INT J CURR SCI 2015, 15S: E 54-62
RESEARCH ARTICLE ISSN 2250-1770

VAM screening studies on *Mucuna pruriens* (L). Dc.

Suresh Kumar J[a]* and M. Jegadeesan[b]

[a]*PG and Research Department of Botany, Government Arts College, Tiruvannamalai, Tamil Nadu, India*

[b]*Department of Environmental and Herbal Studies, Faculty of Science, Tamil University, Thanjavur, Tamil Nadu, India*

**Corresponding author: jayninebiotech@gmail.com*

Abstract

Globally, about 85% of the traditional medicines used for primary healthcare are derived from plants. The plant, *Mucuna pruriens* (L.) DC. Var. utilis (Wall ex Wight) Baker ex Burck., belonging to the Fabaceae family is contain L-DOPA (3,4-dihydroxy phenylalanine) which is a drug for Parkinson's disease. The plant seeds were collected from wild but it was under cultivation. In this study concentrate the variability of VAM infection rates, spore density and species richness of AM fungi associated with this medicinal plant *Mucuna pruriens* and in and around occurring plants which was collected from herbal garden at Tamil university, Thanjavur, Kolli hills and the point Calimere.

Keywords: VAM, *Mucuna pruriens*,

Received: 14th March 2015; Revised: 28th March; Accepted: 15th April;

Introduction

Primitive man might have learnt the art of healing injuries during hunting, accidents and bites of beasts and serpents. He instinctively turned to nature for relief leaf juices and some wild plants. Thus began the exploration of plants as agents of cure. It has been estimated that 1500 out of 2000 drugs are of plant origin others are animal and minerals (Sinha and Jain, 1994). The Indian systems of medicine viz. Ayurveda, Siddha, Unani and Homeopathy predominantly use plant-based raw materials in most of their preparations and formulations. The efficacy of these systems thus mainly depends upon the use of authentic raw materials without residual any toxicity for the manufacture of drugs of these systems. It is always advisable to raise the medicinal plant through organic cultivation practices.

India is one of the twelve mega-biodiversity countries of the World having rich vegetation with a wide variety of plants with medicinal value. In many countries, scientific investigations of medicinal plants have been initiated because of their contribution to healthcare. Herbal medicines have good values in treating many diseases including infectious diseases, hypertension, etc. That they can save lives of many, particularly in the developing countries, is undisputable (Farnsworth, 1988). The medicinal plant *Mucuna pruriens* is an anti Parkinson plant. *Mucuna Pruriens* (Family-Fabaceae), a drug plant commonly called as 'Poonaikali' (Velvet bean). Seeds are astringent and tonic; they possess slight insecticidal activity; the best aphrodisiac, anthelmintic, purgative and nerve tonic; also used to treat cholera, delirium, impotence, leucorrhoea, menorrhagia, spermatorrhoea, urinary troubles, paralysis and for expelling round worms (Ghosal et al., 1969; Kirtikar and Basu, 1980).

The symbiotic arbuscular mycorrhizal (AM) fungi develop on extensive hyphal network and provide water and nutrients to plants. Soil microorganism can influence

the soil structure and play important role for AM fungi colonization in roots. Standardization of this plant is helpful for effective quality and quantity production of secondary metabolism and also increases the biomass. Therefore, this medicinal plant is selected for the present screening investigation as influenced by arbuscular mycorrhizal fungi (AMF).

Materials and Methods

Study site

To assess the native AM fungi from roots and rhizosphere soils of *Mucuna pruriens* which were collected from Herbal garden at Tamil University, Thanjavur Dt,, Kolli hills at Namakkal Dt. and Point Calimere at Nagappattinam Dt., Tamil Nadu, India.

Sample Collection

From each study site, 3-5 healthy plants were selected. The roots of *Mucuna pruriens* and their rhizosphere soil samples were collected at 0-40 cm soil depth (Koske and Halvorson, 1981). Roots of *Mucuna pruriens* was washed thoroughly free of attached soil particles and cut into 1cm bits and fixed in Formalin Acetic Alcohol (FAA) in the field itself (Phillips and Hayman, 1970).

