REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

Biofertilizers are of immense use in today’s farming system, considering the recent concept of sustainable agriculture and increased cost of inorganic fertilizers. Application of microbial inoculants based fertilizers are widely accepted as low cost supplements to chemical fertilizers and have no deleterious effect either on soil health or environment. Biofertilizers applied in combination with inorganic fertilizers reducing the required quantity of fertilizer has been reported in many field, horticulture, medicinal and aromatic crops (Anuradha, 2003). However, review pertaining to the use of bioinoculants in aromatic crops is scanty and in particular very less work has been done in patchouli. Therefore literature pertaining to the effect of biofertilizers and inorganic fertilizers on medicinal and aromatic crops has been reviewed and presented in this chapter.

The aromatic crops are firmly emerging on the scene in Indian agriculture from three different perspectives. First, the traditional health systems under Ayurveda, Siddha and Unani are becoming more and more popular mainly due to the holistic approach to treatment, reduced cost of treatment and least side effects. Second, the herbs were collected from the natural habitat indiscriminately and under minimal supervised environment. As a result, the density of medicinal and aromatic plants in the natural habitat are declining at a faster rate. This over-exploitation of these plant species has led to the cultivation of these under field conditions. Lastly, these crops are emerging as better economic opportunities than the traditional field crops. The price of these crops as raw-material to the pharmaceutical industries has increased substantially. This is also encouraged by the increasing demand of these crops in the world trade. All these have led
to the emergence of medicinal and aromatic crops as alternatives to some of the traditional uneconomic crops, in many parts of India.

2.1 Effect of nutrients on growth and yield in aromatic crops:

The aromatic crops require application of large quantities of chemical fertilizers, it is also observed that this group of crops holds promise for sustainable and organically oriented agriculture due to features such as (i) perennial nature of these crops minimizes tillage and the consequent ill effects on soil physical properties, which act as continuous ground cover and helps in reducing soil erosion (ii) comparatively these crops are less affected by pests and diseases, and the consequent absence or minimum use of pesticides protects environment from pollution and harmful residues and (iii) due to their commercial applications and suitability for value addition they offer diversification in rural enterprises (Prakasa Rao, 1996).

2.1.1 Palmarosa:

*Cymbopogon martinii* is a species of grass in the lemon grass genus best known by the common name palmarosa. Other common names include Indian geranium and rosha or rosha grass. This perennial grass is native to Southeast Asia, especially India and Pakistan, and it is cultivated for its oil. The essential oil of this plant, which contains the active compound geraniol, is valued for its scent and for a number of traditional medicinal and household uses.

Maheshwari *et al.* (1984) reported that N at 30 kg /ha was optimum and reported that application of P$_2$O$_5$ and K$_2$O had no significant effect on the herbage as well as oil yield. Similar results have been observed by Pareek *et al.* (1984) who recommended the use of 10 tonnes FYM per hectare at land preparation together with 40 kg N per hectare.
for optimum herbage (94.5 q/ha) and essential oil yield (107.7 lt /ha) in palmarosa. But a large number of reports of fertilizer use on palmarosa crop suggest the use of nitrogen between 40 and 80 kg together with 40 kg P₂O₅ and K₂O per hectare in different parts of the country (Pareek and Gupta, 1985).

2.1.2. Rosemary:

Rosemary (*Rosmarinus officinalis*) is a woody, perennial, aromatic herb with fragrant evergreen needle-like leaves. It is native to the Mediterranean region. It is a member of the mint family Lamiaceae. Rosemary contains a number of potentially biologically active compounds, including antioxidants such as carnosic acid and rosmarinic acid. Other bioactive compounds include camphor (up to 20% in dry rosemary leaves), caffeic acid, ursolic acid, betulinic acid, rosmaridiphenol, and rosmanol.

In rosemary, Dimri (1994) recommended a dose of 10 tones of FYM, 100 kg of N, 40 kg P₂O₅ and 40 kg K₂O per hectare to obtain high yields. While full dose of P and K was recommended to be applied at the time of planting, N was to be given in three split doses at four monthly intervals.

According to Munnu Singh and Ramesh (2000) application of 150 kg N/ha was found optimum for the good yield of rosemary and the oil quality was not affected by nitrogen and soil moisture regimes, but there was a seasonal influence in oil composition.

2.1.3. Ocimum species:

*Ocimum* is a genus of about 35 species of aromatic annual and perennial herbs and shrubs in the family Lamiaceae, mostly native to the tropical and warm temperate regions. Supply of nitrogen and phosphorus influenced plant height, number of branches, number of leaves and oil yield significantly. Overall, 60 kg N applied with 40 kg P₂O₅ per
hectare proved to be the most economic dose in *Ocimum canum* and *Ocimum americanum* at Jammu as observed by Balyan *et al.*, (1987).

Umesh *et al.* (1993) reported that P had no significant effect on either growth or yield parameters of *ocimum*. The results of six cropping over a period of two years showed that N and K significantly influenced the dry matter accumulation and leaf yield. While the application of 150 kg nitrogen per hectare significantly influenced the essential oil yield, were as application of 75 kg K₂O per hectare improved the yield and yield parameters significantly. Whereas, P₂O₅ had no significant effect either on growth or yield of *ocimum*.

A higher dose of nitrogen (75 kg / ha) recorded a higher oil and herb yield as compared to control in *ocimum* as suggested by Arularasum and Sambandamurthi (1999).

### 2.1.4. Geranium:

*Geranium* is a genus of 422 species of flowering annual, biennial, and perennial plants that are commonly known as the cranesbills. It is found throughout the temperate regions of the world and the mountains of the tropics, but mostly in the eastern part of the Mediterranean region.

Prakasa Rao *et al.* (1985) reported *geranium* to respond well to application of nitrogen fertilizers, while there was no response to applied P and K. It was recommended to apply 150 kg of nitrogen per hectare per year, in six splits at bimonthly intervals and 40 kg each of P₂O₅ and K₂O per hectare per year at the time of planting to obtain an yield of 40 tonnes of herbage per hectare per year which in turn yielded about 40 kg of *geranium* oil at 0.1 per cent recovery.
Rajeswara et al. (1989) reported application of 100 kg N per hectare to significantly increase the total herbage of the crop (19.1 %) and essential oil yield (24.1%) in geranium. Increasing N levels further (>100 kg) did not show any advantage.

On the contrary a significant increase was observed in the herb yield when nitrogen was applied @150 kg / ha (Pandu Shastry et al. 2000).

2.1.5. Davana:

Davana (Artemisia pallens) is an aromatic herb, xerophytic in nature. It is commercially cultivated for its fragrant leaves, flowers and essential oil. Davanone, Davan-Ether, Davana Furane and linalool are the major constituents of davana oil. Methyl cinnamate, ethyl cinnamate, bicyclogermacrene, davana ether, 2-hydroxyisodavanone, farnesol, geranyl acetate, sequiterpene lactones, germacranolides, etc. are also found in the oil.

Farooqi et al. (1991) studied the effect of N and P and their interactions on the growth, yield and oil content in Davana (Artemisia pallens Wall) and observed that the application of nitrogen at 180 kg / ha was responsible for increased herb (16.32 t/ha) and oil yield (21.67 kg/ha), besides increased growth. However, this was on par with nitrogen at 120 kg per hectare, whereas phosphorus did not have any appreciable effect on the growth, yield and oil content.

