# INDUCTION OF GROWTH PROMOTION IN VANILLA THROUGH PLANT GROWTH PROMOTING MICROORGANISMS CONSORTIA

By

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**THESIS**

Submitted in partial fulfilment of the requirement for the degree of



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Kerala Agricultural University



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**2007**

## DECLARATION

I hereby declare that the thesis entitled **'Induction of growth promotion in vanilla through plant growth promoting microorganisms consortia'** 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, associate ship, fellowship or other similar title , of any other university or society.

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## CERTIFICATE

Certified that this thesis, entitled **'Induction of growth promotion in vanilla through plant growth promoting microorganisms consortia'** is a record of research work done independently by **Mrs. Dhanya, V** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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(Dhanya.V)

*Dedicated to*

*My parents, husband and son*

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# *Introduction*

# INTRODUCTION

Vanilla, a tropical climbing orchid, has real economic value in food and related industries owing to its unique flavour and pleasant aroma. It is one of the important spices traded in the global market. The substance chiefly responsible for the fragrance, flavour and pleasant aroma of vanilla bean is vanillin, which is largely used in ice-creams, chocolates, bakery products, puddings, pharmaceuticals, liquors and perfumes preparations.

Now a days, there is great demand for organically produced vanillin because of its quality and naturality. At present, synthetic vanillin is used and in order to increase the availability of natural vanillin, the vanilla production has to be increased. The use of inorganic nutrients to increase the vanilla production is not only expensive but causes environmental hazards. Moreover, the continuous use of plant protection chemicals for pest and disease management has destroyed the biological balance of nature. These chemicals will cause contamination of sources of water, damage to beneficial soil microflora, contamination of food including animal feeds, soil, air and serious health related problems. Under these circumstances, the importance of microbial inoculants for growth enhancement and control of diseases assumes greater role than resorting to chemical means. Further, microbial inoculants are harmless, cheaper and ecofriendly which are used to promote plant vigour, improve yield, control or suppress various diseases caused by soil borne pathogens (Sivaprasad, 2002).Among the several microbial inoculants used, the plant growth promoting microorganisms present in the rhizosphere region are important, which directly benefits the plant by providing nutrients for its growth.

Plant growth promoting microorganisms (PGPM) are abundant in the rhizosphere region and they produce various growth promoting substances, which promote the growth of roots, absorb sufficient nutrients and increase the activity of

beneficial organisms in the rhizosphere. Thus, they enhance the plant growth, control soil- borne pathogens and induce systemic resistance to phytopathogens. Hence, these microbes are used for biofertilization, phytostimulation and biocontrol. Plant growth promoting microorganisms in the rhizosphere mainly includes *Pseudomonas* sp., *Bacillus* sp., *Trichoderma* sp. and orchid mycorrhiza. However, soil often contain inadequate and ineffective beneficial microflora, such as diazotrophs and phosphorous mobilizing microorganisms (Kloepper, 1980). Therefore, the PGPM needs to be inoculated in such soils with inadequate population. Recently, the consortial approach of PGPM has gained importance in enhancement of growth of plants.

The consortia of plant growth promoting microorganisms are known to have a better effect on the plants than individuals and such consortial application are known to closely mimic the natural situation and broaden the spectrum of activity. Therefore, the present study was undertaken to asses the potentiality of plant growth promoting microorganisms (PGPM) consortia in enhancement of growth of vanilla. The study was carried out with the following objectives:

- Isolation of different plant growth promoting microorganisms (PGPM) like *Pseudomonas* sp., *Bacillus* sp., *Trichoderma* sp. and orchid mycorrhiza from the rhizosphere soil of vanilla, collected from different vanilla growing areas of Thrissur and Wynad districts.
- Identification of selected plant growth promoting microorganisms (PGPM).
- *In vitro* evaluation of mutual compatibility among selected plant growth promoting microorganism (PGPM) isolates.
- Screening of plant growth promoting microorganisms (PGPM) consortia for enhancing the growth of vanilla.

# Review of literature

# 2. REVIEW OF LITERATURE

Vanilla (*Vanilla planifolia* Andrews.), is a climbing, shade loving orchid. It is cultivated mainly for its pleasant flavor and aroma. At present, synthetic vanillin is being used instead of natural vanillin and this synthetic vanillin is carcinogenic also. So, in order to increase the availability of natural vanillin, organic cultivation of vanilla has to be promoted.

## 2. 1 Effect of individual PGPM and its consortia on plant growth

Plant growth promoting microorganisms are naturally occurring soil microorganisms that are able to aggressively colonize plant roots and improve plant growth when applied to roots, tubers or seeds (Kloepper *et. al.*, 1980). Improvement of nutrient availability (Suslow and Schroth, 1982) and production of plant growth regulators such as gibberellins, cytokinins and auxin like indole acetic acid (IAA) by the rhizobacteria resulted in significant increase in plant growth (Krause ,1992). Glick (1995) reported that phosphorous solubilization, biological nitrogen fixation, improvement of other plant nutrient uptake and phytohormone production like IAA, gibberellins and cytokinins are some of the mechanism of PGPR that directly influence plant growth. PGPM also directly antagonize soil borne pathogens and stimulate plant growth (Wei *et. al.*,1996). Jubina and Girija (1998) found that inoculation of antagonistic rhizobacteria improved the growth characteristics of black pepper cuttings in terms of shoot length, fresh weight and dry weight. Benizri *et. al.* (2001) reported that PGPR can contribute to the biological control of plant pathogens and improves plant growth. They enhance root development either directly by producing phytohormones or indirectly by inhibiting pathogens through the synthesis of different compounds. The experiment results of Asghar *et. al.*(2002) showed that seeds of *Brassica* species, inoculated with different isolates of rhizobacteria significantly increased plant height ,

stem diameter, number of branches, number of pods per plant , 1000 grain weight, grain yield and oil content over the uninoculated control.

The selected isolates of PGPR evaluated in green house for their efficiency for growth promotion and foot rot suppression in black pepper indicated that bacterial strain IISR-51 promoted growth of black pepper up to 55.15 per cent (Nisha *et. al.*, 2002). Application of rhizobacteria (Pf IISR-51) enhanced growth of black pepper. The better developed root system increased the mineral uptake in plants (Chakraborty *et. al.*,2003). Paul *et.* al.(2003) reported a significant uptake of nitrogen and potassium in black pepper treated with PGPR. They also observed that enhanced nutrient mobilization resulted in enhanced plant vigor. Yan *et. al.* (2003) observed that the inoculated PGPR strains usually have been found to increase the root length and root biomass and the better developed root system may increase nutrient uptake in plants (Khalid, A., 2004). Lucy *et. al.* (2004) reported that the addition of PGPR increased germination rate, root growth, leaf area, chlorophyll content, Mg content, $N_2$ content, protein content, hydraulic activity, tolerance to draught, shoot and root weight and delayed leaf senescence which reflected in higher grain yield of Ashwagandha. Shahida (2007) reported that *Azospirillum*, AMF and *P. fluorescens* enhanced the growth of vanilla and reduced the *Phytophthora* rot incidence in plants by 33 per cent. In a similar study, it was observed that combined application of two antagonists (*T. harzianum* and *P. fluorescence*) had good effect on increasing the yield of chilli. While, combination of more than two antagonists had no effect on yield as the single treatments and control recorded higher yield than these treatments (KSCSTE project report, 2007).