Analysis of soil Physico-chemical properties

Test plant rhizosphere soil samples were collected separately from each study site, and a portion of soil was for analyzed soil texture, PH, EC, OM, N, P, K, Zn, Cu, Mn and Fe at the soil testing Laboratory. The following standard methods (Piper, 1950; Jackson, 1973; Farnsworth, 1988). Form the remaining soil sample, 100 g was used to estimate AM fungal spore number per sample bag.

Root colonization by AM Fungi

Root segments of *Mucuna pruriens* were first washed thoroughly in distilled water and then placed in 10% KOH and heated to 90^{o}C for 15-30 mins. They were then washed in distilled water and immersed in alkaline 3% H_2O_2 for 5-10 mins. They were then washed in distilled water and acidified with 5N HCL for 2-3 mins. The root segments were stained with 0.05% tryptophan blue in lacto phenol for 15-30 mins and the excess stain was removed with clear lacto phenol (Phillips and Hayman, 1975). Root segment were mounted on glass slide with lacto phenol and observed under compound microscope. A minimum of 100 segments for each samples were observed for the assessment of percentage colonization of AM fungi using the following formula,

$$\% \text{ AM colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of root segments observed}} \times 100$$

Isolation and identification

Spores of AM fungi were isolated form rhizosphere soil by the wet sieving and decanting technique (Sarma et al., 1984). Approximately 100 g of individual air-dried rhizosphere soil sample was dispersed in 500 ml of water in a beaker and the suspension was left undisturbed for 15-20 min. The suspension was then decanted through the stack of sieves 180 and 38 μm (arranged in decreasing order of mesh size from top to bottom). Same process was repeated 2-3 times and the residue from each sieve was collected into Petri plates with little distilled water. Intact AM fungal spores were examined and counted under stereomicroscope (Olympus OIC 1629) and identifications were made by observing diagnostic

characteristics such as spore wall, colour, size and type of hyphal attachment according to Schenck and Prez (1990) and Gerdemann and Nicolson (1963) under compound microscope (Nikon-Optiphot-2).

Estimation of AM fungi

The term frequency was used to assess the establishment and survivability of AM fungi in the rhizosphere of the host. Frequency denotes the number of samplings in which spores of a particular AM fungus present during the study period and expressed as percentage (Schenck and Perez, 1990).

$$\text{Percentage of frequency (\%)} = \frac{\text{Number of Sampling in which a particular AM Fungus was recorded}}{\text{Total number of sampling}} \times 100$$

The density and distribution of the AM fungi in the rhizophere soil samples were expressed in term of percentage occurrence. Here is the percentage occurrence formula,

$$\text{Percentage of occurrence} = \frac{\text{Total number of spores of individual AM fungus}}{\text{Total number of spores al AM fungi}} \times 100$$

Table 1. Physico-chemical characteristics of the rhizosphere soils of *M. pruriens* (L.) DC. from three different eco-sites

AM features	Thanjavur (S1)	Kolli Hills (S2)	Point Calimere (S3)
Number of AM fungal spores per 100 g rhizosphere soil	320	310	285
AM fungal root colonization (%)	72	68	63
Number of Species,	6	6	5
Soil type	Sandy loam	Red Sandy loam	Sandy clay loam
Soil pH	7.2	6.4	8.4
EC	1.72	1.95	2.23
Moisture	13%	17%	22%
Organic carbon (%)	1.32	1.85	0.96
Available Phosphorus (kg/acre)	21.4	19.4	23.7
Available Nitrogen (kg/acre)	1.2	3.2	2.4
Available Potassium (kg/acre)	124	131	160
Copper (ppm)	2.5	2.1	4.2
Zinc (ppm)	2.4	2.3	3.8
Manganese (ppm)	5.8	5.2	7.2
Iron (ppm)	2.8	3.1	5.5

Data analysis

The relationship between percent root colonization and spore density were analysed by Karl Pearson's correlation (Udaiyan et al., 1996). From the data obtained the spore density and richness, for each sampled site was worked out according to the standard derivation. The diversity of AM fungi in the entire three study site was assessed based on diversity indices (Zar, 1984).

