REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

Mycorrhizae are non-pathogenic symbiotic soil fungi which invade on or in to the root system of plants. Among the different types of mycorrhizae, Arbuscular Mycorrhizal (AM) fungi have gained much importance in the field of agriculture. It has been found to improve the growth and various other plant parameters providing a scope for improvement in the growth of numerous plant species. It has been found that even in unsterile soils, plants respond to inoculation with efficient strains of AM fungi (Bagyaraj and Manjunath, 1980).

Mycorrhizae are vital for the uptake and accumulation of mineral ions from soil and translocation to hosts because of their high metabolic rate and strategically diffuse distribution in the upper layers of soil. The hyphal network of mycorrhiza serves as a highly efficient extension of the host root system. Bieleski (1973) reported that AM fungi increased the effective absorbing surface of a host root by as much as ten times. Mycorrhizal fungal hyphae extend into the soil, penetrating the zone of nutrient depletion and can increase the effectiveness of absorption of immobile elements like phosphorus, zinc and copper, which do not diffuse readily through soil, by as much as 60 times.

The main advantage of mycorrhiza is its greater soil exploration and increasing uptake of P, N, K, Zn, Cu, S, Fe, Mg, Ca and Mn and their supply to the host roots (Habarte and Manjunath, 1987; Lu and Miller, 1989; Johnson et al., 1991; Kothari et al., 1991; Lambert and Weidensaul, 1991; Li et al., 1991; Smith et al., 1994 a; Selvaraj and Subramanian, 1995 and Abdul Malik, 2000). In
addition to enhancing plant growth, AM fungi exhibit synergistic activities such as biological control of root pathogens, hormone production and greater ability to withstand water stress (Raman and Mahadevan, 1996). They produce enzymes and growth hormones like phosphatases, phytases, auxins, vitamins, cytokinins and other substances that increase rootlet size and longevity (Mehrotra, 2005).

2.1. Distribution of AM fungi in various habitats

The occurrence of AM fungi has been reported in many plant communities such as forests (Thapar et al., 1992; Raman et al., 1993 and Sengupta et al., 1998), grasslands, steppes and prairies (Dhillion, 1992 and Sanders and Fitter, 1992), deserts (Khaliel, 1988), mangroves (Sengupta and Chaudhuri, 1989 and Selvaraj et al., 1994) and sand dunes (Koske and Gemma, 1990 and Rodriguez and Jaiswal, 1999).

Mohankumar and Mahadevan (1988) studied in detail the viability of AM spores in a tropical forest soil. Bhaduria and Yadava (2000) conducted field surveys to determine the status of AM fungal associations in *Acacia nilotica* of various forest nurseries and plantations in alkaline (Usar) soil sites of Manipuri, Uttar Pradesh. They further reported that the mean number of AM propagules and percentage root colonization were greater in plantation samples than in nursery samples.

Dwivedi et al. (2003) studied the qualitative and quantitative distribution of arbuscular mycorrhizal fungal spores in the rhizosphere soils of betel (*Piper betle*) vine in four villages of Sagar, Madhya Pradesh. They recorded seven
species from *Glomus*, four from *Acaulospora* species and one species from the genus *Gigaspora*.

### 2.2. AM fungal association in medicinal plants

AM fungal are present in practically all soils and associated with a great variety of plants of different taxonomic groups (Jeffries, 1987). Originally, medicinal plants in India were reported to be non-mycorrhizal due to the presence of various secondary metabolites (Mohankumar and Mahadevan, 1984). However, roots of field-grown garlic were found to be colonized by AM fungi (Shuja and Khan, 1997).

Rao *et al.* (1988) examined 25 medicinal plant species growing in red sandy loam soil and found that the plants harbored AM fungi in their root systems. Such reports of AM fungal colonization of medicinal plant roots are now supported by Asian mycologists who found the roots of many medicinal plants to be mycorrhizal (Lakshman and Raghavendra, 1990; Sullia and Prabha Sampath, 1990; Sharma and Roy, 1991; Ueda *et al*., 1992; Burni *et al*., 1994 and Ratti and Janardhanan, 1995).

Occurrence of AM fungi in 7 aromatic *Cymbopogan* species cultivated in Jorhat, Assam were noted, in which *C. curatus* showed maximum colonization (82.2%) (Janardhanan *et al*., 1990). The incidence of AM fungal association in rhizomatous tubers and roots of *Gloriosa superba* and the diversity of AM fungi associated with it were reported by Khade and Rodrigues (2003).
The association of AM fungi with the roots and rhizomes of Marsilea minuta, an aquatic fern was reported by Rama Bhat and Kaveriappa (2004). Thirty common herbaceous medicinal plants of 14 different families were studied from four different sites of Thanjavur district and screened for AM colonization in the roots as well as their existence as spores in root zone soils (Rajasekaran and Nagarajan, 2005). Of the 30 species, 26 were positive for AM colonization in roots and totally 20 different AM fungal species belonging to the genera Acaulospora, Endogone, Entrophospora, Gigaspora, Glomus, Sclerocystis and Scutellospora were observed in the rhizospheric soils of the host plants. Also a certain degree of specificity was observed among the different species of AM in their association with the root zone soils of the host plants.

