IMPACT OF BIOAGENTS AND SOIL AMENDMENTS ON THE PERFORMANCE OF PATCHOULI (*Pogostemon patchouli* Pellet.)

By

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THESIS

submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled **"Impact of bioagents and soil amendments on the performance of patchouli (*Pogostemon patchouli* Pellet.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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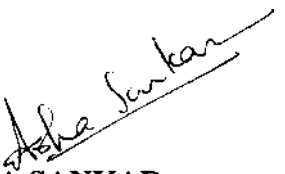
CERTIFICATE

Certified that the thesis entitled" **Impact of bioagents and soil amendments on the performance of patchouli (*Pogostemon patchouli* Pellet).**" is a record of research work done independently by **Miss Smilu Babu** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.



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Smilu Babu

DEDICATED

TO

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Introduction

1. INTRODUCTION

India is considered to be the ancient home of perfumes and aromatic plants. The natural essential oils and their aroma are the most remarkable products of plant metabolism, and these products have influenced human thoughts and emotions since the beginning of our civilization. There is immense demand for the use of native and natural products. Modern advances in chemistry led to the development of technology for separation of odoriferous principles from these aromatic plants.

A herbal renaissance is occurring throughout the world and more and more people are turning to natural health care remedies. The demand for traditional aromatic essential oils in India and abroad is ever increasing. Shortage of natural aromatic means is projected as a major limitation to meet the increasing demand. Policy changes and practices like enhanced trade monitoring, consideration of international trade controls for particular species, improved legislation and law enforcement, enhancement of cultivation efforts and future research works were recommended for its effective delivery to the farmers. To cope with the current trend and to provide good quality oil at required levels, there is an urgent need to scale up production by undertaking commercial cultivation of aromatic plants.

Bioagents and soil amendments have a decisive effect on both qualitative and quantitative components of growth and yield, which are of great significance in aromatic plants. Soil micro organisms play a key role in soil biological activities through mineralisation of organic matter releasing the plant growth nutrients available in them and transforming them to a form that can be readily absorbed by the plants. Thus, these organisms play a predominant role in soil fertility. Also several microbes and bioagents have been identified to have good potential for disease management as they can impart resistance and can produce hormones and vitamins,

which can cause physiological and biological changes. Effective crop husbandry practices like implementing biofertilizer programmes and exploiting naturally occurring micro organisms for controlling debilitating pests and diseases in aromatic plants have generated considerable interest, since they offer possibilities of growing crops in environmentally safe agricultural systems. They also form major components of organic farming concept, currently popular in crop production technology.

Essential oil yielding crops are currently identified as lucrative crops that could successfully introduced as component crops of prevailing cropping systems. Among the aromatic plants, patchouli (*Pogostemon patchouli* Pellet.), belonging to the family Lamiaceae which is used as the source of commercial patchouli oil has emerged as a prospective crop in recent years. A native of Philippines, it grows wild in Malaysia, Indonesia and Singapore, and is cultivated mainly for its oil. The commercial oil of patchouli is obtained by steam distillation of the shade dried leaves, and is one of the most important naturally occurring essential oils used in perfumery industry. Although rarely used as dominant source of fragrance on its own, the oil is widely used to give a solid foundation and lasting character to fragrance. Patchouli oil having notably strong fixative properties, helps to prevent rapid evaporation of a perfume, and thereby promotes tenacity. The basic character of the fragrance, apart from its tenacity, is its dominant woody note, although the aroma possesses other characteristics and is very complex. The oil is generally blended with other essential oils. It is used in a wide range of toilet soaps, scents, body lotion, preshave and aftershave lotions and detergents. Its strong tenacity renders it particularly suitable for heavy perfumes and for imparting a lasting character and strength to lighter perfumes. Patchouli oil is also significant medicinally and also used in aromatherapy, the chief properties being its use as antidepressant, anti-inflammatory, antiseptic, aphrodisiac, astringent, carminative, diuretic and febrifuge.

Having originated in a tropical climate and on account of its ability to thrive under shade, the crop is suitable as a component crop of coconut based cropping system of Kerala. Presently agricultural sector is facing a lot of serious set backs and the value of many crops stands on the brim of drastic reduction of price and instability of demand. Hence there is of great demand and significance for diversified cultivation with remunerable crops like patchouli. Thus there is immense potential for the cultivation of patchouli in the homesteads of Kerala. India produces negligible amounts of this oil, and all its requirements are met through imports. The current demand for patchouli is about 40 t of pure oil and 60 t of formulated oil. Indonesia is the major exporter of this oil, and the recent natural calamity of forest fire in Indonesia led to drastic increase in the price of patchouli oil both in domestic and international markets. Hence, increase in production of patchouli oil within the country can help mitigate the problems of short supply of this oil. Limited availability of quality planting materials is a major constraint in the production of patchouli. Also, crop is prone to nematode attack and incidence of bacterial wilt. A pragmatic solution to overcome these constraints is to introduce improved management practices. Use of bioagents and soil amendments for enhancing field performance of crop plant is generally accepted as a key practice in commercial agriculture. Hence, the present investigations attempt to study the effects of beneficial microbial associations and soil amendments for the sustained performance of the crop in coconut gardens. The basic objectives of the study are

1. To refine vegetative propagation techniques in *Pogostemon patchouli* to generate disease free quality planting materials.
2. To asses the impact of soil amendments, microbial antagonists and beneficial microbial associations on the performance of patchouli with respect to growth, yield and oil quality.

Review of Literature

2. REVIEW OF LITERATURE

Pogostemon patchouli Pellet. belonging to the family Lamiaceae is the source of commercial patchouli oil, which possess a characteristic strong fixative tenacity and used as a blending agent with other essential oil. The plant is a native of the Philippine islands and grows widely in Malaysia, Indonesia and Singapore.

Patchouli oil has a unique position in the market and the oil is in great demand in perfumery. Production of patchouli oil in India is negligible (100-150 kg per year) against the total annual world production of 700-800 tones (Manjunatha *et al.*, 2002). India's share in Patchouli oil production is limited, and so we have to gear up the production to meet the internal demand. Large quantities of patchouli oil is imported at high cost to meet the requirement of our perfumery industry. In order to meet the internal demand of our country for this oil and to make a significant dent in the export trade it is necessary to take up cultivation of this crop on a commercial scale. The first prerequisite for popularizing the crop commercially is the availability of quality planting materials and the provision of better growing conditions. By adopting the ecofriendly technologies like application of bioagents in combination with soil amendments in growth enhancement and disease resistance through better availability of nutrients of crop plants can be achieved. Thus keeping the above facts in view, the present review was focused on the effect of biofertilizers and soil amendments on the growth, yield and quality of horticultural crops with aromatic crops.

2.1 Botany of patchouli

The patchouli plant was first described by botanists and was named *Pogostemon patchouli*. Later, it was identified as *Pogostemon cablin* Benth. a native

of the Philippine islands. The word cablin has been derived from 'Cablam' vernacular name of the plant in Philippines.

The plant is a branched, erect or ascending aromatic herb or under shrub, pubescent with quadrangular stem, about 1 to 1.2 m tall. Leaves are simple, opposite, decussate and pale to purplish green when grown in open but bright green under shade. They are two to four inches long and one and a half inches broad. The margin is slightly lobed and those lobes have crenate serrate teeth, the lobes and apex of the leaf being obtuse. Hairs are in abundance on the under surface along the ribs and give the leaf a pale appearance, these are not closely pressed to the leaf, but stand out a little (Guenther, 1949).

Trichomes on the epidermis of patchouli leaves are the main accumulation sites of essential oil and sesquiterpenes (aroma compounds) and the content is directly proportional to trichome number. (Saggoo and Bir, 1981). Somatic chromosome number of patchouli is 2n is equal to 34. The oil is secreted by specialized glandular cells, both located over the leaf surface and within cortex of the leaf. Glandular cells are closely associated with the photosynthetic cells of the leaf, although some internal glands also occur in stem and even in root tissues.

Flowering has been induced in short day conditions. Flowers are small, usually in spikes in the leaf axils or at the ends of branches. Sepals are united into a tube with four or five unequal teeth closely appressed to a two lipped corolla tube which is white with purple streaks and each lip of corolla has two lobes; lobes of the upper lip are longer than those of lower lip. There are four stamens. Ovary is deeply four lobed, superior with two united carpals with a long style arising from its center. Each lobe of ovary contains a single ovule and the fruit consists of four smooth ovoid

nutlets (Cobley and Steele, 1976). Patchouli is reported to flower only in its natural habitat (Anupkumar *et al.*, 1986).

2.2 Impact of bioagents and soil amendments on yield and quality of crop plants.

2.2.1 BIOAGENTS

Bioagents are the primary active strains of micro organisms. They are used either to fix nitrogen, or to solubilise plant nutrients like phosphates. They can stimulate plant growth through the synthesis of growth promoting substances (Vargcha, 1991). Given below is a comprehensive review of the effect of bioagents, soil amendments and bioregulators tried in the study on vegetative characters, yield and quality8 enhancement in various horticultural crops.

2.2.1.1 Trichoderma

Trichoderma can induce enhanced growth and disease resistance either by direct inhibition of growth of pathogens, or by increasing antagonistic micro organisms and by increasing soil fertility (Osnando and Wando, 1992). Brion *et al.* (1997) found that cellulase production by *Trichoderma* can induce better growth of crop plants. Wingster *et al.* (2002) observed the mode of antagonism of *Trichoderma* as nitrate nitrogen regulation and antibiosis through which it can induce better growth and resistance.

Georgieva (1992) opined that treatment of capsicum plants (*Capsicum annuum)* with trichodermin, a biocontrol preparation from *Trichoderma koningi*

reduced *Verticillium dahliae* wilt by 23 to 35 per cent and increased yield by 30 to 40 per cent compared to control.

Chattopadhyay *et al.* (1993) observed that mint residue amended with starter nutrients (1 per cent nitrogen and 0.5 per cent phosphorus) and microbial culture of *Trichoderma viride* (104 spores per ml) or soil suspension (1:10 soil: water) enhanced herbage and essential oil yield in mint (*Mentha arvensis*). Significant increase in soil available nutrients and plant nutrient uptake was also recorded. Seeds of *Eucalyptus camaldulensis* treated with *Trichoderma viride* were sown in coir pith compost wherein successful control of damping off was obtained. Soil application of the antagonist reduced the incidence of damping off by 95 per cent and increased seedling vigour to a significant level (Kumar and Marimuthu, 1994). Combined application of *Trichoderma* sp. (106 cfu per ml of soil) and a reduced dose of Ridomil (0.25 g ai per m^2) in basil reduced occurrence of *Rhizoctonia solani* and increased biomass production to a significant level (Minuto *et al.*, 1997).

Seed treatment of ginger (*Zingiber officinale*) with mancozeb, carbendazim, *Trichoderma harzianum*, *T. hamatum* and *Gliocladium virens* increased the yield of ginger and reduction in ginger yellowing was also observed in treated plots (Dohroo, 1995). Shelf life enhancement of fresh ginger rhizomes at ambient temperatures by combined treatment of gamma irradiation and *Trichoderma* suspension (108 spores per ml) was reported by Mukherjee *et al.* (1995).

Raguchander *et al.* (1997) found that dipping of banana suckers in a suspension of *T. viride* (106 cfu per ml) along with the application of 500 g wheat bran, three months after planting effectively reduced Fusarium wilt incidence and produced the highest yield. Ginger when treated with *Trichoderma harzianum* and *Pseudomonas fluorescens* for the biological control of rhizome rot resulted in reduced

pathogenecity and increased biomass content (Ram *et al.*, 1997). A similar observation was made by Jayasekhar *et al* (2000) who confirmed the favourable effect of *Trichoderma* in reducing rhizome rot of ginger and increasing yield.

Application of *Trichoderma* with soil ameliorants like neem cake, garlic and mustard extracts resulted in maximum survival of vines against foot rot of black pepper (*Piper nigrum*) coupled with increased nutrient uptake (Hegde and Anahosur, 1998). Cucumber seeds when sown in peat substrate, supplemented with varying levels of *T. viride* resulted in increased fresh and dry weights, plant height, number of nodes and leaf area (Poldma *et al.*, 1998).

Bari *et al.* (2000) studied biological control of black scurf disease of potato using fungal antagonists *Trichoderma harzianum, T. koningi, T. viride* and *Gliocladium virens.* All treatments with biological control agents significantly reduced sclerotium index. Broad casting and tuber coating with *T. harzianum* produced the highest tuber yield.

Tuber dressing of potato with *T. harzianum* as alginate starch formulation supported higher growth parameters and yield. It was proved that biocontrol agents can increase growth parameters and reduce disease incidence (Haggag and Nofal, 2000).

Hazarika *et al.* (2000) observed that inoculation of *T. viride* and *T. harzianum* by seedling dip or soil application in tea (*Camellia sinensis*) reduced mortality of plants from stump rot, besides increased plant growth and dry matter production was also noted.

Rabeendran *et al.* (2000) noted inconsistent growth promotion of cabbage and lettuce with the application of *Trichoderma* isolates. Dipping transplants in spore

suspensions of *T. longipile* and *T. tomentosum* increased leaf area (58 to 71 per cent), shoot dry weight (91 to 102 per cent), and root dry weight (100 to 158 per cent) compared with untreated control. An increase in yield of lettuce was also recorded with the application of *T. longipile*.

Ravi *et al.* (2000) evaluated integrated control of burrowing nematode and found that combined application of 250 g neem cake, 20 g *T. viride* and 10 g carbofuran as the most effective treatment in increasing height of plant, pseudostem girth, leaf area, leaf number and fruit yield of banana. The same treatment combination was also proved effective for the control of nematode population.

Combined application of *T. harzianum* and *Alcaligenes* sp. reduced the incidence of nursery rot of black pepper and increased root and shoot growth in nursery (Anith and Manomohandas, 2001).

Pomegranate cuttings were treated with one month old *T. harzianum* for enhancing rooting. Sixty percent per cent of the cuttings treated with *T. harzianum* were rooted as against 56 per cent in IBA. In addition to rooting, increase in shoot growth and weight of shoots and roots were also recorded (Patil *et al.*, 2001).

Cucumber plants when sown in soils amended with *T. harzianum* resulted in early seedling emergence (30 per cent). These plants exhibited 95 per cent and 75 per cent increase in root area and cumulative root length respectively. A significant increase in dry weight (80 per cent), shoot length (45 per cent) and leaf area (80 per cent) was also observed (Yedida *et al.*, 2001).

Avada and Abdel (2002) observed increased tuber yield in potato with the application of *Trichoderma harzianum* and *Bacillus thuringiensis*.Tomato plants

treated with *Trichoderma* showed significant reduction in nematode population and improved height of plant, shoot weight and root length to a significant level (Devi and Richa, 2002).

Dwivedi and Shukla (2002) studied biocontrol of Fusarium wilt in guava using *Trichoderma* and *Gliocladium* species. They observed *T. viride* as the most effective remedy against *F. psidii* followed by *T. harzianum* and *G. virens*. Minimum infection and maximum plant height was also observed from *T. harzianum* treated plots.

2.2.1.2 Pseudomonas fluorescens

Broadbent *et al.* (1977) listed out the benefits of root colonisation by *Pseudomonas fluorescens* as protection against non parasitic root pathogens, production of biologically active substances like auxins and gibberellins, transformation of unavailable mineral and organic compounds in the available form to plants and nitrogen fixation. (Benhamon *et al.*, 1996) found that *Pseudomonas fluorescens* can induce growth and plant defense mechanisms through the production of siderophores, mineralization of phosphorus and antibiosis. Gutierrezmanero *et al.* (1996) reported that *Pseudomonas fluorescens* have been found to increase the growth and yield by 5 to 10 per cent due to increased soluble phosphorus nutrition and nitrogen fixation, synthesis of growth promoting substances and production of antibiotic like compounds

Growth promotion in tea (*Camellia sinensis*) through the production of siderophores by a fluorescent pseudomonas strain, RRLJ 181 was reported by Kumar and Balamani (1997)

Raguchander *et al.* (1997) observed that dipping of banana suckers in a suspension of *P. fluorescens* (106 cfu per ml) and application of 500 g wheat bran three months after planting effectively reduced Fusarium wilt incidence and produced the highest yield.

Bucki *et al.* (1998) reported that tomato seeds treated with selected isolates of actinomycetes and fluorescent *Pseudomonas* doubled the number of seedlings obtained compared with non treated seeds.

Shanthi *et al.* (1998) noted increased yield in grape vine with the inoculation of *Pseudomonas fluorescens*. Yield increase between 45 per cent at a dosage of 1g per vine and 166 per cent at a dosage of 4 g per vine was recorded. The study also proved that application of *P. fluorescence* on grape vine was effective in suppressing the nematode multiplication as well as giving higher yield.

Kurze *et al.* (1999) opined that application of *P. fluorescens* produced a relative increase in the yield of strawberry between 24 and 174 per cent with reduced incidence of Verticillium wilt by 15 to 59 per cent.

Manoranjitham and Prakasam (1999) observed that treatment of tomato with *Trichoderma viride* (4g per kg of seeds) and *Pseudomonas fluorescens* (5g per kg seeds) resulted in the lowest mortality per cent against damping off, and obtained increased shoot and root length and the highest dry matter content, both in pot and field experiments.Chilli seeds treated with *Trichoderma viride* (4g per kg) and *P. fluorescens* (5g per kg) recorded maximum germination (92.3 per cent), shoot length (4.45 cm), root length (13.5 cm), dry matter production (6.77 mg) and vigour index (1655.67) compared to control (Manoranjitham *et al.*, 1999).

Ginger seeds when treated with *Pseudomonas fluorescens* strain EM 85 along with solarisation decreased the wilt incidence and increased yield up to 29.42 tonnes per hecctare compared to 19.51 t per hectare in control (Anith *et al.*, 2000).

Kharchenko and Ryabchinskaya (2000) observed increased plant growth and yield in black current with the application of *Pseudomonas fluorescens* strain AP-33 and B-3481.

Pre inoculation of *Pseudomonas fluorescens* in tomato showed the least incidence of bacterial wilt and the highest yield of 12.81 quintals per hectare (Minku *et al.*, 2000).

Siddiqui *et al.* (2001) noticed improved growth of tomato with the application of *P. fluorescens*.

Devi *et al.* (2002) observed that seed treatment of okra with *P. fluorescens* (500 or 1000 g per 20 kg seeds) or foliar spray (2 or 4 kg) improved shoot and root lengths and weights. Reduction in nematode population was also observed.

Treatment of pepper (*Piper nigrum*) transplants with *Pseudomonas fluorescens* (Pf 5) effectively increased shoot and root weights of pepper plants grown in artificially sterilized soil. Reduction in *P. capsici* infection was also recorded (Mosa *et al.*, 2002).

Increased yield and biomass production were observed in chilli with the application of *Pseudomonas fluorescens*. (Gehlot and Purohit, 2002). Rajappan *et al.* (2002) observed increased fruit yield in banana with the application of *Pseudomonas fluorescens* pf.1.

(2002) observed increased fruit yield in banana with the application of *Pseudomonas fluorescens* pf.1.

2.2.1.3 VAM

Josaphine (1991) found that application of *AMF* can induce growth and disease resistance through the solubilization of major and minor nutrients, and also by the availability of phosphorus, nitrogen, Fe, Mn, Zn and Ca. Timjone (1992) studied the mechanism of action of *AMF* and found that *AMF* can cause anatomical, physiological and biochemical alternations.

Gupta *et al.* (1991) found enhanced growth and biomass production in palmarosa (*Cymbopogon martinii*) with the association of VAM fungi. Significant increase in phosphorus uptake was noticed in mycorrhizal plants compared with non mycorrhizal plants and a similar mode of potassium uptake was also noticed; indicating that AMF inoculation could enhance the productivity of essential oil bearing grasses. Effects of mycorrhizal symbiosis and soil compaction in *Cymbopogon winterianus* was studied by Kothari and Singh (1996), who observed that *Glomus intraradices* substantially increased root and shoot biomass, root length, nutrient uptake (K, P, Zn and Cu) per unit root length and nutrient concentration in plants compared with uninoculated treatments.

Response of palmarosa to dual inoculation of VAM and Azospirillum was studied by Neelima and Janardhanan (1996a), who found that dual inoculation increased growth, yield and oil content of palmarosa to a significant level over uninoculated control as well inoculation with *Glomus aggregatum* or *Azospirillum brasilense* alone. A study was conducted at CIMAP, Lucknow to analyse the enhancement of growth in palmarosa in association with VAM and the results

showed that inoculation of four *Glomus* species, *Glomus aggregatum, Glomus fasciculatum, Glomus interaradices* and *Glomus mosseae* increased shoot dry weight, P content of root and shoot tissues and cytokinin production to a significant level (Neelima and Janardhanan., 1996b). Influence of VAM on productivity of mint was studied by Khaliq and Janardhanan (1997) who reported that, VAM inoculation in general, improved shoot biomass production of all species although it did not affect the essential oil content or composition of oil in any of the mints examined.

Association of *Glomus fasciculatum* in *sandal* (*Santalum album*) was reported by Krishnamurthy *et al.* (1997) wherein its growth promoting ability in sandal seedlings has been described. Vineeta *et al.* (1997) recorded increased yield of *Mentha spicata* with the application of *Glomus fasciculatum,* which also increased root length, root surface area and leaf area to a significant level as compared to control.

