% to 100% in the plants in summer season and decreased in rainy season. This may be explained by the accumulation of phosphorus in the rainy season due to waterlogging in soil (Mohankumar and Mahadevan 1986).

Setua, Das, Ghosh, *et al.* (2001) revealed that percentage of root colonization and endomycorrhizal spore population was inversely related to soil moisture regime. Similarly, in *T. asthamatica*, at 52.33% moisture content (that is, beyond 45% moisture content), the spore count and percentage root colonization were lesser. This is attributed to the condition that higher moisture levels reduce oxygen tension, thereby affecting the development of the endophyte (Mohankumar and Mahadevan 1988).

Temperature has a strong influence on the activity of VAM fungi (Zhang, Hamel, Kianmehr, *et al.* 1995). Staddon, Heinemeyer, and Fitter (2002) recorded that mycorrhizal fungi respond to increase and decrease in soil temperature. Fitter, Heinemeyer, Husband, *et al.* (2004) also studied the effect of temperature on AM fungi community and found that AM fungi are temperature sensitive. In the plants under study, maximum root colonization and higher VAM spore number was recorded from 20–32°C. This observation is supported by records from several other studies. Germination of mycorrhizal spores occurs more slowly or is entirely inhibited at soil temperature below 18°C (Mohammad, Pan, and Kennedy 1998).

Soil pH influences AM root colonization as well as the growth and phosphatase activity of extraradical mycelium. In the present investigation, extraradical mycelium was formed at higher pH. Similar observations were made by Van Aarle, Olsson, and Soderstrom (2002). The soil pH for the samples studied ranged from 6.1–9.8. A higher soil pH resulted in increase in VAM spore population and percentage of root colonization. A pH of soil between 7.5 and 8.5 was found to be the best salinity level for fungal growth.

Kemp, Adam, and Ashford (2003) correlated percentage of root length with seasons. The percentage colonization was lowest in April/May (50%) and highest in October (70%). Similar observations were made in *T. asthamatica*, where 100% root colonization, maximum VAM spore count, and maximum growth were observed in the month of October.

Maximum number of VAM spores in August and September in *R. serpentina* are in conformity with the observations of Bhatt and Kaveriappa (1999). The lack of correspondence between root colonization and spore maxima during monsoon could be attributed to dormancy of AM spores. The cessation of dormancy in rainy season induces the vegetative phase of AM fungi, resulting in maximum root colonization. Therefore, high percentage of VAM colonization is correlated with active growth of the host plant.

Acknowledgements

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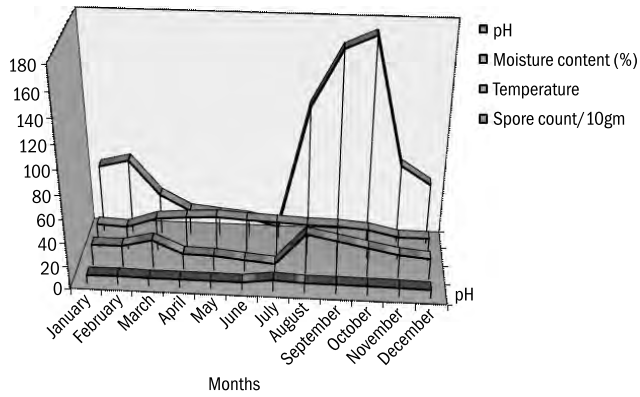

Figure 1(a) Effect of abiotic factors on mycorrhizal spore count in *R. serpentina* in different seasons

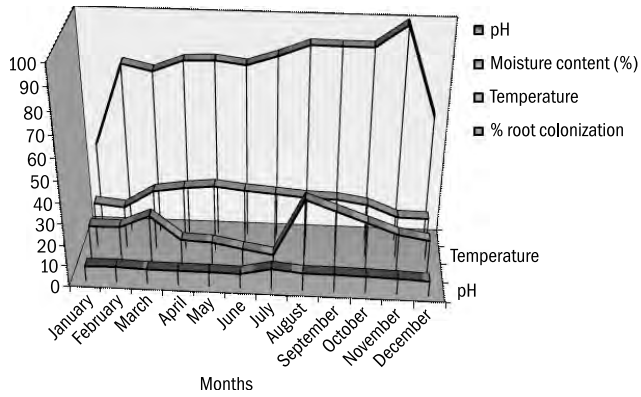

Figure 1(b) Effect of abiotic factors on root colonization in *R. serpentina* in different seasons

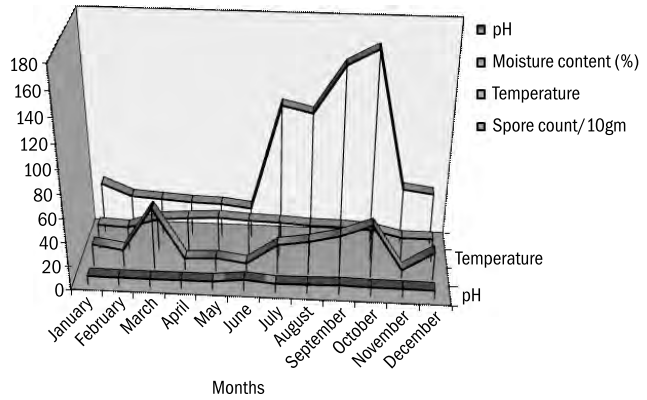

Figure 2(a) Effect of abiotic factors on mycorrhizal spore count in *T. asthamatica* in different seasons

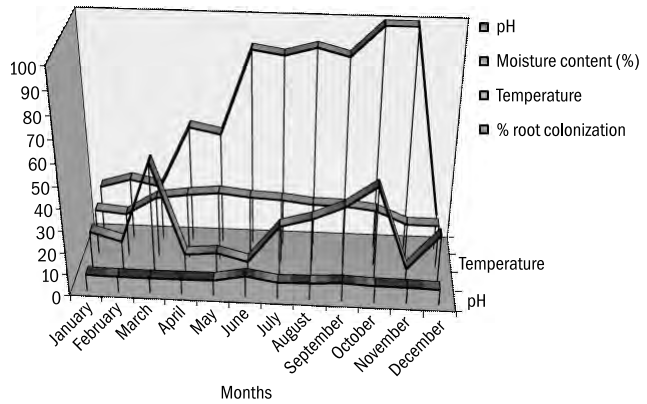

Figure 2(b) Effect of abiotic factors on root colonization in *T. asthamatica* in different seasons

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Table 1 Seasonal variation of mycorrhizal association in *Ranuwolfia serpentina*

