Study Of Arbuscular Mycorrhiza Associated With Some Important Medicinal Plants In Suburban Area Of Mumbai

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Abstract

A mycorrhizal fungus infects the plant roots to form symbiotic associations whereby the fungi give nutrients, water and protection to the plant in exchange for food in the form of carbon. Mycorrhizal diversity is important to maintaining the crop vigor and soil fertility. They play a crucial role in plant nutrient uptake, water relations, ecosystem establishment, plant diversity, and the productivity of plants. Hence, the study of mycorrhizal diversity is needed to understand the roles of the various species of mycorrhizal fungi in a habitat.

The present investigation was carried out to study the prevalence of AM fungi in some medicinal plants growing in tribal belt in and around Mumbai by determining the extent of root colonization, spore density in the rhizospheric soil and the actual species composition associated with each host.

In all 15 medicinal plants, hosts were screened for the presence of AVM fungi. Out of these 11 medicinal plant hosts from different families were found to be mycorrhizal. The roots of the hosts which were not found colonized with AM fungi were *Oroxylum indicum*, *Desmodium gangeticum*, *Bacopa monnieri* and *Zingiber officinale*. The colonization was observed in the form of mycelium, arbuscules, vesicles and chlamydospores. The arbuscular mycorrhizal fungi, spores isolated from different sites were represented by six genera, namely 1 species of *Acaulospora*, 1 species of *Gigaspora*, 8 species of *Glomus* and 2 species of *Scutellispora*.

The AM fungi spores were from five genera and the arbuscular mycorrhizal fungi species were almost same in all the rhizoshperic soil of native medicinal plants (1 species of *Acaulospora* namely *A. appendicula*, 1 species of *Gigaspora* namely *G. gigantea*, 8 species of *Glomus* namely *G. aggregatum*, *G. boreale*, *G. fasciculatum*, *G. geosporum*, *G. heterosporum*, *G. segmentatum*, *G. tortuosum*, *G. radiatum*, 2 spore of *Scutellispora* namely *S. pellucida*, and *S. auriglobosa*.

KEYWORDS: Mycorrhizal fungus, Symbiotic association, Diversity, Rhizospheric soil, Medicinal plants, Vesicles, *Glomus*, *Species* richness, Species distribution

Introduction

Mycorrhizas are the symbiotic association between the plants and fungi (Frank, 1885). In nature the mycorrhizal condition is the rule, and non-mycorrhizal is the exception. The soil fungi that penetrate the roots and form morphologically distinct structures as vesicles and arbuscules within the cortex of the host are called vesicular arbuscular mycorrhizal fungi (VAM fungi). In the past few decades, Arbuscular mycorrhizal (AM) fungi have emerged as potential biofertilisers, a

cheap, environmentally friendly alternative to expensive chemical fertilizers (Srivastava et al., 1996). Several symbiotic groups, phosphorous solubilizers, plant growth promoters, and other such beneficial micro-organisms are reported from different soils. Balanced microbial systems contribute to the sustainability in agriculture, forestry, and range management. Hence, mycorrhiza has a substantial role to maintain the ecology of the soil and to help the plant to absorb more water, mineral and protect the roots from many pathogens.

Mycorrhizas are broadly classified into two types i.e. ectomycorrhiza and endomycorrhiza. The ectomycorrhizal association is common with the forest trees, while endomycorrhizas are common with herbaceous flora. They occur in soil, sand, in crop fields, and are found associated with native plant species and represents different ecological condition. In the rhizospheric soil the arbuscular mycorrhizal fungi (AM fungi) are present in the form of chlamydospores, zygospores and azygospores as resting spores.

The role of AM fungi is well known. They provide important nutrient absorption system for mineral nutrients like nitrogen, phosphorus, potassium and trace elements like zinc, manganese etc. to the plant and also make available soluble form of phosphate. They improve disease resistance, drought resistance and survival of host plant under stress conditions and being used to tolerate transplantation shocks in micro-propagated plants.

Survey of arbuscular mycorrhizal fungi has been carried out from in different parts of world from time to time, vesicular arbuscular mycorrhizal fungi has the potential to influence the ecosystem processes, its potential to determine the plant communities and its ability to induce a wide variety of growth responses in coexisting plant species (Hartnett and Wilson, 1999; Heijden et al., 1998a; 1998b; Klironomos et al., 2000; Sanders et al., 1996). It is estimated that 90% of all higher plants including pteridophytes are mycorrhizal (Trappe, 1987 and Nair, 1988). The root systems under natural forest ecosystem are dominated by angiosperm species which are interconnected by a diverse population of mycelia (Zhao et al., 2001). These mycelia interconnected between different groups of plant under one ecological condition serve in exchange of mineral and nutritional resources (Simard et al., 1997). It is this symbiosis from time immemorial between the arbuscular mycorrhizal fungi and plant community cannot be ignored in a terrestrial ecosystem.