Results and Discussion

Soil physico-chemical parameters of study site

The physico-chemical characteristics of the soil of the three study areas of Tamil Nadu such as Herbal garden-Tamil University, Thanjavur (S1), Kolli hills-Namakkal (S2) and Point Calimere-Nagappattinam (S3) containing soil types were sandy loam, Red sandy loam and sandy clay loam it soil pH were 7.2, 6.4 and 8.4.

There areas moisture content lies between 13-22%. In soil temperature and moisture were in accordance with the climatic changes during different seasons. Edaphic characteristics of samples collected from areas indicated that the soil pH was neutral to alkaline (pH 7.2 and 8.4) in herbal garden at Tamil University and point Calimere respectively, with low to moderate electrical conductivity (ECse 1.72-2.23). The physico-chemical properties of the three site soils containing properties were various ranges especially organic carbon (0.96-1.85%), Phosphorous level (19.4-23.7 kg/acre), Nitrogen (1.2 to 3.2 kg/acre), Pottassium (124-160 kg/acre) and also with other micronutrient contents such as zinc (2.3-3.8 ppm), copper (2.1-4.2 ppm), manganese (5.2-7.2 ppm) and iron (2.8-5.5 ppm). The available nitrogen content of the soils irrespective of the study localities was invariably high (1.2-3.2 kg/acre). The results on the chemical analysis and pH of the soil are presented in Table 1 by the following standard methods (Piper, 1950; Jackson, 1973; Farnsworth, 1988).

Table 2. Colonization % spore density and species richness of AM fungi associated with *M. pruriens* (L.) DC. (Mean of five replicates)

Study site	Root colonization	Vesicles %	Arbuscules %	Total number of AM fungal spores per 100 g soil	Associated AM fungal species *	Positive for AM fungi in the roots
Thanjavur (S1)	96.4	64.2	40	712±9.4	LAGR LASM LINR LMSS SSNS	*Glomous intraradius*
Kolli Hills (S2)	97.3	66.5	52	768±11.2	ASPN LAGR LCTM LFSC LINR LMSS CHTG	*Glomous intraradius*
Point Calimere (S3)	84.8	53.2	32	457 ±8.2	GMRG LASM LFSC LINR CHTG SSNS	*Glomous intraradius*

AM Status of Mucuna pruriens

AM play a pivotal role in plant ecology is based on its widespread occurrence in natural ecosystem (Magurran, 1988). The present study was undertaken to make a detailed examination of AM status of *Mucuna pruriens* collected from three different sites of Tamil Nadu, India in relation to soil physic chemical characteristics. The selection of sampling area and *Mucuna* plants were based on soil characteristics as suggested by (Bergelson and Crawley, 1988). The aim of the present study deals to the symbiotic relations between the selected medicinal plants with the association of AM fungi. The association of AM fungal work has been done in the medicinal plants (St. John and Coleman, 1983), garlic, carrot and gram (Selvaraj, 1989), ginger (Shuja and Khan, 1977) and turmeric (Sharma et al., 1995).

In the present study of the medicinal plant, *Mucuna pruriens* was positive for AM fungal colonization in the roots of both the study sites. This type of studies was carried out (Sampath and Sullia, 1992).

The mycorrhizal treatment was colonized spore density and species richness by the AMF (Table 2). This shows the identity to the AMF can determine the species of AM colonizing the roots varied between as 84.8 and 97.3%. This type of similar observation noted and reported by (Koske, 1987). The sites exposed the infection of the positive AM fungi was *Glomous intraradius.* About 70-90% of land plant species form arbuscular mycorrhiza (Sharma et al., 1986) so, it is obvious that the interaction of plants and the obligate symbiotic arbuscular mycorrhizal (AM) fungi of the Glomeromycota (Schübler et al., 2001) is of major importance for the entire terrestrial ecosystem. In research on AM fungi (hereafter AMF), a fungus named *Glomus intraradices* is the most frequently used member of the Glomeromycota. To date > 1200 publications refer to this species > 130 of which have the name in the title. This wide use resulted from the first AMF established in in vitro root organ culture (ROC) being determined as *G. intraradices* (Chabot et al., 1992).

