SYNOPSIS
INTRODUCTION

India is endowed with a rich wealth of medicinal plants. Although a good proportion of the medicinal plant species do occur throughout the country, peninsular Indian forests and the Western Ghats are highly significant with respect to varietal richness (Parrota, 2001). Medicinal plants are important for pharmacological research and drug development, not only as plant constituents used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Mukherjee, 2003). It is reported that in India, 4,365 ethnic communities, including over one million folk healers, use around 8,000 species of medicinal plants. They are also increasingly becoming economically important due to the growing demand for herbal products in the domestic and global market.

Across the country, the forests are estimated to harbour 90% of the country's medicinal plant diversity and only about 10% of the known medicinal plants of the country are restricted to non-forest habitats. Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products, being non-toxic, having no side effects and are easily available at affordable prices. Due to an increasing demand for medicinal plants and a loss and fragmentation of natural habitats, close to 300 species of Indian medicinal plants have been so far assessed as under threat in the wild (based on International Union for Conservation for Nature (IUCN) (Red List Criteria). Around 1,000 species are
estimated to be facing various degrees of threat across different biogeographic regions in the country (Seth and Sharma, 2004).

Arbuscular mycorrhizal (AM) fungi are a major component of rhizosphere microflora in natural ecosystems and play significant role in the re-establishment of nutrient cycling (Peterson et al., 1985). They can modify the structure and function of plant communities (Douds and Miller, 1999) and may be useful indicators of ecosystem change (McGonigle and Miller, 1996).

Although AM fungi have not been used specifically to increase the production of medicinal compounds in plants, their ability to enhance the plant growth and root health has been demonstrated earlier in many crop species (Maier et al., 1995; Van Loon et al., 1998). The use of microbial association for medicinal crops provides a sustainable approach to improve crop quality and yield. It provides the potential to increase production, value and export of human health enhancing crops and products.

The Western Ghats, a valuable repository for biodiversity after the Himalayas, is one of the 34-mega diversity hot spots of the world. It contains 4000 (27%) of the country's plant species, of which 38% (1500 species) are endemic and also a treasure house of over 8,000 ethnic and endemic varieties belonging to 386 botanical families which accounts for one-fourth of the world's medicinal plants. The high biodiversity of the Western Ghats can be attributed to its varied habitat types ranging from semi-arid grasslands to tropical rainforests. In the recent years, mycorrhizal association in
several plant species from different habitat types of the Western Ghats region in Southern India and Goa have been reported (Appasamay and Ganapathi 1995; Muthukumar et al., 1996; Muthukumar and Udaiyan, 2001; Khade et al., 2002 and Bukhari et al., 2003). However, the species diversity and composition of AM fungal communities from medicinal plants of the Western Ghats, Goa region is largely unknown. Therefore, the present work was undertaken to study the AM fungal diversity in medicinal plant species of Western Ghats of Goa region.

AIMS AND OBJECTIVES:

1. To study root colonization of AM fungi associated with the medicinal plants selected for the study.

2. To isolate, identify and study the diversity of AM fungal spores from the rhizosphere soil of medicinal plants.

3. To identify dominant indigenous AM fungi and their multiplication in the roots of compatible host in pot cultures.

4. To study the histochemical localization of polyphosphate granules and lipid bodies in intraradical mycelium of selected AM fungal species.

5. To investigate mycorrhizal status of medicinal plants selected for the study as influenced by phenology.

6. To study the response of selected AM fungal species on growth of selected medicinal plant species.
METHODOLOGY

1. Roots and rhizosphere soil samples of selected medicinal plant species were collected from different localities in north and south Goa of Western Ghats region.

2. Assessment of AM fungal colonization was carried out in roots of selected plant species by trypan blue staining method (Koske and Gemma, 1989).

3. Quantification of AM fungal colonization in roots was carried out using slide method (Giovannetti and Mosse, 1980).

4. Arbuscular Mycorrhizal fungal spores were isolated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963; Muthukumar et al., 1996) and quantification of spore density was carried out as described by Gaur and Adholeya, (1994).

5. Trap cultures of the above isolated AM fungal spores were carried out by open pot cultures (Gilmore, 1968) using Coleus sp. as host plant.

6. Taxonomic identification of intact and unparasitized AM fungal spores was carried out by using various bibliographies (Almeida and Schenck, 1990; Bentivenga and Morton, 1995; Walker and Vestberg, 1998; Redecker et al., 2000; Morton and Redecker, 2001) and INVAM (International Culture collection of Vesicular Arbuscular Mycorrhizal Fungi) (http://invam.caf.wvu.edu.).

7. Diversity studies were carried out using Simpsons Diversity Index, (Simpson, 1951), and Shannon Wiener Index (Weaver and Shannon, 1949).
8. Histochemical analysis were carried out for the localization of polyphosphate granules and lipid bodies using histochemical stains viz., Toluidine blue O (TBO) (Kumble and Kornberg, 1996), and Sudan Black (McGee-Russell and Smale, 1963).

9. Estimation of phosphorus (P) concentration (ppm) in selected medicinal plant species was carried out using Vanadomolybdate phosphoric yellow colour method (Chapman and Prat, 1961) following dry ash digestion procedure.

10. Mycorrhizal dependency (RMD) (Plenchette et al., 1983) and Mycorrhizal Efficiency Index (MEI) (Bagyaraj, 1994) were calculated for growth response studies in selected medicinal plant species.