Month	Mycorrhizal spore count/10 g of soil	Percentage mycorrhizal root colonization	pH of soil	Temperature of soil	Moisture content of soil
January	58.0 ± 4.32	42.3 ± 1.69	7..5 ± 0.09	21.1 ± 0.36	18.3 ± 2.86
February	64.0 ± 3.55	79.7 ± 6.89	7.7 ± 0.12	19.7 ± 0.25	18.3± 2.35
March	34.7 ± 20.5	76.7 ± 2.49	7.1 ± 0.12	28.1 ± 0.12	24.3 ± 3.29
April	21.3 ± 1.24	81.9 ± 2.68	7.1 ± 0.16	30.3 ± 0.17	13.3 ± 2.35
May	18.7 ± 1.69	82.4 ± 1.69	7.1 ± 0.17	31.6 ± 0.12	12.7 ± 2.49
June	16.3 ± 1.24	80.7 ± 0.94	6.8 ± 0.16	30.1 ± 0.08	10.0 ± 1.63
July	09.7 ± 2.05	85.1 ± 2.59	9.9± 0.05	29.0 ± 0.12	7. 7 ± 2.05
August	114.6 ± 1.24	90.3 ± 0.89	8.4 ± 0.16	27.8 ± 0.25	35 ± 4.08
September	161.3 ± 0.94	90.1 ± 1.06	8.4 ± 0.20	27.5 ± 0.34	30 ± 4.08
October	172.3 ± 2.05	90.1 ± 0.98	8.1 ± 0.12	25.4 ± 0.12	25 ± 4.08
November	68.0 ± 2.16	100 ± 0	7.7 ± 0.08	20.0 ± 0.12	20.0 ± 4.08
December	52.3 ± 1.24	60.7 ± 0.94	7.0 ± 0.21	19.8 ± 0.17	17.7 ± 2.05
Correlation value	0.01	0.293	0.277	– 0.222	*0.800

Note The figures in the table are mean of three replicates (±) standard deviation

* Correlation is significant at the 0.01 level

Table 2 Seasonal variation of mycorrhizal association in *Tylophora asthamatica*

Month	Mycorrhizal spore count/10 g of soil	Percentage mycorrhizal root colonization	pH of soil	Temperature of soil	Moisture content of soil
January	43.0 ± 3.74	24.2 ± 4.23	7.4 ± 0.12	19 ± 2.94	21.0 ± 0.36
February	32.7 ± 3.39	28.3 ± 8.49	7.1 ± 0.12	15 ± 4.08	19.8± 0.17
March	31.0 ± 2.94	25.4 ± 3.18	7.0 ± 0.12	52.3 ± 2.05	28.0 ± 0.09
April	29.3 ± 2.49	53.7 ± 6.43	7.1 ± 0.08	10 ± 4.08	30.0 ± 0.05
May	28.7 ± 2.86	51.0 ± 0.81	7.2 ± 0.08	11.3 ± 2.86	31.4 ± 0.16
June	25.7 ± 1.69	88.5 ± 0.72	9.8 ± 0.05	7.7 ± 2.05	29.9± 0.09
July	114.7 ± 1.24	86.4 ± 4.53	7.5 ± 0.08	25 ± 4.08	29.2 ± 0.17
August	109 ± 0.81	90.1 ± 1.06	7.8 ± 0.12	29 ± 2.94	27.6 ± 0.12
September	148 ± 3.74	86.6 ± 4.71	8.5 ± 0.08	35 ± 4.08	27.4 ± 0.16
October	161.3 ± 1.69	100 ± 0	7.8 ± 0.05	45 ± 4.08	25.7 ± 0.08
November	47.7 ± 2.05	100 ± 0	7. 7 ± 0.05	7.3 ± 2.49	20.0 ± 0.05
December	44.3 ± 2.86	16.7 ± 4.71	7.3 ± 0.08	23.7 ± 2.62	19.8 ± 0.22
Correlation value	0.1	* 0.619	0.178	0.557	0.123

Note The figures in the table are mean of three replicates (±) standard deviation

* Correlation is significant at the 0.05 level

Impact of microbial inoculants on AM colonization in tree legumes

S Dash and N Gupta[1*]

Introduction

Arbuscular mycorrhizal (AM) fungi act as transporters and carriers of various minerals like phosphorus, zinc, manganese, magnesium, copper, and aluminium from soil to the host plants, which help in enhancement of growth and productivity of the plants (Ahanthem and Jha 2007). A wide spectrum of studies have been carried out on their ecology and distribution, their effects on host physiology, biochemistry, and genetics in national and international laboratories. Their bio-fertilizing potential has been well documented with respect to various agricultural, horticultural, and forest plants (Sabarad, Swamy, Patil *et al*. 2007; Li, Smith, Holloway, *et al*. 2006). In the present study, the authors analysed the roots of tree legumes grown in different pots inoculated with mineral solubilizing bacteria and fungi in order to evaluate AM infection and colonization.

Materials and method

A pot experiment was carried out on *Dalbergia sissoo*, *Acacia nilotica*, *Acacia auriculiformis*, *Acacia leucocephala*, and *Adnenthera pavonina* in sandy loam soil. Pre-soaked seeds of the above legumes were sown at 1″ depth of the pot soil. The pot soil was separately inoculated with 25 ml culture of each phosphate solubilising fungi (*Penicillium* sp. [PF1], *Penicillium* sp. [PF2], *Penicillium* sp. [PF3], *Aspergillus* sp. [PF4], *Aspergillus* sp. [PF5], *Aspergillus* sp. [PF6], four iron leaching fungi (*Penicilium* sp. [IF1], *Paecilomyces* sp. [IF4], *Cunnighamella* sp. [IF5], and *Penicillium* sp. [IF6]), and five phosphate solubilizing bacteria (*Streptomyces* sp. [PB1], *Micrococcus* sp. [PB2], *Micrococcus* sp. [PB3], *Micrococcus* sp. [PB4], and *Streptomyces* sp. [PB5]). Watering through misting for 2 hours twice a day was done regularly. Hogland nutrient solution was added to each pot at 25 ml/

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Table 1 Analysis of roots of different tree legumes

Treatment	*Dalbergia sissoo*	*Acacia nilotica*	*Acacia auriculiformis*	*Acacia leucocephala*	*Adnanthera pavonina*
Control	35.18	33.33	0	1.85	0
Penicillium sp. (PF1)	0.92	0	0	0	0
Penicillium sp. (PF2)	12.03	38.8	0	0	17.59
Penicillium sp. (PF3)	0	0	0	3.7	0
Aspergillus sp. (PF4)	3.7	0	0	0	33.33
Aspergillus sp. (PF5)	6.4	0	0	0	38.88
Aspergillus sp. (PF6)	1.85	2.7	0	7.4	36.11
Penicillium sp. (IF1)	10.1	28.43	0	0	12.03
Paecilomyces sp. (IF4)	0	0	0	0	13.88
Cunninghamella sp. (IF5)	25	22.22	0	0	0
Penicillium sp. (IF6)	0.92	7.6	0	0	0
Streptomyces sp. (PB2)	11.1	22.8	0	0	0.92
Micrococcus sp. (PB3)	0	0	0	31.48	0
Micrococcus sp. (PB4)	44.4	27.7	0	0	0
Micrococcus sp. (PB5)	0	4	0	0	0
Streptomyces sp. (PB6)	13.88	73.2	0	0	0

month. Finally, 120-day-old plant roots were taken out for analysis of AM colonization by following KOH digestion and trypan blue staining of 1 cm roots bits of each tree legume (Phillips and Hayman 1970; Kormanic and McGraw 1982). Microscopic observations for the presence of vesicles and arbuscules within the stained roots were recorded and per cent colonization was calculated.