### 2.1.1 Effect of *Pseudomonas* sp.

Rhizobacteria that colonize roots, mostly *P. fluorescens* have emerged as organisms with great potential in plant growth promotion (Burr *et al.*, 1978). Kloepper *et. al.* (1980) reported that certain root colonizing bacteria belonging to fluorescent pseudomonads group such as *P. fluorescens* and *P. putida* promote plant growth. Many

soil microorganisms are antagonistic towards range of plant pathogens and also indirectly promote plant growth. Disease suppression by antagonistic bacteria has resulted in yield increase of a variety of crops. Suslow and Schroth (1982) found that enhanced plant growth caused by *P. fluorescens* is often accompanied by reduction in root zone populations of fungi and bacteria. Beneficial fluorescent pseudomonads and other microorganisms can promote plant growth and induce disease suppressiveness by mechanisms other than siderophores production namely through competition for carbon (Flad and Baker,1985) or through production of hormones ,antibiotics or bacteriocins. Fluorescent pseudomonad strains of PGPR when applied to crop seeds were thought to improve plant growth by displacing or excluding deleterious rhizosphere microorganisms (Schippers *et. al.*, 1987). Dowling and O'Gara (1994) found that *P.fluorescens* induced the production of plant growth regulators like gibberellins, cytokinins and IAA thus, enhancing plant growth and increasing disease resistance.

Sidorenko *et al.* (1996) showed that combined inoculation of *Pseudomonas* with other growth promoting bacteria increased the plant height, biomass and tuber yield in potato. Considerable increase in root length and seedling growth was observed by Lazarovits and Nowak (1997) when they used *Pseudomonas* spp. to promote growth of potato plantlets. Yungchun *et. al.* (1997) found that strains of fluorescent pseudomonads, $FPP_5$ and $FPP_3$ increased the ration of plant height, total weight and root weight of eggplant. Gulati *et. al.* (1999) reported that application of fluorescent pseudomonads significantly increased the growth of strawberry plants. It also increased the shoot length, root length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight and plant biomass. Madhaiyan *et. al.* (1999) found that *P. fluorescens* and *B. subtilis* were effective in improving shoot and root dry weight as well as the P content in vanilla. *Pseudomonas* strains promoted wheat growth in terms of root and shoot length and weight (Srivastava *et. al* 1999). Anith *et. al.* (2000) found that seed treatment with *P. fluorescens* strain EM 85 along with soil solarisation decreased the wilt incidence in ginger and increased the yield compared control plots. The evaluation by Adhikari *et .al.* (2001) confirmed that four strains *viz. P.fluorescens* ($S_3$), *P. tolassii* ($S_{20}$), *P. veronii*

($S_{21}$) and *Sphingomonas trueperi* ($S_{12}$) significantly enhanced plant growth as evidenced by increase in plant height and dry weight of inoculated rice seedlings relative to control. According to Kumar (2001), the seed bacterization of chickpea, eggplant, soyabean and tomato with *P.fluorescence* showed an increased seed germination, shoot height, root length, fresh weight, dry weight and yield. The *in vitro* experiment results conformed that plant growth promotion by *Pseudomonas* strain (RRLJ008) isolated from virgin soils was due to siderophore production, where as the disease suppression was due to the antibiotic substances (Boruah and Kumar, 2002). Application of *P. fluorescens* have been found efficient in promoting the growth of black pepper, which resulted increased number of nods and consequently the number of cuttings (Anandaraj *et. al.*, 2003).

In one of the experiments, Ramamoorthy *et. al.* (2002) found that compared to *P.putida* , *P.fluorescens* increased plant growth in tomato and hot pepper. Nautiyal (2003) found that application of fluorescent *Pseudomonas* enhances the germination and yield of chickpea. In another study, Pal *et. al.* (2003) found that application of *Pseudomonas* enhances plant growth, yield and nutrient uptake. Similarly, Paul *et. al.* (2003) reported that the treatment with *P. fluorescens* resulted in enhanced plant vigor due to the increased nutrient mobilization in the rhizosphere of black pepper. Fluorescent pseudomonads have significant effect on growth and root development of black pepper (Sivaprasad *et. al.*, 2003). Joseph *et. al.* (2003) reported the production of growth promoting substances like indole acetic acid (IAA) and gibberllins by PGPR in *Hevea brasiliensis. P. fluorescencs* significantly increased the growth and biomass production of crop. Thomas and Vijayan (2003) reported that seed coating with and *P. fluorescens* had resulted in increased seed germination and enhanced growth and vigor as expressed by increase in seedling height, root length and leaf area of cardamom seedlings. Sendhilvel *et. al.* (2005) revealed that *P. fluorescens* strain $SVPF_2$ treatment on cowpea seeds increased the germination per cent and vigour index compared to untreated control. According to Paul and Sarma (2005), *P. fluorescens* strains (IISR-6, IISR-8, IISR-11,IISR-13 and IISR-51) could significantly increase the root biomass and root length in treated black pepper. They concluded that the enhanced growth parameters

on root bacterization could be corroborated with the production of plant growth hormones IAA, gibberllic acid produced by these bacterial strains and their phosphate solubilization potential. Shahida (2007) reported that inoculation of PGPM like *Pseudomonas* and *Trichoderma* in vanilla nursery increased the plant biomass.

### 2.1.2 Effect of *Bacillus* sp.

Several species of bacterial genus *Bacillus* have been found predominantly in the rhizosphere of various crops. The resistant endospores that the *Bacillus* spp. provide tolerance to heat and cold as well as to pH extremes, pesticides, fertilizers and storage. The growth promoting activity of *B. subtilis* had been demonstrated by Podile and Dube (1988) on cotton, cucumber, pigeon pea and eggplant. This strain when used as seed treatment increased the yield of carrots by 48%, oats by 33% and peanuts up to 37%. In addition, application of some *Bacillus* spp. has shown increased grain yield and plant biomass accumulation (Broadbent *et. al.*, 1997).Bochow *et. al.* (1999) studied the mode of action of root colonizing *B. subtilis* and showed an increased level of phytohormones and significantly increased activity of beta 1,3-glucanase.Classical phytohormones such as IAA, IBA and Kinetin were also proved. Madhaiyan (1999) found that *B. subtilis* were effective in improving shoot and root dry weight as well as P content in vanilla. Sing *et. al.* (2000) reported increased yield in potato variety Kufri Jyothi when tubers were dipped in *Bacillus* spp. suspension before planting. According to Vivek *et. al.* (2001) inoculation of *Bacillus* significantly increased the growth and yield attributes of potato. They reported the increase in yield and height can be ascribed to production of hormones like IAA, gibberellins and vitamins like biotin folic acid and B group vitamins. Experiments conducted by Sood and Sharma (2001) indicated that *B. subtilis* increased the potato tuber yield from 115 to 268 quintals/hectare and this was at par with 100 per cent NPK treatment. Amruthesh *et. al.* (2003) evaluated seven plant growth promoting *Bacillus* isolates for their efficiency of growth promotion and disease management. *In vitro* analysis of seed germination and vigor index identified enhancement of growth. Assessment under green house condition identified

enhancement in emergence rate, germination per cent age, height, fresh and dry weight of the plants raised from seeds treated with PGPR isolates.

