PRELIMINARY SURVEY OF MICROBIAL COMMUNITIES AND ENZYME ACTIVITIES OF SANDALWOOD RHIZOSPHERES IN DIFFERENT AGRO CLIMATIC ZONES IN KARNATAKA

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MAMATHA, G., JAYANTHI, S., BAGYARAJ, D. J. & SURESH, C. K. 2004. Preliminary survey of microbial communities and enzyme activities of sandalwood rhizospheres in different agro climatic zones in Karnataka. As there were no reports on the microbial profile of sandalwood rhizosphere, a survey was carried out throughout Karnataka for vesicular arbuscular mycorrhizal (VAM) fungi, *Pseudomonas* and *Bacillus*, and other micro-organisms. VAM, in general, showed maximum spore number in rhizosphere soil samples of sandalwood and grass. The least number of spores was observed in fallow soil. Enzyme activities, namely acid and alkaline phosphatases, and dehydrogenase were higher in sandalwood followed by grass rhizosphere soils. The least enzymatic activity was observed in fallow soils. VAM spores of seven species of *Glomus* were isolated.

Key words: Isolation – identification – *Glomus* – population dynamics

MAMATHA, G., JAYANTHI, S., BAGYARAJ, D. J. & SURESH, C. K. 2004. Tinjauan awal komuniti mikrob dan aktiviti enzim rizosfera pokok cendana di zon agro iklim berlainan di Karnataka. Memandangkan tiadanya laporan tentang profil mikrob rizosfera pokok cendana, satu tinjauan dijalankan di Karnataka untuk mengkaji kulat mikoriza arbuskel vesikel (VAM), *Pseudomonas* serta *Bacillus* dan mikrorganisma lain. Pada amnya VAM menunjukkan bilangan spora yang maksimum dalam sampel-sampel tanah rizosfera pokok cendana dan rumput. Bilangan spora yang terendah terdapat dalam tanah rang. Aktiviti enzim iaitu fostat asid serta fosat alkali dan dehidrogenase adalah lebih tinggi dalam tanah rizosfera pokok cendana diikuti oleh tanah rizosfera rumput. Aktiviti enzim adalah terendah dalam tanah rang. Tujuh spesies spora VAM (*Glomus*) diasingkan.

Introduction

Vesicular arbuscular mycorrhizal (VAM) symbiosis is of great importance in plant growth and nutrition (Harley & Smith 1983). Soils of tropical regions are mainly low in available nutrients and moisture (Osonubi *et al.* 1991). VAM fungi can help plants in utilising soil phosphorus (Gianinazzi-Pearson & Gianinazzi 1989), protect them from the detrimental effect of root pathogens (Suresh & Bagyaraj 1984, Grandison & Cooper 1986) and provide resistance against drought. Therefore, there has been considerable interest in the possible utilisation of VAM as inoculum

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in forest tree species. We need to understand the ecology of VAM in forests with established vegetation. Revegetated soils may be valuable sources of inoculum.

Sandalwood belongs to the genus *Santalum* of the family Santalaceae, which comprises shrubs and small trees. Sandal trees are confined to Karnataka and Tamil Nadu. The tree flourishes well in regions where the climate is cool with moderate rainfall, abundant sunshine and long periods of dry weather. It needs a temperature between 12 and 30 °C. The formation of heartwood is best at an altitude of 600 to 900 m and a rainfall of 85 to 135 cm (Srinivasan *et al.* 1992). The purpose of this work was to (a) evaluate the microbial status of the sandalwood trees growing in seven agro climatic zones of Karnataka, (b) study the VAM fungal species associated with sandalwood trees and (c) study the enzymatic activities to correlate microbial load to microbial activity (namely acid and alkaline phosphatases as well as dehydrogenase) in sandalwood rhizosphere, adjacent grass rhizosphere and non-rhizosphere soils.

Materials and methods

Samples

Investigations were carried out in seven agro climatic zones of Karnataka. Rhizosphere soil samples were collected from two or three locations of seven different agro climatic zones of Karnataka, namely central dry zone (Zone IV: red sandy loam), eastern dry zone (Zone V: red sandy loam), southern dry zone (Zone VI: red sandy loam), southern transition zone (Zone VII: red loam and red sandy loam), northern transition zone (Zone VIII: shallow to medium black clay and sandy loam), hilly zone (Zone IX: red clay loam) and coastal zone (Zone X: red lateritic and coastal alluvial). Soil and root samples of sandalwood, adjacent grasses and non-rhizosphere were collected randomly from the upper 15 cm soil using a soil corer. Non-rhizosphere soil samples without any vegetation served as fallow and this provided a measure of the background abundance of VAM population or microbial population.

Microbial count

Populations of groups of micro-organisms in the soil samples were assessed by the standard dilution plate technique (Jensen 1968). The results were expressed in colony forming units per gram of soil sample. Enumeration of the soil micro-organisms was done by the dilution plate method using nutrient agar for bacteria, potato dextrose agar for fungi, Kuster's agar for actinomycetes and Waksmann-77 for *Azotobacter* (Seeley & Van Demark 1975).

Enzyme activities

Enzyme activities such as acid phosphatase, alkaline phosphatase and dehydrogenase of soil were assayed as described by Eivazi and Tabatabai (1977).