Gupta and Janardhanan (1991) studied the effect of mycorrhizal association in palmarosa and found that inoculation of *Glomus aggregatum* in palmarosa caused a two fold growth and three fold biomass production increase as compared with non mycorrhizal plants.

Reddy (1991) reported inoculation of various VAM fungi in mango, citrus and papaya increased the vegetative characters like number of leaves, leaf area, stem diameter and shoot mass. This is of advantage since it can save one or two months in softwood grafting and four to six months in wedge and veneer grafting.

He *et al.* (1994) observed increased plant height up to 90 to 100 per cent and shoot dry weight up to 130 to 200 per cent in tea (*Camellia sinensis*) with the application of *Glomus epigaeum*.

Liang (1995) found inoculation of VAM on one year old seedlings of *Mangifera indica, Litchi chinensis* and *Dimocarpus longan* significantly increased plant height, stem diameter and biomass. Application of VAM also resulted in increased chlorophyll content and photosynthetic rates in leaves and enhanced nutrient uptake.

Silva *et al.* (1996) observed that inoculation of *Glomus intrarardices* increased plant height, leaf area and number of leaves in strawberry seedlings both in green house and field conditions. Inoculated plants produced more runners than the control plants.

Effect of VAM on growth of ginger (*Zingiber officinale*) was studied by Sharma *et al.* (1997) who observed that inoculation with *Glomus mosseae* at 21spores per gram of soil gave the tallest plants, with the highest yield (46.5 gram per pot) and the greatest number of tillers per plant. Inoculation with *Gigaspora margarita* (2.5 g per rhizome) at the time of planting increased plant height, number of leaves and tillers, root weight and yield of ginger.

Growth of young tea shoots in association with VAM was studied by Deori *et al.* (1998) wherein enhanced overall growth and dry weight of tea shoots were observed as a result of the beneficial fungal association.

Fentahan *et al.* (1998) observed combined application of *Azospirillum brasilense*, VAM and 50 kg N in onion resulted in the highest horizontal and vertical diameter. Application of VAM, *Azospirillum brasilense* and 25 kg P produced the highest number of bulbs, shoot dry weight (8.28 g) and leaf number (8.1) Plants treated with VAM matured earlier (116 days) than other treatments. Effect of single or multiple VAM inoculates on growth parameters of tomato were studied by Iqbal

and Mahmood (1998). Results obtained showed that *Glomus mosseae* produced maximum growth of plants followed by *Glomus constrictum* and *Glomus fasciculatum.*

Kichadi and Sreenivasa (1998) observed interaction effect of *Glomus fasciculatum* and *Trichoderma harzianum* in tomato and the results showed that interaction of both fungi not only increased plant growth and yield but also improved P nutrition.

Aparajitha *et al.* (2000) observed the longest shoots in brinjal with the application of VAM at the rate of 300 spores per plant followed by 150 spores. They concluded mycorrhizal plants had longer shoot system than non mycorrhizal plants.

Domey and Berymann (1999) observed differential responses of *Glomus intraradices* and *Glomus albidum* on growth and protein content of *Panax ginseng* and *Panax quinquefolium. Glomus intrardices* rather diminished root and shoot growth of *Panax ginseng* whereas *Glomus albidum* increased the above two parameters in *Panax quinequefolium.*

Jothi *et al.* (2000) studied the effect of pre inoculation of VAM on okra and observed enhanced growth and yield between 212.5 and 343.0 g. Reduction in nematode population was also recorded.

Nagaraju *et al.* (2000) noticed that treatments receiving 100 per cent single super phosphate and *Glomus mosseae* recorded the highest plant growth responses in onion with respect to all biometric and biochemical characters.

Deokar and Sawant (2001) evaluated response of chilli (*Capsicum annuum*) to various biofertilizers and observed that, combined application of VAM at

the rate of 50 gram per pot and with Azotobacter at the rate of 250 gram per pot resulted in maximum growth and nutrient uptake compared to other biofertilizer treatments.

Cabbage seedlings inoculated with *Glomus* species (500 spores per g of soil) resulted in increased plant growth, biomass production and P uptake to a significant level (Nelson and Achar, 2001).

A study was conducted to evaluate the response of coffee to different species of VAM. The results obtained indicated that per cent root colonization, mycorrhizal spore count, plant height, leaf area index and plant dry weight were highest with *Glomus leptotichum* inoculated plants compared to those inoculated with other VAM fungi (Thammaiah *et al.*, 2001).

Kumar and Murugesh (2002) observed tallest plants from ten medicinal plants, those were treated with VAM. Improved seedling growth, dry weight and phosphorus uptake were also recorded with VAM inoculation.

Manjunatha *et al.* (2002) opined that combined application of 75 per cent N, P, 100 per cent K, Azotobacter, Azospirillum and VAM in patchouli (*Pogostemon cablin* Benth.) resulted in the production of superior values for plant height (80.14 cm), number of leaves (357.75), number of branches (22.04), plant spread (76.12 cm), yield of fresh herbage (10.73 tonnes per hectare).

2.2.2 Soil amendments

2.2.2.1 Neem cake

Application of neem cake can increase the growth and yield because of its nitrification regulation property and transfer of nutrients to plants (Skulbhram *et al.*, 1982).

Kumaran *et al.* (1998) found that application of neem cake can add organic carbon, nitrogen and K to the soil, which can create a stable C: N ratio that facilitates better availability of nutrients to plants.

Ram and Prasad (1989) observed that neem cake coated urea at the rate of 126 kg N per hectare when applied to *Mentha arvensis* had given promising results. Increased height of plant, leaf: stem ratio, leaf area index, dry matter production and herbage and essential oil yield were also recorded the highest with the same.

Pandey *et al.* (1992) obtained enhanced yield from *Mentha arvensis* with the application of neem cake at 1 g N per kg of soil. Singh and Singh (1992) recorded better oil yield in *Cymbopogon winterianus* with the application of neem cake coated urea granules up to 150 kg N per hectare. Effectiveness of some nematicides and oil cakes in the management of *Pratylenchus thornei* on *M. citrata, M. piperita* and *M. spicata* was tested by Shukla and Haseeb (1996). They found neem cake as an effective remedy for the control of *P. thornei*. Significant increase in herb weight and oil yield was also recorded with the addition of neem cake. Chakraborty and Dutta (1997) obtained increased flower yield from tuberose (*Polianthes tuberosa*) with the application of garlic and neem cake

Increased tuber yield in potato with the application of lime and neem cake was observed by Singh *et al.* (1993). Study of comparative performance of oil seed cakes in davana (*Artemisia pallens*) showed that neem cake application enhanced the growth and yield of the crop (Pandey, 1994) to a significant level than any other treatments.

Mohanty *et al.* (1995) noticed that pre planting application of neem cake at the rate of 1 tonnes per hectare followed by post planting application of carbofuran

(1 kg ai per ha) 45 days after planting reduced nematode population to a significant level and increased the yield of ginger rhizome.

Sheela *et al.* (1995) recorded increased yield of ginger with the application of neem cake at the rate of 2.5 tonnes per hectare and carbofuran at the rate of 1 kg ai per ha. They also observed significant reduction in nematode population with the same.

Influence of organic amendments on the intensity of Fusarium wilt in banana was studied by Karthikeyan and Karunanithi (1996). Among the organic amendments tested, neem cake at 250 kg per hectare was rated as the most effective treatment for the control of *Fusarium oxysporum* which also produced the highest crop yields.

Sharma *et al.* (1996) recorded increased yield and effective nematode control in tomato with the application of neem cake at the rate of 1.5 tonnes per hectare.

In green house tests, maximum shoot length, fresh and dry weight, root length, fresh and dry root weight and pod yield of okra were recorded with the application of neem cake at the rate of 13.5 g per pot (Ramakrishnan *et al.*, 1997).

Effect of organic and biofertilizers on growth enhancement and total biomass production of papaya was studied by Rani and Sathiamoorthy (1997) who observed the highest growth (37.8 per cent) when 50 per cent N fertilizer was substituted by FYM and neem cake.

Singh *et al.* (1997) found that neem cake coated urea at 60 kg N per hectare had given higher test weight, seed, straw and biological yields in coriander (*Coriandrum sativum* L.) than prilled urea.

Tomato when treated with inorganic and organic fertilizers including neem cake produced good results in terms of growth and yield. Plant height, branches per plant, mean fruit weight and number of fruits per plant were recorded highest with the combination treatment (Kumaran *et al.*, 1998).

Effect of organic manures on nutrient uptake, yield and quality of turmeric (*Curcuma longa* L.) was studied by Sadanandan and Hamza (1998)a. Highest curcumin yield was obtained from plots treated with neem cake at the rate of 287 kg per hectare. In ginger it is found that application of all oil cakes including neem increased, nutrient uptake, rhizome yield and oleoresin content to a significant level (Sadanandan and Hamza 1998b).

Integrated management of root knot nematode in ginger was studied by Vadhera *et al.* (1998). The highest yield and minimum count of nematode population were recorded from neem cake treated plots.

Pereira and Mitra (1999) observed the highest number of fruits per plant in guava with the addition of neem cake. They also recorded the highest yield (17 kg per plant and 38 quintals per hectare) and average fruit weight (96.39 g) with the application of neem cake in two splits along with NPK at the rate of 75, 100 and 75 g per plant.

Nematode management in banana was studied by Jonathan *et al.* (2000) who found application of neem cake at the rate of 1.5 tonnes per hectare, not only reduced the population of nematodes but also enhanced plant height, pseudostem girth, number of leaves per plant, leaf area and yield.

Effect of organic and inorganic manuring on growth, yield and quality of Khasi mandarin was studied by Borah *et al.* (2001). They reported that maximum

yield with appreciable tree vigour and fruit quality was obtained through combinations of organic and inorganic fertilizers. Application of 7.5 kg neem cake, along with 600:300:600 g NPK per plant per annum produced the maximum yield and best quality of Khasi mandarin.

Efficacy of coating treatments on urea application in Nagpur mandarin was studied by Huchche *et al.* (2001). Among treatments, gypsum and neem cake coated urea recorded the highest canopy volume; fruit yield and leaf nutrient content.

Ingle *et al.* (2001) conducted a study on integrated nutrient management in acid lime. They observed that yield and quality of acid lime fruits were significantly improved with the application of neem cake (7.5 and 15 kg) along with chemical fertilizers. Significantly higher yield with better quality fruits were obtained with the application of 600 g N, 300 g P_2O_5 and 300 g K_2O with 15 kg neem cake per plant per year.

Karthikeyan *et al.* (2001) observed an increase in dry shoot and root weights in brinjal with the combined application of *Trichoderma viride* and neem cake.

2.2.2.2 Lime

Application of lime which supplies Ca to the soil also plays an important role in cell division and it is an important component of the cell wall (Fernandes *et al.*, 1974). Saravanan and Nambisan (1995) found that response to addition of lime can induce neutralization of soil acidity leading to better availability of nutrients, which ultimately resulted in increased growth parameters.

Accumulation of dry matter and uptake of micro and macro nutrients in ginger (*Zingiber officinale*) was studied by Haag *et al.* (1990). They observed that

incorporation of poultry manure (4.5 tonnes per hectare) with dolomitic lime (1.8 tonnes per hectare) before planting could increase yield and dry matter accumulation to a significant level.

Effect of lime and phosphorus on American ginseng (*Panax quinquefolius*) was studied by Konsler and Shelton (1990). Ginseng was grown in pots of loam amended with factorial combination of 0, 4.42 or 8.84 kg dolomitic lime per m^3 and 0, 0.16 or 0.32 kg P per m^3. At the end of each growing season, root size was greatest at intermediate liming rate with the highest P rate. But Thomas *et al.* (1990) observed that application of lime had no significant influence on yield and quality of palmarosa oil (*Cymbopogon martini*).

Cymbopogon khasianus when treated with powdered lime at the rate of 2.5, 5, 7.5 or 10 tonnes per hectare produced high herbage dry yield. The uptake of N, P, K and Ca also increased with each increase in liming rate (Choudhury and Bordoloi, 1992). Eucalyptus trees (*Eucalyptus grandis*) treated with Araxa phosphate and lime at the rate of 2 tonnes per hectare had fibres and vessel elements of larger diameter and thicker fibre walls. Total extractives and Ca content were also increased with liming (Andrade *et al.*, 1994). Fastest growth of eucalyptus seedlings with the application of lime at the rate of 500 g per plot was reported by Balagopalan (1997). He concluded that liming on eucalyptus had significant influence on height, growth and dry matter production.

Saravanan and Nambisan (1995) opined that pruning and liming had beneficial effects on fruit yield of hale plum. The highest fruit yield of 39.10 kg per tree was reported with the application of lime at the rate of 75 g per tree.

Beneficial effects of liming in tea (*Camellia sinensis*) was studied by Wilkie *et al.* (1995). More vigorous root system with enhanced nutrient uptake was exhibited by the treated tea plants.

Kotowska (1996) reported increased tuber yield in potato with increasing NPK rate under all liming treatments. Liming increased tuber Ca content, especially at a higher rate than control.

Ortiz *et al.* (1996) observed larger stem diameter and increased foliage production in coffee (*Coffee arabica*) with the application of 10 per cent dolomitic lime.

Robinson *et al.* (1997) recorded better growth and greater bearing capacity of apple trees with the application of lime at the rate of 3 tonnes per hectare along with NPK and B. Application of lime registered highest yield and optimum plant growth in grapes (Smolarz *et al.*, 1997)

Topcuoglu and Yalcin (1997) observed better fruit yield, increased fruit dry matter per cent, fruit hardness and leaf chlorophyll content in tomato with the application of lime.

Chilli (*Capsicum annuum*) when grown in vermiculite based growing media amended with lime at the rate of 12 g per kg produced better plant height, fresh and dry weight, leaf area and stem diameter (Chung *et al.*, 1998).

Engel *et al.* (1998) obtained the best quality apples and highest marketable yield from lime treated plots. Bulb yield of garlic was increased up to 18.16 tonnes per hectare with liming at the rate of 3 tonnes per hectare (Majundar *et al.*, 1998).

They also observed increased sulphur and phosphorus contents with the addition of lime.

Effect of various sources of lime on yield of lettuce, cucumber and tomato was studied by Jarvan (1999) where in increased yield of cucumber and lettuce along with improved taste of tomato were observed.

Suresh and Savithri (2001) observed increased bunch yield of banana up to 26.3 per cent and pulp to peel ratio 4.2 per cent with the application of lime. Soil application of NPK, spraying of micronutrients and liming gave the highest yield (42.5 tonnes per hectare) and pulp to peel ratio (4.1 per cent).

2.2.3 Influence of auxins in root induction of horticultural crops

Selvarajan and Rao (1982) found beneficial effects of IBA on rooting of patchouli (*Pogostemon patchouli* Pellet.) and observed that dipping of two to three nodded cuttings in 1500 ppm IBA had given the highest rooting per cent.

Pillay *et al.* (1982) reported that dipping of 2 node cuttings of *Piper nigrum* in 1000 ppm IBA for 45 seconds gave the highest number of rooted cuttings.

Zeubini (1984) observed significantly better root growth in pepper with the application of IBA. Triacontanol (0.05%), auxin (0.25%) and cytokinin (2 ppm) at biweekly intervals or 2,4-D (0.5%) at monthly intervals were found to enhance growth and yield of patchouli (Tasma and Monko, 1988).

Shridhur and Singh (1990) studied effects of IBA on black pepper and found 80 per cent rooting compared to control (40 per cent). Thimmappa and Bhattacharjee (1990) observed, improved rooting of geranium with the application of

IBA 2000 ppm. They also found that IBA (2000 ppm) treated patchouli cuttings had given the highest rooting per cent and longer roots (Bhattacharjee and Thimmappa, 1991).

It is found that auxin treated plants (IBA at the rate of 1000 ppm) recorded maximum rooting and increased root length in many horticultural crops (Evans, 1991). Significant improvement in rooting of *Pelargonium graveolens* and *Pogostemon patchouli* was recorded with the application of IBA at the rate of 2000 ppm (Bhattacharjee and Thimmappa, 1992).

Singh and Hippalganokar (1992) observed maximum rootability in patchouli with the application of 1000 ppm IBA. Roland (1994) observed the impact of auxins including IBA, IAA and 2,4-D upon the effective rooting of lavender

Ganesh and Sreenath (1997) observed best rooting in coffee with the application of IBA (1 mg per litre). In cocoa soft woodcuttings treated with 6000 ppm IBA rooted best (Hernandez and Leal, 1997).

Patil and Jayanthi (1997) found that rooting of *Rauvolfia micrantha* and *Rauvolfia tetraphylla* were possible both *in vitro* and *ex vitro* with the application of IBA.

Guava shoots when treated with 4500 ppm IBA resulted in the highest rooting per cent (91.6 per cent), the greatest root length (13.4 cm) and the highest number of leaves per layer (34.8) (Bhagat *et al.*, 1998).Suksa *et al.* (1998) observed better root growth of papaya with the application of IBA 10 mM.

Response of hybrid tomato to growth regulators was studied by Singh (1999) and found that IBA at the rate of 500 ppm alone or in combination with NAA produced maximum rooting percent and increased net returns. Response of guava to different concentrations of IBA was studied by Tomar *et al.* (1999) who obtained enhanced rootage and the highest survival rate.

Alobed (2000) found maximum rootability in guava with the application of IBA (3000) ppm and catechol (500 or 1000 ppm). He also noted that combined application of IBA, catechol and cinnamic acid resulted in longer and thicker roots along with enhanced root and shoot growth.

Lavender (*Lavendula stoechas* L.) cuttings treated with IBA resulted in better rooting per cent and the best results were obtained from cuttings treated with 4000 ppm IBA (Ayangolu *et al.*, 2000).

Garcialopez *et al.* (2001) observed the presence of adventitious roots and increased root length in tomato with the application of IBA at 1500 or 3000 ppm. Effect of growth regulators on growth and yield of onion was studied by Singh *et al.* (2001) who observed that application of IBA at the rate of 30 ppm resulted in the highest plant height (63 cm), number of leaves (13.46), neck diameter (2.3 cm), fresh weight of plant (148.92 g), fresh weight of bulb (65.48 g) and yield per hectare (150.80 g) compared with other treatments and control.

Influence of plant growth regulators in mango was studied by Mahabir and Baghet (2001). They found IBA as the most effective treatment in promoting the success and survival of air layers in rooting and growth attributes. Nath and Korla (2001) found that IBA at the rate of 1 ppm gave the tallest plants (46.83 cm) with the highest number of leaves (31.45), the heaviest rhizomes (49.62 g) and the highest

yield (27.13 quintals per hectare) in ginger. Sun *et al.* (2001) noticed that IBA treated tea cuttings exhibited profuse rooting and enhanced shoot growth.

Tea cuttings when treated with 8000 ppm IBA gave the best results with regards to per cent rooting (73.33 per cent), root number (9.83), root length (21.07 cm), shoot length (20.57 cm) and number of leaves per cutting (4.44) than control (Badshah *et al.*, 2002). Kananjia *et al.* (2002) observed highest root length of onion seedlings (2.85 cm) with the application of IBA at the rate of 100 ppm.

Singh (2002) studied the effect of growth regulators in guava and confirmed the effectiveness of IBA in root formation. They also noticed enhanced vegetative growth in guava with IBA.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Impact of bioagents and soil amendments on the performance of patchouli (*Pogostemon patchouli* Pellet.) was conducted in Kerala Agricultural University at the Department of Plantation Crops and Spices and Biochemistry Laboratory, College of Horticulture during the year 2002-2004. The materials used and methodology adopted for the study are described in this chapter.

3.1 Details of the experimental field

3.1.1 Area

Field experiment was conducted in an adult coconut garden under 30 to 35 percent shade.

3.1.2 Location

The experimental field is situated at $10^0$32'N and 76^0 13' E longitudes with an altitude of 22.25 m above Main Sea Level.

3.1.3 Soil

The soil of the experimental plot is deep laterite with clay loam texture of $_P{}^H$ 5.6.

3.1.4 Climate

The area enjoys a warm humid tropical climate. The weather situation during the period under study was normal and in tune with the annual cycle with out any significant variation. The details of the meteorological observations for the period of the experiment are presented in Appendix 1.

The study was carried out in two experiments.

3.2 EXPERIMENT-1

Production of quality planting materials in nursery.

3.2.1 MATERIALS

Two to three noded terminal cuttings of patchouli cultivar Johore were used as the planting materials. Cuttings were raised in nursery in polythene bags of size 10 x 15 cm^2 filled with potting mixture consisting of sand, soil and farm yard manure mixed in equal proportions. There were six treatments including control, *AMF, Pseudomonas fluorescens, Trichoderma,* combination of *Trichoderma* and *Pseudomonas fluorescens* and IBA. The experiment commenced in May 2002.

3.2.2 METHODOLOGY

Nursery was laid out in a completely randomised design with six treatments in three replications with 20 plants in each replication (Table 1).

Table 1. Details of treatments

No.	Treatments	Method of application
T_1	Control	
T_2	IBA 1000 ppm	Quick dip method (15 seconds).
T_3	*Trichoderma*	Applied at the center of polybags at 5 cm depth at the rate of 3 g per bag.
T_4	*Pseudomonas fluorescens*	Applied at the centreof polybags at 5 cm depth at the rate of 3 g per bag.
T_5.	*Trichoderma* + *P.fluorescens*	Applied at the center of polybags at 5 cm depth each at the rate of 2 g per bag.
T_6	*Arbuscular Mycorrhizal Fungi*	Applied at the centre of polybags at 5 cm depth at the rate of 10 g per bag.

