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Isolation and characterization of Arbuscular Mycorrhizal (AM) fungi from the rhizospheric soil of some medicinal plants

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Abstract: The present investigation was carried out to estimate the colony forming units of indigenous fungi and to study the diversity and Arbuscular mycorrhizal (AM) of some medicinal plants. The study was also aimed to estimate and isolate the Arbuscular mycorrhizal (AM) spore population from the rhizospheric soil of some important medicinal plants from the Botanical garden of College campus. All the selected medicinal plants i.e., *Mentha* sp., *Ocimum sanctum*, *Bryophyllum* sp., *Adhatoda vasica*, and *Andrographis paniculata* showed a very good diversity of rhizospheric soil fungi. The highest number of Colony forming unit (c.f.u) was estimated in *Mentha* sp (162/10⁻¹) followed by *Bryophyllum* sp. (156/10⁻¹), *Andrographis paniculata* (91/10⁻¹), *Adhatoda vasica* (72/10⁻¹) and *Ocimum sanctum* (68/10⁻¹). Arbuscular mycorrhizal (AM) spore population was also estimated in all the medicinal plants. The highest number of AM spores was estimated in *Andrographis paniculata* (121/50gm⁻¹) followed by *Ocimum sanctum* (112/50gm⁻¹), *Adhatoda vasica* (94/50gm⁻¹), *Bryophyllum* sp.(89/50gm⁻¹) and *Mentha* sp (75/ 50gm⁻¹). The rhizospheric soil fungal diversity including mycorrhizal fungi (*Glomus* sp.) also was found to be beneficial for increasing the photosynthetic activity and uptake of phosphorus in medicinal plants. Thus, the use of AM inoculum can increase the yield of medicinal plants along with their secondary metabolites to utilize on a sustainable basis as a traditional and herbal medicine for the human welfare.

Key words: Medicinal plants, indigenous fungi, Arbuscular Mycorrhizal (AM) fungi, *Glomus* sp, herbal medicine.

Introduction

Medicinal plants traditionally occupied an important position in rural and tribal lives of India and are considered as one of the most important source of medicine since the dawn of human civilization. Medicinal plants constitute the basis of the population of India and are a great source of income for rural population. There is corresponding evidence in the literature that suggests a correlation between plant bioactivity and the traditional medicinal use (Joner *et al.*, 2003).

Medicinal plants play a central role not only as traditional medicines but also as trade commodities, meeting the demand of distant markets. The compounds derived from medicinal plants form the ingredients of analgesic, antibiotics, laxatives ulcer treatment etc. Plants with ethno pharmaceutical importance are being exploited because of their healing properties, (Barbhuiya and Sharma, 2009). However large-scale harvesting of medicinal plants has already become a major threat to biodiversity. As an alternative, microbes which live in such plants may offer tremendous potential sources of therapeutic compounds (Tamilarasi *et al.*, 2008). Herbal plants are of immense medicinal value for

drugs and pharmaceutical industries. The growth of the herbal plants also depends on the population of soil microorganisms present in their rhizosphere and rhizoplane area of plants as these microorganisms constitute one of the important biotic and ecological factors responsible for plant growth (Koberi *et al.*, 2013).

Fungal populations in the rhizosphere and rhizoplane areas show a qualitative change with age of plants and also with changing environment (Sundar *et al.*, 2011). Sampathkumar *et al.*, (2007) also observed seasonal changes in the composition of AM fungi. It is a very important and intensive interaction take place between the plant and microflora. The diversity and functions of microbes in the rhizosphere, a narrow region around the root, are related to the root exudates, biogeochemical reactions and respiration (Kumar *et al.*, 2010). The rhizosphere contains abundant bacteria, fungi, protozoa and nematodes. Some nematodes are feeding on bacteria and fungi. Root based interactions between plants and organisms in the rhizosphere are highly influenced by edaphic factors. These interactions include signal traffic between

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roots of competing plants and soil microbes and one-way signals that relate the nature of chemical and physical properties to the roots Zhao *et al.*, 2012).