Results and discussion

Analysis of roots of different tree legumes exhibited AM colonization. However, they differed in occurrence of colonization and their patterns. Since the experiment was carried out in natural and unsterile soil, uninoculated plants were also infected and colonized, except for *A. auriculiformis* (Table 1). The treatment of all fungal strains (except IF5, PF3, and PF1) exhibited good AM colonization in the roots of *A. pavonina***.** Infection was absent in plants of bacterial treatment. Uninoculated plants had no AM infection too. Similarly, *A. leucocephala* showed less infection in AM fungi in different plants grown under different treatments, except for PB3 (31.48%). Although plants of *A. leucocephala* grown under treatments of PF4 and IF5 showed optimal growth, their roots did not show any AM infection under these treatments. Most of the inoculated plants of *A. nilotica* exhibited AM colonization. Maximum colonization (73.2%) occurred in plants of PB6 treatment. Almost all treatments showed AM colonization in roots of *D. sissoo***,** except for PF3, IF4, PB3, and PB5 inoculations. The highest per cent colonization was observed in PB4 (44.4%). Control sets showed AM infection and colonization. As compared to fungal inoculation, bacterial inoculation encouraged increase in per cent AM colonization in the roots of *D. sissoo***,** *A. leucocephala***,** and *A. nilotica***.** A limited range of colonization implies low levels of potentiality and infectivity of indigenous AM fungi. It may also reflect the lower potential of forest trees toward AM infection (Rahangdale and Gupta 1999).

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Mycorrhization in medicinal plants

M Singh, P Singh, and D Vyas[*]

Introduction

A discovery of arbuscules in Agalophyton major, an early devonian plant, provides unequivocal evidence that mycorrhizae were established more than 400 million years ago (Remy, Taylor, Hass 1994). Mycorrhiza is the mutualistic symbiosis (non-pathogenic association) between the soil and roots of higher plants (Quilambo 2003). Mycorrhizae are found in a wide range of habitats, usually in the roots of angiosperms, gymnosperms, and pteridophytes. They also occur in the gametophytes of some mosses, lycopods, and psilotes, which are rootless (Mosse, Stribley, and Tacon 1981; Vyas, Dubey, Soni, *et al.* 2007; Vyas, Singh, Mishra, *et al.* 2008). Arbuscular mycorrhizal (AM) fungi associated with plants of Asclepidaceae have been reported.

Gupta, Mohankumar, and Janardhanan (1995) and Soni and Vyas (2007) observed the association of AM fungi with medicinal and aromatic plants. The main benefit for vascular plants having arbuscular mycorrhiza has usually been considered to be increased access to soil nutrients, in particular phosphorus. Apart from phosphorus, AM fungi have shown to be potentially able to take up both organic (Hodge, Campbell, and Fitter 2001) and inorganic nitrogen from the soil (Govindarajulu, Pfeffer, Jin, *et al.* 2005). Borie, Rubio, and Morales (2008) reviewed close relationships between glomalin and soil aggregates found in Chilean soil. Vesicular–arbuscular mycorrhiza (VAM) fungi are essential components of the ecosystem for revegetation of degraded lands and maintenance of soil structure (Caravaca, Alguacil, Barea, *et al.* 2005), thereby reducing the risks of erosion and desertification. Dormancy and distribution patterns of AM fungi spores in the soil have been reported (Bever, Morton, Antonovics, *et al.* 1996; Gemma and Koske 1988; Greipson and El-Mayas 2000; Koske 1987; Koske and Halvorson 1981; Sylvia 1986; Walker, Mize, and McNabb 1982; Zhao 1999). Guadarrama and Alvarez-Sanchez (1999) pointed out that disturbance, but not seasonality, affected the abundance and richness of mycorrhizal spores in a tropical wet forest in Mexico.

The most important and historical account of medicine in the form of "Ayurveda" (2500–900 BC), which is considered as "Upaveda", "Charak Samhita", and "Susruta Samhita", also dealt with plants related to medicine and their use in health management. Currently, cultivation of medicinal plants has increased in order to fulfil the increasing demands of pharmaceutical industries. Thus, based on the above facts, the authors undertook the present study to understand the role AM fungi in association with test medicinal plants so that their biofertilizing potential can be exploited accordingly.

Materials and method

The study was conducted at a semi-natural grassland in Sagar (Madhya Pradesh), located between the latitudes of 23°10′N to 24°27′N and longitudes of 78°4′E to 79°21′E, near the Tropic of Cancer. The climate of this region is subtropical monsoon and the year is divisible into a mild winter (October to February), a hot summer (March to June), and a warm rainy season (July to September).

Three independent samples were taken for the present study (2007/08). Fine terminal feeder roots were collected in polythene bags during rainy and winter seasons from different places and were brought to the laboratory for mycorrhizal assessment. The clearing and staining of roots and estimation of the degree of root colonization was done following the slide method, as described by Giovannetti and Mosse (1980).

Results

During the present study, about 69 plants belonging to 26 families showed mycorrhizal association. Interestingly, not only did the degree of mycorrhization vary but variability in the internal structure of the arbuscules and vesicles was also observed. Correlation between the number of spores and per cent colonization could be drawn. However, variations among the plants of same family were observed. From the results, it is deduced that mechanism of mycorrhization in test plants does not follow any thumb rule.

Two members belonging to family Acanthaceae—*Adhathoda vasica* Nees and *Andrographis paniculata* Nees—showed 206.4 ± 16.32, 296.7 ± 19.56 average spore density (ASD) and 32.3 ± 2.15, 52.6 ± 3.56 per cent root colonization (PRC) respectively. Intermediate arbuscules and globose vesicles with

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hyphae were present, while vesicles with hyphal thread followed by *Arum*-type arbuscules were absent.

Four genera of the family Amaranthaceae were studied—*Achyranthus aspera* Linn, *Alternanthera sessilis* Linn Dc., *Amaranthus spinosus* Linn, and *Trigonella foenumgraeceum* Linn. Among all the test plants, *T. foenumgraeceum* Linn shows greater ASD (345 ± 36.43), mycorrhizal status, PRC (64.7 ± 6.35), Arum-type arbuscules, and intermediate globose/globose vesicles with hyphae. Arbuscules and vesicles were not observed in the other members of the family, and only hyphae were present.