Table 3. Percentage of frequency occurrence AM fungi in the rhizosphere soils of *Mucuna pruriens* at three different sites

AMF Species	Unique Code*	Thanjavur (S1)	Kolli Hills (S2)	Point calimere (S3)	Frequency (%)
Acaulospora *A. bireticulata* *A. scrobiculata* *A. spinosa*	ASPN	_	+	_	33.3
Gigaspora *G. margarita*	GMRG	-	-	+	33.3
Glomus *G. aggregatum* *G.ambosporum* *G. constrictum* *G. fasciculatum* *G. intraradius* *G. mosseae*	 LAGR LASM LCTM LFSC LINR LMSS	 + + - - + +	 + - + + + +	 - + - + + -	 66.6 66.6 33.3 66.6 100 66.6
Scutellospora *S.heterogama*	CHTG	-	+	+	66.6
Sclerocystis *S. sinuosa*	SSNS	+	-	+	66.3
60	80	60	62.9		

* Unique code for AME fungal species (Perez and Schenck, 1990)

AM fungal colonization in the roots of Mucuna pruriens

The screening studies of AM fungal isolated from this plant roots mainly containing five genera they were *Acaulospora, Gigaspora, Glomus, Scutellospora, Sclerocystis.* Among the five genera *Glomous* species were dominated in the infection ratio over other genera (Table 3). The same results were reported in their studies which were the genus, Glomus was dominant at all sites while the genera *Gigaspora*, *Scutellospora* and *Pacispora* were in minority in the roots of cassava (Manihot esculenta Crantz) (Don-Rodrigue Rosin Bi Voko et al., 2013).

Mycorrhizal infection in and around of *Mucuna pruriens*

The nearby appearing medicinal plants roots were examined and tabulated the type of mycorrhizal infection. Few plants exhibited ecto mycorrhizal

infections but endomycorrhizal infection were confirmed with the form of hyphae, vesicles and arbuscules. The importance of ectomycorrhizae in forest systems has been increasingly recognized on the last century. Historically studies as individual mutualisms between a host tree and a fungus, mycorrhizae are now perceived in more encompassing terms as the associations between microscopic fungi and plant roots on which many forest ecosystems are predicted. A diversity of ectomycorrhizal fungi thrive in coniferous and deciduous forests and

Table 4. The type of mycorrhizal infection on some economically / medicinally important plants grown in their natural habitat in and around of *Mucuna pruriens* (L.) DC. of three study areas

Name of the plant	Family	Types of mycorrhizal infection			
		Ecto-mycorrhiza	Endomycorrhiza		
			Hyphae	Vesicles	Arbuscule
Achyranthus aspera	Amaranthaceae	-	+	+	+
Albezzia lebbeck	Mimosaceae	+	+	+	+
Andrographis paniculata	Acanthaceae	-	+	-	-
Aerva lanceolata	Amaranthaceae	-	+	+	+
Asparagus racemosus	Liliaceae	-	+	+	+
Boerrhavia diffusa	Nyctaginaceae	-	+	+	+
Calotropis procera	Asclepedeaceae	-	-	+	+
Catheranthus roseus	Apocyanaceae	-	+	+	+
Cassia fistula	Caesalpiniaceae	+	-	+	+
Cassia tora	Caesalpiniaceae	+	+	+	+
Centella asiatica	Umbelliferae	-	+	+	-
Clerodendron paniculata	Verbinaceae	-	+	+	+
Cucurbita pepo	Cucurbitaceae	-	+	+	-
Cynodon dactylon	Poaceae	-	+	+	+
Cuperus rotundus	Cyperaceae	-	-	-	-
Datura metel. L	Solanaceae	-	+	+	-
Eclipta alba	Compositae	-	+	+	+
Euphorbia hirta	Euphorbiaceae	-	+	+	+
Evolvulus alsinoides	Convolvulaceae	-	+	+	+
Gymnema sylvestris	Asclepediaceae	-	+	+	+
Helianthus sp	Compositae	-	+	+	+
Hevea braseliansis	Euphorbiaceae	+	+	+	+
Indigofera tinctora	Fabaceae	-	+	+	+
Ipomeae batata	Convolvulaceae	-	+	+	+
Ixora coccinea	Rubiaceae	+	+	+	+
Lawsonia alba	Lythraceae	-	+	+	-
Leucas aspera	Labiatae	-	+	+	+
Lycopersicon Lycopersicum	Solanaceae	-	+	+	-
Mirabilis jalapa	Nyctaginaceae	-	+	+	+
Murraya koeniji	Rutaceae	-	+	+	+
Ocimum sanctum	Labiaceae	-	+	+	-
Oldenlandia umbellata	Rubiaceae	-	+	+	+
Phyllanthus neruri	Euphorbiaceae	-	+	+	+
Passiflora sp	Cucurbitaceae	-	+	+	+
Solanum melangena	Solanacea	-	+	+	+
Solanum torvum	Solanacea	-	+	+	+
Scroparia dulcis	Scrophulariaceae	+	+	+	-
Sida cordata	Malvaceae	+	+	+	+
Sida acuta	Malvaceae	+	+	+	+
Tridax procumbens	Compositae	-	+	+	-
Tylophora asmatica	Asclepediaceae	-	+	+	+
Vitis quandrifolia	Vitaceae	+	+	+	-