Acid and alkaline phosphatase

One gram of soil was placed in a 50-ml Erlenmeyer flask. Then 0.2 ml of toluene, 4 ml of modified universal buffer (pH 6.5 for assay of acid phosphatase and pH 11 for assay of alkaline phosphatase) and 1 ml of P-nitrophenyl phosphate solution (made in the same buffer) were added to it. The flasks were stoppered, swirled for a few seconds and incubated at 37 °C in an incubator for 1 hour. After incubation, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added to the flask, mixed well for a few seconds and the supernatant was filtered through Whatman No. 2 filter paper. The yellow colour complex was measured using 1 cm cuvette in a spectrophotometer (Shimadzu UV-visible) at 420 nm. The amount of P-nitrophenol (PNP) released was calculated by referring to a calibration graph. Controls were prepared using the same procedure but 1 ml of P-nitrophenol was added after the incubation period before filtration. Results were expressed as µg PNP/g soil/hour.

Dehydrogenase activity

Two grams of soil and 0.2 g of $CaCO_3$ (AR grade), 1 ml of 2% 2,3,5-triphenyl tetrazolium chloride (TTC) and a column (1 cm) of distilled water were added to screw cap test tubes and incubated at 37 °C for 24 hours. After incubation, the contents were filtered using Whatman No. 2 filter paper with washings of methanol until a colourless filtrate was obtained. The filtrate was made up to 100 ml with methanol in a volumetric flask and the absorbance was read at 485 nm on spectronic-20 using methanol as a blank. The absorbance units were converted to concentrations of triphenyl formazon (TPF) from a standard curve prepared from 5, 10, 15 and 20 ml of TPF. The results recorded were expressed as µg TPF/g soil/hour.

Chlamydospores count

For assessment of spores in the rhizosphere, the soil was subjected to the wet sieving and decanting method (Gerdemann & Nicolson 1963). Fifty grams of root zone soil samples were added to 500 ml water and stirred thoroughly. The suspension was allowed to stand for 1 min and then made to pass through a series of sieves measuring 1 mm, 450 µm, 350 µm, 250 µm, 105 µm and 45 µm arranged one below the other in the same order. Spores from the two sieves of smaller pore sizes were transferred onto a nylon mesh of 45 µm, which was then placed in a plate and counted using a stereomicroscope. Distinct morphological types of mycorrhizal spore were isolated from the root zone soil of sandal, wet sieved and sterilised using 200 ppm of streptomycin sulphate (5 min) and chloramine T 0.2% for 5 min to remove debris. Then the spores were mounted on a glass slide with a drop of lactoglycerol as mounting fluid and covered. Spore shape, colour surface characters and hyphal arrangements were observed under a stereomicroscope. Spore and hyphal dimensions were measured by micrometry and identified

according to species following the keys of Trappe (1982) as well as Schenck and Perez (1988).

Results and discussion

Enzyme activities varied widely between the soils studied (Figures 1, 2 and 3). The narrowest range in enzyme activities was observed for alkaline phosphatase (Figure 2), while the widest range was observed for dehydrogenase (Figure 3). The soil in which the highest activity of enzymes occurred was influenced by the enzyme and the zone. The highest was in sandal rhizosphere, followed by grass rhizosphere and fallow (non-rhizosphere) soils.

The number and kinds of micro-organisms found in the soil are shown in Figures 4, 5, 6 and 7. Among the fungi enumerated, *Rhizopus*, *Penicillium* and *Aspergillus* were dominant in all the soil types. Among the bacteria, *Bacillus* and *Pseudomonas* were found to be greatest in the sandal rhizosphere, followed by grass rhizosphere and fallow soils. Higher numbers of micro-organisms, namely bacteria, fungi, actinomycetes and azotobacter were recorded in soil samples collected from rhizospheres of sandal and grass, followed by fallow. This was correlated with the higher enzyme activities in rhizosphere soil as compared with fallow. This can be attributed to the soil functioning within ecosystem boundaries. Soil quality has been defined as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Doran & Parkin 1994). Dehydrogenase activity has been commonly used as an indicator of biological activity in soils because the biochemical properties of this enzyme are such that free dehydrogenases in soil are not expected (Frankenberger & Dick 1983).

Enzyme assays are useful as indicators of microbial activity; the enzyme activity must not be greatly modified by factors like extracellular enzymes stabilised in soils. Enzymes adsorbed on organic and mineral soil particles or compressed with humic acid are important for the activity of the enzymes (Ruggiero & Radogna 1988). Any factor that influences the soil micro-organisms will indirectly affect soil enzyme activities (McClaugherty & Linkins 1990). Soil enzyme activity may serve as useful indicator for change in the biology and biochemistry of soil due to external management and environmental factors (Dick 1994). In accordance with these studies, the present study showed that soil samples collected from sandalwood and grass rhizospheres had higher microbial population with higher enzyme activities as compared with fallow soil samples. The soil enzyme activities were correlated with microbial numbers. Soil enzyme activities are often used as indices of microbial growth and activity of soils. They play an important role in describing and making predictions about an ecosystem production, quality and interactions among the subsystems.