Plant depends on the ability to communicate with microbes. The converse is also true, many fungi dependent on association with plants that are often regulated by root exudates (Zubek *et al.*, 2013) Root exudates play an active and relatively well documented role in the regulation of symbiotic and protective interactions with microbes. A wide range of organic compounds secreted by plant roots in the rhizosphere provide a food source for microorganisms increasing microbial density and activity in the rhizosphere than in the bulk soil. They helps to solubilise poorly soluble inorganic phosphorous and mineralize organic phosphorous sources and markedly increase plant growth (Smith and Zhu, 2001). The rhizosphere is the region around the root and has high nutrient availability. This is due to the loss of as much as 40% of plant photosynthates from the roots (Guntilaka, 2006). As a result, this region contains large and active microbial populations that may exert beneficial, neutral, or detrimental effects on the growth of plant. The rhizospheric microbial populations play a major role in maintaining root health, nutrient. Such microorganisms are important components of management practices so as to achieve the crop yield. The crop yield refers to the attainable yield that is limited only by the natural physical environment of the crop and its innate genetic potential (Gentil and Jumpponen, 2006).

Plant roots play a major role in providing the mechanical support as well as facilitate water and nutrient uptake. Besides these, plant roots secrete wide variety of compounds that attract soil microbial communities. Such chemicals, called as root exudates are secreted by roots into the soil helps in promoting the plant microbe interactions and inhibiting the growth of the competing plant species (Soka *et al.*, 2014). These exudates may act as attractants or repellants and their composition is dependent upon the physiological status and species of plants and microorganisms. The quality and quantity of root exudates is influenced by microbial activity in the rhizosphere which affects rooting patterns

and the supply of available nutrients to plants. These exudates are metabolized by microbes as C and N sources, and the resulting molecules are utilized by the plants (Smith and Read, 2008). There is a wide range of mechanism through which PGPR may exert their beneficial effect on plants for growth promotion and biocontrol. Production of phytohormone by PGPR help in stimulation of the growth of plant roots (Khan, 2000).

Soil harbours a variety of fungi, and other soil microorganisms. Soil bacteria and fungi play a vital role in various geochemical cycle (BCG) (Trevors, 1998). It is considered as the most dynamic site of biological interactions in nature where various biological and biochemical reactions are performed. Plant growth is directly dependent on soil quality. Soil microorganisms are the living component o soil organic matter and are responsible for mineralization of nutrients, decomposition and degradation or transformation of toxic compounds. Soil helps to maintain the equilibrium of earth's surface environment by supporting vegetation, hydrologic cycle, storage of organic matter etc. It also traps pollutants and participates actively in the oxygen and nitrogen cycles. The soil organisms found at different depths play an important role in recycling of nutrients, soil formation, ecosystem biogeochemistry and degradation of waste product. Growth of plants in the agricultural soil is influenced by various biotic and abiotic factors. Many different physical and chemical approaches have been used by the growers for the management of soil environment for the improvement of plant growth and crop yields (Chanda *et al.*, 2014).

India has one of the richest plant medical cultures in the world. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plants as potential sources medicinal substances. Soil microorganisms constitute world's largest reservoir of biological diversity and are crucial to the functioning of terrestrial ecosystems. The rhizosphere, a narrow zone, adjacent to and influenced by, living plant roots (Ingle *et al.*, 2013) is a site of high microbial activity in and around roots in soil It harbors a great diversity of microorganisms affecting plant growth and

health (Karthikeyan *et al.*, 2009 and Mishra and Shukla, 2014).