A study by Niranjan *et. al.* (2003) revealed that *B. subtilis* IN937b and other *Bacillus* spp. as fresh suspension or powdered formulation significantly enhanced the germination rate of pearl millet seeds compared with untreated controls. Ryu *et. al.* (2003) reported that extracts of the volatiles produced by PGPR strains *B. subtilis* GBO3 and *B. amyloliquefaciens* IN937a induced plant growth promotion at a level similar to that induced by other PGPR strains. Samiyappan (2003) reported that commercial formulation containing *B. subtilis* and *B. amyloliquefaciens* and chitin as carrier was found to be effective for increasing the growth of tomato, tobacco, cucumber and pepper. Sattar *et. al.* (2003) reported that the *B. subtilis* treatment increased the survival of geranium cuttings, initiated early root production and produced higher growth and herb yield. Seed coating with *B. subtilis* had resulted in increased seed germination and enhanced growth and vigor as expressed by increase in seedling height, root length and leaf area of cardamom seedlings (Thomas and Vijayan, 2003).Sunaina and Ajay (2005) reported a large and heavily branched root system in potato plants arising from PGPR (*B.subtilis*) treated experiment. This led to improved uptake of water and nutrients. Gopal *et.al.*(2006) found that inoculation of *Bacillus* APb-1 with other growth promoting bacteria recorded the maximum growth and yield in Ashwagandha.

### 2.1.3 Effect of *Trichoderma* sp.

*Trichoderma* is a well known biocontrol agent, in addition to that it has been implicated to promote plant growth. Baker *et al* (1984) first reported growth promotion of radish in soil by application of *T. harzianum* and *T. viride.* Increased growth by *Trichoderma* spp. was induced by diffusible growth regulating factors produced by these organisms (Windham *et. al* , 1986), increased uptake of nutrients by plant through nutrient mobilization or the control of one or more pathogen (Chang *et.*

*al.*, 1986). Shoots grown in *T.harzianum* inoculated soils were found to be better than that grown in uninoculated field (Windham *et. al.*, 1989).

Vrang *et. al.* (1990) noticed increase in growth and yield of potato when the potato seed tubers were inoculated with *Trichoderma* spp. Sarma *et. al.* (1996) observed that solarized nursery mixture fortified with mycorrhizal propagules in combination with a mixture of *Trichoderma* spp. and *Gliocladium* sp.yielded healthy and robust rooted cuttings of black pepper in nursery. Cruz and Cisterna (1998) found that *T.harzianum* significantly increased the seed germination in *Capsicum annum*. Root length, root dry weight, plant height, leaf number etc. were also increased. Madhaiyan (1999) studied the effect of *Trichoderma* in vanilla and he found that *T. viride*. Increased the shoot and root dry weight over control.

According to Binimol (2000) and Harman (2000), *Trichoderma* spp. can increase the rate of plant growth and development and also produced more robust roots. But, Silva *et. al.* (2000) observed that *T.harzianum* strain T22 reduced the growth of blueberry in pasteurized soil. Lisha *et. al.* (2002) reported that the *Trichoderma* isolates obtained from black pepper rhizosphere showed increased growth promotion by 55-116% as compared to control. In addition to their role as a biocontrol agent, (Sivaprasad,2002) reported that *Trichoderma* spp. will promote plant growth . Application of rhizobacteria and *T. harzianum* was efficient in promoting the growth of black pepper, which resulted in increased number of nodes and consequently the number of cuttings (Anandaraj *et. al,* 2003). Manimala (2003) recorded that treatment of chilli with *T.viride* increased all biometric characters by recording maximum shoot and root length, number of leaves, fresh and dry weight of plant and fruit weight. Vijayaraghavan (2003) also observed that inoculation of *T. viride* in solarized potting mixture increased the height and number of leaves of pepper cuttings in nursery. Shahida (2007) reported that *T.harzianum* gave higher per cent of germination and plant biomass of vanilla cuttings in nursery.

### 2.1.4. Effect of orchid mycorrhiza

Mycorrhiza in vanilla roots were first recorded by Decordenoy (1904), who observed the infection of fungi on the roots adhering to their living supports and suggested that roots obtained their nutrients *via*; the fungus. The mycorrhizal association is reproduced by Tonnier (1954) in flasks by growing vanilla seedlings in agar with *Rhizoctonia*. Warcup and Talbot(1967) found *R repens* as endomycorrhizal fungi on many orchidaceous plants. Mycorrhizas are highly evolved, symbiotic associations between soil fungi and plant roots. The mycorrhizal fungi play an important role in the life cycle of plants of the family Orchidaceae. Smith (1966) observed that orchid mycorrhizal fungi like *Rhizoctonia repens* and *R. solani* were able to utilize and translocate carbohydrates to the orchid plants and there by increasing the plant growth. Numerous isolates of form genus *Rhizoctonia* have been obtained from orchid roots grown in culture and shown to establish a mycorrhizal relationship when allowed to infect germinating orchid seeds (Warcup, 1975). The orchid *Spiranthes sinensis* (Persoon) Ames Var. Amoena (M. bieberstein) Hara is widely distributed in Japan . Alexander and Hardley (1983) has shown that mycorrhizal infections in the orchid *Goodyera repens* bring about enhanced growth rate and increased P concentration within tissues. Orchid mycorrhiza showed improved resistance to draught and environmental stress (Allen and Boosalls, 1983). Terashita(1982) and Masuhara and Katsuya (1992) observed that this adult orchid form mycorrhizal association often with *R. repens* and occasionally with *R. solani.*

Ogoshi *et. al.* (1983) reported that some isolates of binucleate *Rhizoctonia* induce symbiotic germination of some of the Australian orchids. Warcup (1983) has shown that some *R. solani* isolates induced symbiotic germination of seeds of *Microtis unifolia, Prasophyllum regiium* and *Orchis moria*. Abbot and Robson (1984) reported that mycorrhizal inoculation increased the growth and yield of different crop plants by improving P uptake and trace elements particularly when these nutrients are sparingly soluble in soil. It was also showed that orchid mycorrhizal fungi improved

nitrogen nutrition in orchid plants by facilitating the use of certain nitrogen forms that were difficult for the non mycorrhizal plants to exploit (Press, 1986 ).

Association of orchid mycorrhiza and arbuscular mycorrhizal fungi in vanilla was studied in detailed by Madhaiyan (1999).He reported that inoculation of orchid mycorrhiza and AMF significantly increased the shoot and root dry weight of vanilla. There was a significant increase in shoot and root length of vanilla seedlings due to the inoculation of orchid mycorrhizal fungi, AMF or combination of both over control. Madhaiyan *et. al.* (2001) observed that inoculation of orchid mycorrhizal fungi, *R. solani* msk-01 was superior in increasing the colonization per cent age, followed by *R. repens* msk-02 , than uninoculated control. In addition, significant increase in the total carbohydrate, starch content, plant nutrient content (N, P, K) and phosphatase activity (acid and alkaline phosphatase) due to inoculation of *R.solani* and *R. repens.* Madhaiyan *et.al.* (2003) observed the infection of orchid mycorrhiza and peleton formation in the cortical cells, particularly in the outer cortical cells, particularly in the outer cortical cells of vanilla seedlings inoculated with orchid mycorrhiza.

### 2.1.5 Effect of PGPM consortia on plant growth

Benefits of using combination of antagonists have been emphasized by Baker and Cook (1974). Sidorenko *et. al.*(1996) reported that combined inoculation of *Azotobacter, Bacillus* and *Pseudomonas* increased plant height, biomass and tuber yield in potato. Combination of *B.subtilis* along with *Azospirillum* increased the root and shoot length significantly over *Azospirillum* alone (Sankar and Jayarajan,1996).The consortium approach for growth enhancement in plantation crops and spices was suggested by Sarma and Anandaraj (1998).Contradictory to this Chiarini *et. al.*(1998) reported that dual strain inoculation (*Burkholderia cepacia* and *P.fluorescens*) on sorghum did not have any significant effect on plant growth in contrast to the separate inoculation of both strains. He also reported that establishment of large populations of bacterial inoculants on root did not appear to be essential for plant growth promotion.