Launaea pinnatifida Roxb of family Anacardiaceae was studied. It showed 436.3 ± 28.24 ASD, 69.6 ± 3.94 PRC, and presence of Arum-type arbuscules and globose vesicles with hyphae.

Centella asiatica Linn of the family Apiaceae was studied. It showed 312.4 ± 29.61 ASD and 70.3 ± 17.19 PRC. Similar to *L. pinnatifida* Roxb, it showed the presence of Arum-type arbuscules and globose vesicles with hyphae.

The family Apocynaceae was represented by three genera—*Rauwolfia serpentine* Benth ex. Kurz, *Rauwolfia tetraphylla* Linn, and *Catharanthus roseus* (L) G. Don. These three genera differed in their mycorrhizal status. *C. roseus* (L) G. Don. harboured greater number of ASD (783 ± 30.34) and PRC (65.6 ± 6.27), followed by *R. tetraphylla* Linn (711.0 ± 23.08 ASD and 62.4 ± 5.59 PRC) and *R. serpentine* Benth ex. Kurz (544.8 ± 10.62 ASD and 57.6 ± 5.59 PRC). Arum-type arbuscules and globose vesicles with presence of hyphal thread were seen in all three test plants.

Acorus calamus Linn of the family Araceae was also studied. It showed 194.7 ± 13.20 ASD and 24.71 ± 1.96 PRC. Presence of, hyphal thread was observed but arbuscules and vesicles were not seen.

The family Asclepiadaceae was represented by two genera—*Calotropis procera* R. Br. and *Hemidesmus indicus* Linn. Both showed significant difference in mycorrhizal status. In case of *C. procera* R. Br., the spore count was 285.8 ± 22.46 ASD and 50.6 ± 6.02 PRC, the arbuscules were intermediate, and Paris-type vesicles were subglobose with hyphae were present. In contrast, *H. indicus* Linn rhizosphere showed 175.2 ± 9.45 ASD and 14.2 ± 1.89 PRC. Arbuscules and vesicles were absent, while hyphae were present.

The family Asteraceae was represented by three genera—*Eclipta alba* Roxb, *Tridex procumbens* Linn, and *Vernonia cinerea* Less. There was no similarity among the test plants regarding their mycorrhizal status. The rhizospheric soil of *E. alba* Roxb showed 662.4 ± 15.21 ASD, which was higher than the other genera. The PRC recorded was 54.8 ± 3.97, Arum-type arbuscules and hyphae were present but vesicles were absent. *T. procumbens* Linn showed comparatively lesser ASD (468.0 ± 32.72) and the PRC recorded was 61.8 ± 2.87. Intermediate type arbuscules and globose and subglobose vesicles with hyphae were present. *V. cinerea* Less showed 187.0 ± 19.76 ASD, 18.67 ± 2.03 PRC. Presence of globose vesicles and hyphae was observed, while arbuscules were absent.

Differential mycorrhizal status was observed with the representative members of family Caesalpiniaceae—*Cassia tora* Linn and *Cassia occidentalis* Linn. The latter showed comparatively greater ASD (574.0 ± 18.51) than *C. tora* Linn (445.9 ± 36.09). Interestingly, the PRC observed was greater in *C. tora* Linn (82.4 ± 12.14) than *C. occidentalis* Linn (61.2 ± 3.92). Both plants showed intermediate and Paris-type arbuscules. Subglobose and globose vesicles with hyphae were also present.

The family Convolvulaceae was represented by three genera—*Ipomoea aquatic* Forsk, *Convolvulus pluricaulis* Linn, and *Evolvulus alsinoides* Linn. Differentiation in mycorrhizal status was observed in all three plants. *I. aquatic* Forsk harboured greater number of ASD (427.4 ± 24.96), followed by *E. alsinoides* (232.0 ± 21.97) and *C. pluricaulis* (203.0 ± 19.42). Greater PRC was observed for *E. alsinoides* Linn (58.4 ± 2.71), followed by *C. pluricaulis* Linn (43.9 ± 11.3) and *I. aquatic* Forsk (38.4 ± 3.79). In all three test plants, Arum-type arbuscules and hyphae are present with the absence of vesicles.

Two members of the family Cyperaceae—*Cyperus rotundus* R. Br. and *Cyperus scariosus* R—were observed and studied. The latter showed comparatively greater ASD (126.3 ± 3.94) and PRC (14.2 ± 1.42) than *C. rotundus* R. Br. (103 ± 4.36 ASD and 11.3 ± 1.92 PRC). Arbuscules and vesicles were not observed, while only hyphal threads were present in the members of this family.

The family Euphorbiaceae was represented by four genera—*Euphorbia geniculata* Orteg, *Euphorbia hirta* Linn, *Jatropa curcus* Linn, and *Phyllanthus niruri* Linn. The mycorrhizal status among the test plants was somewhat similar except in *E. hirta* Linn, where the ASD was greatest (401.0 ± 31.74), followed by *P. niruri* Linn, *J. curcus* Linn, and *E. geniculata* Orteg. The PRC was greatest in *E. geniculata* Orteg (42.8 ± 3.97), followed by *J. curcus* Linn, *P. niruri* Linn, and *E. hirta* Linn. Except for *P. niruri* Linn, all three plants showed Arum-type arbuscules. Subglobose and globose vesicles were seen only in *E. hirta* Linn and *J. curcus* Linn.

The family Lamiaceae was represented by five genera—*Occimum bassilicum* Linn, *Occimum sanctum* Linn, *Occimum gratissimum* Linn, *Mentha arvensis*

Linn, and *Mentha piperita* Linn. *O. gratissimum* Linn showed greater ASD (415.23 ± 26.48) and higher PRC (72.54 ± 4.89), followed by *M. piperita* Linn (413.4 ± 32.46 ASD and 72.26 ± 22.48 PRC). Minimum ASD and PRC within the family were recorded in *O. bassilicum* L (378.0±23.88 ASD and 56.4 ± 3.21 PRC). Arum-type arbuscules and globose vesicles were seen in all the test plants of the family. However, *O. gratissimum* Linn and *M. arvensis* Linn showed subglobose vesicles; intermediate vesicles were also seen in *O. bassilicum* Linn.

The family Liliaceae was represented by three genera—*Asperagus racemosus* Linn, *Gloriosa superba* Linn, and *Aloe indica* Mill. All three members differed in their spore density and mycorrhization. *A. racemosus* Linn harboured greater number of ASD (222.0 ± 17.72) and PRC (48.4 ± 2.21) than the other genera. Arbuscules and vesicles were not present, although presence of hyphal threads was observed.