2.1.6. Patchouli:

Patchouli is a soil exhausting crop and requires liberal manuring. It responds well to both organic and inorganic manures. A trial conducted in Indonesia indicated that patchouli plants respond well to nitrogen and phosphorus but not to potassium (Adiwingenda et al., 1973).
Lokesh (1979) observed that the application of nitrogen had a significant influence on plant height, stem diameter, primary and secondary branches. Nitrogen at 100 kg per ha was found to be optimum in increasing the herbage yield for patchouli. For soils of poor fertility, a basal dose of 25 kg N, 50 kg P₂O₅ and 50 kg K₂O per hectare was given in the form of urea, super phosphate and muriate of potash at planting. After about 8 weeks, 25 kg N was applied in two split doses (the first immediately after harvest and the balance about two months later) thus a total of 150 kg N was applied per hectare every year (Sarwar et al., 1983).

Application of 150 kg of N, P₂O₅ and 50 kg K₂O per ha was suggested in soils with low fertility in patchouli crop (Hussain et al., 1988). Higher yields in patchouli can be obtained by administering 150: 50: 50 kg N, P₂O₅ and K₂O per hectare (Farooqi and Khan, 1991). Applying 140 to 160 kg N per hectare in four equal splits recorded maximum yield of oil indicating split application being advantageous for yield of herb and oil of the crop as reported by Saha et al., (1992).

Baskar and Putievsky (1978) reported that the application of N at 200 kg per hectare for patchouli gave 6.93 tonnes of fresh herbage per hectare but it was on par with 150 kg N. Singh (1999) found that, out of four levels of nitrogen (0, 25, 50 and 75 kg N / ha) application of 50 kg N per hectare gave optimum herbage and oil yield compared with that of control with no N application.

Singh et al. (2002) studied the influence of irrigation, organic mulch and nitrogen application on its growth, herbage and oil yield and quality of patchouli oil grown under field conditions. Irrigation at 1.0 IW: CPE ratio (irrigation water: cumulative pan
evaporation), 5 t ha\(^{-1}\) distilled waste material of palmarosa or 200 kg N ha\(^{-1}\) produced maximum herbage and oil yields.

2.2. Beneficial microflora promoting plant growth and yield in aromatic crops:

Soil microorganisms play a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients such as N, P and K. It is well-recognized fact that microbial inoculants constitute an important component of integrated nutrient management that leads to sustainable agriculture. In addition, microbial inoculants can be used as an economic input to increase crop productivity; fertilizer doses can be lowered and more nutrients can be harvested from the soil. Biofertilizer is defined as a substance which contains living microorganisms and is known to help with expansion of the root system and better seed germination. A healthy plant usually has a healthy rhizosphere which should be dominated by beneficial microbes. Conversely, in soil dominated by pathogenic microbes, optimum plant growth would not be possible (Ajimuddin, 2002; Anuradha, 2003).

Aromatic and medicinal crops are of immense economic importance in India. The wide range of soil and climatic conditions permit successful cultivation of a large number of such plants. Microbial inoculants, which play an important role in the improvement of plant growth and productivity, ought to be explored for use in the cultivation of aromatic and medicinal plants. At present, reports on the use of bioinoculants for the cultivation of aromatic crops are very limited. However few studies carried out so far revealed the application of certain biofertilizers like *Azotobacter*, *Azospirillum*, phosphate solubilizing bacteria, arbuscular mycorrhiza and other plant growth promoters in aromatic crop
cultivation and in the improvement of their growth and yield (Ramaswamy et al., 1996; Rodríguez and Reynaldo, 1999; Anuradha, 2003; Mahfouz and Sharaf-Eldin, 2007).

2.2.1 Bioinoculants supplying Nitrogen:

Nitrogen is the mineral nutrient most often in short supply for plant nutrition. It represents the mineral fertilizer most applied to agricultural lands. This is because available soil N supplies are generally inadequate for optimum crop production. The world human population is likely to double during next 50 years so also the demand for food. Provision of an adequate supply of fixed N is central to the successful meeting of the food challenge. Fertilizer nitrogen is not only costly, but also the growing awareness of environmental quality and limitations of non-renewable resources is implicated as additional constraints. Biological N\textsubscript{2} fixation has contributed to the productivity in natural and agricultural habitats from an early stage in the development of living matter on the earth (Bagyaraj and Arpana, 2009).

Availability of fixed N in soil is a major determinant of soil fertility and thereby crop productivity. Major contributors of fixed nitrogen to soil are the nitrogen fixing microorganisms and fertilizers. Where as bacteria and blue green algae contribute about 70% of the total nitrogen, the fertilizers account for only 15% of the total (Pandey and Kumar, 1989). Now the major thrust is on biological N fixation in agriculture mainly because (i) N fixers fix atmospheric N directly in the rhizosphere region so that it is easily available for the crop plants and (ii) Nitrogenous fertilizers applied to the field suffer up to 50% loss due to denitrification and leaching. A wise utilization of both the combinations may help us minimize the damage caused by chemicals to the environment.
In this review, the evidence on the effects of application of the biofertilizers on a variety of medicinal and aromatic plants is discussed to bring out whether these provide any advantage to the crop plants.

2.2.2. *Azotobacter* species:

*Azotobacter* fixes atmospheric nitrogen in the rhizosphere. There are different strains of *Azotobacter* each of which has varied chemical, biological and other characters. Some strains have higher nitrogen fixing ability. The genus *Azotobacter* comprises of the species such as *A. chroococcum*, *A. beijerincki*, *A. vinelandii* and *A. paspali*. Among these *A. chroococcum* has been effectively used as biofertilizer (Anuradha, 2003).

Govind Rao *et al.* (1987) observed more microbial population in the rhizosphere of medicinal plants than in non rhizosphere soil i.e the region away from the root zone. Among 25 medicinal plants studied, the highest population of *Azotobacter* was observed in the rhizosphere of *Rauvolfia tetraphylla*.

Field study conducted on palmarosa (*Cymbopogan martini*) revealed that under rain fed conditions, an average increase in the herb yield of 20 and 22% was observed on applying *Azotobacter* and 80 kg N per hectare compared to the control respectively (Maheshwari *et al.*, 1991; 1998).

A pot experiment was conducted on Java citronella by Ramaswamy *et al.*, (1996) to study the influence of *Azotobacter* and *Azospirillum* inoculation in single and mixed culture with the combination of the nitrogen levels (60 and 120 kg /ha). They concluded that these organisms either singly or in combinations with 60 kg /ha nitrogen produced significant increase in crop growth and yield.
Inoculation of palmarosa (*Cymbopogon martini*), *Papaver somnifera*, henbane (*Hyoscyamus niger*) with *Azotobacter* strains increases the yield of essential oil content and alkaloid latex (Sen, 1995). According to them nitrogen fixing bacteria can be used for increasing the yield of aromatic and medicinal plants quantitatively and qualitatively.