3.2.3 Observations in the nursery

Observations on the following parameters were taken at an interval of 15, 30 and 45 days after planting the terminal cuttings in the nursery.

3.2.3.1 Earliness in sprouting - Date of emergence of the first sprout was recorded and mean was calculated.

3.2.3.2 Number of leaves - Total number of leaves produced per plant under each treatment was noticed and mean was calculated.

3.2.3.3 Length of sprout - Length from the base of sprout to the apex was taken and mean length was expressed in centimetre.

3.2.3.4 Earliness in rooting - Date of emergence of roots was noticed for each observational plant and mean was calculated.

3.2.3.5 Length of roots - The length of the longest root was measured separately for each observational plant and mean was tabulated.

3.2.3.6 Number of roots - Total number of roots emerged under each observational plant was recorded and mean was taken.

3.2.3.7 Root volume - Volume of roots were noted separately for each observational plant using a measuring cylinder sufficiently large to hold the roots and expressed in millilitre

3.2.3.8 Estimation of soil microflora of AMF

In order to assess the efficiency of the commercial formulation of AMF, mycorrhizal spores in the sample was checked. Root bits of patchouli were sorted and kept in a solution of Formaldehyde, acetic acid and alcohol at the rate of 1, 5 and 15 ml and kept it over night to loosen them. Then these root bits were taken out and kept in a solution of 10 per cent KOH solution and washed in 2 per cent HCl. Finally the root bits were stained in tripen blue 0.05 per cent. Slides were prepared and colonization of *AMF* was observed through stereo microscope.

Based on these observations the best treatments for raising rooted cuttings of patchouli in the nursery was noted and plants treated with a combination of *Trichoderma* + *Pseudomonas fluorescens* were selected for main field studies.

3.3 EXPERIMENT- 2

Impact of bioagents and soil amendments on the growth and yield of patchouli.

Forty five days old rooted cuttings of the treatments rated best in Experiment No.1, raised from the terminal cuttings of patchouli were transplanted to the main field. The experiment was carried out in a randomized block design with two replications. Details regarding treatments and experiment are given below.

3.3.1

Table 2. Details of experiment

Total number of treatments	16
Number of replications	2
Number of plants per plot	9
Bed size	2 m x 2 m
Distance between beds	30 cm
Plant to plant distance	60 cm x 60 cm
Design	RBD

3.3.2

Table 3. Details of treatments in the main field

T_1	Control (FYM + recommended doses of N, P_2O_5 and K_2Oat the rate of 150:50:50)
T_2	Control + *Trichoderma*
T_3	Control + *Pseudomonas fluorescens*
T_4	Control + *Trichoderma* + *Pseudomonas fluorescens*
T_5	Control + neem cake
T_6	Control + neem cake + *Trichoderma*
T_7	Control + neem cake + *Pseudomonas fluorescens*
T_8	Control + neem cake + *Trichoderma* + *Pseudomonas fluorescens*
T_9	Control + lime
T_{10}	Control + lime + *Trichoderma*
T_{11}	Control + lime + *Pseudomonas fluorescens*
T_{12}	Control + lime + *Trichoderma* + *Pseudomonas fluorescens*
T_{13}	*Control + lime + neem cake*
T_{14}	Control + lime + neem cake + *Pseudomonas fluorescens*
T_{15}	Control + lime + neem cake + *Trichoderma*
T_{16}	Control + lime + neem cake + *Trichoderma* + *Pseudomonas fluorescens*

The experiment was laid out in a mature coconut garden. The land was thoroughly ploughed and tilled. Raised beds of 2 m x 2 m size and 30 cm height were prepared with 30 cm wide channels in between beds. FYM at the rate of 15 tonnes per hectare and NPK at the rate of 25:50:50 kg per hectare were applied to each plot as basal dose at the time of field preparation. Rest of the required amount of nitrogen (125 kg per hectare) was applied in six equal splits at intervals of one month.

Rooted cuttings were transplanted in the bed at a spacing of 60 cm x 60 cm between plants. Irrigation was given immediately after transplanting and subsequently alternate days irrigation was given during the drier months. Timely weed management was also done.

3.3.3 Application of bioagents and soil amendments

The bioagents *Trichoderma* and *Pseudomonas fluorescens* were mixed with sand and applied to the experimental plants. *Trichoderma* at the rate of 2.5 kg per hectare was given to the respective plots at the time of planting and at intervals of 60 days by forking. Application of *Pseudomonas fluorescens* was also done at the rate of 2.5 kg per hectare at the time of planting and at an interval of 45 days. Combined application of *Pseudomonas fluorescens and Trichoderma* was also given at the time of planting at the same rate and at an interval of 60 days. Lime and neem cake were incorporated to their respective plots at the rate of 15 tonnes per hectare in two equal splits at the time of planting as basal dose and three months after planting by forking.

3.3.4 Harvesting

At six months after transplanting when the crop emitted characteristic odour of patchouli and colour of the herbage turned from pale green to yellowish brown harvesting was done in the morning by hand. Young shoots were cut at 20 cm below the apex. A few shoots were left over to ensure resuming of growth for the next harvest. Second harvest was done 3 months after the first harvest. Fresh herbage yield and fresh leaf yield were recorded during each harvest.

3.3.5 Drying

Shade drying was done. Fresh leaves were separated and spread out in thin layers on a hard dry surface in shade, which allowed free circulation of air. During drying, leaves were frequently turned over to ensure proper drying and dry weight was taken till the weight reaches to a constant value for about ten days.

3.3.6 Essential oil extraction

The essential oil from shade dried leaves was extracted by hydro distillation in Clevenger apparatus. Fifty gram of shade dried and finely powdered leaves was taken in the round bottom flask and distilled with 200 ml of distilled water. The duration of distillation was standardised as 6 hours as no further increase in essential oil content was noticed beyond this period. The volatile oil being lighter than water condensed and collected on the top of the oil separator. The volume of oil was noted and the per cent oil recovery in the sample was worked out.

$$\text{Per cent of oil} = \frac{\text{Volume of oil} \times 100}{\text{Weight of sample}}$$

3.3.7 Estimation of nematode population

Incidence of nematode attack was tested by Cobb's sieving technique. 100 g of the soil from patchouli field was collected and soil was allowed to pass through micro sieves of varying size and of the solution was decanted. Finally the number of nematodes present in the soil sample was calculated.

3.3.8 Observations in the main field

Following observations were taken in all plants in the main field at bimonthly intervals.

3.3.8.1 Height of plant - Height of the plant was calculated from the base of the plant to the tip for each treatment and mean was calculated and expressed in cm.

3.3.8.2 Spread of plant - Spread of the plant was measured using a scale in two radial directions viz., North-South and East-West and mean was worked out and expressed in cm.

3.3.8.3 Number of branches - Total number of branches produced per plant was recorded for each treatment and mean was calculated.

3.3.8.4 Number of leaves - Total number of leaves produced per plant was calculated for each treatment and mean was worked out.

3.3.8.5 Leaf area - Leaf area was calculated for each plant by analyzing the sample leaves through the leaf area meter and average leaf area was calculated in cm^2.

3.3.8.6 Fresh herbage yield - The herbage was weighed separately for each treatment and yield per plot was recorded in kg per hectare.

3.3.8.7 Fresh leaf yield - The leaves were separated from the stem for each treatment and the yield per plot was recorded in kg per hectare.

3.3.8.8 Dry leaf yield - Leaves were dried under shade to a constant weight and per plot yield was calculated in kg per hectare.

3.3.8.9 Reaction to pests and diseases – Incidence of pests and diseases if any was recorded in the main field.

3.4 Estimation of physicochemical properties of patchouli oil

3.4.1 Refractive index

Refractive Index was recorded using an Abbe Refractometer. The instrument mainly consists of one telescope and two prisms. A beam of light is reflected through the mirror and oil is kept in between these two prisms. Refractometer is arranged in such a way that the telescope is fixed and the prism box is rotated so as to get the coincidence of the critical ray with the crosswire of the eyepiece. The setting of the prism at this position corresponds to a definite critical angle and therefore to a definite value of refractive index. This is read directly on a scale engraved in the instrument.

3.4.2 Specific gravity

Specific gravity of the essential oil was recorded by using a specific gravity bottle of 10 ml. The bottle was washed with distilled water and finally dried in an oven. The weight of the empty bottle was recorded accurately. The bottle was filled with 10 ml distilled water and weighed again. After removing the water, the bottle was dried. Then 5 ml of the oil was added to the bottle and volume made up to 10 ml by adding distilled water. The difference in weight was noted on that day's temperature.

3.4.3 Gas chromatographic profile

Gas chromatography was carried out to separate the oil mixture in to its component particles by a moving gas phase passing over a stationary sorbent. Oil samples were introduced into the device using a micro syringe with hypodermic needle. The needle was inserted through a self sealing silicon rubber septum and the sample injected smoothly in to a heated metal block at the end of the column. The

detector which is connected at the exit of the separation column sense and measure the amount of the separated components present in the carrier gas stream. The output from the detector was fed to a recorder which makes a pen trace called a chromatogram.

3.5 Statistical analysis

MSTATC package was followed for the statistical analysis. Data relating to different characters were analysed by applying the technique of Analysis of Variance and significance was tested by Duncan's Multiple range Test.

Results

4. RESULTS

4.1 EXPERIMENT 1

Production of quality planting materials in nursery

Data on the effect of various treatments applied in the nursery on the following parameters is presented in Table 4.

4.1.1 Earliness in sprouting

It was observed that sprout emergence was not altered significantly with different treatments. Still a marginal improvement was noticed in T_5, which recorded a mean of 6.30 days for sprout emergence while T_4 recorded 6.60 days and T_1 recorded 7.30 days for sprout emergence. Rest of the treatments (T_2, T_3 and T_6) had taken 7.00days for the emergence of sprouts.

4.1.2 Length of sprout

No significant difference in sprout length was noticed among the treatments. All treatments recorded sprout lengths on par with one another. Among the treatments applied, T_3 recorded the longest sprout with a mean length of 0.45 cm, whereas sprout length was found the lowest with T_1 and T_6 with a mean length of 0.30 cm each.

4.1.3 Earliness in rooting

It was observed that root emergence was also not altered significantly with the application of bioagents. Among the treatments, IBA at the rate of 1000 ppm (T_2) recorded the earliest root emergence in 8.00 days after planting. Treatments T_4 and T_1 recorded 8.30 days and 8.60 days respectively for the emergence of roots.

Table 4. Effect of bioagents on days to sprout, sprout length and days to root in cuttings of patchouli

Treatments	Days to sprout	Length of sprout	Days to root
T1	7.30[a]	.30[a]	8.60[a]
T2	7.00[a]	.35[a]	8.00[a]
T3	7.00[a]	.45[a]	9.00[a]
T4	6.60[a]	.40[a]	8.30[a]
T5	6.30[a]	.40[a]	9.00[a]
T6	7.00[a]	.30[a]	9.00[a]

Table 5 a. Effect of bioagents on number of leaves in cuttings of patchouli

Treatments	Number of leaves per plant		
	15 DAP	30 DAP	45 DAP
T1	3.94[c]	6.22[d]	7.85[e]
T2	4.03[c]	6.44[d]	8.67[e]
T3	4.41[b]	9.87[b]	14.26[c]
T4	4.45[b]	11.83[a]	16.03[b]
T5	4.69[a]	10.60[b]	19.94[a]
T6	4.10[c]	7.96[c]	10.54[d]

Table 5b. Effect of bioagents on the increment of number of leaves in patchouli

Increment in number of leaves			
Treatments	Increment(30 DAP)	Increment(45 DAP)	Average increment
T1	2.28	1.62	1.95
T2	2.44	2.23	2.33
T3	5.46	4.39	4.92
T4	7.38	4.20	5.79
T5	5.91	9.34	7.62
T6	3.86	2.57	3.21

On applying the rest of the treatments (T_3, T_5 and T_6) the cuttings had taken 9.00 days for root emergence.

4.1.4 Number of leaves

Data on the effect of treatments on number of leaves are presented in Table 5a and 5b.

Treatments varied significantly with respect to number of leaves. Among the treatments, T_5 recorded significantly, higher number of leaves (19.94 leaves per plant) over a period of 45 days. The lowest number of leaves was obtained with T_1 (7.85), which was on par with T_2 (8.67).

Increment in number of leaves was found highest in T_5 with an average of 7.62 leaves per plant. The treatment T_5 also recorded the highest leaf increment at 45 DAP (9.34) whereas T_4 recorded the highest leaf increment at 30 DAP (7.38).

4.1.5 Root number

Effect of treatments applied in nursery on root number is studied and presented in Table 6a and 6b. Significant difference in number of roots was observed among the treatments.

The highest number of roots was recorded in T_2 with a mean value of 19.00, 39.48 and 73.20 roots per plant respectively after 15, 30 and 45 days of planting the cutting, which was followed by T_3 and T_4 with a mean root number of 72.05 and 70.43 respectively, 45 days after planting.

The lowest number of roots was obtained in T_6 (42.46) followed by T_1 (47.61) at 45 days after planting. The increment in root number over a period of 45 days was recorded. It was found the highest with T4, which recorded an average

increment of 27.61 roots while T_6 recorded the lowest increment with an average of 17.60 roots. Increment in root number was found the highest with T3 (20.66) at 30 DAP; whereas T4 recorded the highest increment at 45 DAP (35.96).

4.1.6 Root volume

Root volume of experimental plants in nursery at 45 days after planting is presented in Table 7.

Significant treatment difference in root volume was noticed. It was highest with T_5 (316.66g), which was followed by T_4 (308.33g) andT_2 (305.00g). T_6 recorded the lowest root volume (238.33g), which was on par with T_1 (240.00g).

4.1.7 Root length

Table 8a and 8b depict the data on the effect of treatments on the root length of patchouli cuttings in nursery.

All the treatments showed significant variation with regard to root length. Among the treatments, T_5 recorded the highest root length with a mean length of 6.95 cm, which was found on par with the root lengths for T_4 (6.93 cm), T_3 (6.53 cm) and and T_2 (6.50) respectively.

Increment in root length was found the highest in T_5 treated plants with an average of 2.58 cm where as T_1 recorded the lowest increment with an average of 1.86 cm. Increment in root length was found the highest with T_3 (1.74 cm) at 30 DAP; whereas T_5 recorded the highest increment at 45 DAP (3.91 cm).

Table 6a. Effect of bioagents on number of roots in cuttings of patchouli

Treatments	Number of roots		
	15 DAP	30 DAP	45 DAP
T1	8.71[b]	18.30[b]	47.61[c]
T2	19.00[a]	39.48[a]	73.20[a]
T3	18.60[a]	39.26[a]	72.05[ab]
T4	15.20[a]	34.26[a]	70.43[ab]
T5	15.33[a]	35.43[a]	63.46[b]
T6	7.25[b]	21.09[b]	42.46[c]

Table 6b. Effect of bioagents on the increment of number of roots in cuttings of patchouli

Treatments	Increment (30 DAP)	Increment (45 DAP)	Average increment
T1	12.38	26.51	19.45
T2	20.48	32.79	26.52
T3	20.66	33.71	27.30
T4	19.26	35.96	27.61
T5	20.10	28.03	24.06
T6	11.04	24.16	17.60

Table 7. Effect of bioagents on root volume in cuttings of patchouli

Treatments	Root volume (ml)
T1	240.00[c]
T2	305.00[ab]
T3	298.33[b]
T4	308.33[ab]
T5	316.66[a]
T6	238.33[c]

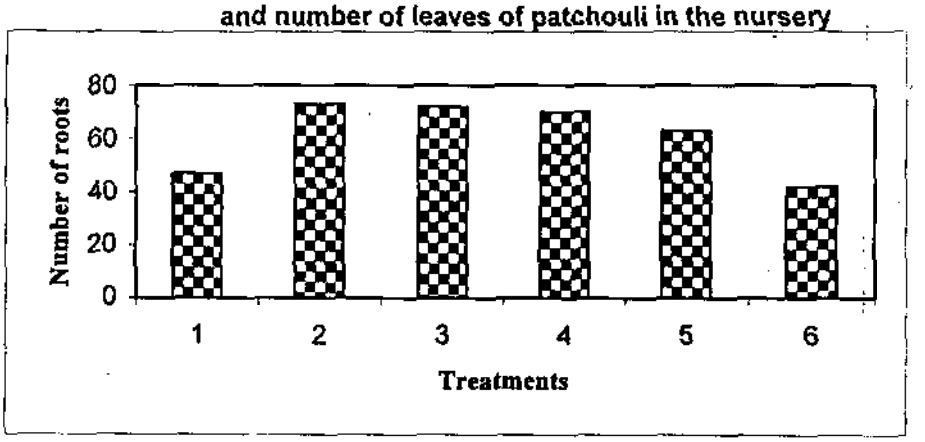
Table 8 a. Effect of bioagents on the root length in cuttings of patchouli

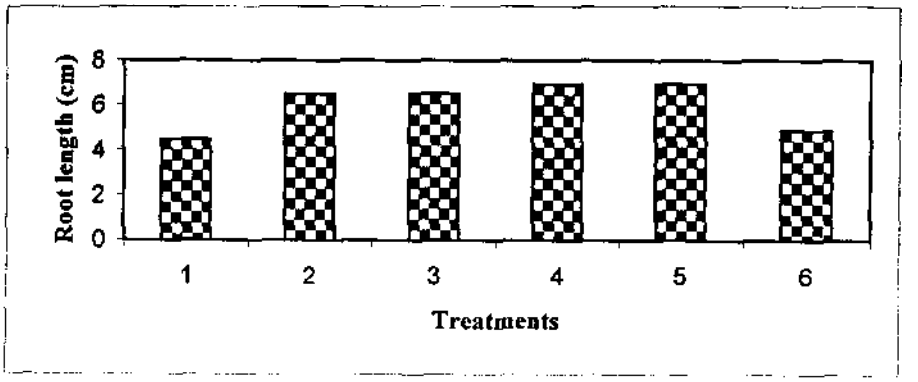
Treatments	Root length (cm)		
	15 DAP	30 DAP	45 DAP
T1	0.76[b]	1.07[c]	4.48[b]
T2	2.05[a]	3.59[a]	6.50[a]
T3	1.84[a]	3.58[a]	6.53[a]
T4	2.07[a]	3.75[a]	6.93[a]
T5	1.79[a]	3.04[b]	6.95[a]
T6	0.93[b]	1.49[c]	4.90[b]

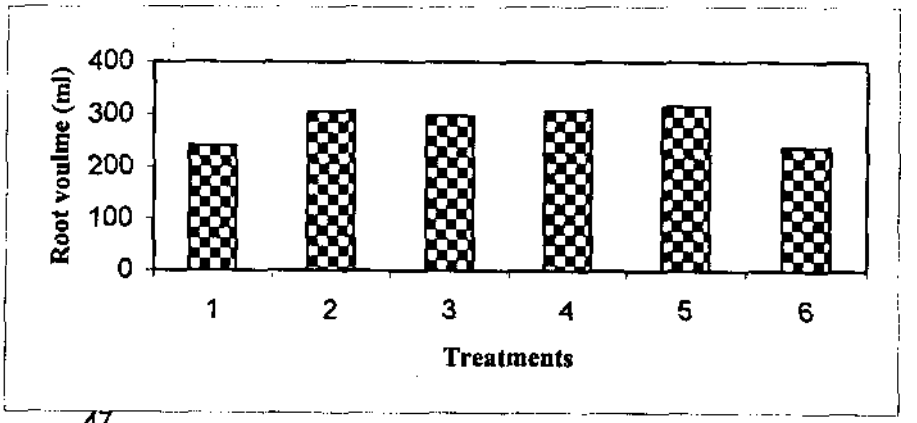
Table 8 b. Effect of bioagents on the increment of root length in cuttings of patchouli

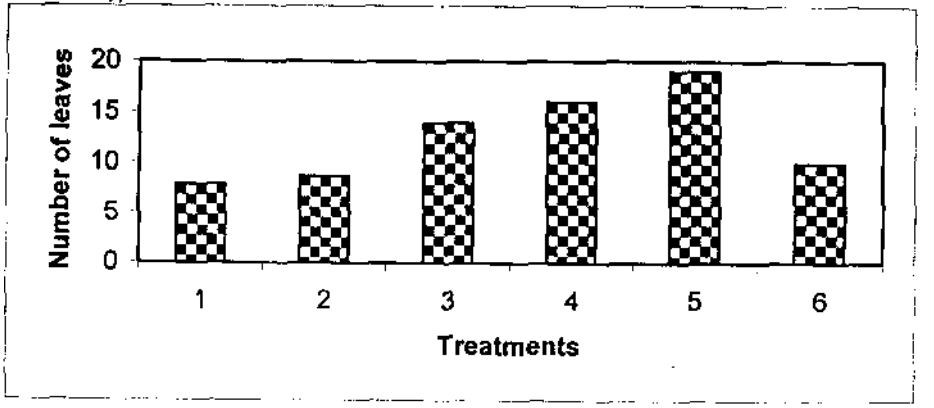
Increment in root length (cm)			
Treatments	Increment at 30 DAP	Increment at 45 DAP	Average increment
T1	0.30	3.41	1.86
T2	1.53	2.91	2.25
T3	1.74	2.94	2.34
T4	1.67	3.18	2.43
T5	1.24	3.91	2.58
T6	0.56	3.40	1.98

Fig.1. Effect of bioagents on number of roots,root length,root voulme and number of leaves of patchouli in the nursery









4.2 EXPERIMENT 2

In the nursery, cuttings applied with the combination treatment of *Trichoderma* + *Pseudomonas fluorescens* were rated the best with respect to majority of the characters like earliness in sprouting, number of leaves produced, root length and root volume.