Kamble *et. al.*(2000) found that individual inoculation of seeds with *Azospirillum, P.fluorescens* and phosphobacteria were more effective than combined inoculation. Sarma *et. al* (2000) has established the biocontrol consortium for black pepper, ginger and cardamom.

The result of the study of Jetiyanon and Kloepper (2002) on mixtures of PGPR for induction of systemic resistance against multiple plant diseases showed that mixtures of PGPR provided greater disease suppression than the individual strains. The *T.harzianum* (IISR1369) and *P fluorescens* (IISR6) treatment combination obtained the maximum disease suppression in black pepper and cardamom. The mutual compatibility of *T.harzianum* and fluorescent *pseudomonas* were studied in order to establish efficient consortium for the management of foot rot of black pepper cost by *P. capsici* (Jisha *et. al.,* 2002). The study revealed that the fungal and bacterial antagonists are compatible.

Field trials with formulations of several PGPR *viz.* LS213 (*B.subtilis* strain GBO3+*B.amyloliquifaciens* strain IN937a), LS254(*B.subtilis* strain GBO3+*B.pumilus* strainSE34),LS255(*B.subtilis* strain GBO3+ *B.subtilis* strain IN937b),LS256(*B.subtilis* strain GBO3+*B.pumilus* strainINR7) and LS261(*B.subtilis* strain GBO3+*B.cereus* strain C4) showed significant increase in tomato and pepper growth. Yield of pepper and tomato also increased (Burelle *et. al.*,2002).

Anandaraj and Sarma (2003) reported that five fluorescent *Pseudomonas* strains when used in combination were showed a synergistic effect in growth and disease control. Karunakaran *et. a*l.(2003) reported that under glass house condition, the treatment combinations *P.fluorescens*+ *B.subtilis* and *P.fluorescens* + *T.viride* were found to be the best for growth of crop plants. Similarly Gopal *et. al.* (2006) reported that the *P fluorescencs* Aps-1in combination with other growth promoting rhizobacteria recorded the maximum growth, fruit, seed and alkaloid yield of Ashwagandha pot culture experiment was carried out by Hemavathi and Navi (2006) to study the effects of inoculation with *G.fasciculatum* and PGPR namely, *B.megatherium* and *P.fluorescens*

on growth and biomass of *Ocimum* under glass house condition. Single and dual inoculation increased the growth and biomass, compared to uninoculated plants. The consortium of all the three organisms was found superior in enhancing plant height, number of branches, herbage yield and P content.

## 2.2 COMPATIBILITY STUDIES

### 2.2.1 Mutual compatibility of *Trichoderma* spp. with rhizobacteria

The advantage of using combination of antagonists ontrol has been emphasized by Baker and Cook (1974). Sarma and Anandaraj (1998) suggested the consortium approach for disease management in plantations and spice crops. Sarma *et. al.* (2000) has established the biocontrol consortium for black pepper, ginger, and cardamom. The maximum disease suppression obtained by treatment combination, *T.harzianum* (IISR 1369) and *P.fluorescens* (IISR 6) in black pepper and cardamom. The mutual compatibility of *T. harzianum* and fluorescent *Pseudomonas* were studied in order to establish efficient consortium for the management of foot rot of black pepper caused by *P. capsici* (Jisha *et. al.,* 2002). The study revealed that the fungal and bacterial antagonists are compatible.Compatibility of *T. viride* with *Azospirillum* under *in vitro* had been proved by Sankar and Jayarajan (1996). They also noted that *Azospirillum* did not inhibit the antagonists under *in vivo* condition and there was a cumulative effect in disease reduction. Shahida (2007) also reported that *Trichoderma* and *P. fluorescens* were compatible with each other.

### 2.2.2 Mutual Compatibility of *Bacillus* sp. and *Pseudomonas* sp.

Compatibility of *Bacillus subtilis* with *Azospirillum* under *in vitro* and *in vivo* had been proved by Sankar and Jayarajan (1996). Sidorenko *et. al.*(1996) reported that combined inoculation of *Azotobacter, Bacillus* and *Pseudomonas* increased plant height, biomass and tuber yield in potato. Sarma *et. al* (2000) has established the biocontrol consortium for black pepper, ginger and cardamom Five bacterial strains which had been proved efficient in suppressing *P. capsici* were studied and found that there was synergistic effect when the strains were used in combination (Anandaraj and Sarma, 2003). In the annual report of KSCSTE project (2007), they showed that *P. fluorescens* and *Bacillus* sp and *P. fluorescens* and *Trichoderma* sp isolated from vanilla rhizosphere are mutually compatible.

Contradictory to the above report, Kamble *et. al.* (2000) found that there was no compatibility between *Azospirillum*, *P.fluorescens* and phosphobacteria selected.

### 2.2.3 Effect of plant protection chemicals on *Pseudomonas* spp.

Elkins and Lindow(1999) reported that the population of *P.fluorescens* A 506 was reduced to 50 per cent when tank was mixed with Terramycin. The fungicide Mancozeb was also found to reduce the population even more than Terramycin.However, Terramycin and Mancozeb had no detrimental effect on *P.fluorescens* A 506 when applied at least five days before or after *P.fluorescens* A 506 application. Positive regulation of *P.fluorescens* by Carbendazim was studied by Guang *et. al.* (1999). The studies revealed that *P.fluorescens* strain $P_{32}$ was not sensitive to Carbendazim *in vitro* . Fungicides Metalaxyl and Copper oxychloride were found to be compatible with *P.fluorescens* (Sabet *et. al.*,2000).

Anandaraj and Sarma (2003) found that the fluorescent *Pseudononas* strains IISR-8, IISR-11 and IISR-51 were compatible with Metalaxyl and Mancozeb .Similarly, Joseph *et. al.* (2003) reported that *P.fluorescens* was compatible with hexaconazole and Mancozeb and hence suitable for combined application. Bhavani (2004) found that potassium phosphonate and lowest concentration of Kocide did not produce inhibition zone in growth of *P.fluorescens* while Bordeaux mixture and Copper oxychloride at all concentrations inhibited the growth of the bacterium. Study by Priya (2005) revealed that Bordeaux mixture at all concentrations completely inhibited the growth of *P.fluorescens,* while Copper hydroxide and Copper oxychloride at different concentrations showed varying percentage of inhibition. It was noticed that as the concentration of fungicides increased, there was an increased inhibition of *P.fluorescens.* In this study, Copper hydroxide was found to be less inhibitory against *P.fluorescens* compared to Copper oxychloride and Bordeaux mixture.