*Azotobacter* is widely distributed in the soils among various land use types like natural forests, coffee plantations and paddy fields. The population was maximum in natural forests during pre monsoon season compared to post monsoon and the major species isolated were *A. chroococcum* and *A. vinelandii* (Vinutha *et al.*, 2004).

Isolates of *Azotobacter* apart from N fixing are also known to promote plant growth. Iqbal Ahmed *et al.* (2005) reported a total of 48 isolates of *Azotobacter* recovered from different crop rhizospheric soils which showed resistance to antibiotics like nitrofurantoin, nalidixic acid, novobiocin, cloxacillin, and chloramphenicol. These isolates showed tolerance to salt (2.5-3.5% NaCl conc.) and pH value (7-10). Thus, exhibiting multi-plant growth promoting activities and elevated tolerance to environmental factors may be suited for further assessment and development as the effective PGPR (Plant Growth Promoting Rhizomicroorganism) inoculants.

Mahfouz and Sharaf-Eldin (2007) observed the application of biofertilizer, which was a mixture of *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus megatherium* applied with chemical fertilizers (only 50% of the recommended dosage of NPK) increased vegetative growth (plant height, number of branches, and fresh and dry weight per plant) compared to chemical fertilizer treatments alone in fennel (*Foeniculum vulgare* Mill.). Further highest oil yield per plant was observed with the treatment of
biofertilizer plus a half dose of nitrogen and phosphorus. With the half dose of chemical fertilizer alone the yield was lowest.

Thus Azotobacter is a promising bioinoculant, N fixer and plant growth promoter when used singly or in combination with other organisms.

2.2.3. Phosphate solubilizing bacteria (PSB)

Phosphorus is an essential element for plant development and growth, making up to about 0.2 % of plant dry weight. Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crops. Plants acquire P from soil solution as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca$^{2+}$, Mg$^{2+}$, Fe$^{3+}$ and Al$^{3+}$, depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants. As a result, the amount available to plants is usually a small proportion. Several scientists have reported the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. The principal mechanism for mineral phosphate solubilization is the production of organic acids. Acid phosphatases play a major role in the mineralization of organic phosphorus in soil. It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms (Rodríguez and Reynaldo, 1999). Production of organic acids results in acidification of the microbial cell and its surroundings. Important microbial strains producing organic acids are Erwinia herbicola, Pseudomonas cepacia, Burkholderia cepacia, Bacillus polymyxa, Aspergillus awamori, Penicillium digitatum etc.
Some of the heterotrophic bacteria and fungi are known to have the ability to solubilize organic form of P from insoluble sources (Bolan, 1991). It has been shown by several workers that *Bacillus coagulans* along with *Glomus mosseae* are the best inoculation for improving the growth and yield of *Withania somnifera* (Earanna, 1998) and *Phyllanthus amarus* (Earanna, 2001).

 Certain isolates of phosphate solubilizing bacteria are known to produce growth hormones. Barea *et al.* (1975) isolated 50 phosphate-dissolving bacteria from rhizospheres of crop plants and examined for IAA, gibberellins and cytokinins. These bacteria possessed phytase activity and 27 could dissolve rock phosphate. Twenty bacteria synthesized all 3 types of plant hormones, 43 produced IAA, 29 formed gibberellins and 45 cultures produced cytokinin-like substances.

2.2.3.1. *Burkholderia cepacia*:

*Burkholderia cepacia* (previously known as *Pseudomonas*) is a nutritionally versatile, Gram negative organism first discovered by Walter Burkholder in 1950. It has attracted intense interest from the agricultural industry as a P solubilizers and possible biological control agent.

The genus *Burkholderia* comprises 29 species, with several of these including *Burkholderia vietnamiensis*, *B. kururiensis*, *B. tuberum* and *B. phynatum* are capable of fixing N\textsubscript{2} (Estrada-de los Santos *et al.*, 2001; Vandamne *et al.*, 2002).

Members of this genus occupy a surprisingly wide range of ecological niches. These bacteria are exploited for biocontrol, bioremediation and plant growth promotion purposes (Mcloughlin *et al.*, 1992; Reddy, 1997).
Several workers have reported that *Burkholderia cepacia* strains can be used to control seedling and root diseases *in vitro* and in field tests, and can replace chemical alternatives. Field tests have shown that such strains can colonize the rhizosphere of several crops, including corn, maize, rice, pea, sunflower and radish, and thereby significantly increase the crop yield, even in the absence of pathogens (Parke *et al.*, 1991; McLoughlin *et al.*, 1992; Bowers and Parke, 1993; Hebbar *et al.*, 1998; Tran Van *et al.*, 2000).

Anandham *et al.* (2007) isolated five phosphate-solubilizing bacteria (PSB) based on their ability to solubilize tricalcium phosphate (TCP) on Pikovskaya's medium. Among the tested bacterial strains *Burkholderia* sp. strain CBPB-HIM showed the highest solubilization (363 μg of soluble P ml⁻¹) activity at 48 h of incubation indicating as a potent P solubilizer. Safety issues regarding human infections, especially in cystic fibrosis patients, have not been solved (Coenye and Vandamme, 2003).

Studies on *B. cepacia* with aromatic and medicinal plants is meager. It has vast range beneficial activities which have to be still exploited on a large scale. Altogether, the use of microorganisms and the exploitation of beneficial plant–microbe interactions offer promising and environmentally friendly strategies for conventional and organic agriculture worldwide.

2.2. 4. Plant growth promoting rhizobacteria (PGPR):

2.2.4.1. *Methylobacterium mesophilicum*

The genus *Methylobacterium* includes a group of aerobic, Gram-negative, pink-pigmented, facultatively methylotrophic (PPFM) bacteria characterized by their ability to
utilize single-carbon compounds like methanol and formaldehyde via the serine pathway, as well as a wide range of multicarbon growth substrates (Green, 1992).

Members of the genus *Methylobacterium* are widely distributed in nature, detected in soil, freshwater and lake sediments, as well as on other solid surfaces (Corpe and Rheem, 1989; Lidstrom and Chistoserdova, 2002). They are known particularly for their close association with plants (Holland and Polacco, 1994; Lidstrom and Chistoserdova, 2002; Sy et al., 2005). Associations of members of *Methylobacterium* with plants range from epiphytic to endophytic and symbiotic relations (Sy et al., 2001; Koenig et al., 2002; Pirttila et al., 2000; Idris et al. (2006).

Madhaiyan et al. (2002) have observed that PPFMs are ubiquitous in nature and found in a variety of habitats. Members of the genus *Methylobacterium* form the major group of PPFMs and occur in the phyllosphere of a variety of plants. They could isolate the PPFMs from phyllosphere of cereals, pulses, oil seeds, vegetables, fruit crops, spices, commercial crops, flower crops, trees and weeds growing under tropical conditions. As such 70 isolates were obtained from the tropical plants.