The family Malvaceae was represented by *Sida cordifolia* Linn and *Sida spinosa* Linn. ASD of 327.0 ± 12.05 and 312.6 ± 30.63 was observed in *S. spinosa* Linn and *S. cordifolia* Linn, respectively. The PRC was greater in *S. cordifolia* Linn (63.0 ± 5.59) than in *S. spinosa* Linn (61.8 ± 2.49). While both test plants showed Arum-type arbuscules and globose vesicles, intermediate globose vesicles were seen in *S. spinosa* Linn.

The family Mimosaceae was represented by *Acacia arabica* Wild, *Acacia catechu* Wild, *Albizzia lebbeck* Benth, and *Mimosa pudica* Linn. Among the four genera, *A. arabica* Wild and *A. catechu* Wild showed almost similar mycorrhizal status, whereas *A. lebbeck* Benth and *M. pudica* Linn showed different mycorrhizal status. *A. arabica* Wild and *A. catechu* Wild showed 225.4 ± 12.32 and 234.6 ± 14.31 ASD and 36.4 ± 2.04 and 39.6 ± 3.03 PRC, respectively. *Arum* type arbuscules, subglobose vesicles, and hyphae were found in the genus *Acacia*. *A. lebbeck* Benth showed 239.6 ± 29.43 ASD, 33.6 ± 3.46 PRC. Presence of Arum-type arbuscules, subglobose, globose or elliptical vesicles was observed. *M. pudica* Linn showed 217.7 ± 19.63 ASD, 41.7 ± 6.42 PRC, lack of arbuscules, and globose and elliptical vesicles with hyphae.

Oxalis corniculata Linn and *Oxalis indica* Linn represented the family Oxalidaceae. In this family, the spore density and PRC recorded was higher than other families. *O. indica* Linn showed 812.36 ± 32.45 ASD and 90.82 ± 4.12 PRC, while *O. corniculata* Linn showed 776.0 ± 30.49 ASD and 86.0 ± 3.09 PRC. Arum-type arbuscules, globose and subglobose vesicles with hyphae were seen in both test plants.

The family Papilionaceae was represented by five genera—*Abrus precatorius* Linn, *Butea monosperma* Lank, *Desmodium gangeticum, Pongamia pinneta* Linn, and *Mucuna puriens* (L)D.C. Among these, *D. gangeticum* showed a greater number of ASD (416.7 ± 26.04) and higher PRC (76.7 ± 13.09) than *M. puriens* (L)D.C (216.3 ± 2.04 ASD and 43.6 ± 6.49 PRC). The other test plants of the family showed ASD and PRC between these two ranges. All the test plants showed Arum-type arbuscules, while *B. monosperma* Lank showed intermediate type arbuscules. Vesicles were not observed in *A. precatorius* Linn. *D. gangeticum*. *P. pinneta* L showed globose and subglobose vesicles with presence of hyphae.

Plantago ovata Forsk represented the family Plantaginaceae. It showed 107.6 ± 4.07 ASD and 14.3 ± 1.32 PRC, with the presence of only hyphal thread.

The family Poaceae was represented by six genera—*Cynodon dactylon* (Linn) Pers, *Cymbopogan martini* (Roxb) Wats, *Apluda mutica* Linn, *Themed quadrivolvis* Linn, *Sporobolus coromandelianus* (Retz) Kunth, and *Diacanthium annulatum* (Forsk) Staff. Among these six genera, *C. dactylon* (Linn) Pers showed maximum ASD (907.0 ± 19.24) and PRC (90.0 ± 3.81). Minimum ASD (589.0 ± 14.45) and PRC (70.0 ± 2.96) was recorded in *T. quadrivolvis* Linn. However, other plants showed ASD and PRC values between that of *C. dactylon* (Linn) Pers and *T. quadrivolvis* Linn. Arum-type arbuscules and globose vesicles are features of the family except for *C. martini* (Roxb) Wats, where Paris-type arbuscules were also observed. No vesicles were formed in the genera of this family.

Ziziphus jujube Mill represented the family Rhamnaceae. It harboured 338.9 ± 34.76 ASD and 52.7 ± 5.04 PRC. It showed the presence of Arum-type arbuscules and globose vesicles with presence of hyphal thread.

The family Rubiaceae was represented by *Rubia cordifolia* Linn, with 113.6 ± 12.31 ASD and 9.43 ± 1.04 PRC. Arbuscules and vesicles were absent and only hyphae were present.

The family Scrophulriaceae was represented by three genera—*Bacopa monniera* Linn, *Leucas aspera* Spreng, and *Leucas cephalotes* Spreng. Among the three genera, the maximum number of ASD and PRC was recorded in *L. cephalotes* Spreng (317.4 ± 24.23 ASD and 62.6 ± 15.64 PRC), followed by *L. aspera* Spreng (312.9 ± 24.32 ASD and 52.0 ± 4.04 PRC) and *B. monniera* Linn (172.2 ± 11.41 ASD and 24.6 ± 3.02 PRC). *L. aspera* Spreng and *L. cephalotes* Spreng showed Arum-type arbuscules and globose to subglobose vesicles. Arbuscules were not present in *B. monniera* Linn, while globose to subglobose vesicles with hyphae were observed.

The family Solanaceae was represented by five genera—*Solanum nigrum* Linn, *Datura metal* Linn, *Solanum xanthocarpum, Withania somnifera* Linn

Dunal, and *Atropa belladonna* Linn, *S. nigrum* Linn showed high spore density (645.7 ± 46.73 ASD) and PRC (86.7 ± 9.43), followed by *D. metal* Linn (613.4 ± 24.86 ASD and 61.2 ± 5.72 PRC). Interestingly, *S. xanthocarpum* showed lesser ASD within the family but high PRC (79.8 ± 8.12), whereas *W. somnifera* Linn Dunal showed high ASD but comparatively lesser PRC. All the test plants showed Arum-type arbuscules, while *D. metal* Linn also showed intermediate type arbuscules. Except for *W. somnifera* Linn Dunal and *A. belladonna* Linn, the plants showed globose vesicles.

Lantana camara Linn and *Vitex negundo* Burn represented the family Verbenaceae. The former showed spore density of 492.6 ± 32.62 and PRC of 74.3 ± 5.62. Paris-type arbuscules. Globose and intermediate globose vesicles with hyphae were present. On the other hand, *V. negundo* Burn showed 275.7 ± 27.31 ASD and 57.6 ± 4.39 PRC, presence of Arum-type arbuscules and globose vesicles with hyphae.

The family Zygophyllaceae was represented by *Tribulus terrestris* Linn, which showed 201.0 ± 19.43 ASD, 30.3 ± 3.03 PRC, absence of arbuscules and presence of subglobose vesicles with hyphae.