2.3. AM status of plants in the Western Ghats

The Western Ghats are one of the 18 hotspots of biodiversity in the world and is comparable to the Himalayas in its diversity of flora. The Western Ghats possess about 4,000 species of rich flora of flowering plants due to remarkable vegetation types, varied climatic conditions and soil types. Mohankumar and Mahadevan (1987) found mycorrhizal associations in 131 out of 178 plant species in Kalakad reserve forest. The occurrence of mycorrhizal fungi in the tropical forest trees of Tamil Nadu was reported by Mohankumar and Mahadevan (1987a).

The mycorrhizal status of the Indian flora is largely unknown. Records exist for the mycorrhizal status of aquatic and marshy plants (Ragupathy et al., 1990 and Sengupta and Chaudhuri, 1990) for plants in the tropical plains
(Ragupathy and Mahadevan, 1993), semi-arid soils (Neeraj and Verma, 1991 and Rachel et al., 1990), sand dunes (Mohankumar et al., 1988) and mangrove (Mohankumar and Mahadevan, 1986). However, only little is known about the mycorrhizal status of plants in the Western Ghats as reported by Appasamy and Ganapathi (1995), Muthukumar et al. (1996) and a few other authors. Raja et al. (1995) reported the mycorrhizal status of 43 pteridophytes from the Palani and the Kodaikanal hills of Western Ghats.

Kannan and Lakshminarasimhan (1989) reported that 48 plant species belonging to 38 families of plants from Point Calimere reserve forest of Thanjavur district differed in their mycorrhizal associations. Muthukumar and Udaiyan (2000b) examined the mycorrhizal association in 71 species of pteridophytes of Western Ghats and reported that 60 species (85%) with AM association were characterized by intraradical and extraradical hyphae, intracellular hyphal coils, inter- or intra-cellular vesicles and/or arbuscules.

Of the 329 plant species examined in the Western Ghats by Muthukumar and Udaiyan (2000a), mycorrhizal infections were observed in 174 plant species. The mycorrhizal colonization was characterized by arbuscules, intraradical hyphae and intercellular hyphal coils with or without vesicles. Mycorrhizas were most frequent in Papilionaceae, Caesalpinaceae, Rubiaceae, Compositae, Boraginaceae, Convolvulaceae, Acanthaceae and Mimosoideae. Forty one percent of the plant roots studied were colonized by “Glomus-type” vesicles and hyphae, not coupled with arbuscules. Their study further indicated the predominance of Glomus over other genera with species like G. aggregatum,
G. geosporum, G. macrocarpum and G. mosseae being widely distributed in different sites.

2.4. Physiology and growth promotion by AM fungi

2.4.1. Physiology of mycorrhizal dependency

Mycorrhization results into an increase in the below ground surface area of the host plants, facilitating more efficient uptake of nutrients. It is especially significant for those plant species, which have a root system not itself adequate to draw optimum quantities of the relatively immobile nutrients, like phosphorus. Plants, which have coarse and poorly branched, scarcely haired or hairless roots or very short root hairs and those which lack specific root response mechanisms to nutrient deficiencies, commonly behave as mycorrhiza dependent. Dependency here means that plants infected by mycorrhiza derive large physiological benefits through improved nutrition of phosphorus and a few other essential nutrients. Physiological basis of mycorrhiza, especially arbuscular mycorrhizal dependency, can be explained almost entirely by improvements in the phosphorus nutrition of plants.

Under appropriate conditions, mycorrhizal plants are credited with 3 to 5 times higher rate of phosphorus uptake per unit root length than non-mycorrhizal plants of the same dependent species (Bolan, 1991; Tinker et al., 1992 and Schachtman et al., 1998). External hyphae of the mycorrhizal roots extend well beyond the phosphorus depletion zone surrounding the absorptive roots, increasing the uptake through explosion of larger volume of soil (Smith and Gianinazzi-Pearson, 1988).
2.4.2. Edaphic factors on AM fungal development

Although AM fungi are known to exhibit little or no host specificity, ecological specificity may exist (McGonigle and Fitter, 1990). AM fungal species may prefer certain habitats to others and earlier studies clearly demonstrate the role of environmental factors and vegetation on AM fungal community composition (Brundrett, 1991). Changes in edaphic factors greatly influence mycorrhizal association. The number of AM spores was found to be increased in summer (Mason, 1964). Hayman (1970) suggested that the increased number of mycorrhizal spores in wheat fields during summer was related to season. Schwab and Reeves (1981) also indicated that in addition to temperature and light, mycorrhizal infection in semi-arid region is greatly influenced by the season.

Both the population size and the diversity of AM spores were higher in the rhizospheres of plants growing in sandy soils. In the rhizospheres of clayey, cultivated soils and aquatic sediments, the occurrence of AM fungi was reduced and their diversity was limited. Spores were rare in permanently water-logged soils and very rare in saline soils. Very few AM fungi were isolated from brackish environments with high salinity (Ragupathy and Mahadevan, 1991).