### 2.2.4 Effect of plant protection chemicals on *Bacillus* spp.

A study by Kim *et. al.* (1988) revealed that Metalaxyl had no effect on *Bacillus* strain AC-1, whereas, Metalaxyl and Copper fungicides in combination inhibited the growth. The result of an experiment conducted by Laha and Venkataraman (2001) showed that *Bacillus* spp. B-44 was compatible with Carbendazim at 500 and 1000ppm concentrations. Guven *et. al.*(2003) reported that the alpha- amylase test system in *B.subtilis* can detect the inhibition by the organometallic fungicides, Maneb and Mancozeb at as low as 0.1ppm. Bhattacharya *et. al.*(2004) reported that Monocrotophos, Azhdirachtin, Imidachloprid and Carbaryl were found to be compatible with *B.subtilis, B.thuringiensis* and *Beauveria bassiana.*

### 2.2.5 Effect of plant protection chemicals on *Trichoderma* spp.

In integrated approach of disease management, Papavizas *et. al.* (1982) observed that biocontrol agents have been used with fungicides without any toxic effect

on antagonists. Use of some strains of *T. harzianum* tolerant to fungicides has also been reported for the integrated control of plant diseases (Papavizas and Lewis, 1981; Papavizas *et. al.,* 1982; Upadhyay and Mukhopadhey, 1986).

Kay and Stewart (1994) observed *T. harzianum* C52 was insensitive to thiram and mancozeb. *T. harzianum* was inhibited to 63 Per cent with 500 ppm mancozeb after 3 days of incubation (Singh *et al.*, 1995). *Trichoderma* spp. gave good growth at lower and medium concentration of Capron and no growth with systemic fungicides like Carbendazim and Benomyl (Ortiz Molinerevo *et.al.*, 1966). Shanmugham (1996) found that Bordeaux mixture completely inhibited the growth of *T. viridae.* Paciulyte *et. al.*( 2000) found that *Trichoderma* spp. were more sensitive to copper oxychloride than copper sulphate. Complete inhibition of *Trichoderma* spp. was observed with Bordeaux mixture and higher percentage of inhibition was recorded with Kocide, Captaf and Kavach indicating incompatibility of these fungicides with *Trichoderma* spp. (Vijayaraghavan, 2003). It was also observed that Indofil M-45 and potassium phosphonate were compatible with *Trichoderma* (Bhavani, 2004).

The compatibility of *Trichoderma* spp. with metalaxyl, mancozeb and potassium phosphonate were reported by many workers (Moeity *et. al.,* 1982; Wongwathanarat and Sivasithamparam, 1997;Shanmugham, 1996; Rajan and Sarma, 1997; May and Kimati, 2000;Akbari and Parakhia, 2001). Rajan *et. al.* (2002) studied the compatibility of eight species of *Trichoderma* with potassium phosphonate and it was revealed that there was no significant effect even at higher concentration. They also observed the increased sporulation over control in the case of *T. aereoviride* and *T. pseudokoningii* with potassium phosphonate.Pandey *et. al.* (2006) found complete inhibition of *Trichoderma* spp. occurred by tebuconazole and hexaconazole showing extremely toxic nature of the fungicides.Shahida (2007) found that among different fungicides, Bordeaux mixture at all concentrations completely inhibited the growth of *T. harzianum* and *T. viride.* While copper hydroxide, copper oxy chloride and mancozeb at different concentrations showed variation in percentage of inhibition. It was found that

both *Trichoderma harzianum* and *Trichoderma viride* were compatible with potassium phosphonate at three different concentrations tested.

# *Materials and methods*

# 3. MATERIALS AND METHODS

The present study on "Induction of growth promotion in vanilla through plant growth promoting microorganisms consortia" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara.

## 3.1 ISOLATION OF PLANT GROWTH PROMOTING MICROORGANISMS

### 3.1.1 Collection of rhizosphere soil samples

Rhizosphere soils of three plants were collected from six locations of Thrissur and Wynad districts. Collected soil was mixed, air dried and then sieved through two mm sieve and filled in polythene bags and used for isolation of plant growth promoting microorganisms. The pH of the soil was also studied.

### 3.1.2 Isolation of plant growth promoting microorganisms

Selected plant growth promoting microorganisms (PGPM) like *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were isolated from the soil by adopting standard protocol (Johnson and Curl, 1972).From each location, predominant bacterial colonies representing typical characters of *Pseudomonas*, *Bacillus* and *Trichoderma* were selected, purified and maintained for further studies. The orchid mycorrhiza was isolated from the roots of vanilla as suggested by Clements. (1986).

#### *3.1.2.1 Isolation of Pseudomonas sp. and Bacillus sp.*

Ten gram of the soil was added separately to 90 ml of sterilized water in conical flask, shaked well and serial dilutions were prepared up to $10^{-8}$dilution. From $10^{-8}$

dilution, one ml was transferred to sterile Petri dishes plated with King's B medium (Appendix I ) and nutrient agar medium (Appendix II ) separately, for *Pseudomonas* sp. and *Bacillus* sp. respectively. The Petri dishes were incubated at 28±2°C for 72 h. Bacterial colonies showing typical cultural characters of *Pseudomonas* sp. and *Bacillus* sp. were selected, purified and maintained for further studies.

### *3.1.2.2 Isolation of Trichoderma sp.*

Serial dilution was prepared as mentioned above, up to $10^{-3}$. From $10^{-3}$ dilution, one ml was transferred to media plated with Martin's Rose Bengal Streptomycin Agar medium (Appendix III). The fungal colonies representing typical characters of *Trichoderma* sp. were transferred to Potato Dextrose Agar (PDA) medium (Appendix IV). Pure cultures of fungi were obtained by hyphal tip isolation method and maintained in PDA slants for further studies.

### *3.1.2.3 Isolation of orchid mycorrhiza*

Orchid mycorrhiza was isolated from roots of vanilla. The roots were soaked in sterile water for three hours and washed thoroughly for removing soil on the root sample. The cleaned roots were cut into segments of two to three centimeter, disinfected with 70 per cent ethyl alcohol and then washed in three changes of sterilized water and placed in Petri dishes containing PDA (Appendix IV) as well as fungal isolating media (FIM) (Appendix V) and incubated in the dark for a period of seven days.

## 3.2 IDENTIFICATION OF BACTERIAL ISOLATES

For the Identification of bacterial isolates cultural and morphological characters were studied.

### 3. 2.1 Cultural characters

Cultural characters like colour of colony, shape of cells, and sliminess of *Pseudomonas* sp. and *Bacillus* sp. were studied by growing them in King's B medium and NA respectively for 48 h. Endospore staining for *Bacillus* sp. was done. Pigment production by *Pseudomonas* sp. was studied on King's B medium and observed under UV light.

### 3. 2. 2 Morphological characters

Huckers' modification of Gram staining was carried out (Hucker and Conn, 1923) to study the Gram reaction and shape of cells of *Pseudomonas* sp. and *Bacillus* sp.

### 3.2.3 Identification of *Trichoderma* sp.

Cultural and morphological characters of the efficient *Trichoderma* were studied and the characters were compared with description for *Trichoderma*. Cultural characters like growth, colour of colony and pigmentation were studied by growing them in dishes plated with PDA. Morphological characters were studied by slide culture technique. Observations were made on shape and size of hyphae, conidiophore ,shape and colour of spores.

## 3.3 PRODUCTION OF INDOLE ACETIC ACID BY PGPM

A loopful of 24 h old bacterial cultures of *Pseudomonas* sp., *Bacillus* sp. and mycelial disc of 6 mm diameter *Trichoderma* sp. were inoculated into LB (Luria Bertani) (Appendix VI) medium and incubated for 24 h on rotary shaker at 28±2°C. After centrifugation at 10,000 g for 15 minutes, two ml of the supernatant was taken and three drops of Ortho-Phosphoric acid were added to the aliquot. It was mixed and four

ml of reagent (one ml of 0.5 M $FeCl_3$ in 50 ml of 35 per cent $HClO_4$) was added and incubated at room temperature for 25 minutes. Then, the absorbance was measured using spectrophotometer at 530 nm against a reagent blank. Standard curve was prepared using different concentrations of IAA (Gordon and Weber, 1951).