+ = Presence; - = Absence

in arctic alpine tundra with woody shrubs, and more are being discovered in tropical ecosystems (Smith and Read, 2008). Ectomycorrhizal symbiosis occurs wherever potential host plants exist, or from another point of view, trees are able to exist in many habitats because of partnerships with ectomycorrhizal fungi. While particular species of mycorrhizal fungi can occur across many forest types, it is becoming evident that particular assemblages (or communities) of mycorrhizal fungi are characteristic of each forest type. Sporocarps are often used to detect diversity and infer abundance of macro fungi (including mycorrhizal species) in forest systems. Studies of mycorrhizal diversity relying on sporocarps in western forests include those for Douglas fir (Norvell and Exeter, 2004; O'Dell et al., 1999; Smith et al., 2002), pine (Gehring et al., 1998), aspen (Cripps and Miller, 1993; Cripps, 2001) and timberline forests (Kernaghan and Currah, 1998; Kerghan and Harper, 2001).

References

Bergelson JM, Crawley MJ (1988). Mycorrhizal infectivity and plant species diversity. Nature 334.

Chabot S, Becard G, Piche Y (1992). Life cycle of *Glomus intraradix* in root organ culture. Mycologia 84: 315–321.

Cripps CL, Miller Jr. (1993). Ectomycorrhizal fungi associated with aspen on three sites in the north-central Rocky Mountains. Canadian Journal of Botany 71: 1414-1320.

Cripps CL (2001). Mycorrhizal fungi of aspen stands: natural occurance and potential applications. pp 285-298. In: Sustaining aspen in western landscapes: Symposium proceedings, WD Shepperd, Binkley D, Bartos DL, Stohlgren TJ, Eskew LG (Editors), USDA, Forest Serv. Proc, RMRS, pp18.

Don-Rodrigue Rosin Bi Voko, Jacob Nandjui, Jean-Marc Drolet Sery, Beaulys Fotso, Jesus Amoua Amoa, Marie-Stephanie Aka Kouadio, Seydou Coulibaly, Sebastien Niamke, Adolphe Zeze (2013). Abundance and diversity of Arbuscular mycorrhizal fungal (AMF) communities associated with cassava (*Manihot esculenta* Crantz) rhizosphere in Abengourou, East Côte d'Ivoire. Journal of Ecology and the Natural Environment 5(11): 360-370.

Farnsworth NR (1988). Screening plants for new medicines. In: Wilson EO, Editor. Biodiversity. National Academy Press, Washington DC, USA, pp 83-97.

Gehring CA, Theimer TC, Whitham TG, Keim P (1998). Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. Ecology 79: 1562-1572.

Gerdemann JW, Nicolson TH (1963). Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Transaction of the British Mycological Society 46: 235.

Ghosal S, Singh S, Bhattacharya SK (1969). Alkaloids of Mucuna pruriens chemistry and pharmacology. Planta Medica 19: 229.

Jackson ML (1973). Soil Chemical analyses, Prentice Hall, New Delhi, India, pp 111-120.