Abdoulaye et al. (2001) reported a fourth rhizobial branch involving bacteria of the *Methylobacterium* genus. *Rhizobia* isolated from *Crotalaria* legumes were assigned to a new species, *"Methylobacterium nodulans,"* on the basis of 16S ribosomal DNA analyses. They demonstrated that these rhizobia facultatively grow on methanol, which is a characteristic of all *Methylobacterium* spp. but a unique feature among rhizobia. Genes encoding two key enzymes of methylotrophy and nodulation, the *mxaF* gene, encoding the α-subunit of the methanol dehydrogenase, and the *nodA* gene, encoding an acyltransferase involved in Nod factor biosynthesis, were sequenced for the type strain,
Plant tests and nodA amplification assays showed that *M. nodulans* is the only nodulating *Methylobacterium* sp. identified so far. Phylogenetic sequence analysis showed that *M. nodulans* NodA is closely related to *Bradyrhizobium* NodA, suggesting that this gene was acquired by horizontal gene transfer.

Bacteria belonging to the *Methylobacterium* genus, are common inhabitants of plants, potentially dominating the phyllosphere population, and are also encountered in the rhizosphere, seeds, and other parts of plants, being versatile in nature. The consistent success of the *Methylobacterium* plant association relies on methylotrophy, the ability to utilize the one-carbon compound methanol emitted by plants. However, the efficiency of *Methylobacterium* in plant growth promotion could be better exploited and thus has attracted increasing interest in recent years (Madhaiyan *et al.*, 2005). Possible mechanisms of plant-growth promotion by *Methylobacterium* include production of phytohormones, such as indole-3-acetic acid (IAA), cytokinins or vitamins (Basile *et al.*, 1985; Koenig *et al.*, 2002; Trotsenko *et al.*, 2001).

Jeounghyun *et al.* (2006) reported effect of *Methylobacterium* spp. on tomato and red pepper. Seeds treated with the *Methylobacterium* strains showed a significant increase in root length compared to uninoculated control and *Methylobacterium extorquens* miaA knockout mutant (treated seeds). Extracts of the plant samples were used for IAA, trans-zeatin riboside (t-ZR), and dihydrozeatin riboside (DHZR) assays by immunoanalysis. The treatment with *Methylobacterium* sp. CBMB20 or CBMB 110 produced significant increases in the accumulation of IAA and the cytokinins, t-ZR and DHZR in the red pepper extracts, whereas no IAA was detected in the tomato extracts, although the cytokinin concentrations were significantly increased. Based on this it can be
concluded that *Methylobacterium* as a plant-growth promoting bacteria could be better exploited.

In addition, members of *Methylobacterium* are associated with nitrogen metabolism of plants by means of bacterial urease (Holland & Polacco, 1992). *Methylobacterium* strains are able to establish efficient nitrogen-fixing symbioses by nodulating legume roots (Sy *et al*., 2001)

Senthilkumar *et al.* (2002) reported the compatibility of *Methylobacterium* sp. Co47 with other bacteria by cross streaking each culture over its appropriate medium. The *Methylobacterium* sp. Co47 was found compatible with all microorganisms used as bioinoculants, viz., *Rhizobium* sp. CoC10, *Azospirillum lipoferum* Az204, *Bacillus megaterium* var. *phospaticum* PSBl, *Pseudomonas fluorescens* PF1, *Trichoderma viride* TV6 and *Aspergillus awamori* PSFl except *Trichoderma harzianum* TH1. All the organisms grew well on glycerol peptone agar, which is also a standard medium for pink pigmented facultative methylotrophs (PPFMs). Similarly, the PPFMs also showed moderate growth in media used for growing other bioinoculants. The compatible nature of these organisms established the potential of PPFMs as a new component to prepare mixed bioinoculants for various crops.

Madhaiyan *et al.* (2005) studied the existence of *Methylobacterium* as a symbiont with sugarcane and its influence on crop growth at various stages. Pink-pigmented facultative methylotrophic bacteria strains isolated from different parts of the sugarcane clone Co86032 showed growth on methanol, and were further confirmed based on the *mxaF* gene encoding the α-subunit of the methanol dehydrogenase by polymerase chain reaction amplification using specific primers. True seeds inoculated with PPFMs had a
higher germination percent and rate of germination than the control. A combined treatment of seed imbibition, soil application and phyllosphere spray increased specific leaf area, plant height, number of internodes, and cane yield. Immunological determination of cytokinin in young and mature leaves significantly increased when the epiphytic population on the leaf surface increased. Trends in sugar qualities in the form of Pol (sucrose) % in cane, Brix % in cane, and commercial cane sugar were similar to that of cane yield. These effects might be mediated by the production or synthesis of plant hormones.

Since Methylobacterium mesophilicum activity as a PGPR is novel, further studies have to be focused towards its exploitation as a bioinoculant in medicinal and aromatic crop plants.

2.2.5. Biocontrol agent:

2.2.5.1. Trichoderma harzianum:

*Trichoderma harzianum* is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds (Haram et al. 1996; Zimand et al. 1996).

Biological control involves the use of beneficial organisms such as *Trichoderma* that promote positive responses by the plant. *Trichoderma* species produce and/or release a variety of compounds, including cell wall degrading enzymes and secondary metabolites, which enhance root development, crop productivity and resistance to biotic and abiotic stresses. There are a variety of fungal species and isolates that have been
examined as biocontrol agents but *Trichoderma* species clearly dominate, perhaps reflecting their ease of growth and wide host range (Whipps and Lumsden, 2001).

*Trichoderma* spp. are free-living fungi that are common in soil and root ecosystems. Recent discoveries show that they are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this layer. They produce or release a variety of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. These root–microorganism associations cause substantial changes to the plant proteome and metabolism. Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Harman *et al.*, 2004).

Mandeel and Baker, (1991) and Couteadier (1992) have reported that competition for carbon, nitrogen and iron has been shown to be a mechanism associated with biocontrol or suppression of *Fusarium* wilt in several systems by non-pathogenic *Fusarium* and *Trichoderma* species Chet *et al.*, 1981 observed directed growth of hyphae of *Trichoderma* to hyphae of *Rhizoctonia solani* prior to penetration.

The final evidence for a role for cell wall-degrading enzymes in biocontrol involves the expression of fungal genes in transgenic plants. For example, an endochitinase from *Trichoderma harzianum* has been transformed into tobacco and
potato and the transgenic plants showed a high level of resistance to a broad spectrum of
diseases (Lorito, 1998).

Apart from being a potent biocontrol agent *T. harzianum* is known to act as plant
growth promoter. Certain isolates of *Trichoderma*, could provide plant growth promotion
in the absence of other major pathogens (Inbar et al., 1994; Whipps, 1997).

Interactions between *Trichoderma* and other soil microorganisms in the root,
rhizosphere and mycorhizosphere region are being reported. Clavet et al. (1989) studied
the interaction of *Trichoderma* species with *Glomus mosseae* and two wilt pathogenic
fungi. Non volatile compounds released by *T. harzianum* inhibited growth of *Fusarium
oxysporum* and *Verticillium dahliae*, whereas hyphae of *T. aureoviridae* affected *F.
oxysporum*. Rosseau et al. (1996) investigated the interaction between *Trichoderma
harzianum* and *Glomus intraradices* to delineate precisely the relationship established
between both the partners. They reported the following events in the interaction between
the two fungi (a) recognition and the local penetration of the antagonist into mycorrhizal
spore (b) active proliferation of antagonists cells in mycorrhizal hyphae and (c) release of
this antagonists through moribound hyphal cells.