## 3.4 SALICYLIC ACID PRODUCTION BY PGPM

A 100µl of 24 h old bacterial cultures of *Pseudomonas* sp., *Bacillus* sp. and mycelial disc of 6 mm diameter *Trichoderma* sp. were inoculated into 25 ml of Casaminoacid broth (Appendix VII) and incubated for 36 h at 200 rpm in dark at 34°C. Then, the ethyl acetate extract (1:3 of cultures in Casaminoacid broth and ethyl acetate) of the cultures were taken and concentrated under vaccum. The salicylic acid concentration was determined by adding 5µl of 2M $FeCl_3$ and three ml of water to one ml of concentrated extract. The absorbance of the purple iron- salicylic acid complex, which developed in the aqueous phase was measured using spectrophotometer at 527nm and compared with a standard curve of salicylic acid dissolved in ethyl acetate (Meyer *et. al.*, 1997).

## 3.5 EFFECT OF *Pseudomonas* sp. AND *Bacillus* sp. ON SEED GERMINATION

Selected *Pseudomonas* sp. and *Bacillus* sp. isolates were bioassayed for their ability to promote seed germination and growth using the method as described by Shende *et. al.* (1977) and Elliot and Lynch (1984) with slight modifications. The seeds (cowpea and sorghum) were surface sterilized with 0.1 per cent mercuric chloride for three minutes separately and washed with three changes of sterilized water.Fourty ml of 48 h old bacterial suspensions were made having $10^{-8}$ cfu and 30 seeds of cowpea and sorghum were added separately to these suspensions. Seeds were kept for 30 minutes in the *Pseudomonas* sp. and *Bacillus* sp. suspensions and decanted. Sterilized Petri plates were poured with 0.8 per cent plain agar and seeds were then placed on the agar plates and incubated at 28±2°C for three days. Three replications were maintained for each

isolate. Seeds treated with sterilized distilled water served as control. Three days after incubation per cent germination, root length and shoot length were recorded.

## 3.6 *IN VITRO* EVALUATION OF MUTUAL COMPATIBILITY AMONG SELECTED PGPM ISOLATES

### 3.6.1 *In vitro* evaluation of mutual compatibility of *Pseudomonas* sp. and *Bacillus* sp.

Mutual compatibility of *Pseudomonas* sp. and *Bacillus* sp. were carried out by cross streaking technique (Oubois *et. al.* 1979). Fourty eight hour old culture of each *Pseudomonas* sp. and *Bacillus* sp. were streaked perpendicular to each other on the mediated plates. The plates were then incubated at 28±2°C and observed daily for the lysis of growth at the juncture point of two isolates for 72 h. Lysis at the juncture point indicated incompatibility.

### 3.6.2 *In vitro* evaluation of mutual compatibility of *Pseudomonas* sp. and *Trichoderma* sp.

Mutual compatibility of *Pseudomonas* sp. and *Trichoderma* sp. studied by dual culture technique on PDA medium. 48 h old cultures of each *Pseudomonas* sp. were streaked parallelly on either side of the plate at one cm away from periphery. The plates were then co- inoculated at the centre with 6 mm diameter mycelial disc of *Trichoderma* sp. Plates inoculated with fungus only served as control. The plates were then incubated at 28±2°C and observed daily for growth. The over growth of *Trichoderma* sp. over *Pseudomonas* sp. and the growth of *Pseudomonas* sp. indicated compatibility.

### 3.6.3 *In vitro* evaluation of compatibility of *Bacillus* sp. with *Trichoderma* sp.

Mutual compatibility of *Bacillus* sp. and *Trichoderma* sp. were studied by dual culture technique as mentioned above. Plates inoculated with fungus only served as

control. The plates were then incubated at 28±2°C and observed daily for growth. The over growth of *Trichoderma* sp. over bacterial culture indicated compatibility.

## 3.7 COMPATIBILITY OF PGPM WITH FUNGICIDES AND INSECTICIDES

Studies on the compatibility of selected PGPM with commonly used fungicides and insecticides in vanilla cultivation were done (Baker and Cook ,1974). Details of the fungicides and insecticides and their concentrations used are given below.

### 3. 7. 1 Compatibility of selected fungicides and insecticides with *Pseudomonas* sp. and *Bacillus* sp.

Sterile filter paper discs of 6 mm diameter were dipped for 10 minutes in various fungicides and insecticides, having different concentrations separately. The discs were placed at the centre of dishes containing King's B medium and NA medium seeded with 48 h old culture of *Pseudomonas* sp. and *Bacillus* sp. Control plates consisted of filter paper dipped in sterile water. Three replications were maintained for each concentration. The inoculated plates were incubated at room temperature and the observations on inhibition zone were recorded after 48 h.

### 3.7.2 Compatibility of selected fungicides and insecticides with *Trichoderma* sp.

Quantity of selected fungicides and insecticides needed to get the desired concentration was added to 100 ml of sterilized molten PDA medium, mixed well and poured in to sterilized dishes @ 20 ml per plate. After solidification of the medium, mycelial disc of six mm diameter from actively growing 72 h old culture of *Trichoderma* sp. was cut and placed at the centre of each Petri dish. Inoculated media without chemicals served as control. Three replications were maintained for each concentration. The inoculated plates were incubated at 28±2°C and observations on

mycelial growth of *Trichoderma* sp. were taken when control plates showed full growth of *Trichoderma* sp.

**Table. 1 Fungicides used for compatibility studies with *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. under *in vitro* condition**

| Sl. No. | Chemical name | Trade name | Concentrations (Per cent) |
|---|---|---|---|
| 1 | $CuSO_4$+ Lime+water | Bordeaux mixture | 0.5, 1.0, 1.5 |
| 2 | Copper oxy chloride | Fytolan 50 WDP | 0.1, 0.2, 0.3 |
| 3 | Copper hydroxide | Kocide 77 WP | 0.1, 0.2, 0.3 |
| 4 | Potassium phosphonate | Akomin 40 | 0.1, 0.2, 0.3 |
| 5 | Mancozeb | Indofil M- 45 | 0.1, 0.2, 0.3 |

**Table .2 Insecticides used for compatibility studies with *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. under *in vitro* condition**

| Sl. No. | Chemical name | Trade name | Concentrations (ai/lit) |
|---|---|---|---|
| 1 | Carbaryl | Sevin 50 WDP | 0.1, 0.2, 0.3 |
| 2 | Chlorpyriphos | Tafaban 20% EC | 4, 5, 6 |
| 3 | Lambda cyhalothrin | Reeva 5% EC | 4, 5, 6 |

## 3.8 SCREENING OF PLANT GROWTH PROMOTING MICROORGANISMS CONSORTIA FOR ENCHANCING THE GROWTH OF VANILLA

Experiment was laid out in the net house of Dept. of Plant Pathology, College of Horticulture, Vellanikkara. Six isolates of *Pseudomonas* sp., *Bacillus* sp. and

*Trichoderma* sp. isolated from Chengaloor ,Mundoor, Vellanikkara,Ambalavayal, Kolvayal and Sultan Bathery, which were found compatible in *in vitro* studies were selected for the experiment. The potting mixture of soil: sand: dried cow dung (1:1:1) was sterilized by autoclaving and filled in polybags of 6" ×8" inch @ 1.5 kg / bag. Three nodded vanilla cuttings were planted in each polybags. Then the isolates were applied individually as well as in consortia from each location. Bacterial suspensions of *Pseudomonas* sp. and *Bacillus* sp. @ 15 ml per polybags and *Trichoderma* sp. grown on rice bran were incorporated @ 3g per polybags. The potting mixture without inoculation of isolates served as control. Commercial isolates of *P.fluorescence* and *T.viride* were also used for comparison. The experiment design followed was CRD and three replications were maintained for each treatment. The plants were irrigated daily with boiled cooled water.