Acid phosphatase, alkaline phosphatase and dehydogenase activities of sandalwood rhizosphere soil were higher than grass rhizosphere. The least enzyme activity of soil was observed in fallow (non-rhizosphere). The plant roots host diverse

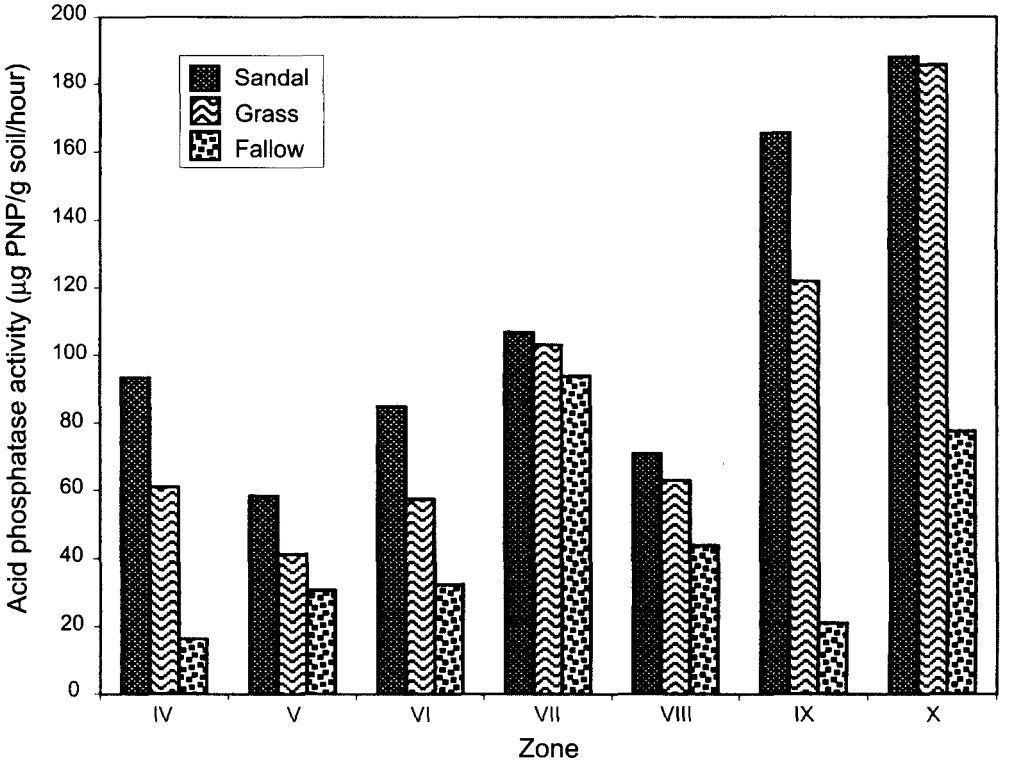

Figure 1 Acid phosphatase activities of sandal and non-sandal rhizosphere soils

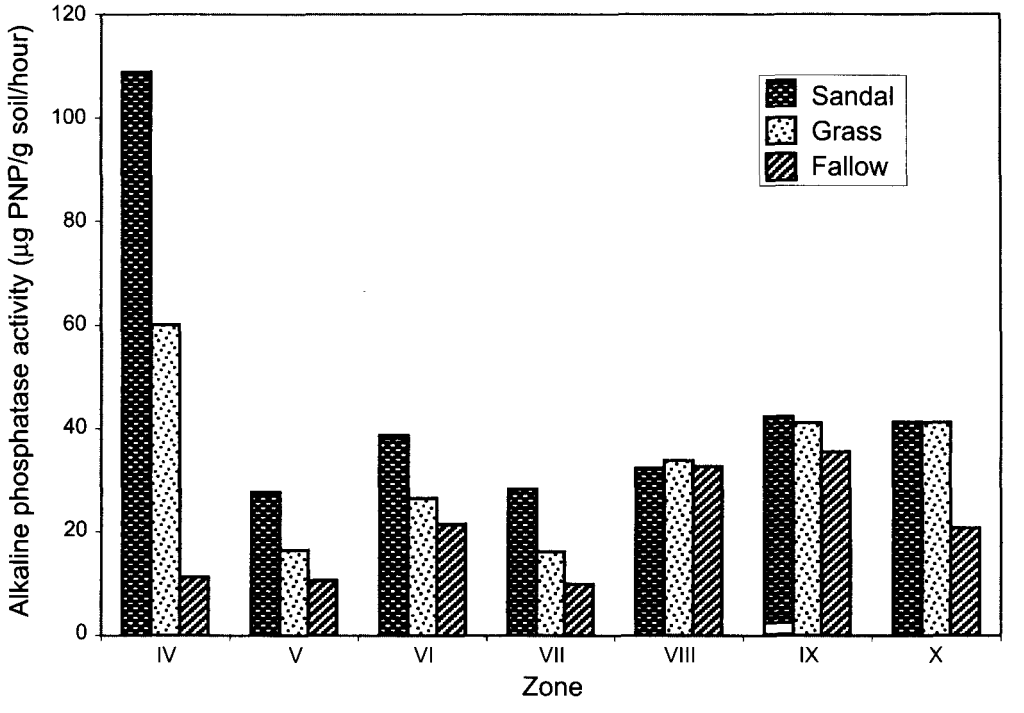

Figure 2 Alkaline phosphatase activities of sandal and non-sandal rhizosphere soils