Since conflicting reports at the beginning of the present investigation regarding occurrence of AM fungi in medicinal plants were found in the AM fungi literature. Very few workers like Lakshman and Raghavendra (1990) and Salveraj and Subranamian (1990) have really attempted to document the status of AM fungi as a survey of medicinal plants growing in their respective topographic regions from India. For the first time we have made an attempt in Mumbai, Maharashtra such kind of survey work for AM fungi associated with the medicinal plants.

Medicinal herbs are known as source of phytochemicals, or active compounds that are widely sought after worldwide for their natural properties. As many medicinal plants selected for this study has pharmaceutical and industrial importance for example, Basil is a useful source of essential oil and has been used for a long time in the perfumery, cosmetic, food and pharmaceutical industry. The studies on association of the mycorrhiza with the native medicinal plant have great importance as it will provide the new source of biofertilizers. They are also responsible to increase the plant vigor; drought resistance and more production of secondary

metabolites as mycorrhiza are responsible to supply the nutrition including water, major and minor elements to the growing medicinal plants.

The reports of mycorrhiza associated with medicinal plants of this region are not available and studied. The main objectives of the present investigation was an attempt to study the occurrence of AM fungi in medicinal plants growing in tribal belt in and around Mumbai by determining the extent of root colonization, spore density in the rhizospheric soil and the actual species composition associated with each host.

The pharmaceutical and industrial use of the selected medicinal plants is as follows:

Andrographis paniculata is commonly called as kadu kirayata or King of bitters, a herbaceous plant of the family Acanthaceae. This plant is a native of India and Sri Lanka. This plant is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots were used for medicinal purposes. . This is one of the promising herbs used in the treatment of several ailments including human cancers and the myriad of symptoms associated with auto immune disorders. The main active principle which is therapeutically known is Andrographolide.

Vinca rosea **(Catharanthus roseus):** The common name is sadafuli or periwinkle. The species has long been cultivated for herbal medicine and as an ornamental plant. In traditional Chinese medicine, extracts from it have been used to treat numerous diseases, including diabetes, malaria, and Hodgkin's disease. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia.

Oroxylum indicum (Arlutetu) seed is used in the traditional Indian ayurvedic medicine. The root bark is also used, administered as astringent, bitter tonic, stomachic and anodyne. It is included in famous tonic formulations, such as Chyawanprash and as a remedy for pains in joints or rheumatism. The decoction of the bark is taken for curing gastric ulcer and a paste made of the bark powder is applied for mouth cancer, scabies and other skin diseases. The seed is ground with fire-soot and the paste applied to the neck for quick relief of tonsil pain.

Phyllanthus emblica (syn. ***Emblica officinalis***),

Indian gooseberry has been used as valuable ingredient of various medicines in India and abroad. It promotes the spontaneous repair and regeneration process of the pancreas occurring after an acute attack. It is a common constituent, and most notably is the primary ingredient in an ancient herbal rasayana called Chyawanprash Amla is one of the most celebrated herbs in the Indian traditional medicine system, Ayurveda. Amla's traditional uses include as a laxative, eye wash, appetite stimulant, restorative tonic, and to treat anorexia, indigestion, diarrhea, anemia, and jaundice. Amla is well known for its high levels of Vitamin C.

Cymbopogon winterianus (Gavati Chaha)
It is commonly known as lemon grass or oil grass. It is a tropical plant from Southeast Asia. Laboratory studies have shown cytoprotective, antioxidant, anti-inflammatory properties. Citronellol is an essential oil constituent from the Cymbopogon winterianus. Citronellol has been shown lower blood pressure and also used in herbal cosmetics.

Clitoria biflora *(Gokarna)*
It is a multipurpose forage legume. It provides bioactive compounds for medicinal use and is also an ornamental plant. The flowers are often used as a food dye or dipped in batter and deep-fried. . Its roots are used in ayurvedic Indian medicine.
Desmodium gangeticum *(*Shalaparni)

It is a general tonic and aphrodisiac, having a calming, sedative effect and is also used to control inflammation, fever and neurological imbalances. Shalaparni is often effective in restoring balance to the system when the other herbs fail. *Desmodium gangeticum* has too many possible beneficial herbal uses to fully enumerate. A few of its uses include: as a bitter, as a tonic, as an antihemorrhoidal and as an antiasthmatic.