Results of the different treatments imposed in the main field on various characters studied are as follows:

4.2.1 Height of plant

Data pertaining to the height of plant after transplanting the rooted cuttings in the main field are presented in Tables 9a and 9b.

After transplanting in the main field significant difference in height of plant was observed among different treatments. Treatment T_{16} registered significantly highest height of plant with a mean height of 65.68 cm, 92.60 cm and 121.16 cm respectively, at 2, 4 and 6 months after transplanting.

Average increment in height of plant was also recorded, which was found the highest with T_{16} (27.74 cm), which also registered the highest height increment at 4 MAP (26.92). Increment in height of plant at six months was found the highest with T_{15} (29. 82 cm).

Plots treated with T_9 yielded shorter plants with a height of 64.16 cm at 6 MAP while the treatment T_5 recorded shorter plants at 2 MAP (36.31 cm) and 4 MAP (50.57 cm) which were on par with control which registered heights of 37.46 cm, at 2 MAP, 51.05 cm at 4 MAP and (69.64 cm) at 6 MAP.

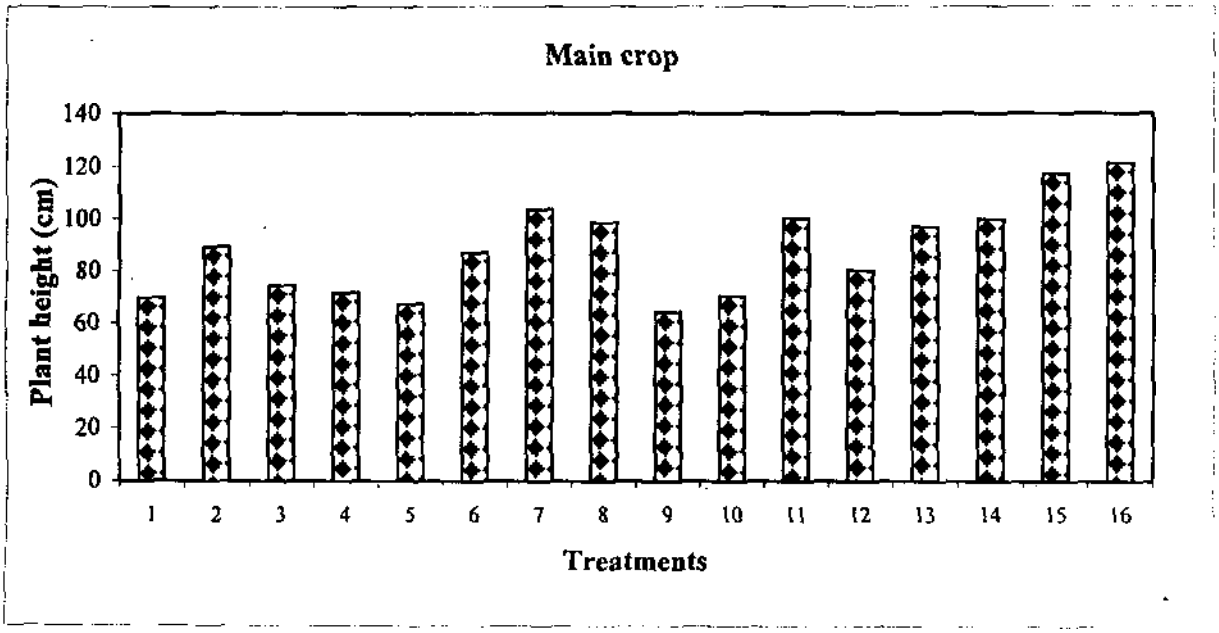
Table 9a. Effect of bioagents and soil amendments on plant height of patchouli

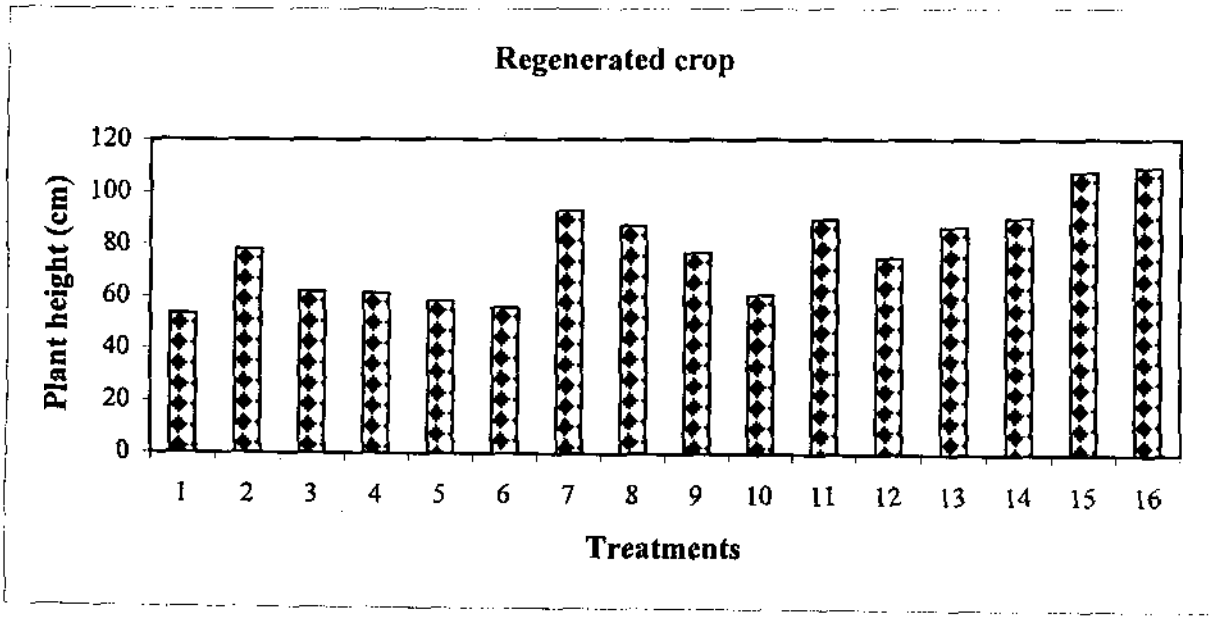
Treatments	Height of plant (cm)			
	Main crop			Regenerated crop
	2 MAP	4 MAP	6 MAP	
T1	37.46^{f}	51.05^{e}	69.64^{e}	53.11^{c}
T2	48.45^{cdef}	64.96^{cde}	89.21^{bcde}	78.09^{bc}
T3	49.10^{bcdef}	66.11^{cde}	74.37^{cde}	61.69^{c}
T4	48.33^{cdef}	64.47^{cde}	71.70^{de}	61.20^{c}
T5	36.31^{f}	50.51^{e}	67.31^{e}	58.17^{c}
T6	41.63^{ef}	58.26^{de}	87.07^{bcde}	55.77^{c}
T7	57.06^{abc}	74.68^{bc}	103.54^{ab}	92.84^{ab}
T8	55.20^{bcde}	74.29^{bc}	98.51^{abc}	87.14^{ab}
T9	49.38^{bcdef}	55.66^{de}	64.16^{e}	76.81^{bc}
T10	42.42^{def}	58.15^{de}	70.29^{de}	60.67^{c}
T11	56.86^{abc}	74.72^{bc}	100.07^{abc}	89.65^{ab}
T12	51.01^{bcde}	67.06^{cd}	80.03^{bcde}	75.03^{bc}
T13	55.51^{abcd}	74.11^{bc}	96.75^{abcd}	86.58^{ab}
T14	54.22^{abcde}	74.99^{bc}	99.73^{abc}	90.04^{ab}
T15	62.45^{ab}	87.31^{ab}	117.13^{a}	107.66^{a}
T16	65.68^{a}	92.60^{a}	121.16^{a}	109.42^{a}

Table 9 b. Effect of bioagents and soil amendments on the increment of plant height of patchouli

Treatments	Increment in height of plant (cm)		
	Increment (4MAP)	Increment (6MAP)	Average increment
T1	13.59	18.59	16.02
T2	16.51	24.25	20.38
T3	17.01	8.26	12.63
T4	16.14	7.22	11.68
T5	13.90	16.80	15.35
T6	16.63	28.80	22.72
T7	17.62	28.86	23.24
T8	19.09	24.21	21.65
T9	6.28	8.50	7.39
T10	15.73	12.14	13.93
T11	17.85	25.35	21.60
T12	16.05	12.97	14.51
T13	18.60	22.74	20.62
T14	20.76	24.74	22.75
T15	24.86	29.82	27.34
T16	26.92	28.56	27.74

Fig. 2 Effect of bioagents and soil amendments on height of plant of patchouli in the main field





Regenerated crop

In the regenerated crop no significant difference in height of plant was recorded among the different treatments. Here also T_{16} recorded the highest plant height with 109.42 cm, which was on par with T_{15} (107.66 cm). In the regenerated phase of growth control plots yielded the shortest plants with a mean height of 53.11 cm. Treatments T_6, T_5, T_{10}, T_4 and T_3 also recorded height of plant on par with that of T_1.

4.2.2 Number of leaves

Data pertaining to the number of leaves per plant in the main field are presented in Tables 10 a and 10b.

Significant difference was observed among treatments with respect to number of leaves per plant. The highest number of leaves at 6 MAP was recorded from T_{16}. (331.87 leaves per plant) which was on par with T_{15} (324.77). This was closely followed by T_{11} with 303.02 leaves per plant.

Leaf production was found the lowest in T_5 plants (205.03), which was on par with T_1 (205.75), at 6 MAP. Average increment in number of leaves was found highest with T_{13} with 132.51 leaves which also registered highest increment of 200.97 leaves over a period of six months whereas T_{14} registered the highest increment of leaves at 4 MAP (81.87).

Regenerated crop

In the regenerated phase, number of leaves did not register any significant difference. In regenerated plants, the greatest number of leaves was recorded from T_{16} (294.35). The treatments T_{15} (288.55) and T_{11} (269.70) were also recorded leaf

number on par with T_{16}. Among the experimental bioagents and soil amendments, T_5 treated plots yielded lesser leaf number (167.81).

4.2.3 Number of branches

Significant difference in number of branches was observed in plants transplanted to the main field. Table11 a and 11 b present the data on number of branches per plant.

Plots treated with T_{14} yielded plants with the highest number of branches (28.65 branches per plant), which was closely followed by T_{16} (27.45). Average increment in number of branches was found the highest with T_{16} (8.54) over a period of four months which also registered the highest increment in number of branches at 6 MAP (12.78) whereas T_8 recorded better increment at 4 MAP (8.57) over a period of two months.

Control plants recorded lesser number of branches with an average of 15.03 branches per plant at the end of 6 months. The lowest increment in number of branches was also observed in control plants which registered an average increment of 4.48.

Regenerated crop

In regenerated plants all the treatments produced number of branches on par with one another and no significant difference was observed. Among the different treatments applied T_{14} recorded more number of branches (30.75), which was followed followed by T_{16} (29.48). In regenerated crop also control plants recorded lesser number of branches, with a mean number of 16.35 branches per plant.

Table 10 a. Effect of bioagents and soil amendments on number of leaves of patchouli

Treatments	Mean number of leaves			
	Main crop			Regenerated crop
	2 MAP	4 MAP	6 MAP	
T1	29.61^{fg}	71.63^{fg}	205.75^{e}	174.11^{bc}
T2	48.98^{def}	114.16^{cdef}	267.82^{bcd}	231.90^{abc}
T3	46.89^{defg}	119.30^{bcde}	267.63^{bcd}	232.38^{abc}
T4	45.31^{defg}	106.71^{cdefg}	249.64^{cde}	213.67^{abc}
T5	38.39^{ffg}	68.61^{g}	205.03^{e}	167.81^{c}
T6	50.56^{cde}	120.46^{bcde}	238.59^{de}	217.81^{abc}
T7	62.14^{bcd}	141.56^{abc}	292.11^{abc}	254.31^{abc}
T8	65.02^{bcd}	145.61^{abc}	296.00^{abc}	256.47^{ab}
T9	39.49^{ffg}	96.78^{efg}	238.59^{de}	237.50^{abc}
T10	55.55^{cde}	136.39^{abcd}	229.31^{de}	228.05^{abc}
T11	80.33^{ab}	161.09^{ab}	303.02^{ab}	269.70^{a}
T12	46.06^{defg}	114.66^{cdef}	256.11^{cd}	218.37^{abc}
T13	27.44^{g}	91.70^{ffg}	292.68^{abc}	257.16^{ab}
T14	54.71^{cde}	136.58^{abcd}	287.74^{abc}	251.39^{abc}
T15	68.25^{bc}	142.66^{abc}	324.77^{a}	288.55^{a}
T16	88.90^{a}	170.40^{a}	331.87^{a}	294.35^{a}

Table 10 b. Effect of bioagents and soil amendments on the increment of number of leaves of patchouli

Treatments	Increment in mean number of leaves		
	Increment at 4 MAP	Increment at 6 MAP	Average increment
T1	42.02	134.07	88.04
T2	65.18	153.66	109.42
T3	72.41	148.33	110.37
T4	61.40	142.93	102.16
T5	30.22	136.42	83.32
T6	69.89	118.13	94.01
T7	79.42	150.54	114.98
T8	80.59	150.38	115.49
T9	57.29	141.81	99.55
T10	80.81	92.92	86.86
T11	80.75	141.95	111.34
T12	68.60	141.45	105.02
T13	64.26	200.97	132.51
T14	81.87	151.15	116.51
T15	74.41	182.10	128.25
T16	81.50	161.47	121.48

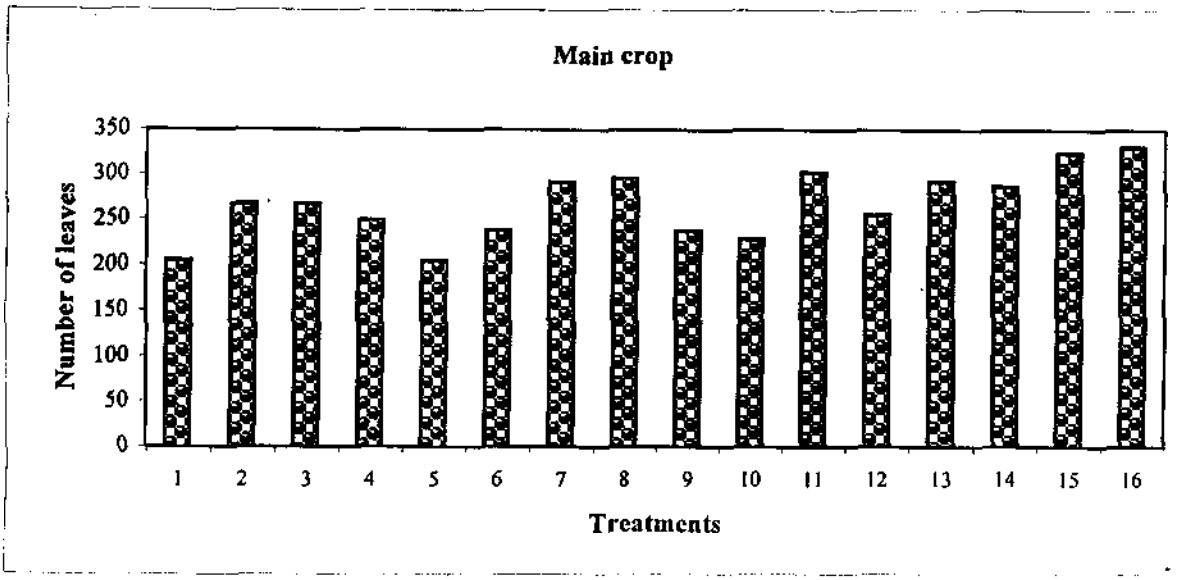
Table 11a. Effect of bioagents and soil amendments on number of branches of patchouli

Treatments	Mean number of branches			
	Main crop			Regenerated crop
	2 MAP	4 MAP	6 MAP	
T1	7.05[b]	13.80[a]	15.03[f]	16.35[d]
T2	7.81[b]	15.60[a]	18.56[cdef]	20.26[cd]
T3	7.51[b]	15.38[a]	19.00[cdef]	20.26[cd]
T4	7.32[b]	14.26[a]	17.36[cdef]	17.88[cd]
T5	7.66[b]	13.91[a]	15.07[ef]	17.00[cd]
T6	7.49[b]	14.99[a]	18.39[cdef]	19.55[cd]
T7	7.95[b]	16.06[a]	22.14[bc]	23.08[bcd]
T8	8.19[b]	16.77[a]	22.05[bcd]	21.05[cd]
T9	7.85[b]	15.47[a]	18.85[cdef]	20.28[cd]
T10	7.57[b]	14.95[a]	15.75[def]	17.35[cd]
T11	7.91[b]	15.95[a]	21.95[bcd]	23.40[bc]
T12	7.30[b]	14.92[a]	17.31[cdef]	18.02[cd]
T13	7.96[b]	16.06[a]	22.49[bc]	23.77[bc]
T14	11.55[a]	19.87[a]	28.05[a]	30.75[a]
T15	7.68[b]	15.51[a]	20.74[cde]	22.09[cd]
T16	11.35[a]	19.09[f]	27.45[ab]	29.48[ab]

Table 11 b. Effect of bioagents and soil amendments on the increment of number of branches of patchouli

Increment in mean number of branches			
Treatments	Increment at 4 MAP	Increment at 6MAP	Average increment
T1	6.78	1.23	4.48
T2	7.79	3.54	5.66
T3	7.86	3.62	5.74
T4	6.94	3.10	5.02
T5	6.25	1.15	3.70
T6	7.49	3.40	5.45
T7	8.11	6.07	7.09
T8	8.57	5.28	6.93
T9	7.62	3.37	5.50
T10	7.37	0.80	4.00
T11	8.04	6.00	7.02
T12	7.62	2.39	5.00
T13	8.38	6.15	7.26
T14	7.74	12.35	8. 04
T15	7.83	5.23	6.53
T16	8.31	12.78	8.54

Fig. 3 Effect of bioagents and soil amendments on number of leaves of patchouli in the main field