**Treatment details**

Design : CRD

Number of Treatments : 27

Replications : 3

$T_1$ : $P_1$ (Chengaloor)

$T_2$ : $P_2$ (Mundoor)

$T_3$ : $P_3$ (Vellanikkara)

$T_4$ : $P_4$ (Ambalavayal)

$T_5$ : $P_5$ (Kolvayal)

$T_6$ : $P_6$ (Sulthan Bathery)

$T_7$ : $B_1$ (Chengaloor)

$T_8$ : $B_2$ (Mundoor)

$T_9$ : $B_3$ (Vellanikkara)

$T_{10}$ : $B_4$ (Ambalavayal)

$T_{11}$ : $B_5$ (Kolvayal)

$T_{12}$ : $B_6$ (Sulthan Bathery)

$T_{13}$ : $Tr_1$ (Chengaloor)

$T_{14}$ : $Tr_2$ (Mundoor)

$T_{15}$ : $Tr_3$ (Vellanikkara)

$T_{16}$ : $Tr_4$ (Ambalavayal)

$T_{17}$ : $Tr_5$ (Kolvayal)

$T_{18}$ : $Tr_6$ (Sulthan Bathery)

$T_{19}$ : $C_1$ (Chengaloor consortia)

$T_{20}$ : $C_2$ (Mundoor consortia)

$T_{21}$: $C_3$ (Vellanikkara consortia)

$T_{22}$: $C_4$(Ambalavayal consortia)

$T_{23}$ : $C_5$ (Kolvayal consortia)

$T_{24}$: $C_6$ (Sulthan Bathery consortia)

$T_{25}$ : Pf (Commercial)

$T_{26}$ : Tv (Commercial)

$T_{27}$ : Control

In the consortia treatments isolates were applied in the same dose as individual treatments. The treatments were given at the time of planting only.

## 3.9 BIOMETRIC OBSERVATIONS

### 3.9.1 Number of days taken for sprouting

Number of days taken for sprouting of vanilla cuttings were recorded 30 days after planting up to six months of planting.

### 3.9. 2 Length of vine

Extension growth of sprout were first recorded one month after planting and there after at monthly intervals for a period of six months. Length of sprout was taken from the base of sprout to tip using a meter scale and expressed in centimeter.

### 3.9. 3 Number of leaves

Number of leaves in the sprout was recorded at monthly intervals from one month after planting for a period of six months.

### 3.9. 4 Girth of vine

Girth of vine at the base of sprout was measured from two months of planting at monthly interval for a period of six months.

### 3.9. 5 Internodal length

Internodal length was taken after the cutting had attained sufficient length after two months. It was taken as an average of three internodal length at monthly interval up to six months.

### 3.9. 6 Fresh weight of shoot

Fresh weight of shoot were recorded six months after planting by uprooting the plants and separating the shoot portion and expressed in grams.

### 3.9.7 Fresh weight of root

Fresh weight of roots were taken after six months of planting by uprooting the plants. The roots were separated, washed thoroughly, airdried and weight was recorded in grams.

### 3.9. 8 Length of root

Length of root were taken from the base to tip of longest root using a meter scale and expressed in centimeter.

### 3.9. 9 Dry weight of shoot

Dry weight of shoot were taken after drying the sample in an oven at 60°C till the constant weight was attained.

### 3.9. 10 Dry weight of root

Dry weight of root was taken after drying the sample in an oven at 60°C till the constant was attained.

## 3.10 ESTIMATION OF TOTAL PHENOL IN PLANT

One gram of the plant sample was taken and extracted in 10 ml ethanol (80 per cent) using mortar and pestle. The homogenate was then centrifuged at 10,000 rpm for 20 minutes and the supernatant was extracted. The residue was extracted with five ml ethanol (80 per cent) and the supernatants were pooled. The pooled supernatant was evaporated to dryness in water bath and the residue obtained was dissolved in five ml distilled water. From that sample, one ml of the aliquot was pipetted in to test tube and volume made up to 3ml with distilled water. To the pipetted sample, 0.5 ml Folin-Ciocateau reagent was added and after three minutes, two ml of $Na_2CO_3$ (20 per cent) was added and mixed thoroughly. The test tube is then placed in boiling water bath for one minute, cooled and the absorbance was measured at 650 nm against a reagent blank. Standard curve was prepared using different concentrations of Catechol.

## 3. 11 ENUMERATION OF PGPM

Microbial population of *Pseudomonas* sp. ,*Bacillus* sp. and *Trichoderma* sp. were enumerated at monthly intervals up to six months after planting on King's B media, nutrient agar (NA), and Martin's Rose Bengal streptomycin agar medium respectively using standard protocol (Johnson and Curl,1972).

## 3. 12 N, P AND K ANALYSIS

The N, P and K in the plant samples were analysed after six months of planting. For this, the whole plant was up rooted, washed-off soil particles and dried in an oven at 60°C till the constant weight was attained .After drying, samples were powdered and was used for the estimation of N,P and K using standard protocols as presented below.

| Nutrient | Digestion Procedure | Method of Estimation | Reference |
|---|---|---|---|
| Nitrogen | Sulphuric acid digestion | Micro Kjeldhal method | Jackson (1964) |
| Phosphorous | $HNO_3$:$HCLO_4$(2:1) Diacid digestion | Vanadomolybdate yellow colour method (Spectrophotometer) | Jackson (1964) |
| Potassium | $HNO_3$:$HCLO_4$(2:1) Diacid digestion | Direct reading (Flame photometer) | Jackson (1964) |

## 3.13 IDENTIFICATION OF MOST EFFECTIVE NATIVE ISOLATES FROM VANILLA RHIZOSPHERE

Based on the above experiment, the most promising native isolates were identified based on the biochemical characters.

### 3.13.1 Biochemical Tests

For biochemical characterization of *Pseudomonas* sp., Potassium hydroxide test, Catalase activity, Starch hydrolysis, Arginine dihydrolase reaction, Nitrate reduction test and Urease test were carried out while for *Bacillus* sp., Potassium hydroxide test, Catalase activity, Starch hydrolysis, Voges-Proskauer test and Glucose- Nutrient agar (one per cent) were done.

#### *3.13.1.1 Potassium hydroxide test (KOH)*

Loopful of bacterial cultures were spread on a clear glass slide. Two drops of three per cent KOH solution was placed over it and thoroughly mixed with the help of a needle. The bacteria as thin thread indicated gram negative bacteria.

#### *3.13.1.2 Catalase activity*

Loopful of bacterial cultures were spread on a clear glass slide. Two to three drops of $H_2O_2$ was placed over the culture and thoroughly mixed with the help of a needle. Appearance of air bubbles indicated positive catalase activity.

#### *3.13.1.3 Starch hydrolysis*

Loopful of bacterial cultures were spot inoculated on Petri dish containing nutrient agar with 0.2 per cent soluble starch. Starch hydrolysis was tested after 48 h of incubation by flooding the agar surface with Lugol's iodine solution. A colourless zone

in contrast to the blue background indicated positive starch hydrolysis (Cappucino and Sherman, 1992).