Sylvia and Williams (1992) studied the effect of soil pH on AM fungi and AM inoculated plants and reported that some AM fungi do not readily adapt to soils with pH unlike that of their soil of origin. They thus concluded that pH constraints AM fungal establishment.
2.4.3. AM fungi and plant growth response

AM associations enhance plants acquisition of nutrients by increasing the effective surface area of the root system. Mycorrhizae are especially important for plant survival and growth, when the soil has low concentrations of available nutrients, especially phosphorus. Mycorrhizal roots are able to obtain more nutrients from deficient soils than roots that are non-mycorrhizal because hyphal strands exploit a greater volume of soil than roots alone (Smith and Gianinazzi-Pearson, 1988).

The primary role of arbuscular mycorrhiza is to increase the absorption and translocation of essential ions that are relatively immobile, i.e., those ions that normally diffuse slowly towards the root, yet are needed in high quantity, such as phosphate, ammonium and nitrate. Elements that have been found to be translocated through the hyphae include P, Zn, S, Ca and N (Cooper and Tinker, 1981 and Liu et al., 2000). The direct uptake of elements such as N (Ames et al., 1983) and Zn (Bowen et al., 1974) was demonstrated by the use of radioactive isotopes of these nutrient elements, and it revealed the involvement of AM fungi in the uptake of these nutrients to the host plant to some extent.

2.4.3.1. Phosphorus uptake

Phosphorus (P) is one of the major elements utilized by plants, largely used in membranes, cell division, nucleic acids and high energy compounds. P is present as either organic or inorganic form in the soil. Inorganic phosphate (PO$_4^{3-}$) ions form the main source of available P to the plants. But majority of
inorganic P is in insoluble form. It is considered that hyphae of AM fungi utilize
the insoluble form of nutrients (Harley and Smith, 1983 and Hayman, 1983).

AM fungi produce a range of phosphatase enzymes by means of which
phosphates are taken up into cells for incorporation into nucleic acids and
phospholipids are stored as polyphosphates in vacuoles (Joner and Johansen,
1999). Further studies on the transport of P, including uptake of P from the soil,
translocation through the hyphae and transfer across the host fungus interface and
physiological aspects of nutrient uptake in mycorrhiza has been reported by many
authors (Bolan, 1991; Smith et al., 1994; George et al., 1995 and Jacobsen,
1999).

An increased concentration of P in mycorrhizal plants compared to non-
mycorrhizal plants has been reported in *Griselinia littoralis* (Baylis, 1959), onion
(Gray and Gerdemann, 1969), *Citrus* species (Kleinshmidt and Gerdemann,
1972), barley (Benians and Barber, 1972), chilli (Sreeramulu and Bagyaraj, 1986)
and in many other plants.

2.4.3.2. Uptake of plant nitrogen, potassium and other nutrients

Arbuscular mycorrhizal fungi do not fix nitrogen (N) at all, but actually
assist plants in uptake of P, which in turn affect the uptake of other nutrients such
as N. The ability of mycorrhizal roots to utilize N sources has been attributed to
the indirect effect associated with an improved P nutrition.

Nitrogen has been reported to stimulate as well as suppress root
colonization by AM fungi. There have been several reports on the suppression of
root colonization by N (Buwalda and Cole, 1982). Hayman (1983) showed that N fertilizers had a large negative effect on the mycorrhizal colonization following the application of 300 kg N as (NH₄)₂SO₄. Chambers *et al.* (1980) reported that both NO₃⁻ and NH₄⁺ depressed root colonization by AM fungi and suggested that the suppressive effect of NH₄ was due to drop in rhizosphere pH. In contrast, N application has been reported to increase AM fungal colonization in lettuce (Hepper, 1983). Nitrogen uptake by AM fungi from the soil may be influenced by a number of factors; predominant are the available ionic forms of nitrogen (NH₄⁺ and NO₃⁻). Factors such as organic matter content, soil texture, microbial mineralization and nitrification can greatly influence N uptake via extramatrical hyphae (Arines, 1990).

Harley and Smith (1983) reported that the increased plant growth resulting from AM symbiosis is associated with increased nutrient uptake by the hyphae from the soil. It has been proved that a hyphal network associated with the roots of a living plant can capably infect the roots of other plants growing in its vicinity (Chiariello *et al.*, 1982; Francis and Read, 1984 and Newman, 1988).

The role of AM fungi in the uptake of K, Ca, Mg and S is little known. The ability of the extramatrical AM fungal hyphae to uptake and transport K has been demonstrated in pot culture by George *et al.* (1992). Significant differences in the growth response of soybean to different geographical isolates of *G. mosseae* seemed to be more related to import of K rather than P nutrition of the host (Bethlenfalvay *et al.*, 1989). The hyphal uptakes of Ca (Rhodes and Gerdemann, 1978) and SO₄²⁻ (Cooper and Tinker, 1978) have been shown
through supplying radioisotopes ($^{54}C$ and $^{35}O_4$). The authors further concluded that the uptake and transport rates of Ca were very low compared to phosphorus.