Sida acuta is well known in India as Bala, is a weed that grows wild in wastelands and along roadsides. The plant though seasonal, is available throughout the year. It is valued as a reliever of stomachache, and is a useful remedy in chronic bowel complaints.

Cyclea peltata (Pahadvel)
The paste of roots and leaves is applied over wound, skin infections, snake bites etc. For head ache, the juice of the roots can be applied as nasal drops. It is also use in poisonous bites, skin infections, wound and ulcer, urinary complaints like cystitis and urinary obstruction diarrhea, hemorrhoids, and digestive problems.

Boerhavia diffusa (Punarnava)
It is commonly known as punarnava meaning that which rejuvenates or renews the body. It is taken in herbal medicine for pain relief and other uses. It is believed to improve and protect eyesight. *Boerhavia diffusa* has diuretic properties and is used by diabetics to lower blood sugar. It has shown antibacterial activity, mainly against Gram-negative bacteria. Punarnavine, an alkaloid isolated from *Boerhaavia diffusa* has been shown in vitro anticancer, antiestrogenic, antiamoebic and immunomodulatory activity. It is also useful in biliousness, blood impurities, leucorrhoea, anaemia, inflammations, heart diseases, asthma, alternatives etc. The leaves are useful in dyspepsia, tumors, spleen enlargement, and abdominal pains.
Aegle marmelos (Bel)
It is commonly known as Bel belonging to the family Rutaceae. A number of chemical constituents and various therapeutic effects of *A. marmelos* have been reported by different workers. It has been used in ethnomedicine to exploit its medicinal properties including antidiabetic, antiulcer, antioxidant, antimalarial, anti-inflammatory, anticancer, radio-protective, antihyperlipidaemic, antifungal, antibacterial and antiviral activities.

Bacopa monnieri (Brahmi)
Bacopa is an herb used for memory and mental enhancement. Many mind formulas include this herbal extract combined with other herbs and nutrients used for memory and mind enhancement.. It is considered diuretic, nervous tonic, heart tonic, Immuno-modulator, adaptogen, cerebral activator, anti-asthmatic, anti-epileptic, anti-ulcer, antispasmodic, anti-allergic, anti-depressant and anti-oxidant. Regular intake of Bacopa monniera tea improves learning ability and high reasoning capacity in both children and adult people

Centella asiatica

Centella grows along ditches and in low, wet areas. Because the plant is aquatic, it is especially sensitive to pollutants in the water, which are easily incorporated into the plant. It has been used for: wound healing, better circulation, memory enhancement, cancer, vitality, general tonic, respiratory ailments, detoxifying the body, treatment of skin disorders (such as psoriasis and eczema), revitalizing connective tissue, burn and scar treatment, anti-stress, anti-anxiety, an aphrodisiac, immune booster etc.

Premna obtusifolia *(*Agnimantha)
Premna is a small to medium-sized tree. The roots are sweet, bitter, astringent, acrid, cordial, thermogenic, anodyne, anti-inflammatory, cardiotonic, laxative, stomachic, expectorant, depurative, digestive, carminative, febrifuge, antibacterial and tonic. Roots are useful in inflammations, cardiac disorders, cough, asthma, bronchitis, leprosy, skin diseases, flatulence, anorexia, constipation and fever. Roots are the ingredients in Dushmula, well-known Ayurvedic medicine. The root contains a yellow coloring matter, tannin and an essential oil which is used for the treatment of colic.

Zingiber officinale **(Ginger or Adrak)**
Ginger is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae). Ginger produces a hot, fragrant kitchen spice. Mature ginger roots are fibrous and nearly dry. Ginger has been used for stomach upset, motion sickness, nausea, and vomiting.

Material & Methods:
The present experiment has been carried out to study the occurrence of AM fungi in medicinal plants growing in tribal belt in and around Mumbai in monsoon. The aim of the study was to determine the extent of root colonization, spore density in the rhizospheric soil and the actual species composition associated with each host the rhizospheric soil from Keshav Shrushti, Utthan, Bhyander, Dist- Thane and Mumbai.