Arbuscular Mycorrhizal (AM) fungi interact either directly with other soil organisms or they may influence these organisms indirectly by affecting host physiology that could change root morphology, physiology and patterns of exudation into the mycorrhizosphere. Mycorrhizae form mutualistic symbiotic relationships with plant roots of more than 80% of land plants including many important crops and forest tree species (Radhika and Rodrigues, 2010). Seven kinds of mycorrhiza: arbutoid mycorrhiza, ectomycorrhiza, endomycorrhiza or arbuscular mycorrhiza, ecto-endomycorrhiza, ericoid mycorrhiza, monotropoid mycorrhiza, and orchidoid mycorrhiza have been recognized (Brundrett, 2009).

Endomycorrhizal fungi are inter and intracellular and penetrate the root cortical cells and form finger like branched structures called arbuscule and vesicles to be known as vesicular arbuscular mycorrhiza (VAM). In some cases no vesicles are formed and they are known as arbuscular mycorrhiza (AM). Arbuscular Mycorrhiza (AM) mycobionts forming symbiosis with approximately 90% (>200000) of the terrestrial plant communities. They are essential components of soil biota and are found in almost all ecological situations particularly those supporting plant communities with high species diversity. So far more than 170 species of AM fungi have been recorded and described (<http://invam.caf.wvu.edu/>). AM fungi belong to nine genera: *Acaulospora*, *Archaeospora*, *Enterophospora*, *Gerdemannia*, *Geosiphon*, *Gigaspora*, *Glomus*, *Paraglomus*, and *Scutellospora* (Raja, 2006 and Schwartz, 2006).

According to Tamilarasi *et al.*, (2008), the total heterotrophic actinomycetes and fungus were enumerated from the rhizosphere and non - rhizosphere soil of 50 selected locally available medicinal plants in and around Bharathiar University. In all the plants, populations of microorganism were higher in the rhizosphere soil than in the non-rhizosphere soil. Of the medicinal plants, the maximum rhizosphere effect was observed in *Annona squamosa* and the minimum effect was seen in *Eclipta alba* and

Cassia auriculata. Among the fungal isolates *Rhizopus* was found to be higher in number followed by *Aspergillus*, *Penicillium*, *Mucor* and *Fusarium*. According to Srivastava and Kumar (2013), rhizosphere and rhizoplane of most of the plants is always rich in various populations of microorganisms. The microorganisms, on a continuous scale forms parasitic to mutualistic association with plants. The present study deals with the investigation of fungal population in the roots vicinity of *Aloe vera*, *Argemone maxicana*, *Abutilon indicum*, *Amaranthus polygamus* and *Achyranthus aspera*. All these five species found to grow in wild and northern plains of India. A total number of 37 species of fungi were isolated and the number of fungi was found to be maximum in rhizospheric region than in the non-rhizosphere region. Maximum number of fungal species were found in *Abutilon indicum* (11) followed by *Aloe vera* (9), *Achyranthus aspera* (9), *Amaranthus polygamus* (8) and *Argemone maxicana* (7). Furthermore, *Glomus mosseae* and *Acaulospora laevis* were found dominant mycorrhizal species in the rhizospheric soil of all five medicinal plant species. Medicinal plants are associated with a broad variety of microbial contaminants mainly Bacteria and Fungi. The study confirmed that the biodiversity of mycoflora differs in rhizosphere and rhizoplane of selected medicinal herbs (Corpetta *et al.*, 2005 and Mishra and Shukla, 2014.).

According to Chanda *et al.*, (2014), AM fungi are widespread and are found from arctic to tropics in most agricultural and natural ecosystems. They play an important role in plant growth, health and productivity. They increase seedling tolerance to drought, high temperatures, toxic heavy metals, high or low pH and even extreme soil acidity. The cultivation of medicinal and herbal plants has assumed greater importance in recent years due to their tremendous potential in modern and traditional medicine. They are also used as raw materials for pharmaceutical, cosmetic and fragrance industries. Indian system of medicine (ISM) uses 25,000 species belonging to more than 1000 genera. Comparatively very less attention has been given for the conservation of some of these rare and endangered medicinal plants which are extensively used by the tribes of Assam. So, AM fungi can play an effective role in the conservation of some valuable medicinal plants where *Glomus* sp.

was found to be widely used for the increase yield of important medicinal plants. This review summarizes the data from recent studies to elucidate the potential use of AM fungi for promoting growth and disease resistance in medicinal plants found in southern part of Assam, which in turn provide a natural enhancer for the commercial production of traditional drugs from various important plants.