Various reports attribute the enhancement of Zn and Cu uptake by AM plants to the uptake and transport of the nutrients by the external hyphae to the host plant. The hyphal contribution to the uptake of Zn was found to be 16 to 25% compared to 13 to 20% for P in maize plants grown in calcareous soil (Kothari et al., 1991). In the same soil, Li et al. (1991) demonstrated that the delivery of Cu from the hyphal compartment was 52 to 60% of the total Cu uptake under restricted rooting space. The role of AM fungi on boron (B) nutrition of the host plant is lacking. AM fungi may decrease boron concentrations in host plants (Kothari et al., 1991). Treeby (1992) indicated that AM fungi may facilitate the uptake of Fe in acidic soils. Ramarao and Manoharachary (1992) reported enhanced levels of Cu, Fe, Mn and Zn in roots and shoots of Mycorrhiza inoculated *Tectona* and *Terminalia* in 90 day old seedlings. An increase in the uptake of Fe by AM plants has also been reported by Raju et al. (1987).

2.4.3.3. Plant pigments

Studies on AM fungi inoculated plants have showed increased chlorophyll and carotenoid contents than uninoculated plants. Allen (1981) reported that leaves of *G. fasciculatum* inoculated tomato seedlings showed increase in total chlorophyll and carotenoid contents. Even after drought conditions, there are reports of higher chlorophyll concentrations in AM plants than uninoculated ones (Allen et al., 1981; Auge et al., 1987, Mathur and Vyas, 1995 and Gemma et al.,
1997). *G. aggregatum* inoculated *Alpinia* plants and *G. macrocarpum* inoculated *Coleus* plants were found to exhibit increased levels of total chlorophyll and carotenoid content in the leaves significantly than the uninoculated control plants as reported by Mani (2006).

2.4.3.4. Plant biochemical constituents

Krishna and Bagyaraj (1983) reported that the protein and amino acid contents were higher in *G. fasciculatum* inoculated *Arachis hypogaeae* roots. The protein content of root extracts of mycorrhizal inoculated plants of tobacco and onion were found to be much higher than non-mycorrhizal root extracts (Dumas et al., 1989). The report of Sharma and Kothari (1992) indicate that increase in protein content of AM colonized plants increases the synthesis of chlorophyll and growth rate. Even in drought conditions, the total protein concentrations of AM plants have been found to be higher than non-mycorrhizal plants (Ruiz-Lozano et al., 1995 and Subramanian and Charest, 1995 and 1998).

Studies on the lipid metabolism of AM fungi are very limited. Cooper and Losel (1978) showed that roots infected by *G. mosseae* contain significantly more lipid than the uninfected roots. The mycorrhizal roots of *Allium cepa*, *Trifolium repens* and *Lolium perenne* had large amounts of total lipid than the non-mycorrhizal roots. Gasper et al. (1997) characterized and quantified lipids and fatty acids in alfalfa during the development of *G. versiforme* in the alfalfa roots. A net increase in the total lipids in the root was observed to be proportional to the development of AM fungal colonization. Krishna and Bagyaraj (1984) reported an increase in phenolic content of roots of *Arachis hypogaeae* colonized by AM
fungi *G. fasciculatum*. Further reports by Mani (2006) also confirm a significant increase in the total phenolic content of roots and rhizomes of *Alpinia* and roots and leaves of *Coleus* plants inoculated with AM fungi.

2.4.3.5. **Plant enzyme activity**

The acid and alkaline phosphatase activities of AM inoculated plants have been found to be higher than in uninoculated plants. The presence of AM specific alkaline phosphatase activity in *Allium cepa* and *Plantanus occidentalis* plants inoculated with *G. mosseae* has been reported by Gianinazzi - Pearson and Gianinazzi (1976). Smith and Gianinazzi - Pearson (1987) found higher alkaline phosphatase activity in the *G. mosseae* infected roots of *Allium cepa*. Anita *et al.* (1988) noted higher acid phosphatase activity in the *G. fasciculatum* inoculated roots of *Trigonella* species.

Tarafdar and Claassen (1988) indicated that alkaline phosphatase in soil was solely of microbial origin, while plant roots produce only acid phosphatase (Tarafdar, 1989). The increased alkaline phosphatase activity in the mycorrhizosphere might be due to the release of this enzyme by AM fungi or other microorganisms, whose activities are enhanced by the mycorrhizosphere effect (Bethlenfalvay and Franson, 1988). The increase in alkaline phosphatase in AM plants grown in sterile conditions is attributed to AM fungi as plants secrete only acid phosphatase, and is further confirmed by the absence of other microbes in sterile soil (Tarafdar, 1989). The phosphatase activities in Glory lilly plants inoculated with *G. geosporum* and PGPRs were found to be significantly higher than uninoculated plants (Elango, 2004).
Peroxidases are enzymes which are involved in cell wall reinforcement during plant reactions to pathogens (Dixon and Harrison, 1990 and Collinge et al., 1994). Higher peroxidase activity has been demonstrated in mycorrhizal than in non-mycorrhizal roots (Spanu and Bon-Fante Fasolo, 1988). Peroxidase activity associated with epidermal and hypodermal cells has been reported to increase in mycorrhizal roots (Gianinazzi and Gianinazzi - Pearson, 1992). The increase in levels of polyphenol oxidase was observed in rhizomes and roots of *Alpinia galanga* and roots and leaves of *Coleus amboinicus* plants as influenced by *G. aggregatum* and *G. macrocarpum* respectively (Mani, 2006).