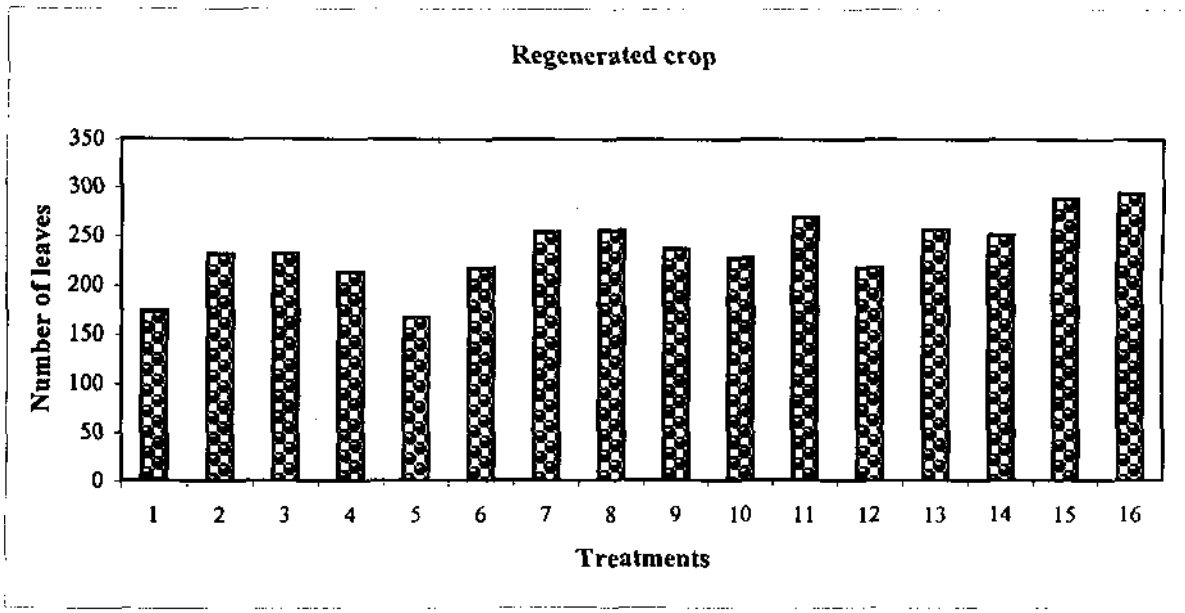
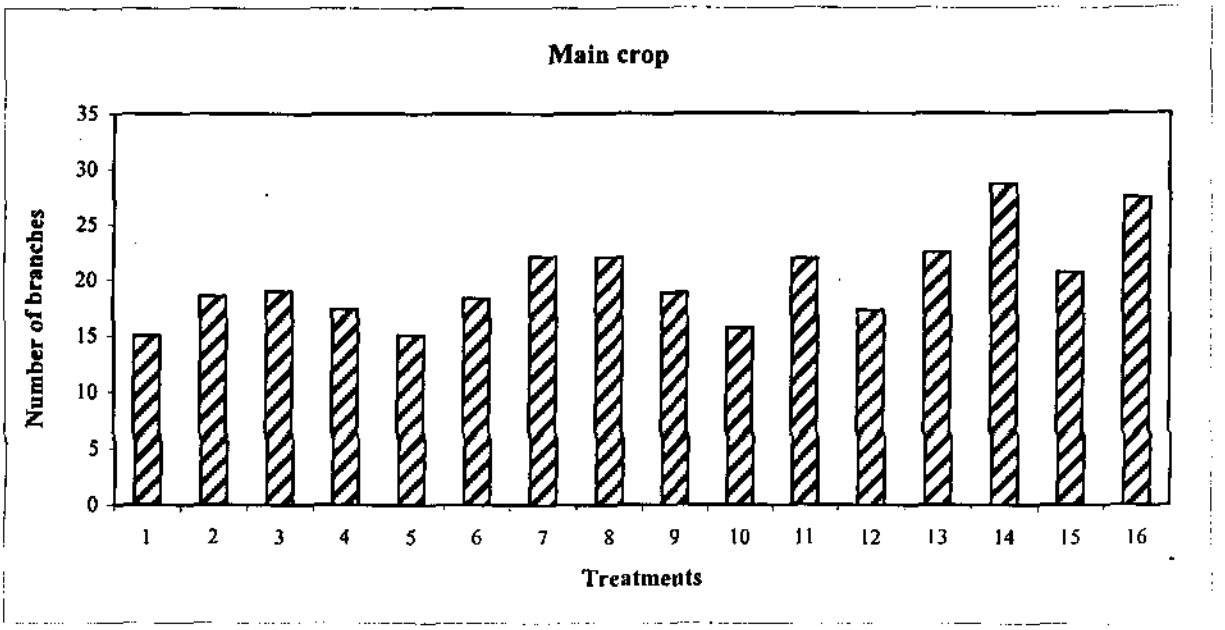
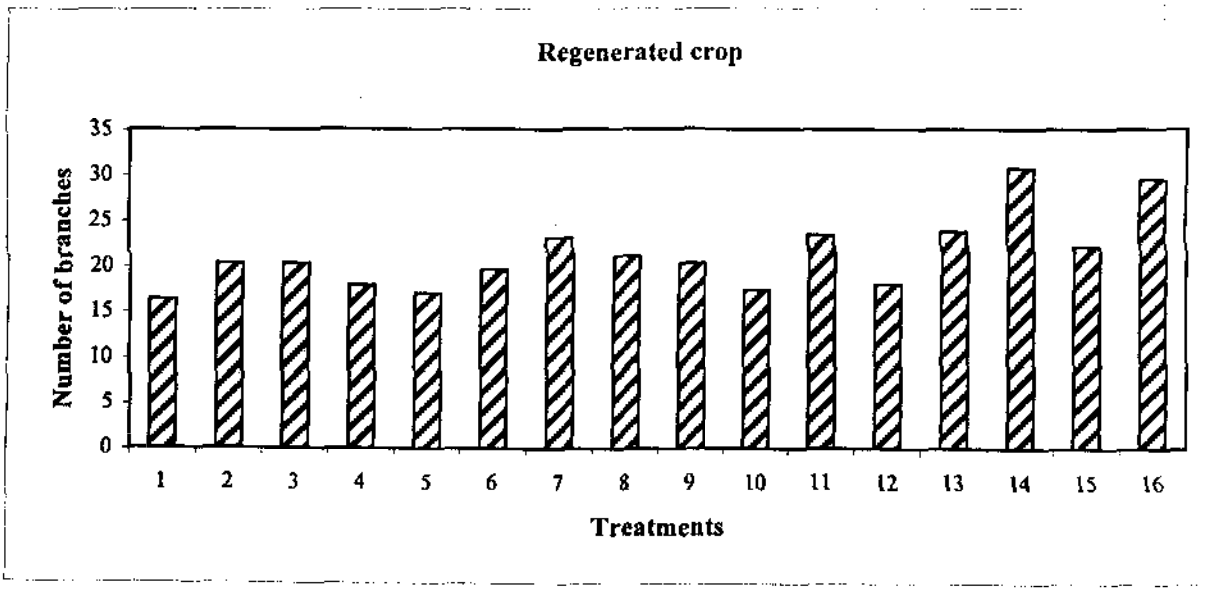


Fig. 4 Effect of bioagents and soil amendments on mean number of branches of patchouli in the main field





4.2.4 Plant spread

Significant difference in plant spread was recorded among the various treatments.

Plant spread was the highest with T_{14} in both N-S (78.21 cm) and E-W (76.81 cm) directions which was closely followed by T_8, T_{15} and T_{11}. An increment in plant spread was also recorded from fourth month to sixth month after planting .T14 treated plots recorded the highest increment of 16.40 cm in N-S direction and 13.54 cm in E-W direction whereas T_3 recorded lesser increment both in N-S (4.96cm) and E-W (6.07 cm) directions. Plant spread was recorded the lowest from control plants, which registered an average spread of (61.01 cm) in N-S direction and (62.37 cm) in E-W direction

4.2.5 Leaf area

Significant difference in leaf area was observed among different treatments. Mean value for the leaf area of experimental plants were studied and presented in Table 13.

Leaf area was found the highest with T_8 (32.81cm^2), which was superior to all other treatments. This was followed by treatments T_{16} (32.10cm^2) and T_{15} (31.76cm^2).

The lowest leaf area was observed in plants treated with T_7 (22.49cm^2), which was closely followed by T_5 with an average leaf area of 22.85 cm^2. Control plants registered an average leaf area of 28.25 cm^2.

Table 12 a. Effect of bioagents and soil amendments on plant spread of patchouli

Mean plant spread (cm)				
Treatments	4 MAP		6 MAP	
	N-S	E-W	N-S	E-W
T1	50.12ef	50.32^{def}	61.01^{def}	62.37^{cde}
T2	57.71^{de}	59.71^{bc}	70.17^{abc}	72.11^{ab}
T3	56.51^{de}	50.50^{de}	61.47^{de}	66.57^{de}
T4	52.11^{e}	51.11^{cde}	64.65^{cde}	63.35^{cd}
T5	55.17^{de}	54.79^{cd}	65.51^{bc}	64.37^{cd}
T6	52.28^{e}	54.66^{cd}	65.01^{bcd}	67.72^{c}
T7	61.71^{bc}	63.50^{ab}	72.48^{ab}	73.81^{ab}
T8	65.31^{a}	62.11^{ab}	76.21^{a}	74.91^{a}
T9	60.21^{bc}	58.20^{bc}	77.21^{a}	75.51^{a}
T10	52.52^{d}	54.21^{cd}	65.19^{bcd}	63.29^{cd}
T11	61.41^{bc}	62.40^{bc}	75.21^{a}	73.10^{ab}
T12	58.49^{bcd}	57.48^{bc}	66.98^{bc}	62.72^{cd}
T13	60.31^{abc}	62.86^{bc}	73.17^{a}	75.81^{a}
T14	61.81^{abc}	63.27^{ab}	78.21^{a}	76.81^{a}
T15	62.21^{ab}	60.81^{abc}	74.21^{a}	71.81^{ab}
T16	64.21^{a}	64.21^{ab}	72.11^{ab}	71.20^{ab}

Table 12 b. Effect of bioagents and soil amendments on the increment of plant spread of patchouli

Increment in mean plant spread (cm) at 6 MAP		
Treatments	N-S	E-W
T1	10.88	12.04
T2	12.46	12.4
T3	4.96	6.07
T4	12.53	12.24
T5	10.34	9.57
T6	12.73	13.05
T7	10.77	10.31
T8	10.90	12.80
T9	17.00	17.31
T10	12.67	9.21
T11	13.79	10.69
T12	8.49	5.241
T13	12.86	12.95
T14	16.4	13.54
T15	11.99	11.00
T16	7.94	6.99

Table 13. Effect of bioagents and soil amendments on leaf area of patchouli

Treatments	Leaf area (cm^2)
T1	28.25^{bcd}
T2	31.20^{abc}
T3	31.51^{abc}
T4	28.67^{bcf}
T5	22.85^{f}
T6	28.84^{de}
T7	22.49^{f}
T8	32.81^{a}
T9	26.63^{de}
T10	23.88^{ef}
T11	27.51^{cde}
T12	31.51^{abc}
T13	27.67^{cde}
T14	27.00^{de}
T15	31.76^{ab}
T16	32.10^{ab}

Fig. 5 Effect of bioagents and soil amendments on spread of plant of patchouli in the main field

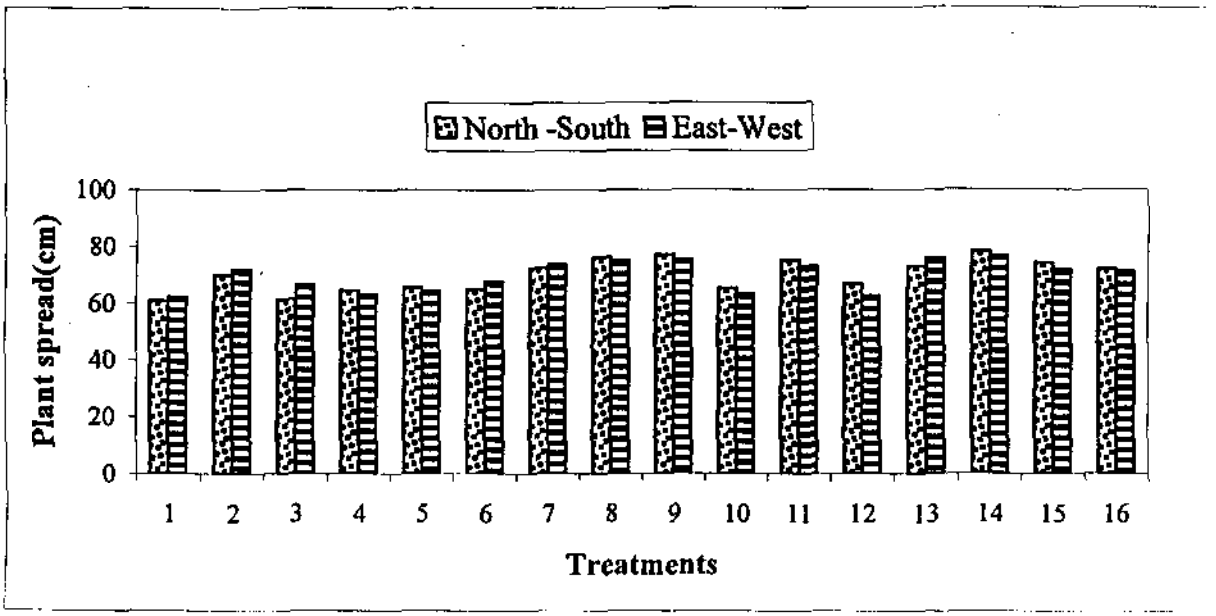
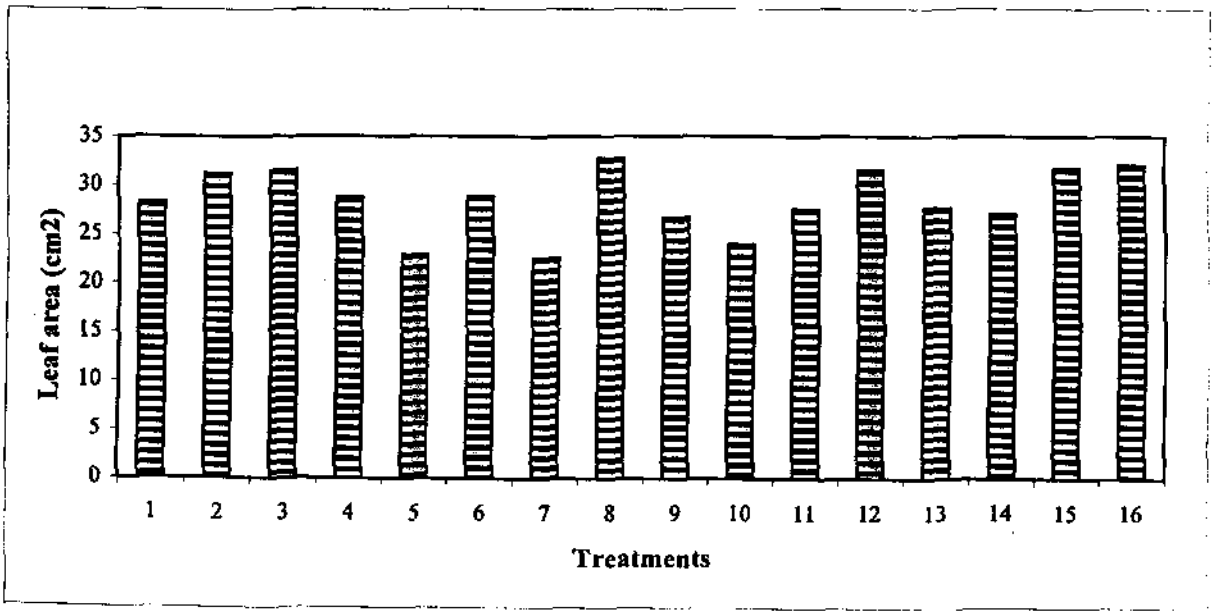


Fig. 6 Effect of bioagents and soil amendments on leaf area of patchouli in the main field



4.2.6 Fresh herb yield per hectare

Treatments applied in the main field crop showed significant differences among one another with regard to average fresh herb yield, which is presented in Table 14.

Fresh herb yield per hectare was found the highest in T_{16} treated plots (6247.50 kg per hectare), which was found superior to all other treatments. Treatments T_{14}(6022.50 kg per hectare), T_8 (5995.00 kg per hectare, and T_{15} (5970.00 kg per hectare) also recorded higher values of herb yield, which were found on par with one another.

The lowest fresh herb yield was obtained in control plots, which yielded on an average herb yield of 4102.50 kg per hectare. This was closely followed by T_9 (4270.00 kg per hectare), T_5 (4282.50 kg per hectare and T_{13} (4352.50 kg per hectare) and T_3 (4390.00 kg per hectare).

Regenerated crop

In regenerated crop, no significant difference in fresh herb yield was observed. All the treatments yielded fresh herb on par with one another. Among the treatments applied T_{14} registered the highest fresh herb yield (2480.00 kg per hectare) per plot where as T_9 recorded the lowest fresh herb yield of 1550.00 kg per hectare.

4.2.7 Fresh leaf yield per hectare

The data on the effect of treatments on average fresh leaf yield is presented in Table15.

Significant difference in fresh leaf yield was recorded among treatments applied in the crop in main field. Treatment T_{16} recorded the highest fresh leaf yield (4407.50 kg per hectare), which was followed by T_8 (4200.00 kg per hectare). Control plots yielded lower fresh leaf with an average yield of 2520.00 kg per hectare, which was closely followed by T_5 (2695.00 kg per hectare), T_9 (2707.50 kg per hectare), T_3 (2807.50 kg per hectare) and T_{13} (2822.50 kg per hectare).

Regenerated crop

In regenerated crop the treatments exhibited no significant difference with respect to fresh leaf yield. Among the treatments T_{16} and T_{14} recorded values significantly superior to the rest of the treatments.The treatment T_{16} recorded the highest fresh leaf production with a yield of 1772.50 kg per hectare, which was on par with T_{14} (1742.50 kg per hectare).

Fresh leaf yield was recorded the lowest from T_9 treated plots, which yielded on an average 942.50 kg fresh leaf per hectare. This was on par with the fresh leaf yield obtained from T_{13} treated plots (955.50 kg per hectare). Control plots yielded 1002.50 kg per hectare of fresh leaf.

4.2.8 Dry leaf yield per hectare

Significant treatment difference in dry leaf yield was observed both in main crop and regenerated crop. Among the various bio agents and soil amendments applied T_8 recorded the greatest dry leaf yield (940.43 kg per hectare). Treatments T_{16}, T_{14}, T_{15}, T_6, T_{12} and T_4 also recorded dry leaf yield on par with that of T_8. Dry leaf yield was the lowest in plots treated with T_5 (562.28 kg per hectare), which was on par with T_1 (607.75 kg per hectare) and T_9 (617.93 kg per hectare).

Regenerated crop

In regenerated plants T_{14} treated plots yielded the highest dry leaf (555.00 kg per hectare), which was on par with the dry leaf yield obtained from T_{16} treated plots (552.05 kg per hectare). The lowest dry leaf yield was obtained from T_9 (282.50 kg per hectare) and control plots recorded an average dry leaf yield of 312.50 kg per hectare.

4.2.9 Oil content

Data on the effect of treatments on oil content is presented in Table 17.

There was significant difference among the treatments with regard to oil content. Among the treatments T_8 recorded the highest oil content (4.10 per cent) indried leaves, which was followed by T_4 (3.90 per cent). T_{10} recorded the lowest oil content of 2.15 per cent) which was on par with T_7, $T_{11}$and T_1. Rest of the treatments (T_2, T_3, T_5, T_6, T_9, T_{12}, T_{13}, T_{14}, T_{15} and T_{16}) registered oil contents between 3 and 4 per cent.

4.2.10 Oil yield per hectare

Among the treatments T_8 recorded the highest oil yield per hectare with a mean oil yield of 38.55 kg, which was followed by T_4 (33.35 kg per hectare). The lowest per plot oil yield was recorded from T_7 with a mean oil yield of 16.10 kg per hectare. (Table 17)

Table 14. Effect of bioagents and soil amendments on fresh herb yield of patchouli

Fresh herb yield (kg ha^{-1})		
Treatments	Main crop	Regenerated crop
T1	4102.50^{f}	1622.50^{a}
T2	4655.00^{cdef}	1890.00^{a}
T3	4390.00^{ef}	1730.00^{a}
T4	5472.50^{abcd}	2095.00^{a}
T5	4282.50^{ef}	1557.50^{a}
T6	5570.00^{abc}	2110.00^{a}
T7	4540.00^{def}	1772.50^{a}
T8	5995.00^{ab}	2115.00^{a}
T9	4270.00^{ef}	1550.00^{a}
T10	5145.00^{bcde}	1925.00^{a}
T11	5472.50^{abcd}	2022.50^{a}
T12	5497.50^{abc}	2045.00^{a}
T13	4352.00^{ef}	1630.00^{a}
T14	6022.50^{ab}	2480.00^{a}
T15	5970.00^{ab}	2230.00^{a}
T16	6247.50^{a}	2275.00^{a}

Table 15. Effect of bioagents and soil amendments on fresh leaf yield of patchouli

Fresh leaf yield (kg ha^{-1})		
Treatments	Main crop	Regenerated crop
T1	2520.00^{e}	1002.50^{bc}
T2	3067.50^{de}	1272.50^{abc}
T3	2807.50^{e}	1065.00^{bc}
T4	3772.20^{abc}	1307.50^{abc}
T5	2695.00^{e}	1012.50^{bc}
T6	3820.00^{abc}	1400.00^{abc}
T7	2952.00^{de}	1150.00^{bc}
T8	4200.00^{ab}	1520.00^{ab}
T9	2707.50^{e}	942.50^{c}
T10	3532.50^{cd}	1372.50^{abc}
T11	3742.50^{bc}	1432.50^{abc}
T12	3787.50^{abc}	1417.50^{abc}
T13	2822.50^{e}	955.50^{c}
T14	4185.00^{abc}	1742.50^{a}
T15	4120.00^{abc}	1182.50^{bc}
T16	4407.00^{a}	1772.50^{a}