Interaction of AM fungi with the Medicinal Plants

Mycorrhizal colonization resulted in increased accumulation of nutrients, chlorophyll, carotenoids, sugars and proteins. This was further confirmed from the presence of spores belonging to different VAM fungal species in the rhizosphere soils. VAM inoculation significantly increased the uptake of N, P and, but most markedly increased of P uptake. The effects of inoculation with vesicular arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* on the root colonization, growth, essential oil yield and nutrient acquisition in three cultivars of menthol mint (*Mentha arvensis*), *Ocimum sanctum*, *Catharanthus roseus*, *Coleus forskholii* and *Cymbopogon flexuosus* (Karthikeyan *et al.*, 2009; Zhao *et al.*, 2012). In the VAM inoculated plants, the percentage of VAM associations were observed significantly higher than the control one (Sagar and Kumar, 2009). The present investigation was carried out to study the diversity and colony forming units of rhizospheric soil, Arbuscular mycorrhizal (AM) spore population and identification and characterization of AM spores up to genus level from 5 medicinal plants viz., *Mentha* sp., *Ocimum sanctum*, *Bryophyllum* sp., *Adhatoda vasica* and *Andrographis paniculata* (Table 1).

Materials and Methods

A. Sample collection

For the isolation of fungal colonies from rhizospheric soil of medicinal plants, soil was collected from rhizospheric region of five medicinal plants viz- *Mentha* sp., *Bryophyllum* sp., *Adhatoda vasica*, *Andrographis paniculata* and *Ocimum sanctum* from G.C. College campus. The soil adhered to the root was collected to sterile polyethylene bags and transferred aseptically to the laboratory.

B. Isolation of Arbuscular Mycorrhizal (AM) spores

The serial dilution technique is followed for the isolation and enumeration of indigenous fungi from rhizospheric soil in potato dextrose agar. Spore extraction from the soil was carried out using the Wet Sieving and Decanting Technique of Gerdemann and Nicolson (1963). Sieve of sizes ranging from 63 to 250 μm were used to separate the spores from the soil samples. During this process care was taken to retain the structures associated with spores such as attached hyphae, hyphal terminus, bulbous suspensor, etc. as such centrifugation in sucrose and or vigorous washing of the spores was avoided. Roots and detritus from the soil were gently sieved to examine the attached hyphae, which may have clinging spores. Sieved spores were placed in water and observed with a stereomicroscope under reflected light. Plastic petriplates were used to count spores because the base of the plate was flat and the disk also was hydrophobic. Dishes which were 85 μm across in diameter were used. Spore characteristics those were recorded were spore colour (using reflected light), type of hyphal attachment, general nature of the spore contents, dull on shiny spore surface, general spore shapes, etc.

C. Diagnostic slide

The isolated spores were mounted on glass slide using Polyvinyl Alcohol-Lactic acid Glycerol (PVLG) as mountant. The spores were then observed for their distinguishing morphological characters such as size, shape, wall characteristics etc., under a compound microscope (100-1000X).

D. Spore Identification

Spores were identified according to the manual of identification of AM fungi by Schenck and Perez (1990). The INVAM worksheet was used for diagnosing the spores. Additional spores not included in the manual were identified as per the description given in the INVAM website (<http://invam.caf.wvu.edu/>). The characteristics used in the identification of genus include spore colour, size and shape, spore walls number, colour, thickness and ornamentation, hyphal attachment, shape and type of occlusions, etc.