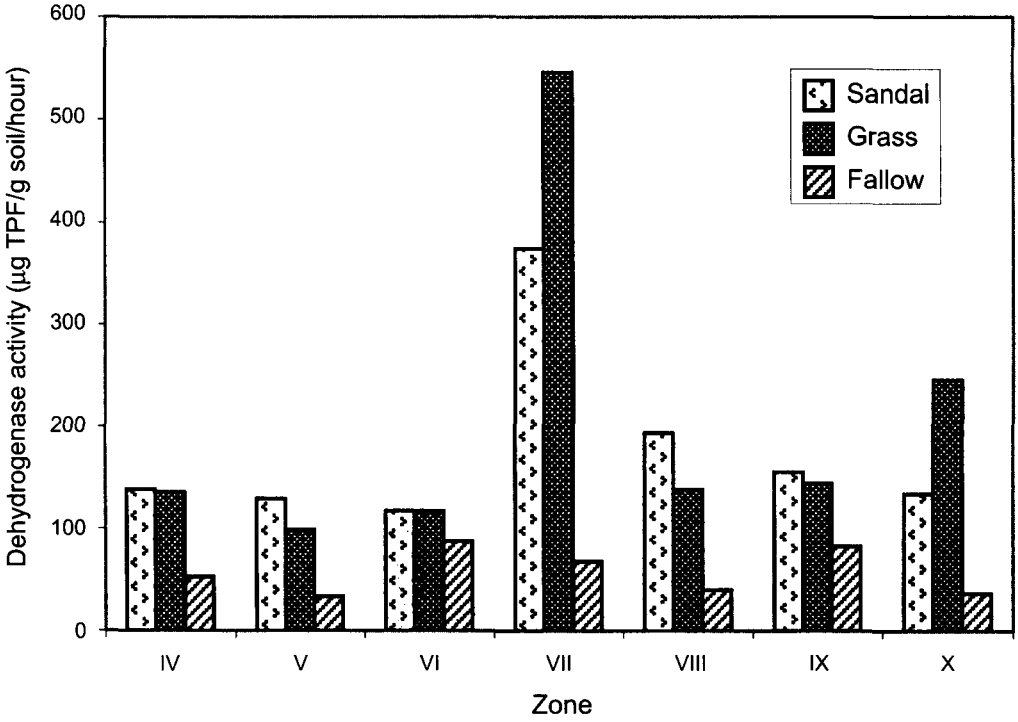

Figure 3 Dehydrogenase activities of sandal and non-sandal rhizosphere soils

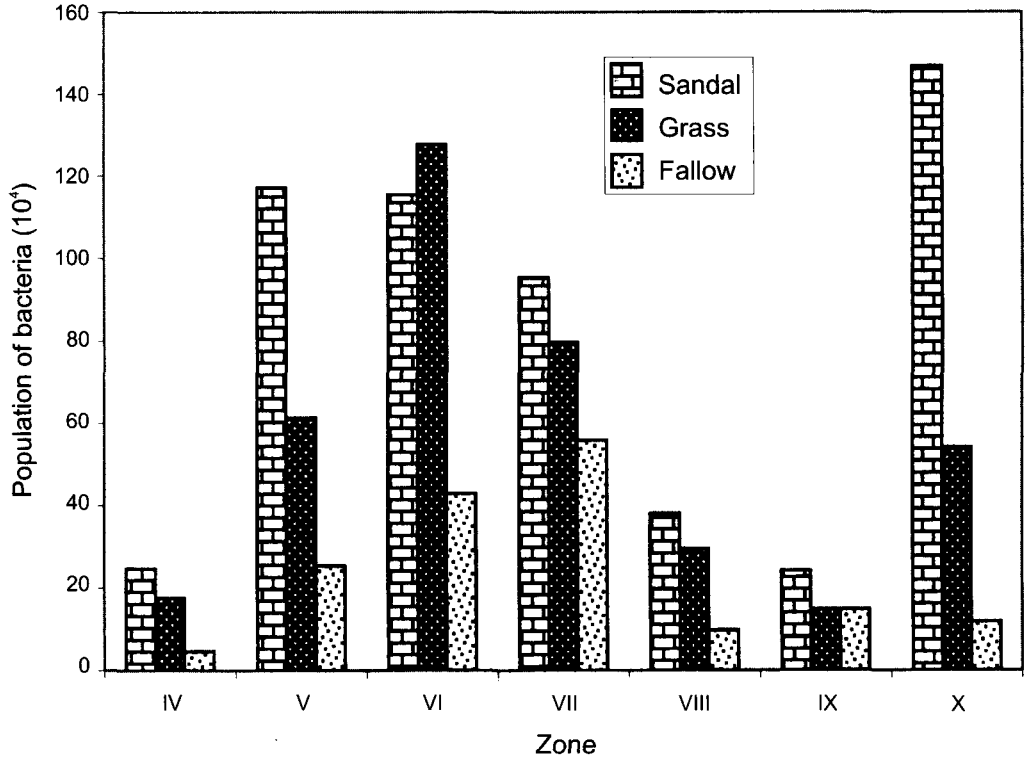

Figure 4 Population of bacteria of sandal and non-sandal rhizosphere soils

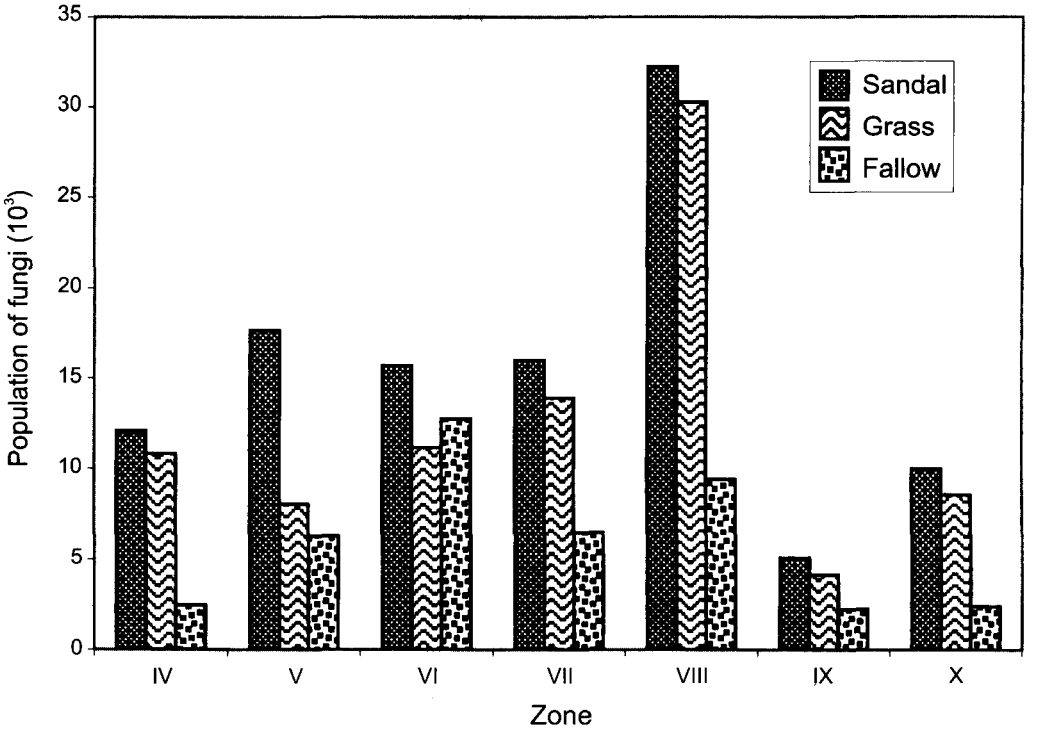

Figure 5 Population of fungi of sandal and non-sandal rhizosphere soils

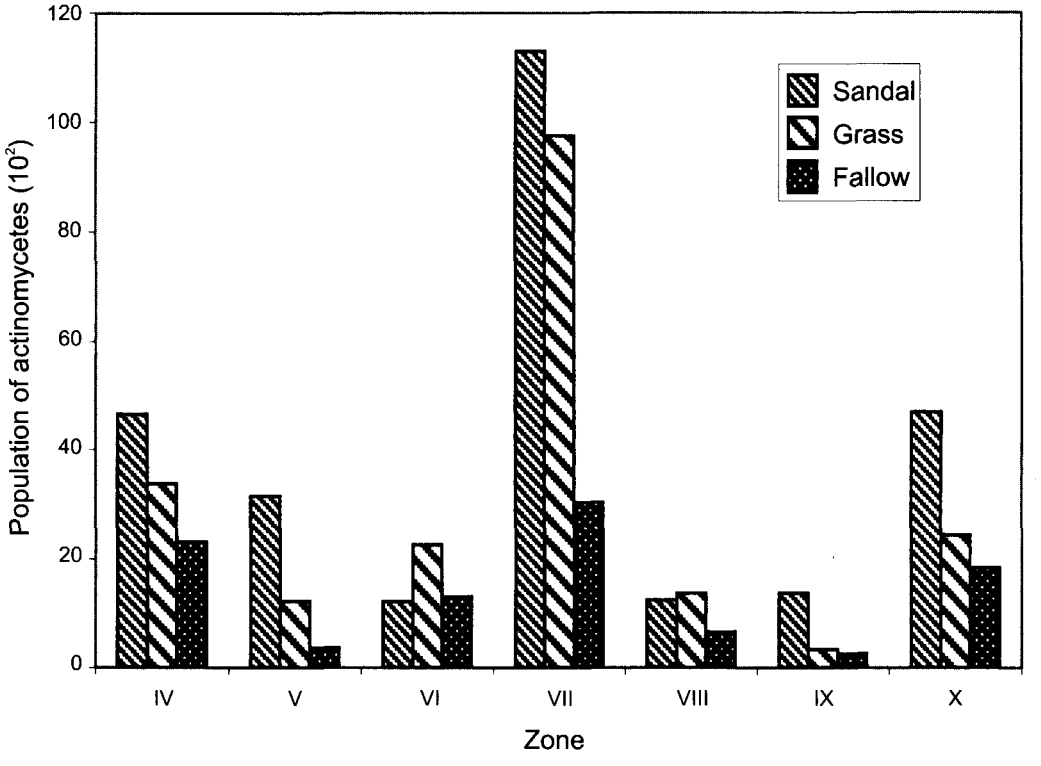

Figure 6 Population of actinomycetes of sandal and non-sandal rhizosphere soils

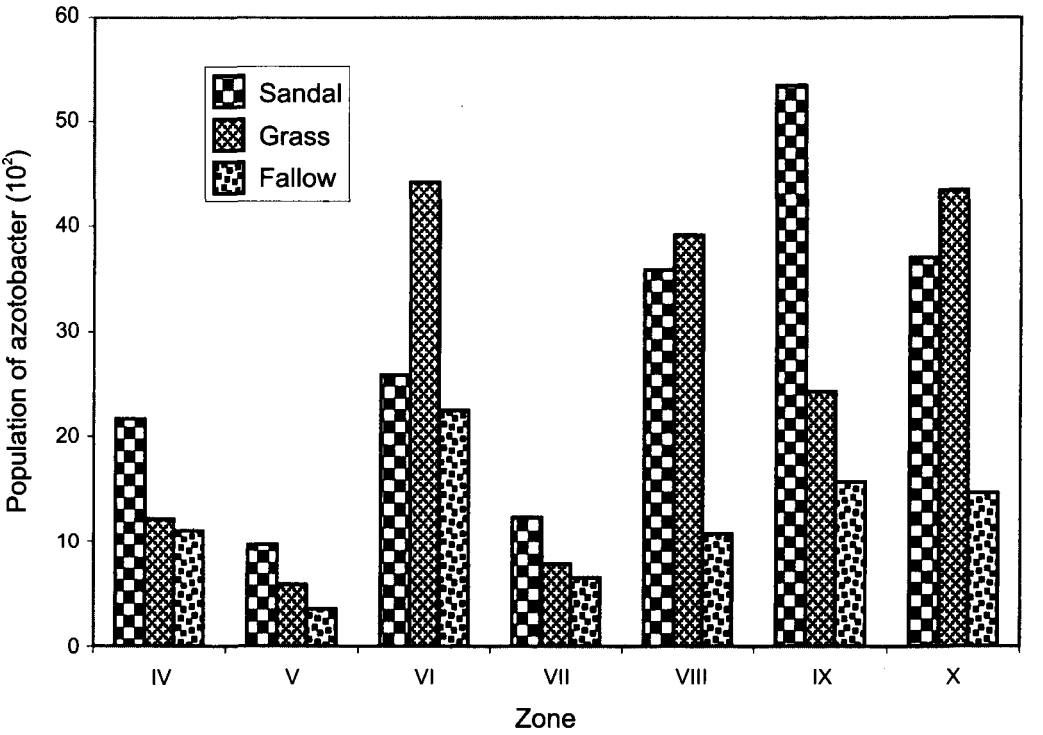

Figure 7 Population of azotobacter of sandal and non-sandal rhizosphere soils

groups of micro-organisms that play an important role in root development. Both the micro-organisms and plants release enzymes into the environment (Juma & Tabatabai 1988). The type of vegetation cover largely influences acid phosphatase, an important soil enzyme that is often found in the rhizosphere soils of plants. Acid phosphatase from the surface of roots hydrolyses organic phosphate and releases inorganic phosphates and this activity is many times higher in roots colonised by mycorrhizal fungi (Ho & Zak 1979). Alkaline phosphatase activity is believed to be entirely derived from