Kerghan G, Harper KA (2001). Community structure of ectomycorrhizal fungi across an alpine/subalpine ecotone. Ecography 24: 181-188.

Kernaghan G, Currah R (1998). Ectomycorrhizal fungi at tree line in the Canadian Rockies. Mycotaxon 69: 39-80.

Kirtikar KR, Basu BD (1980). Indian Medicinal Plants, vol. 1, Second Ed., B Singh, MP Singh, India.

Koske RE, Halvorson WL (1981). Ecological studies of vesicular- arbuscular mycorrhizae in a barrier sand dune. Canadian Journal of Botany 59: 1413-1422.

Koske RE (1987). Distribution of AM mycorrhizal fungi along a latitudinal temperature gradient. Mycologia 79: 55-68.

Magurran AE (1988). Ecological Diversity and its measurement, Princeton University Press, New Jersey, USA, pp 192.

Norvell LL, Exeter RL (2004). Ectomycorrhizal epigeous basidiomycete diversity in Oregon Coast Range *Pseudossuga menziesii* forests-Preliminary observations. In: Fungi in forest ecosystems: systematic, diversity and ecology, Edited by CL Cripps. Memories of The New York Botanical Garden 89: 159-189.

O'Dell TE, Ammirati JF, Schreiner EG (1999). Species richness and abundance of ectomycorrhical basidiomycete sporocarps on a moisture gradient in a Tsuga heterophylla zone. Canadian Journal of Botany 77: 1699-1711.

Phillips JM, Hayman DS (1970). Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transaction of British Mycological Society 55: 158-160.

Piper CS (1950). Soil and Plant Analysis, The University of Adelaide Press, Adelaide, Australia, pp 368.

Sampath P, Sullia SB (1992). The occurrence of VAM fungi in the scale leaves of turmeric. Mycorrhiza News 14: 5.

Sarma VAKP, Krishnan P, Budihal SL (1984). Soil Analytical Methods for Soil Survey Investigations, National Bureau of Soil Survey and Land use Planning, ICAR, Regional Centre, Bangaluru, India.

Schenck NC, Perez Y (1990). Manual for the identification of VAM fungi [NC Schenck, Y Perez (Eds.)], INVAM, University of Florida, Gainesville, Florida, USA, pp 241.

Schüßler A Schwarzott D, Walker C (2001). A new fungal phylum, the Glomeromycota: Phylogeny and Evolution. Mycological Research 105: 1413-1421.

Selvaraj T (1989). Studies on Vesicular-arbuscular mysorrhizae of some crop and medicinal plants, Ph.D. Thesis, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India, pp 120.

Sharma SK, Sharma GD, Mishra RR (1986). Records on *Acaulospora* sp from India. Current Science 55: 77-79.

Sharma S, Dohroo NP, Raina JN (1995). Effect of VAM inoculation on dry weight, mycorrhizal inoculation efficiency and phosphorus contents of ginger. Indian Journal of Plant Pathology 13: 60-61.

Shuja N, Khan AG (1977). Occurrence and characteristics of VA mycorrhizae in garlic, carrot and gram. Islamabad Journal of Science 4: 5-8.

Sinha S, Jain S (1994). Natural Products as Anticancer Agents. Progress in Drug Research 42: 53-132.

Smith JE, Molina R, Huso MM, Luoma DL, Mc-kay D, Castellano MA, Lebel T, Valac Y (2002). Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudossuga menziesii)* in the Cascade

Range of Oregon, USA. Canadian Journal of Botany 80: 186-204.

Smith SE, Read DJ (2008). Mycorrhizal symbiosis. 3rd Edition, Academic Press, Cambridge, UK.

St. John TV, Coleman DC (1983). The role of mycorrhizae in plant ecology. Canadian Journal of Botany 61: 1005-1014.

Udaiyan K, Kaethiykeyan A, Muthu kumar T (1996). Influence of edaphic and climatic factors on dynamics of root colonization and spore density of vesicular-arbuscular mycorrhizal fungi in *Acacia farnesiana* Wild Trees 11: 65-71.

Zar JH (1984). Two-sample hypotheses: Biostatistical Analysis, Ed. 2. Englewood Cliffs, NJ Prentice-Hall, Inc., pp 122-149.