Table 1: Taxonomic data and medicinal properties of selected plants

S.No.	Name of medicinal plants	Vernacular name	Family	Medicinal properties
1.	<i>Mentha sp.</i>	Pudina	Lamiaceae	Stomachic, bad breath, diarrhoea.
2.	<i>Ocimum sanctum</i>	Tulsi	Lamiaceae	Coughs, asthma, bronchitis.
3.	<i>Bryophyllum sp.</i>	Patharkuchi	Crassulaceae	Dysentery, blood pressure, cardiac problem.
4.	<i>Adhatoda vasica</i>	Adusa	Acanthaceae	Leprosy, blood disorders, vomiting.
5.	<i>Andrographis paniculata</i>	Kalmegh	Acanthaceae	Headache, fever, fatigue



Table 2: C.F.U of isolated fungi from rhizospheric soil of medicinal plants in different dilutions

Sl.No.	Name of the medicinal plants	Local name	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
1	<i>Mentha sp.</i>	pudina	162	58	10	4
2	<i>Ocimum sanctum</i>	Tulsi	68	32	18	6
3	<i>Bryophyllum sp.</i>	Patharkuchi	156	78	22	12
4	<i>Adhatoda vasica</i>	Adusa	72	45	18	8
5	<i>Andrographis paniculata</i>	Kalmegh	91	53	31	15

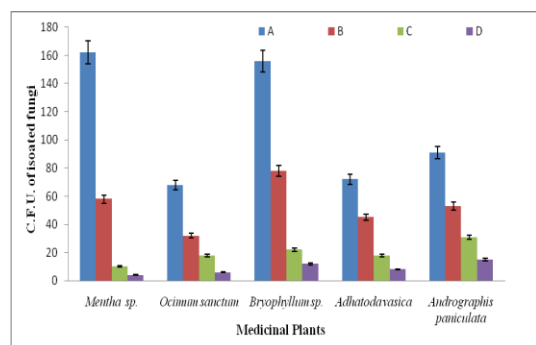


Figure 1: C.F.U. of isolated fungi from rhizospheric soil of medicinal plants in (different dilutions A= 10⁻¹, B= 10⁻², C= 10⁻³, D=10⁻⁴)

Table 3: Arbuscular mycorrhizal (AM) spore population (50gm⁻¹) of medicinal plants

S.No.	Name of the medicinal plants	Arbuscular Mycorrhizal (AM) spore population (50gm ⁻¹)
1	<i>Mentha sp.</i>	75±0.5
2	<i>Ocimum sanctum.</i>	112±0.3
3	<i>Bryophyllum sp.</i>	89±0.4
4	<i>Adhatoda vasica.</i>	94±0.2
5	<i>Andrographis paniculata</i>	121±0.6

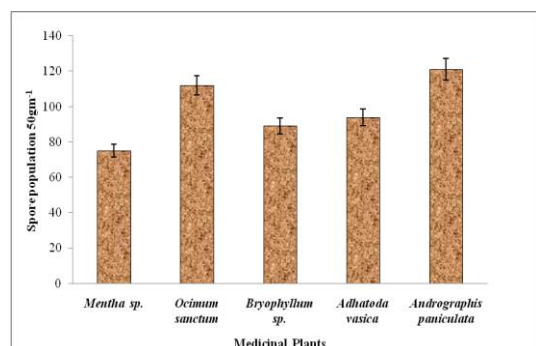


Figure 2: Arbuscular mycorrhizal (AM) spore population (50gm⁻¹) of medicinal plants

Results and Discussion

All the medicinal plants show a very good diversity of rhizospheric soil fungi. *Mentha sp.*, *Ocimum sanctum*, *Bryophyllum sp.*, *Adhatoda vasica*, *Andrographis paniculata*. The highest number of Colony forming unit (c.f.u) was estimated in *Mentha sp* (162/10⁻¹) followed by *Bryophyllum sp.* (156/10⁻¹), *Andrographis paniculata* (91/10⁻¹), *Adhatoda vasica* (72/10⁻¹) and *Ocimum sanctum* (68/10⁻¹)

(Table 2). The presence of 16 species of fungi also isolated from rhizosphere soil of the medicinal plants like *Centella asiatica* and *Ocimum sanctum* (Barik *et al.*, 2010). The importance of microbial diversity and their role in overall growth, development and quality of various medicinal plants (Johansson *et al.*, 2004).