Discussions

The results obtained from the present study suggest that all the plants exhibit mycorrhizal association. However, the degree of per cent root colonization and presence of arbuscules and vesicles varies from one plant to another. It is evident from the results that there is no correlation between these factors (per cent root colonization and presence or absence of arbuscular and vesicular structures). Hence, it can be deduced that each plant has its own preference towards mycorrhization. Several environmental factors such as soil moisture, temperature, pH, nutrients (especially nitrogen and phosphorus), organic matter, and changes in plant community composition can influence the composition of VAM fungal species (Johnson, Tilman, and Wedin 1982; Jansa, Mozafar, Kuhn, *et al.* 2003). The diversity and abundance of VAM fungal species are generally reduced by disturbance in ecosystems (Lovelock and Ewel 2005). In semi-natural conditions, management practices (such as burning, moving and fertilization) decrease local biodiversity and threaten ecosystem productivity and sustainability of nutrient cycling, subsequently reducing AM fungal diversity (Bentivenga and Hetrick 1991; Eom, Hartnett, and Wilson 1999).

The presence of arbuscules is normally used to designate AM associations, although the presence of hyphae or vesicles is also evidence of these associations. Generally, arbuscules are die within 15 days of their formation and are therefore sometimes not found in older roots. This may be attributed to the inactiveness of roots when samples were collected, whereas vesicles are considered as storage organs produced in the older regions of infection. *Arum*-type colonization was dominant among all the test plants examined. Since vegetation of the sampling site was typical of a natural forest, Arum-type dominance may be a general characteristic of most vegetation in this region. O'Connor, Smith, and Smith (2002) also observed *Paris*-type colonization, which is rare among AM plants examined in the southern Simpson Desert in Australia. It is well known that most cultivated herbs grow without any shading from *Arum*-type AM fungi (Smith and Smith 1997). It has also been shown that AM morphological type of fungi is controlled by the host plant (Gerdemann 1965). A strong link between plant identity and AM morphology was observed at the present field site, where AM morphological type fungi was determined mostly at the plant family level. However, some inconsistency of morphological type occurred at the family level in previous studies (Vyas, Singh, Mishra, et al. 2008).

During the present study, all conditions except root exudation are similar because the plant weeds were collected from the natural forest of Pathariya hill, Sagar. Thus, any influence of root exudation would have been negligible on mycorrhizal association in test plants. Moreover, grazing might influence the root colonization among these plants. It has been reported that root exudation by plants influences the occurrence of mycorrhizal species (Walker, Bais, Gratewold, *et al.* 2003). Similarly, it has also been reported that grazing influences mycorrhizal associations (Eom, Wilson, and Hartnett 2001). In earlier studies (Dwivedi, Yadav, Vyas, *et al*, 2003, 2004; Soni and Vyas 2007; Vyas and Soni 2004; Vyas, Mishra, Singh, *et al.* 2006; Vyas, Singh, Mishra, *et al.* 2008), occurrence of mycorrhizal association and per cent root colonization was observed in a number of plants. Chaurasia and Khare (1999) reported the occurrence of VAM fungi and root colonization in few grasses and forbs from Sagar. Greater colonization of VAM fungi in roots of *C. dactylon* (Linn) Pers, followed by *O. indica* Linn and *C. tora* Linn, may play an important role for its sustainable ecosystem.

Conclusion

As is evident from the above discussion, *C. dactylon* (Linn) Pers, a member of the family Poaceae, not only harbours maximum number of spores but also colonizes 90% of the root cortex. Although *O. indica* Linn of the family Oxalidaceae harbours comparatively lesser number of spores, it colonizes the same area of roots (90%). On the other hand, *C. roseus*

(L) g. Don of the family Apocynaceae, which harbours 783.0 ± 30.34 ASD, is able to undergo 65.6% ± 6.27% colonization. In spite of the difference in degree of colonization, the three plants share common internal structures (that is, Arum-type arbuscules and globose vesicles with presence of hyphae). *A. indica* Mill of the family Liliaceae, *C. rotundus* R. Br. of Cyperaceae, and *P. ovata* Forsk of Plantaginaceae

Table 1 Mycorrhization in medicinal plants

Family	Test plant	ASD (Mean ± SD)	Root mycorrhization			
			PRC	A	V	H
Acanthaceae	*Adhathoda vasica* Nees	206.4 ± 16.32	32.3 ± 2.15	I	_	+
	Andrographis paniculata Nees	296.7 ± 19.56	52.6 ± 3.56	A	G	+
Amaranthaceae	*Achyranthus aspera* Linn	141.0 ± 34.72	10.0 ± 10.49	_	_	+
	Alternanthera sessilis Linn Dc.	169.4 ± 10.53	14.0 ± 8.34	_	_	+
	Amaranthus spinosus Linn	145.0 ± 29.13	18.4 ± 5.95	_	_	+
	Trigonella foenumgraeceum Linn	345.7 ± 36.43	64.7 ± 6.35	A	IG, G	+
Anacardiaceae	*Launaea pinnatifida* Roxb	436.3 ± 28.24	69.6 ± 3.94	A	G	+
Apiaceae	*Centella asiatica* Linn	312.4 ± 29.61	70.3 ± 17.19	A	G	+
Apocynaceae	*Rauwolfia serpentine* Benth ex. Kurz	544.8 ± 10.62	57.6 ± 5.59	A	G	+
	Rauwolfia tetraphylla Linn	711.0 ± 23.08	62.4 ± 559	A	G	+
	Catharanthus roseus (L) G. Don.	783.0 ± 30.34	65.6 ± 6.27	A	G	+
Araceae	*Acorus calamus* Linn	194.7 ± 13.20	24.71 ± 1.96	_	_	+
Asclepiadaceae	*Calotropis procera* R. Br.	285.8 ± 22.46	50.6 ± 6.02	I/P	SG	+
	Hemidesmus indicus Linn	175.2 ± 9.45	14.2 ± 1.89	_	_	+
Asteraceae	*Eclipta alba* Roxb	662.4 ± 15.21	54.8 ± 3.97	A	_	+
	Tridex procumbens Linn.	468.0 ± 32.72	61.8 ± 2.87	I	G, IG	+
	Vernonia cinerea Less	187.0 ± 16.76	18.67 ± 2.03	_	G	+
Caesalpiniaceae	*Cassia tora* Linn	445.9 ± 36.09	82.4 ± 12.14	I/H	SG, G	+
	Cassia occidentalis Linn	574.0 ± 18.51	61.2 ± 3.97	I/P	SG	+
Convolvulaceae	*Ipomoea aquatic* Forsk	427.4 ± 24.96	38.4 ± 3.79	A	_	+
	Convolvulus pluricaulis Linn	203.0 ± 19.42	43.9 ± 11.3	A	SG	+
	Evolvulus alsinoides Linn	232.0 ± 21.97	58.4 ± 2.71	A	_	+
Cyperaceae	*Cyperus rotundus* R. Br.	103.4 ± 4.36	11.3 ± 1.92	_	_	+
	Cyperus scariosus R. Br.	126.3 ± 3.94	14.2 ± 1.42	_	_	+
Euphorbiaceae	*Euphorbia geniculata* Orteg	212.0 ± 17.54	42.8 ± 3.97	A	_	+
	Euphorbia hirta Linn	401.0 ± 31.74	36.0 ± 3.08	A	SG, G	+
	Jatropa curcus Linn	212.4 ± 10.04	42.3 ± 2.25	A	G	+
	Phyllanthus niruri Linn	264.0 ± 19.18	40.0 ± 5.88	_	_	+
Lamiaceae	*Occimum bassilicum* Linn	378.0 ± 23.88	56.4 ± 3.21	A	G, IG	+
	Occimum sanctum Linn	390.42 ± 24.12	60.56 ± 3.54	A	G	+
	Occimum gratissimum Linn	415.23 ± 26.48	72.54 ± 4.89	A	G, SG	+
	Mentha arvensis Linn	390.3 ± 29.49	69.7 ± 19.76	A	G, SG	+
	Mentha piperita Linn	413.4 ± 32.46	72.26 ± 22.48	A	G	+
Liliaceae	*Asperagus racemosus* Linn	222.0 ± 17.72	48.4 ± 2.21	_	_	+
	Gloriosa superba Linn	119.2 ± 13.25	12.3 ± 2.15	_	_	+