Fig. 7 Effect of bioagents and soil amendments on fresh herb yield of patchouli

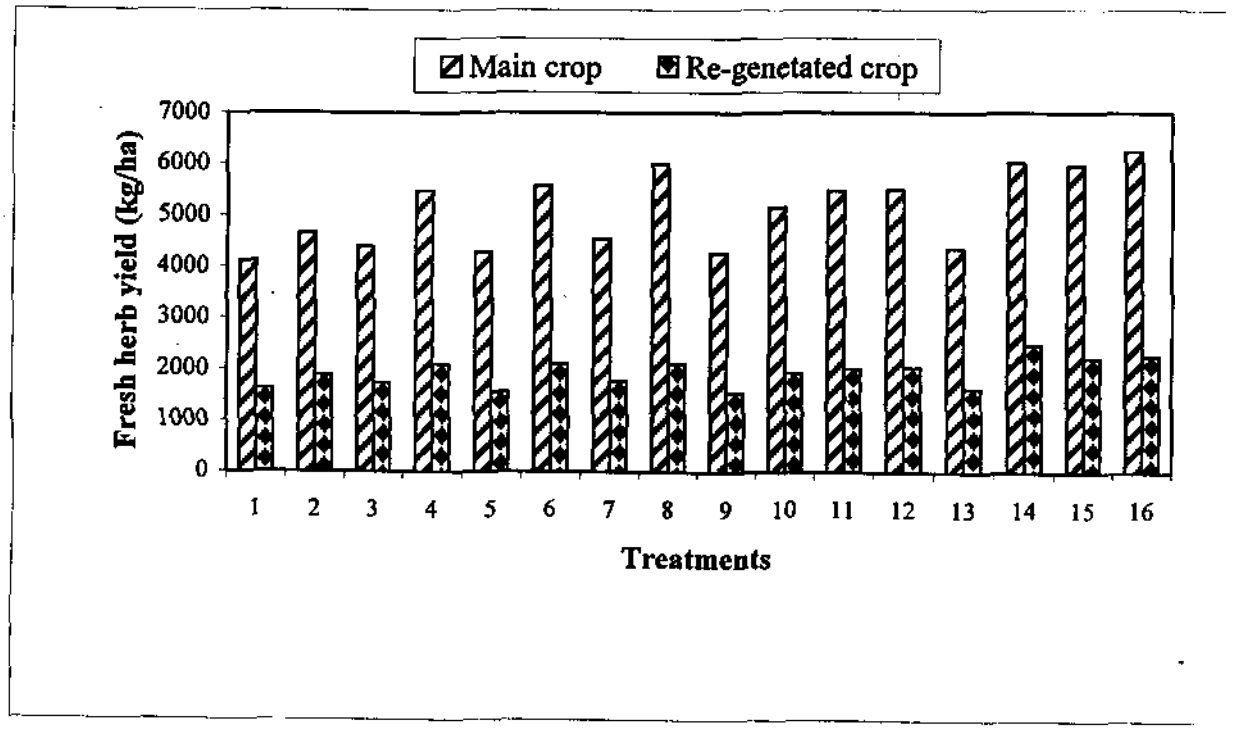
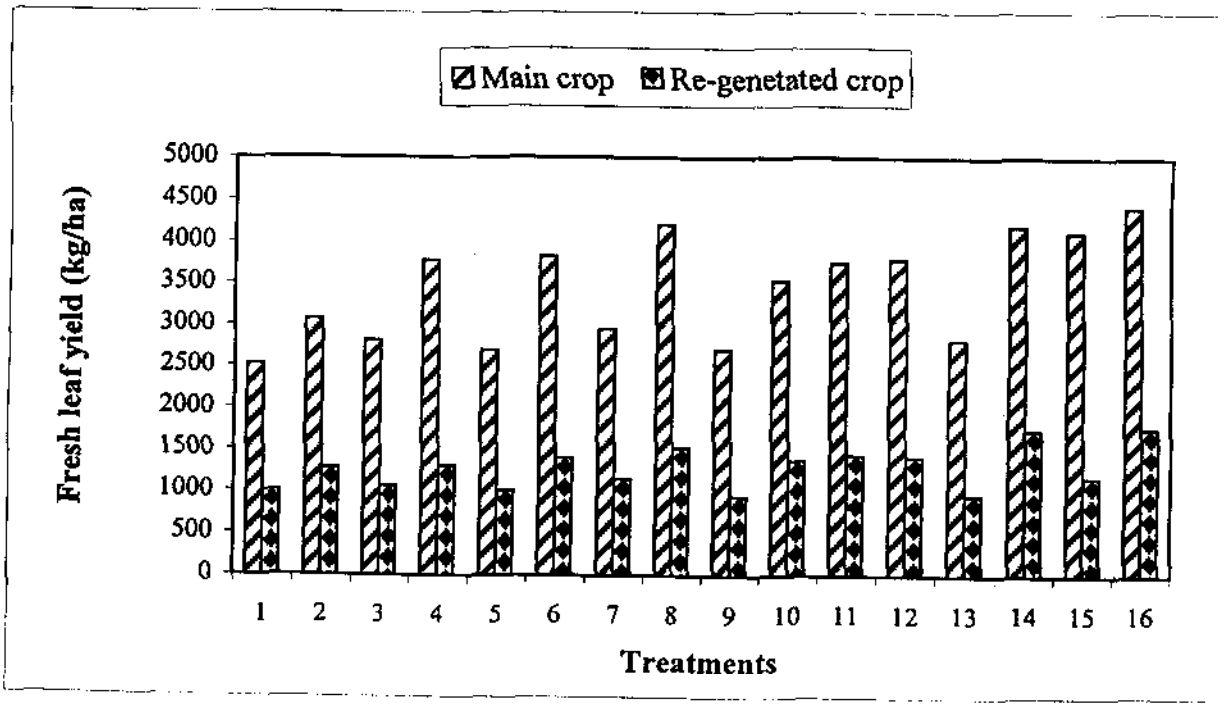


Fig. 8 Effect of bioagents and soil amendments on fresh leaf yield of patchouli



4.2.11 Physicochemical properties of patchouli oil

4.2.11.1 Specific gravity and refractive index

In the present study specific gravity and refractive index of patchouli oil from various treatments were recorded. It was found that specific gravity of patchouli oil range between 0.940 and 0.953 and refractive index between 1.5032 and 1.5150 at 31^{0c}

4.2.11.2 Gas chromatographic analysis

The results of gas chromatographic profile revealed significant variation among the treatments for the number of components present in the oil sample. Number of peaks and per cent of common peaks of gas chromatography profile have been analysed and are summarised in Table 18.

From the table it is observed that when the number of peaks increase the per cent of major components in the oil decrease. Numbers of peaks were recorded the highest from T_5 (control+ neem cake), which recorded total of 10 peaks which gave moderate oil yield of 7.23 g per plot. High number of peaks were also obtained from treatments T_8 (9), T_{16} (8) and T_4 (8). Treatments T_6, T_7 and T_{14} yielded moderately higher peaks in the gas chromatographic profile. Among the treatments neem cake applied plots registered comparatively higher number of peaks representing higher number of oil components. Less number of peaks in the oil was recorded in T_9, T_2, T_{15} and T_{10}.Oil samples from lime induced plots recorded lower number of peaks except T_{16}

4.3 Incidence of pests and diseases

Three insect pests, leaf feeding caterpillar (*Pronomis profusalis* Warren.), leaf roller *(Herpatogramma licarsisalis*) and *Protista moesta* were found to attack patchouli. Spraying 0.2 per cent Roger effectively controlled the above pests. Nematode population in the soil of patchouli field was found insignificant. Incidence of bacterial wilt and nematode attack were not recorded from the main field.

Table 16. Effect of bioagents and soil amendments on dry leaf yield of patchouli

Dry leaf yield (kg ha^{-1})		
Treatments	Main crop	Regenerated crop
T1	607.75^{d}	312.50^{hij}
T2	688.03^{bcd}	370.00^{efgh}
T3	642.50^{cd}	330.00^{ghij}
T4	855.51^{a}	382.50^{defg}
T5	562.28^{d}	315.00^{hij}
T6	870.50^{a}	412.50^{cde}
T7	657.94^{cd}	350.00^{fghi}
T8	940.43^{a}	465.00^{bc}
T9	617.93^{d}	282.50^{j}
T10	802.50^{abc}	402.50^{def}
T11	847.82^{ab}	430.00^{cde}
T12	857.90^{a}	435.00^{bcd}
T13	641.41^{cd}	297.00^{ij}
T14	803.30^{a}	555.00^{a}
T15	902.50^{a}	492.50^{b}
T16	935.00^{a}	552.05^{a}

Table 17. Effect of bioagents and soil amendments on per cent oil content and oil yield of patchouli

Treatments	Oil content (%)	Oil yield (kg ha^{-1})
T1	2.90^{abc}	17.60^{cd}
T2	3.30^{abc}	22.70^{bc}
T3	3.05^{abc}	19.60^{cd}
T4	3.90^{ab}	33.35^{ab}
T5	3.20^{abc}	18.08^{cd}
T6	3.15^{abc}	27.40^{abc}
T7	2.55^{abc}	16.10^{e}
T8	4.10^{a}	38.55^{a}
T9	3.00^{abc}	18.55^{cd}
T10	2.15^{c}	17.25^{de}
T11	2.65^{abc}	22.32^{bcd}
T12	3.30^{abc}	28.30^{abc}
T13	3.20^{abc}	20.47^{bcd}
T14	3.30^{abc}	30.12^{ab}
T15	3.45^{abc}	31.12^{ab}
T16	3.40^{abc}	31.80^{ab}

Table 18. Gas chromatographic profile

Treatments	Number of peaks	Peak area (cm^2)	Oil content (%)	Oil yield ($kg\ ha^{-1}$)
T1	6	87.61	2.90	17.60
T2	3	93.21	3.30	22.70
T3	5	87.99	3.05	19.60
T4	8	84.91	3.90	33.35
T5	10	82.94	3.20	18.08
T6	7	85.19	3.15	27.40
T7	7	84.21	2.55	16.10
T8	9	82.31	4.10	38.55
T9	2	95.81	3.00	18.52
T10	4	93.24	2.15	17.25
T11	6	88.91	2.65	22.35
T12	5	89.91	3.30	28.30
T13	5	88.23	3.20	20.47
T14	7	85.14	3.30	30.12
T15	3	97.62	3.45	31.12
T16	8	83.38	3.40	31.80

Fig. 9 Effect of bioagents and soil amendments on dry leaf yield of patchouli

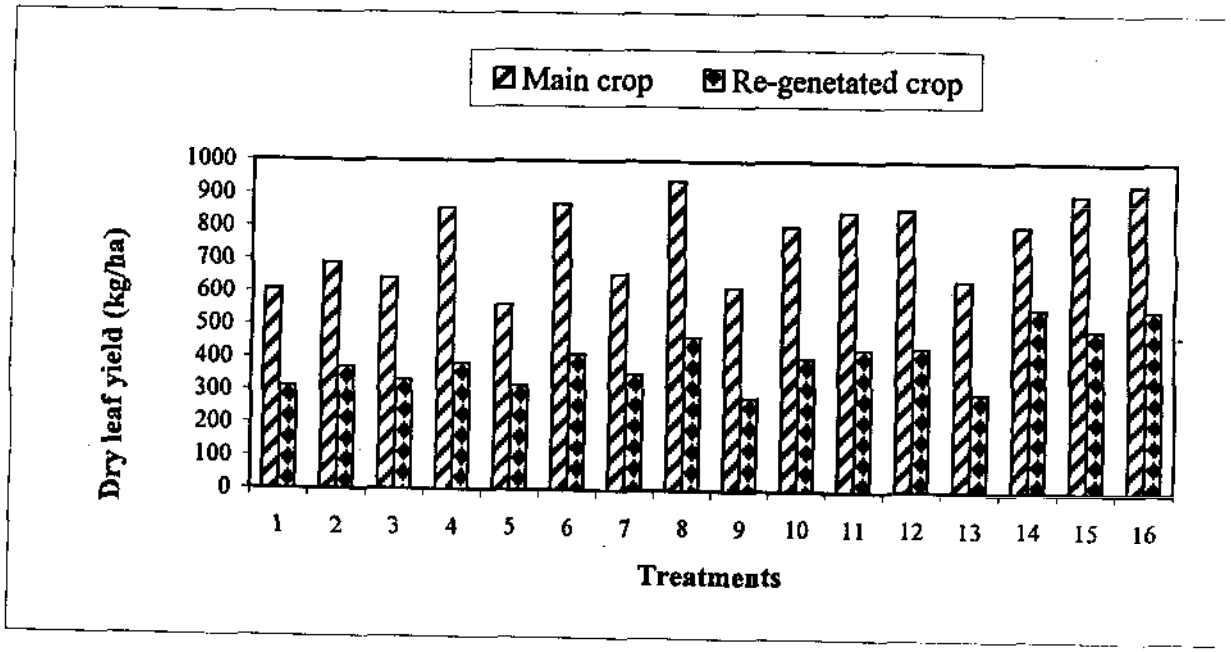


Fig. 10 Effect of bioagents and soil amendments on oil content of patchouli

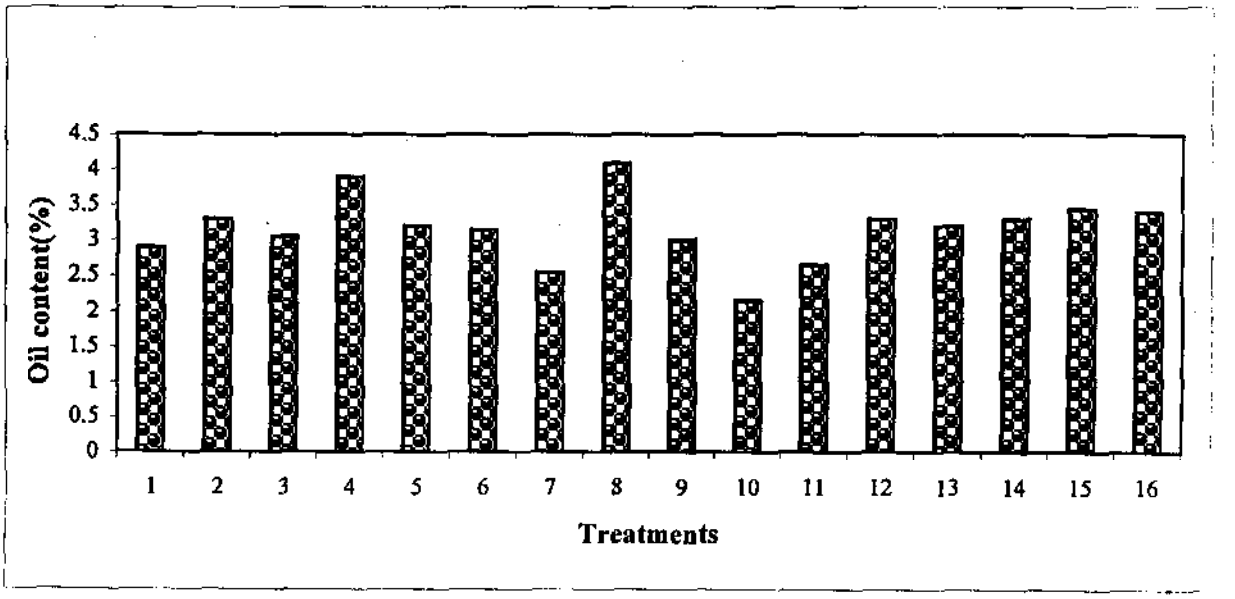
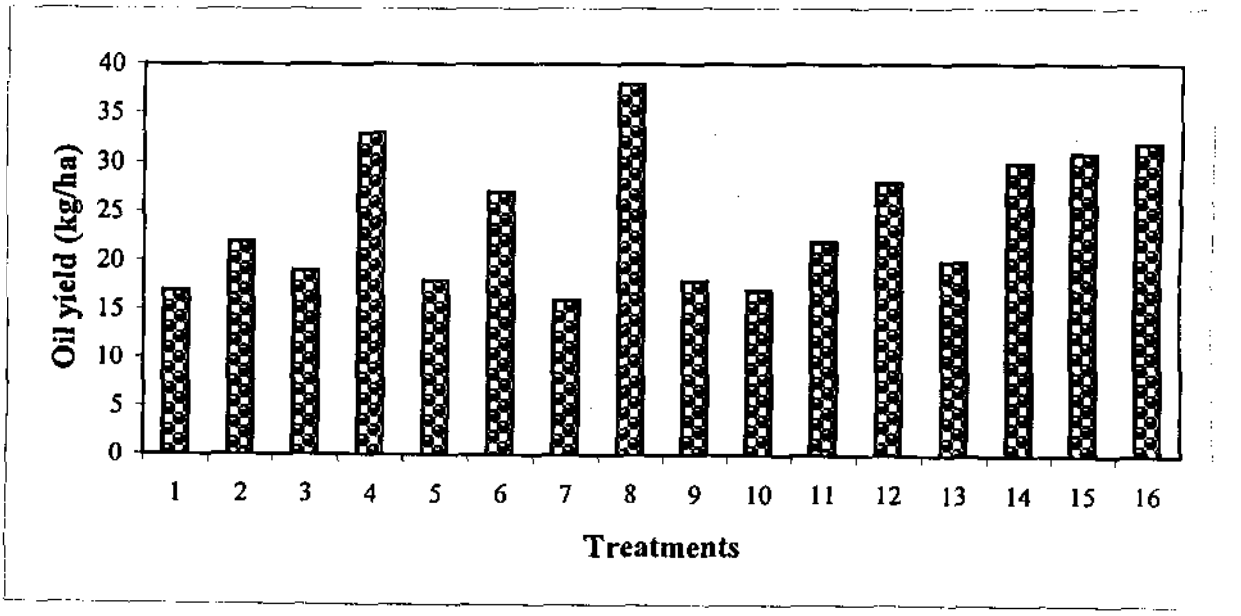


Fig. 11 Effect of bioagents and soil amendments on oil yield of patchouli



Discussion

5. DISCUSSION

The research results obtained in the study on "Impact of bioagents and soil amendments on the performance of patchouli (*Pogostemon patchouli* Pellet.)" conducted during 2002-2004 in the Medicinal and Aromatic Plant garden of the department of Plantation Crops and Spices of College of Horticulture of Kerala Agricultural University, Vellanikkara are discussed in this chapter. The study was conducted to evaluate the effect of different biofertilizers, soil amendments and microbial antagonists on the performance of patchouli with special reference to growth, yield and quality.

5.1 Experiment I

5.1.1 Effect of treatments on production of quality planting materials

In nursery, patchouli responded well to the application of various treatments. In nursery stage, significant difference was observed among the treatments with respect to characters such as number of leaves, root number, root volume and root length.

Combined application of *Trichoderma* and *Pseudomonas fluorescens* in nursery recorded the earliest sprout emergence, highest number of roots, longest roots and highest root volume. Sprout emergence was recorded the earliest with T_5 (6.30 days), which was on par with T_4 (6.60 days). These results are in conformity with the findings of Chinnaswamy (1967) and Fernandes *et al.* (1974). *Trichoderma* can induce growth enhancement and resistance either by direct inhibition of growth of pathogens, or by increasing antagonistic micro organisms and by increasing soil fertility (Osnando and Wando, 1992). *Pseudomonas fluorescens* can induce growth and plant defense mechanisms through the production of siderophores, mineralization of phosphorus and antibiosis (Benhamon *et al.*, 1996). Combined application of

Trichoderma and *Pseudomonas fluorescens* enhanced faster sprout emergence because of the suitable soil rhizosphere and nutrient status which facilitated earlier crop emergence and better growth (Reviresta, 1996)

The highest leaf number (19.94 leaves per plant) in 45 days old patchouli plants was registered in nursery with the incorporation of *Trichoderma* and *Pseudomonas fluorescens*. Similar results were also recorded by Sethuraman and Muthuswamy (1994) in tomato and Hazarika *et al.* (2000) in tea. This is attributed to the improved soil properties and greater nutrient availability to plants. (Zone, 1996). Cellulase production by *Trichoderma* can induce enhanced growth stimulation as reported by Brion *et al.* (1998).

The highest root length and root volume were also observed in plants treated with *Trichoderma* and *Pseudomonas fluorescens*. (6.95 cm and 316.66g). This was followed by the treatment wherein *Pseudomonas fluorescens* was applied which registered longer roots (6.93 cm) and higher root volume (308.33 g). Broadbent *et al.* (1977) found that application of *Pseudomonas fluorescens* significantly influenced the root characters like number and length of primary and secondary roots and the benefits from root bacterization were listed as protection against non parasitic root pathogens, production of biologically active substances like auxins and gibberellins, transformation of unavailable mineral and organic compounds in the available form to plants and nitrogen fixation. Bashan and Levanomy (1990) found additive action hypothesis of the above functions wherein probably more than one mechanism participated in association. Young *et al.* (1991) found good correlation between induction of root elongation and production threshold concentration of growth hormones and disease escape mechanisms, which was suggested to be attributed due to the involvement of bacteria derived plant growth regulators. Patil *et al.* (2001) observed increased root length, root number and root

volume in pomegranate with the application of *Trichoderma*.The highest root volume was also recorded with the combined application of *Trichoderma* and *Pseudomonas fluorescens* because of the increased root length, as it contributes directly to root volume. Growth improvement by the combined application of *Trichoderma* and *Pseudomonas fluorescens* could be due to their cumulative and synergistic action on nutrient uptake and production of growth promoting substances as suggested by Quensigva (2001). The present study also confirmed the superiority of the combined treatment of *Trichoderma* and *Pseudomonas fluorescens* in improving the root length and root volume of patchouli plants in nursery.

In the present study root number was recorded the greatest with IBA at the rate of 1000 ppm, which recorded 73.20 roots per plant. Plant growth regulators have been known to stimulate growth activities and terpenoid biosynthesis. Some of the plant growth regulators that stimulate the growth parameters, simultaneously influences the inter-relationship between the primary and secondary metabolism leading to the increased biosynthesis of secondary product (Agnus ,1992).

Successful regeneration of roots largely depends on the presence of auxin. The crucial role of auxin in root regeneration has been demonstrated by Skoog and Tsui as early as in 1948.

Excellent rooting of patchouli with IBA has already been discussed by Selvarajan and Rao (1982) and Bhattacharjee and Thimmappa (1991). Similar results were reported by Chauhan and Reddy, 1971 in plum; Thimmappa and Bhattacharjee, 1990 in geranium and Nath, 2000 in Assam lemon.

The treatments did not register any superiority with respect to sprout length. Among treatments plants applied with *Trichoderma* recorded the highest

Plate 1. Effect of bioagents on rooting of Patchouli cuttings

Plate 2. Effect of 1) *AMF* and 2) IBA on rooting of Patchouli cuttings

sprout length of 0.45 cm. Generally control plants and AMF treated plants showed poor performance in sprout emergence, sprout length, root length and leaf production. Poor performance shown by AMF may be due to the lower count of AM fungi observed in the patchouli root bits. Control plants showed the lowest sprout length, number of leaves and root length with late emergence of sprout.

5.2 Experiment 2

Impact of bioagents and soil amendments on the growth, yield and quality of patchouli in the main field

5.2.1 Impact of bioagents and soil amendments on height of plant

The application of bioagents and soil amendments revealed their supremacy in improving all the growth parameters in the main field. Use of organic amendments improves physical properties of the soil and balances the nutrient availability to plants and boosts up production and quality of the crop. Bioagents are of microbial origin, which contain living cells of microbes that mobilize nutritionally important elements to available form through biological processes (Burton, 1967).