*Trichoderma virens* GL-3 combined with *Burkholderia cepacia* provided stands
of pepper (*Capsicum annuum* L.) greater protection used in the presence of a mixture of
up to four soil-borne pathogens (Mao et al., 1998).

2.2.6. Arbuscular mycorrhizal (AM) fungi:

Many microorganisms form mutualistic symbiosis with plants. Among these the
most widespread mutualistic symbiosis is the arbuscular mycorrhiza, formed between
Arbuscular Mycorrhizal (AM) fungi and vascular flowering plants. These associations
occur in terrestrial ecosystems throughout the world and have a global impact on plant phosphorus nutrition. The arbuscular mycorrhiza is an endosymbiont in which the fungus inhabits the root cortical cells and obtains carbon provided by the plant while it transfers mineral nutrients from the soil to the cortical cells. Development of symbiosis involves the differentiation of both symbionts to create novel symbiotic interfaces within the root cells. AM fungi play an essential role in plant growth, plant protection and soil quality. They are ubiquitous in the soil and can form symbiosis with most terrestrial plants including major crops, cereals, vegetables and horticultural plants.

German botanist Albert Bernard Frank in 1885 introduced the Greek word mycorrhiza, which literally means “fungus root”. Most plant roots form mycorrhizal associations of one kind or the other fungus in soil. These mycorrhizal fungi perform the function of root hairs. Mycorrhizal association generally enhances the growth and vigor of the host plants. In addition the mycorrhizal plants have greater tolerance to toxic heavy metals, root pathogens, drought, high soil temperatures, soil salinity, adverse soil pH and transplantation shock. Because of their widespread occurrence in nature and their numerous benefits to plants, these fungi are currently attracting much attention in agricultural, horticultural and forestry research. Though there are different mycorrhizal associations, the most common type occurring in all ecological situations, especially in tropics, is the arbuscular mycorrhiza (Barea and Jeffries, 1995). Increased plant growth because of AM colonization is well documented (Bagyaraj and Varma 1995).

2.2.6.1. Benefits of arbuscular mycorrhizal fungi:

Improved plant growth due to inoculation of soil with AM fungi has been demonstrated especially under P deficient conditions (Mosse, 1973). The growth
improvement is mainly because of enhanced P uptake. They also improve the uptake of other elements like Zn and Cu (La Rue et al., 1975; Krishna et al., 1982), K (Powell, 1975) and N and Ca (Ross and Harper, 1970; Pai et al., 1993). AM fungi are involved in increased water uptake (Allen, 1982; Graham and Sylvertsen, 1984). AM fungi are also known to protect the plants from root invading organisms such as parasitic nematodes, phytopathogenic fungi and bacteria since the occupation of root cortex by AM fungi reduces or prevents colonization of that zone by the pathogens (Bagyaraj, 1984; Price et al., 1990). In addition the mycorrhizal plants have shown greater tolerance to toxic heavy metals, saline soils, high soil temperature and to transplant shock than non-mycorrhizal plants.

Sanders and Tinker (1971) explained the mechanism of P uptake from soil by AM fungi. The hyphae of the mycorrhizal fungi form better distributed surface for absorbing P from the soil solution than the roots alone. Goerge et al. (1992) showed that arbuscular mycorrhizal hyphae have a role in translocation of PO4 and N from the soil zones several cm away from roots in cough grass or white clover. Similar increase in P uptake by AM inoculated plants was observed in Citronella java (Kothari and Singh, 1996), bergamot mint (Kothari et al., 1999) and pepper (Demir, 2004). Increased plant biomass because of AM fungi inoculation has been reported in aromatic plants like palmarosa (Gupta and Janardhanan, 1991), eucalyptus (Oliveria et al., 1995) and bergamot mint (Kothari et al., 1999).

Gupta et al. (2002) observed that the AM-inoculated mint plants depleted the available N, P and K in the rhizosphere soil as compared to non-inoculated control plants. The extent of nutrient depletion was greater for P than N and K. They concluded that the
AM inoculation could significantly increase the root colonization, growth, essential oil yield and nutrient acquisition of mint (*Mentha arvensis*).

Toussaint *et al.* (2008) investigated the potential of the AM fungus *Glomus mosseae* to protect basil (*Ocimum basilicum*) against *Fusarium oxysporum* f.sp. *basilica*. It was hypothesised that *G. mosseae* could confer a bioprotective effect against *Fusarium* as a result of increase in leaf rosmarinic (RA) and caffeic acids (CA) or essential oil concentrations. *Glomus mosseae* conferred a bioprotective effect against *Fusarium* by reducing plant mortality to 20% compared to 33% in non-mycorrhizal (NM) plants. This bioprotective effect was not related to improved phosphorus nutrition, as AM and NM plants treated with *Fusarium* had similar shoot P concentrations (6 and 8 mg g$^{-1}$ dry weight, respectively). Both AM and NM plants treated with *Fusarium* had similar leaf and root RA and CA concentrations. Further, phenolic or essential oil concentrations were not increased in plants treated with the AM fungus and *Fusarium*. Therefore, the bioprotective effect conferred by *G. mosseae* was not a result of increase in the phytochemicals tested in this study. However, under the AM symbiosis, basil plants treated with *Fusarium* had lower methyleugenol concentrations in their leaves than NM plants treated with the pathogen.

These fungi are not culturable. Mass production in the roots of host plants and used as inoculants, it improves hardness in seedlings and helps to withstand transplant shock.

### 2.3. Screening for efficient strain of arbuscular mycorrhizal fungi:

The AM fungi show a preferential colonization to hosts, and thereby, the extent to which a host is benefited depends on the fungal species involved in the symbiosis (Abbott
and Robson, 1982). The existence of inter and intraspecific variations among the plant species involved in relation to their Phosphorus requirement and the ability of the host to translocate the native soil Phosphorus further determines the efficacy of these fungi. Thus it is essential to screen for an efficient AM fungus for a particular host in order to harness the maximum benefit from the fungus.

In general, mycorrhizal inoculation results in a significant increase in plant height, number of branches, biomass and P content of crop plants. This can be attributed to the increased uptake of nutrients by AM fungi. The benefit conferred on to the host is related mainly to the extent of the colonization of host roots by the fungus. AM fungi show preferential colonization with the hosts (Vasantha krishna et al., 1995) and extent to which the host is benefited depends on the fungal species involved in symbiosis (Abbott and Robson, 1982). Hence the need to screen for an efficient AM fungi for inoculating, commercially important plants is being stressed upon (Bagyaraj and Varma, 1995). However, studies on selection of efficient fungi among aromatic crop plants are scanty. Effort has been made to present the work done on medicinal and other related crop plants.

Enhanced growth and biomass was observed in *Glomus aggregatum* treated palmarosa plants (Gupta and Janardhanan, 1991). Improved plant height and spread because of AMF inoculation has been reported in coleus (Boby and Bagyaraj, 2003).