the microbial population in soils (Dick 1994). There was a correlation between acid as well as alkaline phosphatase activities and soil microbial numbers in this study. Gomez-de-Guinan and Nageswara (1996) found no significant correlation between fungal population and alkaline phosphatase activity in the plant-free soil fraction, but a significant correlation was observed between root zone fungi and acid and alkaline phosphatase activities. This suggests that the rhizosphere-associated mycoflora play an important role in phosphorus transformation.

It is interesting to know that the mycorrhizal fungi interact with a wide range of other soil micro-organisms in the root, rhizosphere, mycorrhizosphere and in the bulk soil. These interactions, with specific functional capabilities such as inhibitory or stimulatory, competitive or mutualistic may influence plant growth (Lindemann 1988, Fitter & Garbaye 1994, Earanna *et al.* 1998). From the present investigations, all sandalwood plants showed the presence of VAM. However, the spore counts

varied from one region to another as depicted in Figure 8. Table 1 shows the occurrence of seven types of VAM fungi spores in the rhizosphere of sandal soil samples. This is the first assessment of VAM fungi spore status of tropical forest tree species, sandal, from seven agro climatic zones of Karnataka. This study showed that higher number of mycorrhizal spores was observed in rhizosphere of sandal and grass. Even as the majority of VAM species are distributed in diverse habitats, certain species may show a slight degree of ecological specificity (McGonigle & Fitter 1990). The present study shows *Glomus* as the dominant genus from all the seven agro climatic zones of Karnataka (Table 1). The concept of mycorrhizosphere is a hypothesis that mycorrhizae exert a strong influence on the micro-organisms of the rhizosphere. This causes a new microbial equilibrium to be established due to the changes in root exudates (Lindemann 1988). Barea *et al.* (1975) pointed out that since root growth and metabolism are altered by infection with VAM fungi, it is likely that the root exudates and in turn the rhizosphere population be affected. Bagyaraj and Menge (1978) also observed high populations of bacteria and *Azotobacter* in the rhizosphere of tomato plants infected by *Glomus fasciculatum* and *Azotobacter*.