Among the treatments, control + lime + neem cake + *Pseudomonas fluorescens* + *Trichoderma* recorded the highest plant height (121.16 cm) in main crop, which was followed by T_{15} (117.13cm).

The suggested reason for this acceleration is the influence of nitrogen, chief constituent of protein, essential for the formation of protoplasm which leads to cell division and cell enlargement. Moreover nitrogen is an important component of aminoacids and co-enzymes which are of considerable biological importance in the physiological growth of crop plants. This was reported by Bakly (1974) and Rathore *et al.* (1985). An extra amount of nitrogen is obtained with the application of

Pseudomonas fluorescens and neem cake, which might have caused the elongation of cells ultimately resulting in increased plant height (Dobereinger and Day, 1986).

The role of efficient phosphorus solubilising micro organisms assumes greater importance for augmenting crop productivity (Gaur, 1990). Gutierrezmanero *et al.* (1996) reported that *Pseudomonas fluorescens* have been found to increase the growth and yield by 5 to 10 per cent due to increased soluble phosphorus nutrition and nitrogen fixation, synthesis of growth promoting substances and production of antibiotic like compounds. Lemanceaug and Alabouvette (1993) and Glick (1995) also put forward similar views in their experiments on oil yielding crops and field crops.

Increment in plant height with the application of *Pseudomonas fluorescens* was also reported by many workers (Rangaswamy and Morachan, 1974 in sorghum; Ascon *et al.*, 1978 in palmarosa; Berkholst, 1989 in rose; Manonmani, 1992 in Jasmine; Velmurugan, 1998 in marigold). Increment in plant height is attributed to the rapid meristamatic ability in plants due to availability of nitrogen as reported by Crowther (1935). Efficacy of neem cake on growth and development was discussed by Alam *et al.* (1980) who reported that the favourable effect of neem cake is due to its nitrification regulation property and also by its complementary effect on the growth and multiplication of plant growth promoting micro organisms. Role of nitrogen in favoring the growth of crop plants especially through organic sources has been well documented by Wallace (1971) and Sorin and Tanaka (1991). Influence of neem cake as an organic supplier of nitrogen in increasing the plant height was reported by Ram and Prasad (1989) in *Mentha arvensis*, Pandey (1994) in davana and Jonathan *et al.* (2000) in banana. Role of nitrogen in the synthesis and translocation of phytohormones especially auxins might have played a major role in

the elongation of cells, which necessarily, enhanced the height of plants (Curiear, 2001).

Compatibility of neem cake to fungal antagonists especially to *Trichoderma* was already detected and it is observed that besides adding organic carbon and K to the soil, neem cake can also increase the residual fertility status of the soil (Zonquial *et al.*, 1994).

Efficacy of *Trichoderma* in increasing plant height has been reported by Poldma *et al.* (1998) in cucumber, Hazarika *et al.* (2000) in tea, Ravi *et al.* (2000) in banana, Devi and Richa (2002) in tomato and Dwivedi and Shukha (2002) in guava. *Trichoderma* can induce better growth and resistance through nitrate nitrogen regulation and antibiosis (Wingster *et al.*, 2002).

Application of lime which supplies Ca to the soil also plays an important role in cell division and it is an important component of the cell wall (Fernandes *et al.*, 1974). Positive influence of lime in increasing plant height was reported by Balagopalan (1997) in eucalyptus and Chung *et al.* (1998) in chilli. Liming can induce better soil fertility through the addition of major and minor nutrients to the soil (Chew *et al.*, 1980). Liming increases the nitrate content of the soil, which is an essential constituent of cell division and cell elongation (Gelminy *et al.*, 1997). Liming can also induce neutralization of soil acidity and can add Ca and K to the soil, which can enable the plants to grow well (Flurette *et al.*, 1998).

In regenerated crop also the treatment T_{16} showed the best performance with respect to plant height with a mean height of (107.66cm). The increment in plant height in this treatment is also attributed to the above discussed factors as seen in the main crop.

Many scientists have already reported the favourable effects of combined application of bioagents on crop growth. Meyer and Lindermann (1986), Fentahan *et al.* (1998) and Kichadi and Sreenivasa (1998) observed the significance of combined application of treatments in augmenting crop production. The present study also confirmed that application of biofertilizers and soil amendments along with inorganic manures and FYM recorded better growth characters with respect to plant height. Among treatments T_1, T_9 and T_5, which lacked both *Pseudomonas fluorescens* and *Trichoderma* recorded shorter plants because of the imbalanced supply or availability of nutrients as compared to the combined application of bioagents and soil amendments.

5.2.2 Effect of bioagents and soil amendments on number of leaves

The highest leaf production (331.87 leaves per plant) was recorded in plants treated with lime, neem cake, *Trichoderma* and *Pseudomonas fluorescens*, followed by treatments in which lime, neem cake and *Trichoderma*were applied along with the control (324.77 leaves per plant). This might be due to the complementary effects of plant growth promoting ability of saprophytic antagonists in the amended soil (Bickmore *et al.*, 1969). Being a part of protoplasm nitrogen plays key role in the build up of new cells and chlorophyll synthesis (Aleem, 1970). Application of neem cake can increase the production of leaves because of its nitrification regulation property and transfer of nutrients to plants (Skulbhram *et al.*, 1982).

Influence of neem cake in augmenting higher rate of leaf production has already been reported by Jagadale *et al.* (1985) in betel vine, Acharya and Padhi (1988) in betel vine, Kumar *et al.* (1988) in china aster and Roul (2000) in lavender. Improved growth parameters as obtained by neem cake application might be due to the nitrification regulation process and which makes available all the applied nitrogen

to plants for a longer period. These results corroborate with the findings of Reddy and Rajendraprasad (1975) Muthuswamy *et al.* (1977) Shilendranath and Rao (1979) and Sieman (1996).

In the treatment, T_{16}, recorded as superior with respect to leaf production, inclusion of *Pseudomonas fluorescens* might have influenced enhanced cell multiplication and biomass production as was observed in sage by Changway and Nelson (1991).

Rao and Vasanthakumar (1989) observed significant correlation between green leaf yield and uptake of phosphorus in patchouli which is a factor accounting for favorable effect of *Pseudomonas fluorescens* on leaf production. Lambart and Joos (1990) reported inconsistent field performance of lavender with *P. fluorescens*. Diao *et al.* (1992) recorded maximum number of leaves in oats with the incorporation of *P. fluorescens*. Duijiff *et al.* (1994) in carnation also reported similar results explaining that siderophores produced by *Pseudomonas* sp. can stimulate chlorophyll synthesis and biomass yield.

Zone (1996) observed significant increase in the uptake of plant micronutrients with the application of *P. fluorescens* which could also explain its positive influence in enhancing growth and yield. Ascon *et al.* (1976) in lavender, Cook and Rovira (1976) in peas, Defago (1990) in tobacco and Weller (1998) in wheat have also reported similar results.

Influence of *Trichoderma* in augmenting leaf production was already discussed by Borthakur and Dutta (1992) in tea, Sethuraman and Muthuswamy (1994) in tomato and Hazarika *et al.* (2000) in tea. In the present study also,

Trichoderma enhanced leaf production in the experimental plants as is evidenced in treatment T_{16}.

Liming plays a major role in leaf production which has been reported by Mascarehas *et al.* (1976), and Andrews and Bellad (1987). Response to addition of lime might be due to the neutralization of soil acidity leading to better availability of nutrients, which ultimately resulted in increased growth parameters (Saravanan and Nambisan, 1995).

Among the treatments, plants applied with neem cake alone along with recommened doses of NPK and FYM recorded lesser number of leaves (205.03), which was on par with control (205.70).

In regenerated crop also T_{16} recorded the highest number of leaves (294.35) which was on par with T_{15} (288.55) and T_{11} (269.70). T_5 (control + neem cake) recorded the lowest number of leaves both in main crop and regenerated crop. The reduction in leaf production may be due to the lack of optimum availability of nutrients, which were provided to other plots through the application of other soil ameliorants.

5.2.3 Effect of bioagents and soil amendments on number of branches

Number of branches per plant was recorded the highest in plants applied with lime, neem cake , *Pseudomonas fluorescens* along with control . The same treatment yielded 28.65 branches per plant in main crop which was closely followed by plants receiving all the soil amendments and bioagents, yielding 27.45 leaves per plant. Among treatments control plants recorded lowest number of branches with an average of 15.03 branches per plant.

Plate 3. Field view of Patchouli at two months after transplanting

Plate 4. T_{16} at two months after transplanting

Increment in branches in the above mentioned treatments could be attributed to activation of biofertilizers, which in turn would have increased the availability of nutrients. This was reported by Amrithalingam and Balakrishnan (1988) in chilli, Manonmani (1992) in jasmine and Saha *et al.* (1992) in patchouli.

Plant growth promotion by *P. fluorescens* is well demonstrated in many crops. Manonmani (1992) in jasmine, Vasanthi (1994) in *Jasminum grandiflorum*, Sadashivam (1995) in dolichos bean, Manoranjitham and Prakasam (1999) in tomato, and Mosa *et al.* (2002) in pepper recorded increased number of branches with the application of *P. fluorescens*. Production of IAA or auxin like substances has been attributed to the plant growth promoting effect of most of the *fluorescent pseudomonas* as reported by Suslow (1982), and Schippers *et al.* (1987).

Kloepper (1996) and Lazaorvitz (1999) attributed the direct effects of growth stimulation by phosphate solubilising bacteria to the activation of host defense mechanism and improved nutrition. Application of *P. fluorescens* can also result in increased P uptake as is observed by Gerretsen (1948), Pikovoskaya (1948) and Bowen and Rovira (1966). A concomitant increment in N and P was observed as a result of *Pseudomonas* inoculation as reported by Rangaswamy and Morachan (1974) and Judith *et al.* (1996).

Krochmal and Samuels (1970) in field crops, Kumaran *et al.* (1998) in tomato and Singh and Kumar (2000) in mint recorded the highest number of branches with neem cake application. Bakly (1974) found that nitrogen plays an important role in the various growth phases of crop plants. Application of neem cake can add organic carbon, nitrogen and K to the soil, which can create a stable C:N ratio that facilitates better availability of nutrients to plants (Kumaran *et al*,. 1998).

Liming can depress the activity of heavy metals and creates better soil environment for the growth. Andrews and Bellad(1987) in palmarosa and Chung *et al.* (1998) in chilli recorded highest number of branches with liming. Increased availability of total extractives and Ca was also recorded with liming. Andrade *et al.* (1994) in eucalyptus and Choudhury and Bordoloi (1992) in *Cymbopogon khasianus* reported the availability of N, P, K and Ca with liming. Kotowska (1996) and Rakesh *et al.* (1996) recorded increased availability of Ca and P with liming.

In regenerated crop, plants receiving lime, neem cake and *Pseudomonas fluorescens* registered the highest number of branches (30.75) whereas control plots recorded the lowest number of branches both in main crop and regenerated crop which yielded an average of 15.03 branches per plant in main crop and 16.35 branches per plant in regenerated crop. This is because the advantages attributed to the bioagents and soil amendments applied, with respect to growth is obviously lacking in control plants.

5.2.4 Influence of bioagents and soil amendments on plant spread

Spread of a plant gives the area occupied by the plant under the growing environmental conditions. Quantitative characters like number of leaves, number of branches and leaf area directly contributes to plant spread. Plant spread recorded was also the highest in plants applied with lime, neem cake and *Pseudomonas fluorescens* along with control in both N-S (78.21 cm) and E-W (76.81 cm) directions which was closely followed by T_8, T_{15} and T_{11}. It was recorded the lowest from control plants which registered 61.01cm in N-S direction and 62.37 cm in E- W direction.

Plant spread was found significantly influenced by the application of *Pseudomonas*. Berkholst (1989) in rose, Merinapremkumari (1991) in horticultural crops, Diao *et al.* (1992) in oats and Vasanthi (1994) in *Jasminum grandiflorum* also

reported the highest plant spread with the application of *Pseudomonas fluorescens*. Kumar *et al.* (1998) found that in pyrethrum treatments which provided N and P increased the plant spread in East-West direction, while P alone affect the spread of the plant in North-South direction. According to Hegde *et al.* (1984) liming also perform nitrification regulation, which might have resulted in increased plant spread.

5.2.5 Influence of bioagents and soil amendments on leaf area

The highest leaf area was recorded in the treatment, Control + Neem cake + *Trichoderma* + *Pseudomonas fluorescens*, which recorded an average leaf area of 32.81 cm^2. This was followed by treatments T_{16} (32.10 cm^2) and T_{15} (31.76 cm^2), whereas T_7 plants recorded the lowest leaf area (22.49 cm^2). Hadas and Okon (1987) reported that application of biofertilizers can produce growth hormones which in turn might have caused the increment in length and breadth of leaves leading to increased leaf area.

Application of bioagents and organic amendments recorded increased leaf area in patchouli because of the better utilization of sun light and nutrient availability. Pareek and Sethi (1985) and Bhasker (1996) have also reported increased leaf area in patchouli with the application of biofertilizers and organic amendments.

In the present study, application of neem cake favorably influenced enhancement of leaf area. Jagadale *et al.* (1985) and Acharya and Padhi (1988) in betelvine have also recorded increased leaf area with the application of neem cake. Role played by nitrogen a main component of neem cake in the increment of leaf area was already discussed by Kanapathi (1974), Okeke *et al.* (1979) and Wouch (1995). This favourable effect of nitrogen is because of the better photosynthate accumulation and better translocation of the products to sink (Khanda and Dixit, 1995).

Diao *et al.* (1992) in oats, Vasanthi (1994) in *Jasminum grandiflorum*, Remesh *et al.* (1998) in cashew have also recorded increased leaf area with the inoculation of *Pseudomonas fluorescens*. Increased leaf area by *Pseudomonas* is because of its nitrogen regulation properties (Steuward 1999). Influence of *Trichoderma* in leaf area increment was already reported by Poldma *et al.* (1998) in cucumber, Rabeendran *et al.* (2000) in cabbage and lettuce, Ravi *et al.* (2000) in banana and Yedida *et al.* (2001) in cucumber.

5.2.6 Influence of bioagents and soil amendments on fresh herb and fresh leaf yield

Fresh herb yield and fresh leaf yield were recorded the highest with treatment receiving lime, neem cake, *Trichoderma* and *Pseudomonas fluorescens* which recorded herbage yield of (6247.50 kg per hectare) and leaf yield of (4407.50 kg per hectare). Compared to control treatments T_8, T_{15} and T_{14} also recorded comparatively better fresh herb yield and fresh leaf yield. Increment in yield due to the combined application of soil amendments and bioagents may be due to the complementary effect of plant growth promoting ability of saprophytic antagonists which acted synergistically when added simultaneously and also by its mass multiplication regulated by organic amendments and increased soil fertility (Christapher, 1991). Ravichandran (1991) opined that production of photosynthates due to the application of biofertilizers and its effective utilization might have been the reason for increased biomass. This was strongly supported by Manonmani (1992) and Mariappan (1992).

Role of *Pseudomonas fluorescens* in increasing the yield and biomass content was recorded in many crops (Raguchander *et al.*, 1997, Bucki *et al.*, 1998, Shanthi *et al.*, 1998, Anith *et al.*, 2000).

Rao and Vasanthakumar (1989) stated that the whole plant yield as well as the green leaf yield in patchouli is significantly correlated to phosphorus content. It is found that increment in yield by PSB is because of its nutrient production. Production of antibiotics, vitamins, hormones, volatile and antimicrobial substances and siderophores and proliferation of other beneficial micro organisms in the soil also contribute to yield increase in crops on application of PSB (Kolarova *et al.*, 1967, Rosales *et al.*, 1995, Lazarovitz, 1999, Mondal *et al.*, 2000).

Neem cake also plays significant role in chlorophyll production and it facilitates the provision of availability of optimum dose of nitrogen. Nitrification regulation property and increased sulphur content of neem cake and easy transfer of nutrients to plants contribute to its potential to increase yield (Skulbhram *et al.*, 1982). Varshney (1991) obtained increased fresh herb yield in patchouli with higher doses of nitrogen.

Krochmal and Samuels (1970) opined that increment in nitrogen rate tend to increase weight of stems and leaves which in turn resulted in increased herbage yield. Prabhakar *et al.* (1979) and Sathianatham (1982) also obtained similar results.

Enhanced growth and herbage yield in tea was recorded by Borthakur and Dutta (1992) and Hazarika *et al.* (2000) with the inoculation of *Trichoderma*. It may be because of its growth stimulating effect and increased soil fertility through the availability of nitrogen.

Andrews and Bellad (1987) and Choudhury and Bordoloi (1992) in *C. khasianum* recorded increased herbage yield with liming. Liming can induce larger amounts of soluble organic compounds, which in turn might have resulted in increased biomass yield (Tapen *et al.*, 1993).

Generally patchouli plants which received higher dose of nutrients in combination with biofertilizers produced the highest number of leaves, which can be mainly attributed to the better growing condition that prevailed in the visibility of root zone due to the application of fungi and bacteria helping the plant to absorb more nutrients (Kumaraswamy and Madalageri, 1989 and Naik *et al.*, 1995). Manjunatha (2002) could obtained an increase of around 30 per cent fresh herb yield in patchouli with biofertilizer application.

The dry leaf yield was found significantly influenced by different treatments. Among the treatments plants receiving neem cake, *Trichoderma* and *Pseudomonas fluorescens* along with control recorded the highest dry weight of leaves (940.43 kg per hectare) per plot which was followed by T_{16}, T_{14}, T_{15}, T_6 and T_{12}, whereas plants receiving only neem cake along with control recorded the lowest dry leaf yield which was on par with T_1 and T_9. Drying is a process which is primarily governed by internal factors rather than external factors. Increment in fresh weight and rate of moisture loss can also contribute to dry weight. Owusubennoah and Mossae (1979) opined that in majority of agricultural crops, increased fresh weight and dry weight were recorded with the application of microbial fertilizers. In regenerated plants those receiving lime, neem cake and *Pseudomonas fluorescens* along with control treated plots yielded the highest dry leaf and the lowest dry leaf yield was recorded from plants receiving only lime along with control.

5.2.7 Effect of bioagents and soil amendments on oil yield and oil content

The variation in oil yield and oil content were significant among the treatments. In patchouli, oil yield and oil content varies from 2.5-3.5 per cent on shade dry basis and an average of 2.55 per cent may be considered satisfactory (Vasanthakumar *et al.*, 1989). Maheswari *et al.* (1993), and Bhasker *et al.* (2001) have also confirmed that oil yield in patchouli varies from 2.5 to 3.5 %.

Plate 5. Field view of Patchouli at four months after transplanting

Plate 6. Field view of Patchouli at six months after transplanting

In the present study, plants receiving *Trichoderma*, *Pseudomonas fluorescens* and neem cake along with the recommended doses of NPK and FYM registered the highest oil yield (38.55 kg per hectare) and per cent oil content (4.15 per cent) which was followed by the treatment where lime and neem cake were excluded, with an oil yield of 33.35 kg per hectare and oil content of 3.90 per cent. Varshney (1991) opined that the formulation and accumulation of essential oil are predominant during the active growth period and nitrogen has a positive effect up to a certain level of growth and herbage yield of essential oil in crops where oil is synthesized and accumulated in leaves as in the case of patchouli. In this experiment T_{10} registered the lowest per cent oil content (2.15%) and lowest oil yield was recorded from T_7 (16.10 kg per hectare).

Role of nitrogen in augmenting the oil yield was reported by many scientists as is observed by Sharma *et al.* (1977) and Hazarika *et al.* (1978) in palmarosa, Singh and Singh (1979) in mentha sp., Singh *et al.* (1983) in citronella java, Rao *et al.* (1983) and Singh *et al.* (1983) in mint, Rao *et al.* (1985) in geranium, Saha *et al.* (1992) in patchouli, Farooqi *et al.* (1994) in *Majorana hortensis*.

The increment in oil yield by nitrogen through organic sources might be due to more leaf area, size of epidermal cells and number of oil glands per unit leaf area (Gonzalezalonso, 1955). Datta and Virmani (1964) stated that nitrogen can increase leaf to stem ratio which in turn can affect oil content, since leaves contain about 10-12 times more oil then stem in patchouli.

Rao *et al.* (1983) stated that P fertilization resulted in vigorous growth of plants and increased the herbage and essential oil yields. Similar results were reported by Singh *et al.* (1983), Saha *et al.* (1992) and Farooqi *et al.* (1994). In the present

study also, increased P uptake as influenced by application of *Pseudomonas* might have contributed to increased oil yield.

Beneveiste (1984) found significant influence of fertilizers on the oil yield and quality of patchouli oil. Content and quality of patchouli oil are also influenced by many factors such as cultivar, soil and agroclimatic conditions, status of leaf and mode of distillation (Bhasker and Vasanthakumar, 2000).

5.2.8 Physico chemical properties

5.2. 8.1 Specific gravity and refractive index

Major physicochemical properties like specific gravity, refractive index were tested. Patchouli grown under areas with almost similar backgrounds recorded specific gravity as 0.9532 and refractive index as 1.5034 at 32^{0c} (Vasanthakumar et al. 1989). In this experiment also specific gravity and refractive index of patchouli oil were studied and specific gravity is recorded with in a range of 0.940 to0.953 and refractive index as1.5032 to 1.5150 at 31^{0c}.