Contd...

Contd...

Family	Test plant	ASD (Mean ± SD)	Root mycorrhization			
			PRC	A	V	H
	Aloe indica Mill	97.8 ± 6.43	16.7 ± 1.32	_	_	+
Malvaceae	*Sida cordifolia* Linn	312.6 ± 30.43	63.0 ± 5.69	A	G	+
	Sida spinosa Linn	327.0 ± 12.05	61.8 ± 2.49	A	G, IG	+
Mimosaceae	*Acacia arabica* Wild	225.4 ± 12.32	36.4 ± 2.04	A	SG	+
	Acacia catechu Wild	234.6 ± 14.31	39.6 ± 3.03	A	SG	+
	Albizzia lebbeck Benth	239.6 ± 29.43	33.6 ± 3.46	A	SG, G, E	+
	Mimosa pudica Linn	217.7 ± 19.63	41.7 ± 6.42	_	G, E	+
Oxalidaceae	*Oxalis corniculata* Linn	776.0 ± 30.49	86.0 ± 3.09	A	G, SG	+
	Oxalis indica Linn	812.36 ± 32.45	90.82 ± 4.12	A	SG, G	+
Papilionaceae	*Abrus precatorius* Linn	331.2 ± 22.42	54.3 ± 3.22	A	_	+
	Butea monosperma Lank	275.3 ± 18.92	45.7 ± 5.09	I	G	+
	Desmodium gangeticum	416.7 ± 26.04	76.7 ± 13.09	A	SG, G	+
	Pongamia pinneta Linn	363.9 ± 29.32	67.4 ± 17.64	A	G, SG	+
	Mucuna puriens(L)D.C.	216.3 ± 20.04	43.6 ± 6.49	A	_	+
Plantaginaceae	*Plantago ovata* Forsk	107.6 ± 4.07	14.3 ± 1.32	_	_	+
Poaceae	*Cynodon dactylon* (Linn) Pers	907.0 ± 19.24	90.0 ± 3.81	A	G	+
	Cymbopogan martini (Roxb) Wats	860.0 ± 15.82	84.0 ± 4.19	A/P	_	+
	Apluda mutica Linn	609 ± 15.56	75.0 ± 2.34	A	G	+
	Themed quadrivolvis Linn	589 ± 14.45	70.0 ± 2.96	A	G	+
	Sporobolus coromandelianus (Retz) Kunth	898 ± 16.67	79.0 ± 3.64	A	G	+
	Diacanthium annulatum (Forssk) Staff	665 ± 17.23	80.0 ± 2.88	A	G	+
Rhamnaceae	*Ziziphus jujube* Mill	338.9 ± 34.76	52.7 ± 5.04	A	G	+
Rubiaceae	*Rubia cordifolia* Linn	113.6 ± 12.31	9.43 ± 1.04	_	_	+
Scrophulariaceae	*Bacopa monniera* Linn	172.2 ± 11.41	24.6 ± 3.02	_	G, SG	+
	Leucas aspera Spreng	312.9 ± 24.32	52.0 ± 4.04	A	SG	+
	Leucas cephalotes Spreng	317.4 ± 24.23	62.6 ± 15.64	A	G	+
Solanaceae	*Solanum nigrum* Linn	645.7 ± 46.73	86.7 ± 9.43	A	G	+
	Datura metal Linn	613.4 ± 24.86	61.2 ± 5.72	A/I	G	+
	Solanum xanthocarpum	447.8 ± 40.43	79.8 ± 8.12	A	G	+
	Withania somnifera Linn Dunal	561.0 ± 41.30	58.0 ± 11.52	A	_	+
	Atropa belladonna Linn	459.5 ± 3.25	53.1 ± 1.02	A	_	+
Verbenaceae	*Lantana camara* Linn	492.6 ± 32.62	74.3 ± 5.62	P	G, IG	+
	Vitex negundo Burn	275.7 ± 27.31	57.6 ± 4.39	A	G	+
Zygophyllaceae	*Tribulus terrestris* Linn	201.0 ± 19.43	30.0 ± 3.03	_	SG	+

not only show lesser AM fungi spores density but also lesser per cent of colonization.

However, *A. aspera* Linn of Amaranthaceae shows 10% root colonization, followed by *C. rotundus* R. Br. (11.3%) and *G. superba* Linn (12.3%) respectively. All three plants belonging to three different families share common internal features such as lack of arbuscules and vesicles on the mycelium, which was poorly distributed among the root cells. This data suggests a relationship between species of AM fungi and the host plant, which regulates the ASD and PRC.

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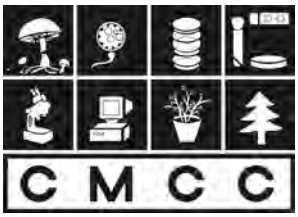

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Biofertilizers regulation: the need of the hour

*Shanuja Beri, and Alok Adholeya**

India is the second most populous country in the world, and its cultivable land resource is shrinking day by day. Moreover, the country has witnessed yield stagnation, despite the use of increased doses of chemical fertilizers, deterioration of soil organic stock, low fertilizer use efficiency, nutrient removal and low replenishment to the soil, and imbalance in NPK ratio among other deleterious effects. To meet the food, fibre, fuel, fodder, and other needs of the growing population, the productivity of agricultural land has to be increased. This necessitates the increased use of agricultural inputs like quality seeds, water, and agro-chemicals in a sustainable manner. Moreover, fertilizers have to be added judiciously. Chemical fertilizers, though an important plant nutrient, have to be augmented by economical and environmental viable options such as biological alternatives.