Soil and plant root sampling: Three different plants of the same species were randomly selected to perform the study. About 100 gm soil along with the roots was collected from approximately 15-30 cm depth from each plant. The soil and the plant root samples were transported to the laboratory in the pre-sterilized polythein bags and stored at 6 ^{o}C (± 2) until processed. The samples were mixed together to form composite sample and three replicates were taken for the analysis from each composite sample.

a) Processing of roots for assessing the existence of root colonization:
Root colonization was observed by rapid clearing and staining technique (Phillips et al. 1970). Roots samples of the selected medicinal plants were stored in FAA were first washed with tap water repeatedly for complete removal of FAA traces and then placed in a small glass vial. These root samples dipped in 10% (w/v) KOH were then digested in autoclave. 0.05% trypan blue in lactophenol stain was added to the above processed roots for several hours. Roots were finally observed under the compound microscope for AM fungi colonization. Quantification of AM fungal colonization was carried out using the grid-line intersection method and expressed as percentage root colonized. The stained roots were mounted on microscopic slides and the segments were examined by light microscopy. Percent root colonization was determined using

formula as mentioned under:
% Root Colonization = (Number of positive segments / Number of segments observed) x 100

b) Extraction of spores or propagules:
The spores were isolated from rhizospheric soil by using wet sieving and decanting method (Gerdemann and Nicolson, 1963). The spores were quantitatively and quantitatively estimated from known quantity of air dried soil samples by following Gaur and Adholeya (1994). The VAM fungal spores collected on filter paper (Whatman filter paper No.1) after wet sieving and decanting they were observed under stereoscopic binocular microscope. These spores were picked through needle and mounted on glass slide in polyvinyl alcohol-actophenol mountant (Koske and Tessier, 1983). VAM spore identification was done by using the "Manual for the identification of VA mycorrhizal fungi" of Schenck and Perez (1990), Morton et al. (1990) and http://www.invam.caf.wvu.edu.

Result and Discussions:
In the present investigation, all 15 medicinal plant hosts were screened for the presence of AVM. Out of these 15 medicinal plants, many hosts from different families were found to be mycorrhizal except 4 medicinal plants (Table-I). The roots of the hosts which were not found colonized with AM fungi were *Oroxylum indicum*, *Desmodium gangeticum*, Bacopa *monnieri* and Zingiber *officinale*. The colonization was observed in the form of mycelium, arbuscules, vesicles and chlamydospores.

The arbuscular mycorrhizal fungi, spores isolated from different selected sites, represented by six genera, namely *Acaulospora* with 2 species, 1 species of *Gigaspora*, 10 species of *Glomus* and 3 species of *Scutellispora*.

The spore diversity and population was observed comparatively low. The AM fungi spores were from five genera and the arbuscular mycorrhizal fungi species were almost same in all the rhizoshperic soil of native medicinal plants (2 species of *Acaulospora* namely *A. appendicula* and *A. denticulate*. 1 species of *Gigaspora* namely *G. gigantea*, 10 species of *Glomus* namely *G. aggregatum, G. boreale, G. jasciculatum, G. geosporum, G. heterosporum, G. segmentatum, G. tenebrosum, G. tortuosum, G. radiatum* and *G. boreale*, 3 spore of *Scutellispora* namely *S. pellucida, S. arenicola* and *S. auriglobosa* (Table 1). *Glomus* species was recorded as dominant root symbionts.

In the present investigation the mycorrhizal mycelium–root associations and formation of vesicle were common then the formation of arbuscle and chlamydospore .The rhizosperic soil collected from all the locations was more or less neutral to alkaline. In this soil, *Glomus* spp is the most dominant fungi and frequently observed. *Glomus* is associated with all the host species. In different hosts, it is represented by as much as 08 species and many of the hosts harboured more than one species.

The dominance of *Glomus* species in alkaline soil was also reported by many workers (Mosse1973, Gautam et al 2009). Bargali (2011) claimed that the number of plants possessing vesicles was higher than plants bearing arbuscules. These results suggested that roots of majority of the plants colonized were mature as vesicles are storage organs and generally produced in the

older region of the infection. Our result correlates with their results. In the present investigation only one plant i.e. *Andrographis paniculata* shows arbuscules (6.6%) along with vesicles and mycelial association. Tejavathi et al. (2011) reported the positive correlation between percent mycorrhizal colonization and plant growth in Andrographis *paniculata*. All the morphological parameters assessed were significantly influenced by AM fungal association both in normal and micro-propagated plants.