2.5. AM fungi and Plant Growth Promoting Rhizomicroorganisms (PGPRs)

Beneficial microorganisms referred as PGPRS (plant-growth promoting rhizomicroorganisms) enhance plant growth through numerous mechanisms including the protection of roots against infection by minor and major pathogens (Whipps, 1997 and 2001), enhancing the availability of nutrients to the host plant, lowering of the ethylene level within the plant or by the enhanced production of stimulatory compounds, such as plant growth regulators (Antoun and Prevost, 2005). Many common heterotrophic microorganisms have the ability to solubilize inorganic P into soluble form (Gaur, 1990) through the secretion of organic acids (Gaur, 1988 and 1990). Phosphorus solubilizing microorganisms are useful in the utilization of rock phosphate and mineralization of organic P into soluble form. The P solubilizers also produce fungistatic and growth promoting substances (Mishustin and Naumova, 1962). Some P solubilizing microbial species include *Pseudomonas striata*, *Bacillus coagulans*, *B. circulans*, *B. megaterium*,
B. subtilis, Micrococcus, Flavobacterium, Penicillium digitatum, Trichoderma sp. etc (Subba Rao, 1999).

Barea et al., (1975) first suggested the possibility of an interaction between mycorrhizal fungi and phosphate solubilizing bacteria (PSB), based on observations of growth synergism on corn plants dually inoculated with AM fungi and P solubilizing Pseudomonas species and Agrobacterium species. They found that PSB, inoculated onto seeds or seedlings maintained high populations for a longer period in the rhizosphere of mycorrhizal than non-mycorrhizal roots of lavender and maize.

Inoculation of clover with PSB and G. mosseae together significantly increased the dry weight over inoculation with either of these organisms alone (Delorenzini et al., 1979), especially when the plants were fertilized with rock phosphate. They also reported enhanced root colonization by AM fungi in the presence of PSB. Dual inoculation of lavender plants with the AM fungus and PSB also increased plant dry matter and P uptake in soils amended with rock phosphate (Azcon et al., 1976). Such positive interactions between PSB and AM fungi on plants have also been reported by Azcon - Aguilar and Barea (1978) and Krone et al. (1987).

AM fungi are well known for their ability to improve P nutrition of plants (Hayman, 1975 and Tinker, 1982). It was thought that the mycorrhizal hyphae contribute to the solubilization of mineral phosphate by their production of respiratory carbon dioxide and by excretion of organic acids (Paul and Clark,
1988), thus improving P nutrition of host plants. However, findings of Raj et al. (1981) on the impact of *Glomus fasciculatum* and a non phytohormone producing strain of the phosphate solubilising bacteria (PSB) *Bacillus circulans* on phosphate solubilization and growth of finger millet and P uptake from P\(^{32}\) labeled tricalcium phosphate and superphosphate indicated that AM fungi do not solubilize unavailable forms of P. They further showed that interactions between PSB and AM fungi resulting in increased plant growth could be attributed to the production of plant hormones or vitamins by PSB, rather than to P solubilization (Barea et al., 1975).

Barea et al. (1976) demonstrated that a majority of the PSB tested for production of hormone, produced indole acetic acid, gibberellins and cytokinins. Many PSBs are also vitamin producers and their numbers are proportionally higher in the rhizosphere soil. Baya et al. (1981) found that a large percentage of PSBs produced vitamins such as B\(_{12}\), riboflavin, niacin, pantothenate and biotin. Even though Bagyaraj (1984) suggested the role of vitamin synthesizing PSB in mycorrhizal development their exact role on AM fungal development could not be clarified by him.

2.6. Effect of AM fungi and PGPRs on medicinal plants

A considerable number of bacterial species exert a beneficial effect on plant growth. Mostly they are associated with the plant rhizosphere and hence called "rhizobacteria". This group of bacteria was found to be synergistic for plant growth and has been termed as "plant growth promoting rhizobacteria" (PGPR) (Kloepper et al., 1991). PGPRs normally include a range of organisms
that live in close association with plant roots, and also comprises of a wide
variety of microbes that have the ability to promote the growth of plants
following inoculation into seeds and subterranean plant parts (Kloepper et al.,
1988).