11. Estimation of andrographolide, the main secondary metabolite in *Andrographis paniculata* was carried out using HPLC analysis (Pholphana et al., 2004)

**OBSERVATIONS:**

The first chapter deals with study of AM fungal association in selected wild and cultivated medicinal plants growing in the Western Ghats area of Goa State. The objective of this study was to survey the medicinal plants for AM fungal association. In all, a total of 36 plant species from north and south Goa areas of Western Ghats were selected for the study. A total of 30 plant species were found to be mycorrhizal...
and characterized by the presence of hyphae, vesicles and arbuscules. Absence of colonization was observed in *Commelina benghalensis*, *Physalis minima*, *Adathoda vasica*, *Murraya koenigii*, *Piper nigrum* and *Euphorbia pulcherrima*. Maximum root colonization was recorded in *Azadirachta indica* and *Cajanu*s sp. (100%) while it was minimum in *Alpinia galanga* (8.33%).

The second chapter deals with diversity of AM fungal species in selected medicinal plants growing in the Western Ghat areas of Goa State. The objective of this study was to record the spore density and AM fungal diversity in medicinal plants. Forty-two AM fungi belonging to five genera *viz.*, *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora* and *Ambispora* were recovered from the rhizosphere soil samples. No significant positive correlation was found between percent colonization and spore density. Spore density varied from 1197 spores (*Hemidesmus indicus*) to 14 spores (*Eclipta alba*). Simpson’s and Shannon Weiner Diversity Index studies carried out in north and south Goa exhibited less variations in both the sites indicating a stable and a diverse plant community.

The third chapter deals with taxonomy of AM fungi associated with medicinal plants. The objective of this study was to identify the dominant AM fungal species. The study revealed that *Glomus* was the most dominant genera with *Glomus fasciculatum*, as the most dominant species followed by *A. scrobiculata*. Trap cultures of AM fungal spores were prepared using pot cultures with *Coleus* sp. as host plant.
In all, a total of seven AM fungal species viz., *A. laevis, A. scrobiculata, Glomus aggregatum, G. fasciculatum, G. geosporum, Gigaspora albida* and *Scutellospora gregaria* were recovered from trap culture which were later used to prepare monospecific cultures.

The fourth chapter deals with histochemical localization of polyphosphate granules and lipid bodies in the intraradical mycelia of two selected AM species. The objective of this study was to locate the accumulation of polyP granules and lipid bodies in the intercellular hyphae in roots of *Coleus* sp. inoculated with monospecific cultures of *Gigaspora albida* Schenck & Smith and *Glomus clarum* Nicolson & Schenck. Accumulation of polyP granules was located in the intercellular hyphae in roots inoculated with *Gi. albida* which stained pinkish purple in Toluidine blue O at pH 1. The study revealed accumulation of lipid bodies in the form of droplets of varying sizes in the intraradical hyphae and spores of *G. clarum*, which stained bluish black in Sudan Black.

The fifth chapter deals with AM fungal status of medicinal plants as influenced by its phenology. The objective of this study was to record the variations in sporulation of AM fungi, and to study P concentrations in three herbaceous medicinal plants viz., *Rauwolfia serpentina* Benth, *Catharanthus roseus* L. and *Andrographis paniculata* Nees growing in wild. Results revealed that all the plant species were colonized by AM fungi but varied in the extent of colonization and
sporulation during different growth stages. Twenty AM fungal species were identified from the rhizosphere soil samples. *Glomus fasciculatum* followed by *G. geosporum*, *G. maculosum* and *A. scrobiculata* were found to be the dominant AM fungal species in terms of relative abundance and frequency of occurrence at different growth stages. The results of the present study revealed that increase in the P concentration during flowering stages of *A. paniculata* and *C. roseus* is directly related to the presence of arbuscules as flower initiation required extra uptake of P.

The sixth chapter deals with the response of AM fungal inoculation on the growth of *Andrographis paniculata*, an important medicinal plant. The experiment consisted of seven treatments viz., Un-inoculated control (sterilized soil), Un-inoculated control (unsterilized soil) viz., *Scutellospora biornata* Spain, Sieverding & Toro, *Acaulospora scrobiculata* Trappe, *Gigaspora albida* Schenck & Smith, *Scutellospora calospora* (Nicolson & Gerdemann) Walkers & Sanders and *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske. The study revealed that plants grown in unsterilized soil showed high Mycorrhizal Dependency (MD), and Mycorrhizal Efficiency Index (MEI) followed by *Gi. albida*, whereas the maximum concentration of andrographolide (secondary metabolite) was recorded in plants inoculated with *Gi. albida* compared to other treatments and control.
CONCLUSION:

The present study revealed a rich and stable diversity of AM fungi associated with medicinal plant species of Western Ghat areas of Goa. The study also showed an increase in the P concentration due the presence of arbuscules which corresponds to active P accumulation in wild medicinal plant species. The growth response studies in A. paniculata on inoculation with AM fungi enhanced plant growth and increased the concentration of secondary metabolite. The concentration of andrographolide varied among the treatments and was independent of P level and is mainly due to colonization and plant defense response induced by the presence of AM fungi and not by the nutritional status of the plant.

BIBLIOGRAPHY:


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