Rupam Kapoor *et al.* (2002) studied the effects of application of two AM fungi *Glomus macrocarpum* and *G. fasciculatum* on shoot biomass and concentration of essential oil in *Anethum graveolens* L. and *Trachyspermum ammi* L. Results revealed significant variation in effectiveness of the two AM fungal species. AM fungal inoculation in general, improved the growth of the plants. On mycorrhization, the
concentration of essential oil increased up to 90% in dill and 72% in carum over their respective controls. *Glomus macrocarpum* was more effective than *G. fasciculatum* in enhancing the oil concentration. The levels of limonene and carvone were enhanced in essential oil obtained from *G. macrocarpum*-inoculated dill plants, while *G. fasciculatum* inoculation resulted in a higher level of thymol in carum.

The effect of association of two AM fungi, *Glomus macrocarpum* and *G. fasciculatum*, on the concentration and composition of essential oil in coriander (*Coriandrum sativum*) was studied by Kapoor *et al.* (2002). VAM inoculation increased the essential oil concentration in fruits by as much as 43%. Although significant variation in effectiveness of the two fungal species was observed, the quality of essential oil was significantly enhanced on mycorrhization. Gas chromatographic characterization of essential oil showed increased concentration of geraniol and linalool in plants inoculated with *G. macrocarpum* and *G. fasciculatum* respectively.

Two AM fungi *Glomus macrocarpum* and *Glomus fasciculatum* significantly improved growth and essential oil concentration of *Foeniculum vulgare* Mill as observed by Rupam Kapoor *et al.* (2004). However, AM inoculation of plants along with phosphorus fertilization significantly enhanced growth, P-uptake and essential oil content of plants compared to either of the components applied separately. Among the two fungal inoculants, *G. fasciculatum* registered the highest growth at both levels of phosphorus used with up to 78% increase in essential oil concentration of fennel seeds over non-mycorrhizal control.

The plant biomass is an important parameter for selecting a fungus for its symbiotic efficiency. A study was conducted to know the effectiveness of eleven AM
fungi on the medicinal plant *Coleus forskohlii*. The extent of growth, P and forskolin status varied with the AM fungi used. Based on the plant biomass, P uptake and forskolin content per plant, *Glomus bagyarajii* was found to be the best AM symbiont for inoculating *C. forskohlii*, the next best being *Scutellospora calospora* (Gracy Sailo and Bagyaraj, 2005).

Ulfath Jaiba *et al.* (2006a) studied the efficacy of eleven different arbuscular mycorrhizal fungi on long pepper (*Piper longum*). Increased spike weight and dry biomass was observed in plants treated with *Glomus bagyarajii* compared to others in the study. Similarly, increased plant growth, biomass and phosphorus uptake was observed in *Stevia rebaudiana*, a medicinal plant when inoculated with *Glomus macrocarpum* which performed best when compared with eleven different AM fungi used in the study (Chitra and Balakrishna, 2006).

Tharun *et al.* (2006) studied the efficacy of eleven arbuscular mycorrhizal (AM) fungi on kalmegh (*Andrographis paniculata*) under glass house conditions. Kalmegh seedlings raised in presence of AM fungi generally showed an increase in plant growth and andrographolide (active ingredient) concentration over those grown in the absence of the inoculation of soil with AM fungi. The extent of improvement by AM fungi varied with the species of AM fungi inhabiting the roots of kalmegh seedlings. Considering the various parameters and andrographolide concentration of the plants, it was observed that *Glomus leptotichum* and *Glomus intraradices* are the two best fungi for inoculating kalmegh.
2.4. Interaction of arbuscular mycorrhizal fungi and beneficial microorganisms:

Many bacteria are known to be able to stimulate plant growth through direct or indirect interactions with plant roots. In addition, most plant roots are colonized by mycorrhizal fungi and their presence also generally stimulates plant growth. However, the beneficial traits of root-colonizing bacteria and fungi have been studied separately. Studies are being concentrated on the synergistic effects of bacteria and mycorrhizal fungi with respect to their combined beneficial impacts on plants.

Azcon et al., (1976) conducted a pot experiment to study the interactions between AM fungi and phosphate-solubilizing bacteria in a low-phosphate alkaline soil amended with 0, 0.1 and 0.5% rock phosphate. *Endogone* and two other bacteria able to solubilize rock phosphate *in vitro* and produce plant growth regulating substances were used as inocula. Lavender plants with mycorrhiza plus bacteria took up more total P than plants with either *Endogone* or bacteria separately at each concentration of rock phosphate. Plants not inoculated with bacteria or *Endogone* derived no benefit from the rock phosphate.

It has been reported that there is enhanced AM fungal colonization levels in roots in the presence of PGPR. Association of *Pseudomonas putida* with indigenous AM fungi resulted in a clear growth enhancement of clover plants (Meyer and Linderman, 1986), suggesting that some PGPR may have properties that support both mycorrhizal establishment and function.

According to Ratti and Janardhanan (1996) when palmarosa plants were inoculated with *Glomus aggregatum* and *Azospirillum brasilense*, the latter stimulated VAM root colonization and spore production in the root zone soil. Further N content of
leaf tissue of these plants were higher. However, P content was higher in *G. aggregatum* inoculated plants compared to plants inoculated with both the organisms.

The response of two selected medicinal plants *viz.*, *Phyllanthus amarus* and *Withania somnifera* to inoculation with AM fungi and plant growth promoting rhizomicroorganisms studied, revealed that *P. amarus* yield can be improved through inoculation with *G. fasciculatum* plus *B. coagulans* or *Trichoderma harzianum* while *Glomus mosseae* plus *Bacillus coagulans* inoculation showed to be best for improving growth and yield of *W. somnifera* (Earanna, 2001).

Neem seedlings responded well to triple inoculation with *Glomus mosseae*, *Azotobacter chroococcum* and *Bacillus coagulans* with maximum plant biomass, N, P, Zn and Cu uptake, biovolume index and quality index resulting in healthy vigorous growing seedlings. It also increased the mycorrhizal root colonization and spore numbers in the root zone soil of inoculated plants. The activity of soil enzymes like acid phosphatase and dehydrogenase also recorded the highest under green house conditions (Sumana *et al.*, 2003).

Dual inoculation with *Glomus bagyarajii* and *Trichoderma harzianum* improved the growth and spike yield in *Piper longum* as reported by Ulfath Jaiba *et al.* (2006b). The AM fungus was inoculated singly and in combinations with PGPRs *viz. Azotobacter chroococcum, Bacillus coagulans, Bacillus megatherium, Pseudomonas flouresense, Trichoderma harzianum* and *Piriformospora indica*. Similar observations were observed by inoculation with AM fungi and PGPRs in medicinal plants like *Calamus thwaitessi* and *Adhatoda vasica* by Lakshmipathy *et al.*, (2002) and Anatha Naik and Earanna, (2006) respectively.
Chitra and Balakrishna, (2007) observed the co-inoculation of *Azotobacter chroococcum* with *Glomus macrocarpum* to be the best for inoculating Stevia followed by combination of *G. macrocarpum* plus *Trichoderma harzianum* among the different combinations of AM fungi and five different PGPRs used viz. *Azotobacter chroococcum*, *Bacillus coagulans*, *Bacillus megatherium*, *Pseudomonas fluorescens* and *Trichoderma harzianum*.