Koolhaas and Rowen (1937) recorded specific gravity of patchouli oil as 0.962 to 0.971 at 15°C and refractive index as 1.5072 to 1.5100 at 20^{0c}. Seychellus (1937) recorded specific gravity of patchouli with in the range of 0.940 to 0.980 and refractive index as 1.505 to 1.510 at 20^{0c}. Gildemeister and Hoffmann (1938) recorded specific gravity of patchouli, as 0.966 to 0.995 at 15^{0c}. Guenther (1949) recorded the specific gravity of patchouli oil with in the range of 0.967 to 0.972 at 15^{0c} and refractive index as 1.5090 to 1.5100 at 20^{0c}. British Standard Institution (BS 2999/10:1965) suggests the specific gravity of patchouli oil as 0.952 to 0.980 and refractive index as 1.505 to 1.512 at 20^{0}c. According to Essential Oils Association of USA (EOA No.23) patchouli oil should meet the standards of specific gravity with in

a range of 0 .950 to 0 .975 at 25^{0c} and refractive index as 1.5070 to 1.5150 at 20^{0c}. International Organisation for Standardisation specifies the specific gravity of patchouli oil as 0.955 to 0.983 at 25^{0c} and refractive index as 1.5070 to 1.5150 at 20^{0c}. Akila and Tewari (1984) recorded the specific gravity of patchouli as 0.9532 and refractive index as 1.5040 at 31^{0c}. Vasanthakumar *et al.* (1989) recorded specific gravity as 0.9736 and refractive index as 1.5034 at 25.8^{0c}. Shankaranarayan (2002) also obtained similar results in patchouli.

5.2.8.2 Gas chromatographic profile of patchouli oil

The gas chromatographic method for quantitative determination of patchouli alcohol provided a method for the standardization of its components (Kang *et al.*, 1998).

A chemical profile of patchouli oil is given by Lawrence (1981). Accordingly true patchouli oil consists of 1% terpenes, 50% sesquiterpenes, 30-40% of patchouli alcohols and related alcohols. Dung *et al.* (1999) detected more than 16 compounds of which 11 were identified as alpha beta and delta patchoulene, beta elemene, beta caryophyllene, alpha and delta guaiene, seychellene, alpha bulnesene, delta cardinene, pogostol and also that patchouli alcohol contributes 32-37% which is the most odour intensive constituent of the oil. Yang *et al.* (1996) also observed almost similar results and they identified 20 major components. The essential oil quality in patchouli in determined by patchouli alcohol content (Sarma and Kanjilal, 2000).

Gas chromatographic results showed significant variation with respect to the application of treatments. Number of components present in the oil sample varied significantly among treatments. Among treatments T_5, T_8, T_{16} and T_4 recorded the highest number of peaks and moderate to high oil yield. Number of peaks recorded

the highest in T_5 (control + neem cake), which recorded total of 10 peaks. Treatments T_6, T_7, and T_{14} recorded moderately higher number of peaks. Among treatments neem cake treated plots recorded better results. It may be due to the fact that neem cake possesses the ability of the slow release of nutrients to plants. Neem cake treated plots also recorded moderate oil yield and major components. Enhancement of growth in the main field also gives stronger support to this result. It is due to the reason that neem cake may remit in slow and steady release and availability of nutrients to the plants. Generally combined application of bioagents and soil amendments recorded an increased amount of oil yield and oil components in a different pattern. Field studies both in vegetative and qualitative characters also points out the relevance of combined application of treatments.

It is observed that when the number of peaks increases, the per cent of major components of the oil decreases. Area normalisation by the detector recorded highest area with T_{15} (97.62%).

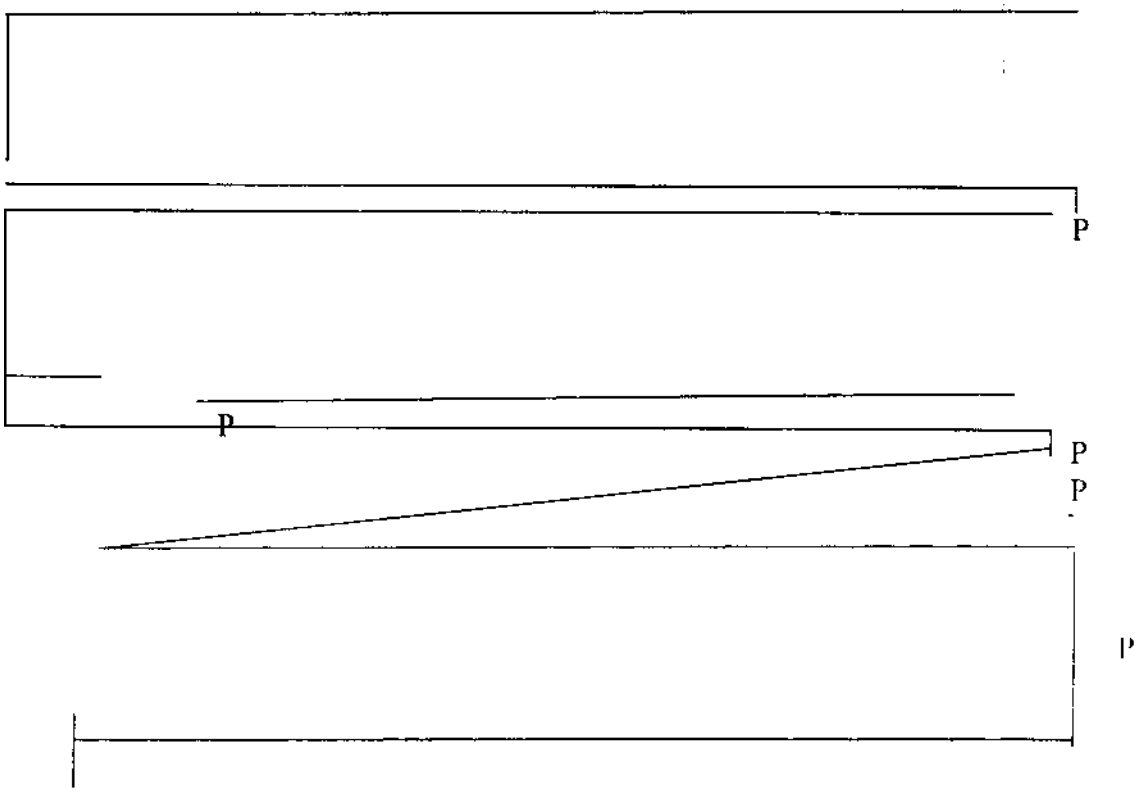
Treatments receiving *Trichoderma*, *Pseudomonas fluorescens*, neem cake and their combined applications recorded comparatively higher number of peaks and oil yield. Both *Trichoderma* and *Pseudomonas fluorescens*possess the ability to cause enhanced nutrient availability to plants at a faster rate. This type of increment on the availability of nutrients is controlled by neem cake remitted in a state in which plant develops a physiological status of increased oil yield and higher number of oil components.

Less number of components in the oil was recorded in T_9, T_2, T_{15} and T_{10}. Oil samples from lime induced plots recorded lower number of peaks except T_{16}. The combination T_{16} is expressing moderately higher number of peaks because of the combined application of lime along with *Pseudomonas fluorescens* and *Trichoderma*

in which phosphorus and nitrogen availability were increased which in turn might have caused moderate increase in oil per cent and higher number of components in the oil. Therefore, it can be inferred that lime treatment has no effect in this regard. Control and lime application alone showed almost similar pattern of results with respect to oil yield and quality. Among the treatments T_{16}, T_8 and T_{14} recorded better performance in all the growth parameters including oil components present in their representative oil samples. Results of both field and lab analysis points out the compatibility of both neem cake with other bioagents and combined application of these bioagents and soil amendments recorded their significant effect upon vegetative as well as qualitative characters.

Gas chromatographic profile also points out the impact of bioagents and soil amendments on qualitative characters like oil components and oil yield. Guenther (1949) opined that patchouli oil contains 40-45% sesquiterpenes and the rest by patchouli alcohol. Oil contains small amounts of benzaldehyde, an alcohol with a rose like fragrance, a ketone with orris like odour, two bases possessing a strong benumbing odour, azulene and sesquiterpene alcohol. ß patchoulene, gamma guaiente, alpha bulnesene, terpene cadinene, benzaldehyde and patchouli alcohol have been identified chromatographically. It was supported by Bates and Slagel (1962) and Koul and Nigam (1966). Maheswari *et al.* (1993) examined the oil of Johore and found that the cultivar met the requirement of ISO 3757.1978 in oil quality and oil content. Gas chromatographic analysis showed that regenerated plants produced an essential oil comprising a full set of patchouli sesquiterpenes (Kegeyama *et al.*, 1995).

Fig.12 Gas chromatographic profile of T_5R_1

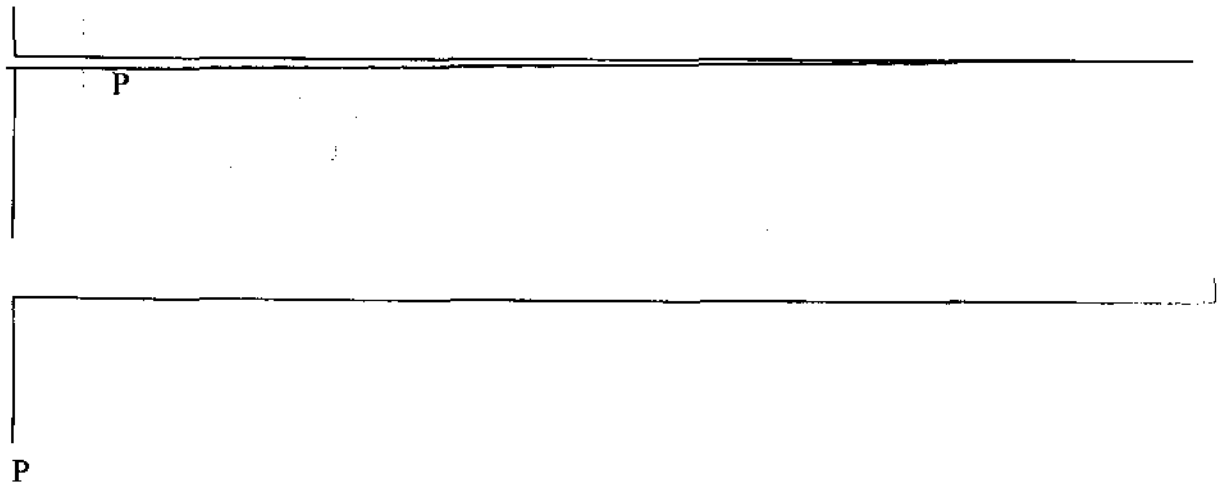


Number of peaks 5

Fig.13 Gas chromatographic profile of T_9R_1

1

T_9R_1



Number of peaks-2

Summary

6. SUMMARY

An experiment was conducted during May 2002-03 in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara to assess the impact of bioagents and soil amendments on the growth, yield and quality of patchouli. The study was conducted in two experiments. The bioagents include *Trichoderma, Pseudomonas fluorescens*, and VAM along with the growth hormone IBA and soil amendments consisted of lime and neem cake.

- In the first experiment of the study in which impact of treatments on production of quality planting materials in the nursery was studied, it was observed that the growth parameters like number of roots, number of leaves, root length and root volume were significantly influenced by bioagents and the growth hormone IBA. Remarkable increase in root number was noticed in plants receiving IBA at the rate of 1000 ppm at the time of planting which recorded 73.20 roots per plant.

- In the nursery number of leaves was found significantly enhanced by the combined application of bioagents with the highest leaf number (19.94 leaves per plant) being observed in those plants which received *Trichoderma* and *Pseudomonas fluorescens* at the rate of 2 g each at the time of planting.

- Bioagents had a significant role in improving root length and root volume of plants. Plants receiving combined application of both *Trichoderma* and *Pseudomonas fluorescens* at the rate of 2 g each at the time of planting recorded the highest root length of 6.95 cm and root volume of 316.66 g.

- In the present study bioagents had no significant influence on earliness in rooting, earliness in sprouting and sprout length. The plants receiving IBA at the rate of 1000 ppm rooted earlier (6.30 days) and *Trichoderma* treated plants recorded the highest sprout length (0.45 cm) while the combined application of *Trichoderma* and *Pseudomonas fluorescens* recorded earliest sprouting in 6.30 days after planting the terminal cuttings.

- Treatment rated best in the nursery (combined application of *Trichoderma* and *Pseudomonas fluorescens*) was selected for planting in main field to study the impact of bioagents and soil amendments on the performance of patchouli with special reference to growth, yield and quality. The results revealed that bioagents and soil amendments significantly influenced both vegetative and yield characters.

- Combined application of all the bioagents and soil amendments (control + lime + neem cake + *Trichoderma* + *Pseudomonas fluorescens*) recorded the highest height of the plant, number of leaves, fresh herb yield and fresh leaf yield. The same treatment also resulted in increased overall vegetative growth with significant positive influence on yield.

- Combined application of lime + neem cake + *Trichoderma* + *Pseudomonas fluorescens* along with FYM and recommended doses of NPK significantly influenced growth parameters like height of the plant and number of leaves. This treatment recorded the highest plant height of 121.16cm and number of leaves (331.87 leaves per plant) in main crop. In regenerated crop also the same combination of treatments recorded highest plant height (109.42 cm) and the highest number of leaves (294.35 leaves per plant).

- The bioagents and soil amendments had a positive influence on number of branches which is directly related to plant spread. The treatment receiving lime, neem cake and *Pseudomonas fluorescens* along with the recommended dose of FYM and NPK recorded the highest value of 28.65 branches per plant and maximum plant spread of 78.21 cm in N-S direction and 76.81 cm in E-W direction. In regenerated crop also this same treatment recorded the highest number of branches (29.48).

- Significant superiority in leaf area (32.81 cm^2) was observed for treatments consisting of neem cake + *Trichoderma* + *Pseudomonas fluorescens* along with FYM + NPK application at six months after planting.

- Fresh herb and leaf yield varied significantly in main crop with the treatments, while no significant difference was noticed among the treatments in regenerated crop with respect to fresh herb yield and fresh leaf yield. In main crop combined application of all the bioagents and soil amendments including control + lime + neem cake + *Trichoderma* + *Pseudomonas fluorescens* recorded the highest fresh herb yield (6247.50 kg per hectare) and fresh leaf yield (4407.50kg per hectare). In regenerated crop plants though the treatments registered non-significant difference, plants receiving lime + neem cake + *Pseudomonas fluorescens* in addition to FYM and NPK recorded the highest fresh herb yield of 2480.00 kg per hectare, while treatments receiving the above combination along with *Trichoderma* recorded the highest fresh leaf yield (1772.50 kg per hectare).

- Dry leaf yield was also influenced by the bioagents and soil amendments applied. Plants treated with FYM + NPK + neem cake + *Trichoderma* + *Pseudomonas fluorescens* recorded maximum dry leaf yield in main crop

(940.43 kg per hectare). In regenerated crop, dry leaf yield was recorded highest from control + lime + neem cake + *Pseudomonas fluorescens* treated plots with an yield of 555.00 kg per hectare.

- Qualitative characters like oil content and oil yield were found significantly influenced by various treatments. The highest oil content of 4.1 % was obtained from plants treated with neem cake+*Trichoderma*+*Pseudomonas fluorescens* along with FYM and NPK.

- Physicochemical properties like specific gravity, optical rotation were tested and it was observed that patchouli oil obtained from experimental plants recorded a specific gravity between 0.940 and 0.953 and refractive index between 1.5032 and 1.5150 at 32^{0c}.

- Gas chromatographic profile was studied and found significant variation in oil components with respect to treatments. Quality of the oil was influenced by bioagents and soil amendments. Control and lime application alone showed almost similar pattern of results with respect to oil yield and quality. Plots treated with combination of treatments in general recorded comparatively high quality patchouli alcohol and better yield. Among the treatments, neem cake treated plots recorded the highest number of oil components while plots treated with lime recorded the lowest number.

- Incidence of pests and diseases were also observed and three insect pests, leaf feeding catter pillar (*Pronomis profusali* warren.), leaf roller *Herpatogramma licasisalis*) and *Protista moesta* were found attacking patchouli in the field. Spraying of 0.2 per cent Roger effectively controlled the pest incidence. Incidence of nematode attack and bacterial wilt were not observed in the main field, in the control as well as in the experimental plots.

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* Originals not seen

Appendices

Appendix 1

PARTICULARS	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY
Mean maximum ^{O} C	29.8	28.9	31.1	30.8	31.8	32.3	33.2	34.7
Mean minimum ^{O} C	23.1	22.9	23.0	23.2	23.2	23.4	22.1	22.9
Highest maximum ^{O} C	31.0	31.4	33.0	33.4	33.2	33.4	-	35.3
Lowest minimum C	21.4	21.0	21.6	22.5	22.3	16.8	19.5	21.6
Mean R.H Morning%	94	94	92	92	92	82	72	66
Mean R.H Evening %	74	78	62	74	60	45	34	43
Mean R.H %	84	86	77	83	71	45	50	63
Rainfall mm	354.2	506.6	124.0	387.7	22.1	0.0	0. 0	162.1
Rainy days	21	19	8	19	3	0	0	5
Evaporation Mm	94.6	93.4	125.5	96.2	124.9	198.8	229.1	152.9
Sunshine Hours	105.7	96.8	233.4	136.4	189.2	270	291.2	258.0
Mean Sunshine Hours	3.4	3.1	7.8	4.4	6.3	8.7	9.4	9.2
Mean Wind Speed Km	3.8	3.8	3.7	3.3	4.7	8.1	8.6	5.1
Soil temperature ^{O} C 5 cm depth -	26.2	25.5	27.3	26.6	26.2	25.5	26.1	27.3

IMPACT OF BIOAGENTS AND SOIL AMENDMENTS ON THE PERFORMANCE OF PATCHOULI (*Pogostemon patchouli* Pellet.)

By

SMILU BABU

ABSTRACT OF THE THESIS

submitted in partial fulfilment of the requirement for the degree of

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Faculty of Agriculture
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ABSTRACT

An experiment entitled "Impact of bio agents and soil amendments on the performance of patchouli" was conducted at the Department of Plantation crops and Spices, College of Horticulture, Vellanikkara during 2002-04 to elucidate the effect of application of bioagents, soil amendments and bioregulators on growth, yield and oil quality of patchouli. The study was also aimed at refining vegetative propagation technique to generate disease free quality planting materials. The investigations were conducted in two experiments.

Results of the first experiment, " Impact of bioagents on the production of quality planting materials in the nursery "revealed that bioagents had a significant influence on the growth characters of the crop in the nursery stage wherein significant differences were observed among the treatments with respect to characters such as number of leaves, number of roots, length of roots and root volume. Root characters were markedly influenced by bioagents and bioregulators. Root length and root volume were found the highest for the treatment receiving combined application of *Trichoderma* and *Pseudomonas fluorescens* at the rate of 2 g each at the time of planting. This treatment also recorded earliest sprout emergence in 6.3 days after planting the terminal cuttings in the nursery.

Terminal cuttings which were treated with IBA at the rate of 1000 ppm at the time of planting came to rooting earlier in 8 days after planting in the nursery. Root number was also recorded the highest with the same treatment.

The highest leaf number in 45 days old patchouli plants was recorded in nursery with the incorporation of *Trichoderma* at the rate of 2 g per bag. This treatment also recorded the highest sprout length of 0.45 cm.

Earliness in sprouting, length of sprout and earliness in rooting were not found influenced by bioagents in nursery experiment.

Plants raised in the nursery by the combined application of *Trichoderma* and *Pseudomonas fluorescens* rated the best in the nursery were selected for main field experiment. Impact of bioagents and soil amendments on growth, yield and oil quality were studied in the main field and results obtained revealed that treatments

involving combined application of bioagents and soil amendments significantly influenced the growth and yield parameters of the crop plant.

Plant height was the highest with the combined application of lime, neem cake, *Trichoderma* and *Pseudomonas fluorescens* along with the application of recommended doses of FYM and NPK both in main crop and regenerated crop. Compared to control, this treatment also improved vegetative characters like number of leaves and yield attributes like fresh herb yield and fresh leaf yield both in main crop and regenerated crop.

Combined application of control, lime, neem cake and *Pseudomonas fluorescens* along with the recommended doses of FYM and NPK recorded highest number of branches and greatest plant spread. Leaf area also was found varying significantly among treatments. Application of neem cake, *Trichoderma* and *Pseudomonas fluorescens* in addition to the recommended doses of FYM and NPK recorded the highest leaf area. Dry leaf yield recorded was also the highest with the same treatment.

Oil yield and oil content in the plant were found to be significantly influenced by bioagents and soil amendments. The plants treated with a combination of neem cake + *Trichoderma* + *Pseudomonas fluorescens* along with the recommended doses of FYM and NPK recorded highest values of oil yield and oil content.

Physicochemical properties of the essential oil of patchouli were analysed wherein it was observed that specific gravity of oil between 0.940 and 0.953 and refractive index was recorded between 1.5032 and 1.5150 at 31^{0c}.

Gas chromatographic profile of oil samples were analysed and significant variation with respect to the application of various treatments was observed. Number of components present in the oil sample varied significantly among treatments. It is observed that when the number of peaks increases, the percentage of major components in the oil decreases. Treatments receiving neem cake along with NPK and FYM recorded maximum number of components in the oil sample. It is found that neem cake can add optimum level of nutrients to the soil at a slow and steady rate which finally resulted in the increment of oil components.