In the present investigation maximum plant shows mycelia-root colonization and the formation of vesicles. Out of 15 plants 11 shows mycelial colonization (73 %) and 9 plant shows formation of vesicles (60 %) and only 2 plants showed chlamydospore formation (13%). *Oroxylum indicum*, *Desmodium gangeticum*, *Bacopa monnieri*, *Zingiber officinale* does not show any type of mycorrhizal associations at all during the period of investigations.
The level of AM fungal association depends on root morphology, metabolism and rate of plant growth (Warner & Mosse, 1980) as well as on specific soil plant system in term of chemical nature of root exudes (Koske, 1985). In addition to these factors, pH of the soil may also play important in controlling AMF root colonization and spore population but it was found to be ineffective in the present investigation as the pH remained within narrow range of 5.0 to 8.5. In natural system Guadarrama & Alvarez-Sanchez (1999), Bohrer & Amon (2004) observed that seasonal fluctuations of mycorrhizal associations were closely related to plant phenology. The maximum spore population in the present investigation was observed during rainy season which coincides with flowering time of the all three plants. It might be correlated with the fact that during this period most photosynthatase is allocated to roots and rhizomes, which helps fungal symbiont to produce more spores (Gemma & Koske 1988, Wallen 1980).

Mycorrhizal colonization in roots occupying a defined volume of soil will depend on a balance between root and fungal activity (Koide, 1993) which is influenced by several factors including soil properties, root phenology, predation, local disturbance and propagule availability (Brundrett, 1991). It is also apparent that rainy season may considered as the best season for the propagation of medicinal plants by the application of AM fungi as bio-inoculants particularly for plants under threat.

Conclusion:
Out of 15 medicinal plant hosts screened for the presence of AM fungi 11 medicinal plant hosts were found to be mycorrhizal. The colonization was observed in the form of mycelium, arbuscules, vesicles and chlamydospores. The spore diversity and population was observed comparatively low. The AM fungi spores were from five genera and the arbuscular mycorrhizal fungi species were almost same in all the rhizoshperic soil of native medicinal plants (1 species of Acaulospora namely *A. appendicula*, 1 species of *Gigaspora* namely *G. gigantea*, 08 species of *Glomus* namely *G. aggregatum*, G. *boreale*, *G. fasciculatum*, *G. geosporum*, *G. heterosporum*, *G. segmentatum*, *G. tortuosum*, *G. radiatum* and 2 spore of *Scutellipora* namely *S. peltucida*, and *S. auriglobosa*. The capacity of VAM fungi to act as biofertilizers, bioregulators and bioprotectors has repeatedly been demonstrated. These associations help to maintain the general plant vigour under a variety of adverse and inhospitable ecological conditions.

Therefore based on this preliminary, investigation it was not possible to assess the host specificity in detail of medicinal plants to AM fungi colonization. It is therefore there is a bright scope for further details study for understanding the host specificity of AM fungi species on

medicinal plant and their effect on enhancement of secondary metabolites active principles.

Table 1: Medicinal plant Root colonization, chlamydospores and AM fungi in the rhizospheric soil.

S.N.	FAMILY	BOTANICAL NAME	COMMON NAME	AM FUNGI COLONISATION				AM FUNGAL SPCIES
				M	A	V	C	
1.	Acanthaceae	*Andrographis paniculata*	Kalmegh	+	+	+	-	*Acaulospora appendicula*
2.	Apocynaceae	*Vinca rosea*	Sadafuli	+	-	+	-	*Gigaspora gigantea,*
3.	Bignoniaceae	*Oroxylum indicum* Vent.	Arlutetu	-	-	-	-	*Glomus aggregatum*
4.	Euphorbiaceae	*Emblica officinalis* Gaertn.	Amla	+	-	+	+	*G. boreale*
5.	Graminae	*Cymbopogon winterianus*	Citronella	+	-	+	-	*G. fasciculatum,*
6.	Leguminosae	*Clitoria biflora* Dalz.	Gokrana	+	-	+	-	*G. heterosporum,*
7.	Leguminosae	*Desmodium gangeticum* DC.	Salvan	-	-	-	-	*G. segmentatum*
8.	Malvaceae	*Sida acuta*	Bala	+	-	-	-	*G. tortuosum,*
9.	Menispernnoceae	*Cyc!ea peltata* (Lann.)	Pahadvel	+	-	+	-	*G. geosporum,*
10.	Nyctaginaceae	*Boerhaavia diffusa*	Punarnava	+	-	-	-	*G. radiatum*
11.	Rutaceae	*Aegle marmelos* Correa.	Bel	+	-	+	-	*Scutellospora pellucida,*
12.	Scrophulariaceae	*Bacopa monnieri* (Linn.)	Nirbrahmi	-	-	-	-	*Scutellospora auriglobosa*
13.	Umbelliferae	*Centella asiatica* (Linn.)	Brahmi	+	-	+	-	
14.	Verbenaeae	*Premna obtusifolia*	Agnimantha	+	-	+	+	
15.	Zingiberaceae	*Zingiber officinale* Rose.	Jangali ginger	-	-	-	-	

M= Myellium; V= Vesicle; A=Arbuscle; C= Chlamydospore

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