A pot experiment was carried out by Vinutha et al. (2007) to study the effects of inoculation of an arbuscular mycorrhizal fungus (*Glomus fasciculatum*), a free living N₂-fixer (*Azotobacter chroococcum*) and a phosphate solubilizing fungus (*Aspergillus awamori*) alone and in combination on growth, biomass and biochemical constituents of *Ocimum sanctum* [O. tenuiflorum]. Single, dual and triple inoculations enhanced the growth, biomass and biochemical constituents of the plant compared to uninoculated plants. Triple inoculation produced maximum effect.

Earanna and Bagyaraj (2008) conducted pot experiments to study the response of *Withania somnifera* inoculated with AM fungi and PGPRs. Plants inoculated with AM fungi either singly or in combination with PGPRs (*Bacillus coagulans* and *Trichoderma harzianum*) significantly increased the growth, biomass, nutrient uptake and mycorrhizal root colonization compared to uninoculated plants. Similar attempt was made by Dayala Doss et al. (2008) to study the effect of AM and PGPR on growth parameters in exotic medicinal plant, *Pulicaria* sp. Dual inoculation with *Glomus fasciculatum* and *Bacillus coagulans* showed maximum growth, thus confirming the dual inoculation performed better.
2.5. Integrated nutrient management using biofertilizers and chemical fertilizers in crop plants:

The continued use of chemical fertilizers and manures for enhanced soil fertility and crop productivity often results in unexpected harmful environmental effects, including leaching of nitrate into ground water, surface run-off of phosphorus and nitrogen run-off, and eutrophication of aquatic ecosystems. Integrated nutrient management systems are needed to maintain agricultural productivity and protect the environment. Microbial inoculants are promising components of such management systems. Efforts are being done in using microbial inoculants, including plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi for increasing the use efficiency of fertilizers. Studies with microbial inoculants and nutrients have demonstrated that some inoculants can improve plant uptake of nutrients and thereby increase the efficiency of applied chemical fertilizers and manures. These proofs of concept studies will serve as the basis for vigorous further research into integrated nutrient management in agriculture.

For sustainable crop production, integrated use of chemical and organic fertilizer has proved to be highly beneficial. Several researchers have demonstrated the beneficial effect of combined use of chemical and organic fertilizers to mitigate the deficiency of many secondary and micronutrients in fields that continuously received only N, P and K fertilizers for a few years, without any micronutrient or organic fertilizer.

Shivalingappa (1998) studied the influence of biofertilizers on tuberose and reported that the treatment combination of NPK at 75% along with *Azotobacter*, *Azospirillum* and AM fungi resulted in maximum plant height, more number of branches, higher leaf area, higher number of tillers and number of spikes per plant.
Manjunath et al. (2002) conducted a field experiment to study the effect of biofertilizers on growth, yield and essential oil content in Patchouli with different biofertilizers (Azotobacter, Azospirillum, Phosphorus Solubilising Bacteria (PSB) and AM fungi in combination with inorganic fertilizers. The results revealed that 75% NP + 100 K + Azotobacter + Azospirillum + VAM recorded significantly superior values for plant height, number of leaves, number of branches, plant spread, leaf area, yield of fresh herbage and essential oil yield and was effective in saving of fertilizers to the extent of 25 per cent.

Combination of 75 % NP and 100% K along with AM fungi, Azotobacter and a PSB recorded a significantly higher plant height, number of leaves, number of branches, plant spread, leaf area, yield of fresh herbage and essential oil yield in sweet basil (Ocimum basilicum) as recorded by Ajimuddin, 2002.

A consortium of Azotobacter, Azospirillum and AM fungi along with reduced levels of nitrogen and phosphorus (50 % N, P and 100% K) recorded maximum net returns and was found to be highly remunerative and economical. A field investigation conducted on rosemary by Anuradha, (2003) revealed maximum growth and essential oil yield indicating that high yield could be obtained by supplementing the recommended dose of chemical fertilizers with biofertilizers.

A field experiment was conducted by Chand et al. (2006) for seven years continuously to evaluate the influence of combined applications and organic and chemical fertility buildup and nutrient uptake in a mint (Mentha arvensis) and mustard (Brassica juncea) cropping sequence. Results indicated that integrated supply of plant
nutrients through farmyard manure (FYM) and fertilizer NPK, along with *Sesbania* green manuring, played a significant role in sustaining soil fertility and crop productivity.

Mahadevaswamy *et al.* (2006) conducted field experiment to study the effect of *Azospirillum*, AM fungi and PGPR strains on growth and yield of Ashwagandha. Increase in plant height, number of branches, number of berries and biomass was obtained by triple inoculation.

Arpana and Bagyaraj (2007) conducted a field investigation to know the influence of *Glomus mosseae* and *Trichoderma harzianum* singly and in combination on growth and yield of kalmegh (*Andrographis paniculata*) at two levels of P fertilizer application i.e. at the recommended level and 75% of the recommended level. The plant height, plant spread, number of branches per plant, number of leaves per plant, leaf area, plant dry matter, plant P content and andrographolide concentration were significantly higher in plants inoculated with both the organisms, at both the levels of P as compared to uninoculated plants. Thus indicating that inoculation with *G. mosseae + T. harzianum* not only improved growth, biomass yield, P nutrition and andrographolide concentration of kalmegh but also helped in saving 25% of P fertilizer application.

Mahfouz and Sharaf-Eldin (2007) conducted field experiments to study the effects of biofertilization on growth, fruit yield, and oil composition of fennel plants. Application of *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus megatherium* applied with chemical fertilizers (only 50% of the recommended dosage of PK) increased vegetative growth (plant height, number of branches, and herb fresh and dry weight per plant) compared to chemical fertilizer only. Nitrogen, phosphorus, and potassium levels in the plant tissue increased when soil was inoculated by nitrogen-fixing bacteria,
phosphate-dissolving bacteria, and a mixture of all strains, respectively. Essential oil content in the fennel fruits was increased due to inoculation compared to the half dose of chemical fertilizer.

Improved growth, biomass, nutrient uptake and mycorrhizal root colonization was observed when *Withania somnifera* was inoculated with *Glomus mosseae*, *Bacillus coagulans* and *Trichoderma harzianum* under field conditions (Earanna and Bagyaraj, 2008).

Gharib *et al.* (2008) conducted a pot experiment to determine the effect of compost and biofertilizers on the growth, yield and oil constituents of marjoram (*Majorana hortensis*). Forty five days old seedlings were transplanted to soil treated with 15 and 30% aqueous extracts of compost and/or biofertilizers (mixture of *Azospirillum brasilienes*, *Azotobacter chroococcum*, *Bacillus polymyxa* and *B. circulans*) in addition to the recommended NPK doses as control. Use of combined treatment of biofertilizers gave better results for all the studied traits than those obtained from either nitrogen fixers (*A. brasiliense*, *A. chroococcum* and *B. polymyxa*) or alone (*B. circulans*). The oil percentage and yield per plant for three cuttings was almost two fold higher on fresh weight basis as a result of aqueous extracts of compost at low level + bio-fertilizers compared to control, indicating that combinations of low input system of integrated nutrient management could be beneficial to obtain relatively good yields of essential oil.