The colony forming units (c.f.u) in all the medicinal plants significantly varied. *Bryophyllum sp* showed highest fungal diversity followed by *Andrographis paniculata*, *Bryophyllum sp*, *Mentha sp* and *Ocimum sanctum* (Figure 1). The symbiotic interactions of fungi, bacteria help in the mineral activity of the roots and thereby improving host plant nutrient uptake he nutrient and space competition with the pathogenic bacteria, changing anatomical structure and the morphology of roots, balancing the host plants endogenous hormones, activating the host plants defence system and restoring the balance of host rhizosphere soil conditions (Srivastava *et al.*, 2013).

Arbuscular mycorrhizal (AM) spore population was also estimated in all the medicinal plants. The highest number of AM spores was estimated in *Andrographis paniculata* (121/50gm⁻¹) followed by *Ocimum sanctum*(112/50gm⁻¹), *Adhatoda vasica* (94/50gm⁻¹), *Bryophyllum sp.*(89/gm⁻¹) and *Mentha sp* (75/ 50gm⁻¹). (Table 3; Figure 2). Medicinal plants with VAM fungus is responsible for significant increase in biomass production (dry wt. of root and shoot). The reason may be due to the formation of external mycelium around the roots by VAM fungi. Similar improved growth response was also observed in 10 medicinal plants when inoculated with three AM fungal species (*G. mossae*, *G. fasciculatum* and *G. monosporum*) for their efficiency (Sampathkumar *et al.*, 2007 and Gaur *et al.*, 2011; Sinigani and Yaganeh, 2017).



A: Microscopic view of AM spores *Glomus* sp. (*Mentha* sp. and *Andrographis paniculata*)



D: *Glomus* sp. (*Bryophyllum* sp.) E: *Glomus* sp. (*Ocimum sanctum*)

Plate 1: Some dominant Arbuscular Mycorrhizal (AM) spores isolated from the Medicinal Plants

Among the isolated genera of AM fungi, *Glomus* was the most dominant AM genus isolated during the present investigation (Plate 1). Similar findings were reported already by different workers who found that plants inoculated with mycorrhiza (*Glomus* sp.) showed significant increase in the growth over non-mycorrhizal plants and also had higher percent of phosphorus over non-mycorrhizal plants after six months field survey. *Glomus aggregatum* and *Glomus fasciculatum* were predominantly present and associated with all the *Ocimum* species as reported by Kumar *et al.*, (2010) and Chanda *et al.*, (2014) and Sinemani & Yeganeh. (2017).

Conclusion

Medicinal plants are used as traditional herbal medicines and are increasingly being used by people for primary health care system. Thus, the cultivation of medicinal plants should be increased to maintain a regular supply and to support their increasing demand by the use of Mycorrhizal fungi for sustainable medicinal plants productivity. The rhizospheric soil fungal diversity including mycorrhizal fungi also beneficial for increasing the photosynthetic

activity due to increased uptake of phosphorus in medicinal plants.

The beneficial role of rhizosphere AM fungi (*Glomus* sp.) is to enhance the tolerance to various biotic and abiotic stresses, thereby increase the growth of medicinal plants. In improving the socio-economic and cultural status of native people for collection of medicinal plants and their mycobionts like AM inoculum to increase the yield of medicinal plants along with their secondary metabolites to utilize these natural resources on a sustainable basis as a traditional and herbal medicine for the human welfare.

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