The role of AM fungi and PGPRs in improving the plant growth is well
documented (Lakshman, 1992 and Murthy et al., 1998). However, information on
the use of these beneficial microorganisms in medicinal plants is meager.
\textit{Andrographis paniculata} when subjected to dual inoculation with \textit{G. mosseae}
and \textit{T. harzianum} showed increase in the plant growth, biomass and alkaloid
content (Arpana, 2000). \textit{Coleus aromaticus} when inoculated with \textit{G. fasciculatum}
and PGPR recorded an increase growth, biomass and P content (Earanna et al.,
2001). The response of \textit{Phyllanthus amarus} to inoculation with \textit{G. fasciculatum}
and PGPRs either singly or in combination were studied under field conditions
(Earanna et al., 2003). Dual inoculation of \textit{G. fasciculatum} with either
\textit{B. coagulans} or \textit{T. harzianum} enhanced the plant growth and biomass of
\textit{Phyllanthus amarus} (Earanna et al., 2003).

2.7. Medicinal plants and their importance

Higher plants are still “the sleeping giants of drug development”, a
virtually untapped reservoir of potentially useful sources of drugs that will
continue to serve mankind in the twenty first century as they have done since the
dawn of history (Farnsworth, 1984). Indian flora is very rich in medicinal plants.
During the last two decades over 3,000 plants had been screened in India for their
biological activities. Bulk production of plant drugs has become an important
aspect of Indian pharmaceutical industry. Some of the drugs which are manufactured today include morphine, codeine, papaverine, thebaine, emetine, quinine quinidine, digoxin, caffeine, hyoscine, hyocyamine, atropine, xanthotoxin etc. (Agarwal and Paridhavi, 2007).

2.8. Phytochemistry of medicinal plants

Plant derived medicines have been a part of the human health care for thousands of years in Asian countries. Plant based medicines were commonly used in India and China. At the same time, indigenous people of the rest of the planet were also utilizing plants for curing diseases. The phytochemical composition and antimicrobial potential of plants has been investigated by many authors.

Different kinds of secondary metabolites such as alkaloids, terpenoids, steroids, coumarins, flavonoids and other compounds in the aqueous and organic solvent extracts of medicinally important plant *Ageratum conyzoides* was reported by Vyas and Mulchandani (1984), Gonzales *et al.* (1991), Mensah *et al.* (1993) and Menut *et al.* (1993).

Choudhary and Choudhary (1986) carried out qualitative phytochemical screening for saponin, syringin, leucoanthocyanin, napthoquinone and HCl/methanol test in *Cassia siamea, C. roxburghii, C. fistula, C. tora, C. sophora, C. occidentalis, Bauhinia variegata, Tamarindus indica, Delonix regia, Parkinsonia aculeata* and *Caesalpinia pulcherrima* of the family
Caesalpinaceae. Giuliano et al. (1996) isolated three antimicrobial isoflavanones namely desmodianones A, B and C from Desmodium canum (Leguminosae).

Phytochemical studies were carried out in the ethanolic extracts of 45 Indian medicinal plants by Iqbal and Arina (2001). Qualitative phytochemical tests and thin layer chromatography of plant extracts demonstrated the presence of common phytocompounds including tannins and flavonoids as major active constituents.

A novel abietane diterpenoid, indigoferabietone was isolated from the stems of I. longeracemosa and the structure was established by using spectral techniques (Thangadurai et al., 2002). A new compound indigocarpan and the known compound mucronulatol were isolated from chloroform extracts of I. aspalathoides by Selvam et al. (2004).

Preliminary phytochemical analysis of Indian medicinal plants Abrus precatorius, cesalpinia pulcherrima and Delonix regia revealed the presence of alkaloids and saponins (Jigna Parekh and Chanda, 2007).

2.9. Antimicrobial activity of medicinal plants

The antibacterial activity of garlic (Allium sativum, L.) has been observed by Johnson Micheal and Vaughn Roose (1969). Maiti et al. (1985) showed that the aqueous and alcoholic extracts of old bark of Eugenia jumbolana were antibacterial against Salmonella typhi, Shigella dysentriae and Salmonella boydi.
Rios et al. (1987) studied the *in vitro* activity of 81 plants that were used as antimicrobial agents against six bacterial strains including *S. aureus*. They recorded 30 plant extracts which showed good activity against the tested organisms. Lovelli and Fuller (1987) had reported antibacterial activity of *Allium cepa* against *S. aureus, E. coli, B. cereus, B. megaterium* and *B. subtilis*. Gandhi and Ghodekar (1988) reported that ginger (*Allium sativum*) has the property of inhibiting or destroying lactic acid bacteria and contaminants of milk. Ginger (*Zingiber officinale*) has been reported to be effective against the growth of many gram-positive and gram-negative bacteria (Mascolo et al., 1989).

The utilization of medicinal plants and their metabolites for the treatment of diseases have long been reported. About 40 percent of drugs came to the attention of the world only because of their use in traditional medicine (Abeleson, 1990). Francisco et al. (1990) tested phenolic compounds from three species of *Helichrysum* (*H. decumbens, H. stoechas* and *H. italicum*) for antimicrobial activity against *E. coli* and fungi (*Pencillium* species, *Cladosporium herbarum* and *Phytophthora capsicii*). The results revealed that phloroglucinal derivatives showed eight times more antibacterial activity than the acetophenone derivative.