The present investigation revealed that VAM fungi were most common. They occurred in sandalwood trees growing in seven agro climatic zones of Karnataka.

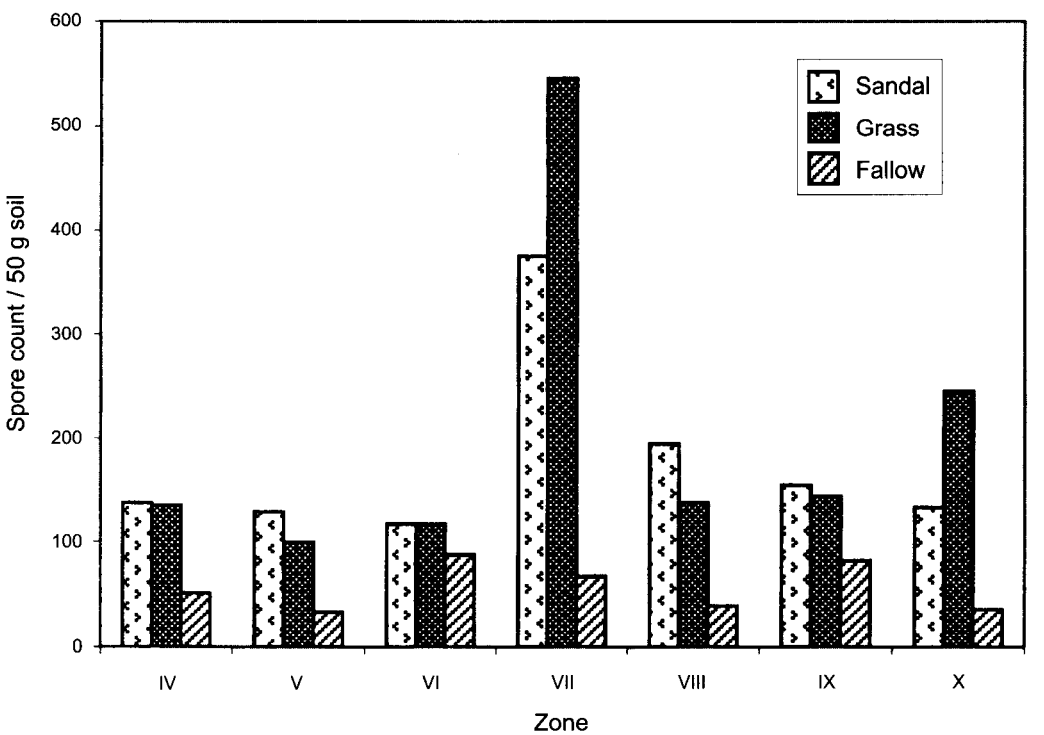

Figure 8 Spore count of sandal and non-sandal rhizosphere soils

Table 1 The distinct morphological types of mycorrhizal spore isolated from the root zone soil of sandal from seven different agro climatic zones of Karnataka

Zone	Dominant species	Important character
Zone IV (Central dry zone)	*Glomus hoi*	Spores are globose to subglobose, borne singly in soil, brown in colour, spore dimension 131.1 × 105 µm; with fracturing and sloughing of the surface of the outer wall and inner light yellow membranous wall; size of the spore wall 4.4 µm.
Zone V (Eastern dry zone)	*Glomus mosseae*	Spores are spherical to oval, brown in colour, spore dimension 108 × 112.8 µm; surface smooth to dull roughened, spore walls two, thin outer and thick inner wall. Size of the spore wall 9.6 µm.
Zone VI (Southern dry zone)	*Glomus caledonium*	Chlamydospores globose yellow to brown in colour. Spore size 108.1 × 126.5 µm; spores filled with deep yellow oil globules; size of the spore wall 14.4 µm thick.
ZoneVII (Southern transition zone)	*Glomus deserticola*	Sporocarps absent, globose in shape, spores borne singly, reddish brown, spore dimension 86.4 × 84 µm, smooth surface to dull roughened. Single laminated wall. Size of the wall 4.8 µm.
Zone VIII (Northern transition zone)	*Glomus etunicatum*	Spores are globose, borne singly in soil, brown in colour, spore dimension 101.2 × 101 µm; with smooth outer wall and laminate inner wall, spore wall size 7.2 µm.
Zone IX (Hilly zone)	*Glomus fulvum*	Spores are yellowish brown in colour, borne singly in soil, characteristically oblong spore dimension 144 × 96 µm; spore wall size 12 µm.
Zone X (Coastal zone)	*Glomus clarum*	Spores formed singly in soil, yellow in colour, subglobose spore dimension 172.8 × 115.2 µm; spore content hyaline, spore wall complex, outer wall hyaline to yellow in colour and thick inner wall, spore wall size 4.8 µm.