Biofertilizers are biotechnological products that use living organisms from the plant's own environment. These soil and root inhabiting micro-organisms have the property of mobilizing plant nutrients from unavailable to available forms to increase the productivity of the soil and/or crop. The methods used by such micro-organisms include nitrogen fixation, phosphorus solubilization, and nutrient mobilization. These micro-organisms are also known as microbial fertilizers and display an ability to grow well in artificial media with genetic stability. They are generally carrier based, which could be solid or liquid, and allow the biofertilizer to remain viable for the given shelf life period. Biofertilizers have an ability to tolerate environmental stress and can persist in soil stress.

Although biofertilizers hold much promise, they were initially inefficient as there were no quality checks in place and the market was overrun by products with different levels of efficiency. They were first commercially produced in 1964 with Rhizobium produced by Nitragin, which was imported to India. This biofertilizer was soon produced by the Indian Agricultural Research Institute (IARI), Tamil Nadu Agricultural University (TNAU), Coimbatore, and Jawaharlal Nehru Krishi Vishwavidyalaya (JNKVV), Jabalpur. A national project on biofertilizers was initiated in 1990 and large-scale demonstrations were organized during 1991 and 2001. The government began taking an avid interest in the production of biofertilizers and began promoting it by offering incentives to production units. As a result, hundreds of units were set up, which began producing not only Rhizobium but also Azotobacter, Azospirillium, phosphate solubilizers (PSBs), blue-green algae, and Azolla.

Biofertilizers also faced many constraints in their efficiency on the field. The carrier used was lignite and invariable sterility was maintained. Moreover, ambient temperatures are very high and there was lack of cold chain storage systems for effective storage of the biofertilizers till they reach the fields. As a result of these disadvantages, including a slower response as compared to chemical fertilizers, biofertilizers proved inefficient to win over the end users. Due to offers of some policy and subsidy incentives, many producers churned out spurious products due to which farmers began to lose faith in biofertilizers. The professed quantity and viability was not a reality in the product and it was contaminated with other micro-organisms. Some measures were brought into place to improve the quality of biofertilizers, including introduction of sterile liquid carriers, temperature-tolerant strains, consortia of inoculants, and better formulations resulting in a longer shelf life. Moreover, the government increased the financial support to units producing sterile liquid

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inoculants and provided a subsidy if the unit produced biopesticides as well. All biofertilizers were made exempt from excise duty and, in certain states, sales tax. These measures helped increase the production and subsequent use of biofertilizers to 20 000 MT; however, quality issues always remained least addressed.

Recognizing the urgent need for not only increasing the production but of producing high quality biofertilizers, the Government of India brought biofertilizers under statutory control system by amending the Fertilizer Control Order (FCO) in 2005. This laid down the basic guidelines that the biofertilizer product and the process of production would have to follow. At present, it is applicable on Rhizobium, Azotobacter, Azospirillum, and PSBs, with mycorrhiza being the most recent addition in November 2010. Other biofertilizers such as consortia are not yet covered and are free from regulatory framework, although efforts are underway to develop specifications, standards, and testing protocols for remaining biofertilizers. According to the FCO, the mandatory requirements for the production units are as follows.

- Registration of manufacture.
- Registration of dealership.
- Registration for trade and retail sales.
- Adherence to standards.
- Need to fulfil all requirement under Essential Commodity Act and Packaged Commodity Act.

Specifications that need to be adhered to by different biofertilizers, the minimum shelf life duration, and tolerance limit are mentioned.

The respective state directors of agriculture are controllers of the Act in that particular state. Field officers are designated, who can collect the samples from manufacturing site, dealers, retailers or farmers and send the samples to laboratories for analysis. Seven laboratories under the central government and eight laboratories under the state governments have been identified to carry out the analysis. Action against defaulters has been laid out as warning with directions to withdraw that batch, suspension of license, termination of license, prosecution, and fine or imprisonment or both as punishment.

Strict observance to the regulations by production units, monitoring of product and process by the inspectors and the subsequent penalities meted out to defaulters need to be followed. The market is swamped with biofertilizer products, which at times live up to the claims of the producers, but by and large these claims are not substantiated by the performance of the product, making the need for regulations and quality control even more imperative. The continued faith of growers in the benefits of biofertilizers needs to be sustained and the onus lies with the producers to ensure the product reaching the market is that of the declared quality. Although the government has now laid out regulations and specifications, ensuring to their adherence is the issue that has to be taken up with vigour as these regulations will have no value unless they are implemented. Personnel from the government or the recognized regulatory body will have to be trained by experts of the field in the methodologies required for quality check of the production of the inoculum. Alternatively recognized and certified laboratories can be assigned to carry out the quality checks to assist the government inspectors. The regulation should be ensured to encompass both process control as well as quality control of the end product. A process control procedure would help address any doubts and remedial steps to be taken in time before further processing of the inoculum. Such measures will ensure that the finished product, which reaches the market, adheres to the quality parameters even as a formulation and can claim robustly of all the benefits professed.

Field demonstrations should be laid out using regulated and quality product, along with the farmers' active participation such that the faith in the product is built up. These demonstrations can be done with the KVKs and other local bodies giving end-users more confidence in the product.

In conclusion, biofertilizers are live products and require care in storage and application for best results. Their use must be emphasized along with chemical fertilizers and organic manures. The quality of the product needs to be scrutinized such that the benefits that the biofertilizers impart actually reach the farmer. Although regulations and control acts are in place, active participation of the government, research agencies, and local bodies is required to make sure that the biofertilizers deliver what they promise. An independent regulatory body is a must, similar to the TRAI or any other successful example. Besides, the government may consider in enforcing an independent act such as the Biofertilizer Control Act to distinguish the biofertilizer sector from the chemical fertilizer sector.

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:

- *Acta Oecologica*
- *Agricultural Sciences in China*
- *Aquatic Botany*
- *Ecological Engineering*
- *Ecological Indicators*
- *Environmental Pollution*
- *European Journal of Soil Biology*
- *Fungal Ecology*
- *Fungal Genetics and Biology*
- *Journal of Hazardous Materials*
- *Journal of Plant Physiology*
- *Mycorrhiza*
- *Mycosphere*
- *Plant Physiology and Biochemistry*
- *Scientia Horticulturae*
- *Soil Biology and Biochemistry*

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3–8 April 2011

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Dr Sylvie Cranenbrouck, Université catholique de Louvain, Earth and Life Institute (ELI), Mycothèque de l'Université catholique de Louvain (MUCL), Center of Study on AM Monoxenics (CESAMM), Croix du Sud n°3 bt 6, 1348 Louvain-la-Neuve, Belgium

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1–6 May 2011

International Training on In Vitro Culture of Arbuscular Mycorrhizal Fungi (Session-2)
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