A study on the antimicrobial activity of *Nigella sativa* seeds (black cumin) was carried out by Hanafy and Hatem (1991) using diethyl ether extract against Gram-positive bacteria *S. aureus* and gram-negative bacteria *P. aeruginosa, E. coli* and *Candida albicans*. Alade and Irobi (1993) investigated water, ethanol, chloroform and hexane extracts of *Acalypha wilkesiana* leaves for *in vitro* antimicrobial activities, and the results revealed that the aqueous extract
was found to be static in action while ethanol extract was uniformly bactericidal in effect.

Olga Batista et al. (1995) isolated a new abietane diterpene, 16-acetoxy 7-alpha, 12-dihydroxy 8, 12-abietadiene 11, 14-dione, from the acetone extract of the root of *Plectranthus hereroensis*. This compound showed antibacterial activity against *S. aureus, V. cholerae* and antiviral activity against Herpes simplex type II virus. Antibacterial activity of few aromatic herbs namely *I. caryophyllus, Prymus vulgaris, C. zeylanicum* and *Cuminum cyminum* extracted with hexane, against *S. aureus, E. coli* and *B. subtilis*, was reported by Agnihotri and Ashok Vaidya (1996).

Seven species of *Argyranthemum* (Asteraceae) were studied for antimicrobial and cytotoxic activities against gram-positive and gram-negative bacteria. Among them, *A. adauctum, A. foeniculaceum* and *A. frutescans* showed antimicrobial activity against gram-positive and gram-negative bacteria (Antonio et al., 1997). Xing-Cong Li et al. (1997) investigated methanol extract of *Ceanothus americanus* for antimicrobial activity against some of the oral pathogens (*Streptococcus mutans, Actinomyces viscosus, Porphyromonas gengivalis* and *Pervotella intermedia*).

Around 34 plant species belonging to different families selected on the basis of folklore medicinal reports were assayed for antibacterial activity against *E. coli, Enterobacter aerogenes, Proteus vulgaris* and *Pseudomonas aeruginosa* (Perumalsamy et al., 1998). Of these, 16 plants showed antibacterial activity.
Among them Cassia fistula, Terminalia arjuna and Vitex negundo showed significant antibacterial activity against all the bacterial pathogens. Antibacterial studies of 35 different Indian spices have been carried out against B. subtilis, E. coli and S. cerevisiae by Minakshi et al. (1999). Of the spices surveyed, clove, cinnamon, nutmeg, garlic and celery had potential antimicrobial activity against the tested microorganisms.

Pradhan et al. (1999) reported the antibacterial potential of the two new phenolic compounds viz., 3,4-dihydroxyphenyl ethanol glucoside and 3, 4-dihydroxy 6-N ethyl aminobenzamide, isolated from green pepper (Piper nigrum). These two compounds inhibited the growth of S. typhimurium, S. aureus, B. cereus and E. coli. The antibacterial and antifungal activities of the aqueous, petroleum ether and methanol extracts of Indigofera dendroid leaves have been studied and demonstrated by Esimone et al. (1999). Antibacterial activity was found in the ethanolic extract of Streblus asper leaves (Sopit Wongkhan et al., 2001). The extract possessed a selective bactericidal activity against Streptococcus mutans. It had no effect on E. coli, S. aureus, P. aeruginosa, Serratia marcescens, K. pneumoniae and Candida albicans.

In vitro microbicidal activity of essential oils of Coleus aromaticus and C. zeylanicus was tested against seven bacterial (B. subtilis, B. megaterium, E. coli, S. aureus, Proteus vulgaris, Pseudomonas aeruginosa and Xanthomonas campestris) and eight fungal species. Of the two oils tested, the oil of C. zeylanicus was found to be active against a wide spectrum of bacteria and fungi (Deena et al., 2002). The crude methanol extract from leaves of Mallotus
peltatus was found to be active against S. aureus, S. faecalis, B. subtilis, E. coli and Proteus mirabilis as reported by Debprasad et al. (2002).

Elizabeth (2003) examined the antimicrobial activity of Caesalpinia digyna against Staphylococcus aureus, Salmonella typhii, S. typhimurium, E. coli, P. aeruginosa and Candida albicans. The fruit extract of C. digyna showed broad-spectrum antimicrobial property and was detected to be more effective than the seed extract. Antimicrobial activities of Pithecolobium avaremotoma bark extract and methanol extract of Benincasa hispida were reported by Dubey (2003) and Natarajan et al. (2003) respectively.

Ashok Kumar et al. (2004) investigated the antimicrobial activity of ethanolic extract of the dried fruit of Terminalia arjuna against Streptococcus faecalis, Staphylococcus aureus, Salmonella typhimurium and Vibrio cholerae. The aqueous extract and various solvent (methanol, chloroform and hexane) extracts obtained from stem bark of Suregada angustifolia were tested by agar well diffusion method against 12 human pathogenic bacteria for antibacterial activity. Maximum antibacterial activity was observed in the order of chloroform, hexane and methanol extracts (Venkatesan et al., 2005).