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References

BAREA, J. M., AZCON, R. & HAYMAN, D. S. 1975. Possible synergistic interaction between endogone and phosphate solubilizing bacteria in low phosphate soil. Pp. 409–417 in Sanders *et al.* (Eds.) *Ectomycorrhiza.* Academic Press, New York.

BAGYARAJ, D. J. & MENGE, J. A. 1978. Interactions between a VAM and *Azotobacter* and their effects on rhizosphere microflora and plant growth. *New Phytologist* 80: 567–573.

DICK, R. P. 1994. Soil enzyme activities as indicators of soil quality. In Doran D. C. *et al.* (Eds.) *Defining Soil Quality for a Sustainable Environment.* American Society of Agronomy Special Publication No. 35. American Society of Agronomy, Madison.

DORAN, J. W. & PARKIN, T. B. 1994. *Defining Soil Quality for Sustainable Environment.* American Society of Agronomy Special Publication No. 35. American Society of Agronomy, Madison.

EARANNA, N., SURESH, C. K., MALLIKARJUNAIAH, R. R. & BAGYARAJ, D. J. 1998. Response of *Coleus aromaticus* to VA mycorrhiza and some plant promoting microorganisms. *39th Annual Conference of Association of Microbiologists of India.* 5–7 December 1998. Mangalore.

EIVAZI, F. & TABATABAI, M. A. 1977. Phosphatases in soils. *Soil Biology and Biochemistry* 9: 167–172.

FITTER, A. H. & GARBAYE, J. 1994. Interaction between mycorrhizal fungi and other soil organisms. *Plant and Soil* 159: 123–132.

FRANKENBERGER, W. T., JR. & DICK, W. A. 1983. Relationships between enzyme activities and microbial growth and activity indices in soil. *Soil Science Society of American Society* 47: 945–949.

GERDEMANN, J. W. & NICOLSON, T. H. 1963. Spores of mycorrhizal endogone species extracted from the soil by wet sieving and decanting. *Transactions of British Mycological Society* 46: 235–244.

GIANINAZZI-PEARSON, V. & GIANINAZZI, S. 1989. Phosphorus metabolism in mycorrhizas. Pp. 227–241 in Boddy, L., Marchant, R. & Read, D. J. (Eds.) *Nitrogen, Phosphorus and Sulfur Utilization by Fungi.* University Press, Cambridge.

GOMEZ-DE-GUINAN, Y. G. & NAGESWARA, I. 1996. Effect of rhizosphere of peanut (Papillomiaceae) on the fungal flora and the activity of phosphatases. *Journal of Soil Science* 32: 214–220.

GRANDISON, G. S. & COOPER, K. M. 1986. Interaction of vesicular-arbuscular mycorrhizae and cultivars of alfalfa susceptible and resistant to *Meloidogyne hapla. Journal of Nematology* 18: 141–149.

HARLEY, J. L. & SMITH, S. E. 1983. *Mycorrhizal Symbiosis.* Academic Press, London.

HO, I. & ZAK, B. 1979. Acid phosphatase activity of six ectomycorrhizal fungi. *Canadian Journal of Botany* 79: 1203–1205.

JENSEN, V. 1968. The plate count method. Pp. 154–170 in Irg, G. & Parkinson, D. (Eds.) *The Ecology of Soil Bacteria.* University Press, Liverpool.

JUMA, N. G. & TABATABAI, M. A. 1988. Phosphatase activity in corn and soybean roots: conditions for assay and effects of metals. *Plant and Soil* 107: 39–47.

LINDEMANN, R. G. 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology* 78: 366–371.

MCCLAUGHERTY, C. A. & LINKINS, A. E. 1990. Temperature response of enzymes in two forest soils. *Soil Biology and Biochemistry* 22: 22–33.

MCGONIGLE, T. P. & FITTER, A. H. 1990. Ecological specificity of vesicular arbuscular mycorrhizal associations. *Mycological Research* 94: 120.

OSONUBI, D., MULONGOY, K., AWOTOYE, O. O., ATYEE, M. O. & OKALI, D. U. U. 1991. Effect of ectomycorrhizal and vesicular arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings. *Plant and Soil* 136: 131–143.

RUGGIERO, P. & RADOGNA, V. M. 1988. Humic acids-tyrosinase interactions as a model of soil humic enzyme complexes. *Soil Biology and Bichemistry* 14: 428–433.

SCHENCK, N. C. & PEREZ, Y. 1988. *Manual for the Identification of VA Mycorrhizal Fungi.* University of Florida, Florida.

SEELEY, H. W., JR & VAN DEMARK, P. J. 1975. *Microbes in Action.* Taraporevala D. B., Bombay.

SRINIVASAN, V. V., SIVARAMAKRISHNAN, V. R., RANGASWAMY, C. R., ANANTHAPADMANABHA, H. S. & SHANKARANARAYANA, K. H. 1992. *Sandal.* Indian Council of Forestry Research and Education, Dehradun.

SURESH, C. K. & BAGYARAJ, D. J. 1984. Interaction between vesicular-arbuscular mycorrhiza and root knot nematode and its effect on growth and chemical composition of tomato. *Nematologia Mediterrean* 12: 31–39.

TRAPPE, J. M. 1982. Synoptic key to the genera and species of zygomycetous mycorrhizal fungi. *Phytopathology* 72: 1102–1108.