A investigation on Davana (*Artemisia pallens*) was carried out to study the influence of nitrogen, phosphorus and biofertilizers on growth, yield and essential oil constituents in ratoon crop of davana. The study revealed that application of nitrogen 93.75 kg ha$^{-1}$ and phosphorus 93.75 kg ha$^{-1}$ along with *Azospirillum* gave the better plant
height, number of laterals, fresh and dry weight of shoot, dry matter production, fresh herbage yield and essential oil yield. The maximum fresh and dry weight of root was obtained by application of nitrogen 93.75 kg ha\(^{-1}\) and phosphorus 93.75 kg ha\(^{-1}\) along with AM fungi. Application of inorganic nutrients and biofertilizers had no significant effect on essential oil constituents (Senthil Kumar et al., 2009).

This clearly suggests that application of AM fungi and other beneficial soil microflora can improve productivity of a wide range of crops. Its potential can be best exploited in medicinal and aromatic crops, which could be grown well under soils of poor fertility and marginal soils (Prakasa Rao, 1996).

### 2.6. Glomalin secretions in soil by arbuscular mycorrhizal fungi.

Glomalin is an amino polysaccharide or glycoprotein formed by combination of a protein from the mycorrhizal fungus with sugar from plant root exudates (Allison, 1968). Glomalin acts like a glue to cement together microaggregates into macroaggregates and improve soil structure. Glomalin initially coats the plant roots and then coats soil particles. The fungal "root-hyphae-net" holds the aggregates intact and clay particles protect the roots and hyphae from attack by microorganisms. Roots also create other polysaccharide exudates to coat soil particles. They are formed by AM fungi, and are beneficial to most crop plants. They are produced in large amounts and found in all soils. They do not dissolve in water and also resistant to decay. Glomalin protect hyphae from nutrient loss, glue together soil aggregates, stabilizes aggregates, reduces wind and water erosion, increases water infiltration, increases water retention near roots, improves nutrient cycling and improves root penetrationg compaction.

Rillig, (2002) examined the effects of five plant species on soil aggregate water stability. The five species were from the same natural grassland, and were grown in
monoculture plots in the field. They found significant differences in soil aggregate water stability (1–2 mm size class) for the five plant species examined, and corresponding differences in plant cover, root weight and length, AM fungi soil hyphal length, and glomalin concentrations. Root length, soil glomalin, and percent cover contributed equally strong paths to water-stable aggregation. The direct effect of glomalin was much stronger than the direct effect of AMF hyphae themselves, suggesting that this protein is involved in a very important hypha-mediated mechanism of soil aggregate stabilization.

2.7. Quality components of patchouli oil:

Not much information is available about the constituents which impart the patchouli oil its characteristic unique but strong odor. The oil is said to contain about 97% of compounds which have almost no influence on its aroma. Of these, 40 to 45% belong to sesquiterpene group and the balance seems to consist of patchouli alcohol. The oil contains small amounts of benzaldehyde, eugenol, cinnamic aldehyde an alcohol with a rose like fragrance, a ketone, azulene and a sesquiterpene alcohol. β- patchoulene, gammaguaiente, α-bulnesene, α-terpene cadiene, benzaldehyde and patchouli alcohol have been identified chromatographically (Guenther, 1952; Bates and Slagel, 1962; Koul and Nigam, 1966).

The main constituents of patchouli oil are patchouli alcohol (33.7%), alpha-patchouline (22.2%), beta-patchouline (13.75%), alpha-bulnesene (25%), elemene (6.08%), and beta-caryophyllene (20.64%). Other constituents are D-copaene (2.01%), beta-garjunene (0.04%), delta-guaiene (2.34%), patchouli oxide (0.06%), guaiene oxide (0.11%), bulnesene (0.14%), caryophyllene oxide (0.30%), nor-patchoulenol (1.18%), pogostol (0.44%), and patchouli pyridine (0.44%). However, the constituents differ
considerably depending upon the strain and geographical area (Akhila and Tewari, 1984; Lawrence, 1980; Sugimura et al., 1990).

Maheshwari et al. (1993) examined the oil of the fine cultivars grown at IIHR, Bangalore and noted that the Johore, Malaysian and Indonesian cultivars met the requirement of ISO 3757: 1978 whereas Java and Singapore cultivars did not fulfill optical rotation and ester value requirement. The GC profile of the Java and Singapore oils were also different from others.

A crystalline fraction of norsesquiterpeneic alcohol called norpatchoulenol has also been isolated and is said to be the true odour carrier of the patchouli oil. However, in spite of modern scientific and olfactive techniques of analysis available, it is still not known precisely which components or combination of components are primarily responsible for odour of patchouli oil which has unique character and it is primarily for this reason that the oil has so far defied all attempts at accurate synthesis (Angadi and Vasantha kumar, 1995).

2.8. Studies on economics of cultivation of aromatic crops using biofertilizers:

Maheshwari et al. (1991) revealed that under rainfed and partially irrigated conditions the extra net returns per hectare on oil production were maximum i.e Rs. 12,410 and Rs. 17,025 respectively using 80 kg N per hectare respectively in palmarosa. On the other hand with a little expenditure compared to control on Azotobacter a handsome extra net income of Rs. 11,240 and Rs. 15,295 per hectare may be earned from the respective swards.

In tuberose the nitrogen application @ 150 kg per hectare gave higher cost – benefit ratio (1: 2.76) and was closely followed by N @100 kg per hectare (1: 2.13).
Inoculation with biofertilizers (*Azotobacter* and *Azospirillum*) alone was found to give slightly more cost–benefit ratio in the range of 1: 3.40 to 1: 3.73. The study also revealed that nitrogen application @100 kg per hectare should be supplemented with *Azotobacter* to give the cost–benefit ratio of 3.10 and *Azospirillum* at same rate to give the ratio of 3.41 respectively, where as nitrogen @ 150 kg per hectare supplemented with *Azospirillum* gave a cost–benefit ratio of 2.96 in tuberose (Wange *et al*., 1995).

The use of biofertilizer (*Azotobacter*) at par with 40 kg N per hectare and expenditure on use of inorganic fertilizer, costing Rs. 300 per hectare price level on the growth, yield and alkaloidal composition of Opium poppy (Pareek *et al*., 1996). Application of 20 kg N + 20 kg P + 2 kg *Azotobacter* per hectare was found to be highly remunerative and economic dose by recording maximum net returns of Rs. 23,727 per hectare per year in palmarosa as worked out by Maheshwari *et al*., (1998).

Ajimuddin (2002) observed that application of *Azotobacter*, PSB and AM with 75 %NPK of the recommended dose of fertilizer (160: 80: 80 kg NPK/ha) produced the highest herb yield (54.83 t/ha), oil yield (168.13 l/ha) and net profit (1: 2.75) compared to all other treatments. Therefore could be recommended for the commercial cultivation of sweet basil.

The biofertilizers (*Azotobacter* + *Azospirillum* + AM) along with reduced levels of N and P (50%N, P and 100% K) was found to be highly remunerative and economical dose by recording maximum net returns per hectare per year. The cost benefit level levels of NPK fertilizers varied from 1: 3.06 (control) to higher cost benefit ratio of 1: 4.86 in the treatment combination with 50% NP, full K along with three biofertilizers in rosemary (Anuradha, 2003).