The antimicrobial activity of crude methanol extract of Terminalia bellerica dry fruit was tested by disc diffusion method against nine human microbial pathogens [Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhii, S. typhimurium, Escherichia coli (entero-pathogen), E. coli (uropathogen), Candida albicans, Pseudomonas aeruginosa and Yersinia enterocolitica]. The
results showed that *Terminalia bellerica* was highly effective against *S. aureus* with lower MIC values. Their potential broad-spectrum activity indicated in the study was attributed to some biochemical alterations induced by *Terminalia bellerica* (Elizabeth, 2005). Kalorey et al. (2005) studied antibacterial activity of aqueous extract of *Butea frondosa* against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli* and *S. typhii*. Antimicrobial activity on various parts of *Tridax procumbens* (Asteraceae) was tested against *Bacillus cereus*, *Salmonella typhii Pseudomonas species*, *E. coli* and *Shigella dysenteriae* (Udaya kumar et al. (2005).

Hexane, methanol and water extracts of leaf, stem and roots were used to evaluate the antibacterial activity of *Clitoria ternatea* (Fabaceae) by Malabadi et al. (2005). Of them, the methanolic extracts showed the highest activity and no activity was recorded with water extract. Hexane and methanolic extracts of root showed the highest and significant antibacterial activity against both gram-positive and gram-negative bacteria. However no activity was recorded with the stem extracts.

The antibacterial activity of *Elaeodendron glaucum* has been tested against various human pathogens by Samuthirakani et al. (2006). The solvents used for extraction were petroleum ether, benzene, chloroform, methanol and distilled water. The methanol extracts showed antibacterial activity against all the nine chosen bacterial species namely *E. coli*, *P. aeruginosa*, *S. aureus*, *S. typhii*, *S. marcescens*, *K. pneumoniae*, *Enterobacter aerogenes*, *Proteus vulgaris* and *B. subtilis*. Antibacterial studies using heated and unheated aqueous extracts of
Ginger (*Zingiber officinale*) and Turmeric (*Curcuma domestica*) have been performed by Mausumi Paul *et al.* (2006) against *E. coli*, *B. subtilis* and *S. aureus*. The activity of heated extracts was found to be statistically greater than that of the unheated extracts for both the plants, used alone or as a mixture, there of against all the three bacterial strains used in the study as test organisms.

Sivakumar and Alagesa Boopathi (2006) investigated the ethanolic and methanolic soluble and insoluble fractions of two different forms of the seed extract of *Abrus precatorius* for their Minimum Inhibitory Concentration (MIC) against *S. aureus* and *B. subtilis*. Methanol soluble and insoluble fractions of both forms showed moderate activity against gram-positive organisms and did not show antibacterial activity against the gram-negative organisms, *E. coli* and *S. typhii*.

Lei *et al.* (2007) investigated various organic and aqueous extracts from leaves of *Indigofera suffruticosa* (Fabaceae), obtained by infusion and maceration, for their antibacterial activity and antifungal activities. They further reported that most of the extracts were devoid of antifungal activities and aqueous extract from the leaves showed strong inhibitory activity against the gram-positive bacteria, *S. aureus*.

Antibacterial screening of hexane, ethyl acetate, water and ethanol extracts of *Erythrina senegalensis* (Fabaceae) were done by Bako and Madhu (2007). The results showed that the ethyl acetate, ethanolic and water extracts had maximum activity on *S. aureus*, *E. coli* and *P. aeruginosa*. The hexane extract
showed no activity against any of the organisms whereas the other extracts showed antibacterial activity. Phytochemical and antibacterial investigations of crude extracts of the bark of *Vitellaria paradoxa* have been performed by Bako et al. (2007). The result showed the presence of saponins, tannins, reducing sugar, phlobatanins, cardiac glycoside and morphine alkaloid. The water, ethanol and ethyl acetate extracts had inhibitory effects on *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*.

The antibacterial effect of methanol and aqueous extract of *Abrus precatorius* (Fabaceae), *Cardiospermum halicacabum*, *Gmelina asiatica* and *Caesalpinia pulcherrima* (Caesalpinaceae) were evaluated against bacterial strains *B. cereus*, *S. aureus*, *Enterobacter aerogenes*, *E. coli* and *K. pneumoniae* by Jigna Parekh and Chanda (2007). The results showed that the most susceptible gram-positive bacterium was *B. cereus* and the most susceptible gram-negative organism was *K. pneumoniae* and the most active antibacterial plant was *C. pulcherrima*. Methanol extract of almost all the plants exhibited antibacterial activity.

The alcohol, butanol, diethyl ether, benzene and ethyl acetate fractions of *I. aspalathoides* were tested for antibacterial activity against *M. tuberculosis* by Rajkapoor et al. (2007). The results showed that the alcohol extract produced 48% inhibition of the organism (maximum activity for antimycobacterial). These studies clearly indicated that the extract exhibited antimycobacterial activity.