### *3.13.1.4 Arginine dihydrolase reaction*

Five ml aliquots of sterilized Thornley's semi solid medium (Appendix VIII ) were stab inoculated with *Pseudomonas* sp. The surface of the medium was sealed with sterile liquid paraffin to a depth of one cm. The tubes were incubated at 28±2°C and observed daily for seven days. A change in colour of the medium to red indicated arginine hydrolase activity (Thornley, 1960).

### *3.13.1.5 Nitrate reduction test*

Sterilized nitrate broth medium (Appendix IX ) in test tubes were inoculated with 24 h old cultures of *Pseudomonas* sp. The test tubes were then incubated at 28±2°C and tested for the reduction of nitrate up to 15 days. The test was performed by adding few drops of Griess -Llosvay's reagent consisting of sulphanilic acid (0.8 per cent in 5 M acetic acid ) and dimethyl alpha-naphthyl amine(0.5 per cent in 5 M acetic acid ) to the nitrate broth culture. If no pink or red colour developed, it indicated that nitrate was present as such or reduced to ammonia and free nitrogen. Few Zinc crystals were added to ensure whether the negative reaction was due to the reduction of nitrate beyond the nitrite level. If the broth became pink or red, it indicated that the nitrate was present without reduction (Hayward *et. al*,1990).

### *3.13.1.6 Urease test*

A 90 ml aliquots of Christensen's urea agar medium (Appendix X ) was dispensed in 250 ml conical flasks and autoclaved. To each flask, 10 ml of 20 per cent sterilized urea solution was added and dispensed in sterilized test tubes in 5 ml quantities and slants were prepared. The tubes were inoculated and observations recorded

periodically. A change in colour of the medium from yellow to pink or red indicated urease production (Christensen, 1946).

#### *3.13.1.7 Voges – Proskauer test*

A 0.6 ml of alpha- naphthol solution (5 per cent in 95 per cent ethanol) and 0.2 ml of 40 per cent aqueous solution of KOH were added to one ml of *Bacillus* sp. culture in nutrient broth. The mixture was shaken for few minutes and allowed to stand for two hours. A crimson colour of the nutrient broth indicated positive reaction.

#### *3.13.1.8 Glucose -Nutrient agar (One per cent)*

The stabbing of the bacterial suspension was done in one per cent glucose-nutrient agar ((Appendix XI ). A thick brown, often rugose growth on the surface of the medium indicated positive.

### 3.13.2 Identification of *Trichoderma* sp

Cultural and morphological characters of the efficient *Trichoderma* were studied and the characters were compared with description for *Trichoderma*. Cultural characters like growth, colour of colony and pigmentation were studied by growing them in dishes plated with PDA. Morphological characters were studied by slide culture technique (Riddle,1950).Observations were made on shape and size of hyphae, conidiophore, length and breadth of phialide and nature shape, size and colour of spores.

# Results

# 4. RESULTS

The results of the studies on "Induction of growth promotion in vanilla through plant growth promoting microorganisms consortia" are presented in this chapter.

## 4.1 ISOLATION OF PLANT GROWTH PROMOTING MICROORGANISMS

### 4.1.1 Collection of rhizosphere soil samples

Rhizosphere soil samples were collected from major vanilla growing area of Thrissur and Wynad districts.

Table 3. Locations selected and pH of the soil samples collected

| District | Locations | pH of soil |
|---|---|---|
| Thrissur | 1. Chengaloor (CHL) | 5.5 |
| | 2. Mundoor (MDR) | 4.9 |
| | 3.Vellanikkara (VKA) | 4.9 |
| Wynad | 4. Ambalavayal (ABL) | 5.1 |
| | 5. Kolvayal (KVL) | 5.3 |
| | 6. Sulthan Bathery (SBY ) | 5.5 |

### 4.1.2 Isolation of plant growth promoting microorganisms

Selected plant growth promoting microorganisms (PGPM) like *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were isolated from the soil by adopting standard protocol (Johnson and Curl, 1972).

#### *4.1.2.1 Isolation of Pseudomonas sp. and Bacillus sp.*

Six *Pseudomonas* sp. and *Bacillus* sp. isolates were selected based cultural characters like colour of colony, and sliminess of the colony. The smooth, slimy colonies exhibiting fluorescence under UV light and dry, wrinkled and cream coloured colonies were also selected. They were purified and mass multiplied for further studies.

#### *4.1.2.2 Isolation of Trichoderma sp.*

Six *Trichoderma* sp. were isolated from each location and they were purified and mass multiplied for further studies.

#### *4.1.2.3 Isolation of orchid mycorrhiza*

The orchid mycorrhiza couldn't be detected in the root sample of vanilla.

## 4.2 IDENTIFICATION OF BACTERIAL ISOLATES

The bacterial isolates were identified based on their cultural and morphological characters.

### 4. 2.1 Cultural characters

The bacterial colonies which was smooth, slimy, exhibiting fluorescence under UV light and produces yellowish green pigments on King's B media was identified as *Pseudomonas* sp. and the dry, wrinkled and cream coloured colony which grew well on nutrient agar and showed central endospore was identified as *Bacillus* sp.

### 4. 2. 2 Morphological characters

Gram reaction and shape of the bacterial cells were also studied. *Pseudomonas* sp. was Gram negative rod and *Bacillus* sp. was Gram positive rod with central endospre.

### 4.2.3 Identification of *Trichoderma* sp.

The *Trichoderma* sp. was identified based on the cultural and morphological characters. The mycelial growth of fungi was rapid, light green to bright green, hyaline, smooth and septate. Chlamydospores were mostly globose, smooth and 6-12µm in diameter. Conidiophores were loose tuft and produced numerous side branches especially at the lower portion. Phialides consisted of whorls up to five, short, skittle shaped, narrower at the base and bulged at the middle and attenuated abruptly into sharp pointed neck. Phialides were 10-12 µm long and 3- 4 µm width. Phialospores were single and accumulate. They were sub globose or short ovoid, smooth, pale green, much darker in mass. Based on these characters the isolate was identified as *Trichoderma* sp.

## 4.3 PRODUCTION OF INDOLE ACETIC ACID BY PGPM

Production of indole acetic acid (IAA) by selected PGPM were tested and the results are given in the (Table 4, Fig.1).The maximum IAA production was by $T_6$ (0.099 mg $ml^{-1}$) followed by $T_{10}$ (0.077 mg $ml^{-1}$).Among *Trichoderma* sp., $T_{15}$ produced maximum IAA (0.064 mg $ml^{-1}$).The lowest rate of production of IAA was by $T_{13}$ (0.031

mg $ml^{-1}$).

Table.4 Evaluation of PGPM for indole acetic acid (IAA) and salicylic acid production

| Isolates | IAA (mg $ml^{-1}$) | Salicylic Acid ($\mu g\ ml^{-1}$) |
|---|---|---|
| $T_1$ | 0.054 | 12.7 |
| $T_2$ | 0.063 | 11.6 |
| $T_3$ | 0.063 | 22.2 |
| $T_4$ | 0.064 | 9.2 |
| $T_5$ | 0.057 | 27.9 |
| $T_6$ | 0.099 | 14.5 |
| $T_7$ | 0.071 | 11.2 |
| $T_8$ | 0.068 | 19.5 |
| $T_9$ | 0.067 | 15 |
| $T_{10}$ | 0.077 | 14.9 |
| $T_{11}$ | 0.064 | 20.2 |
| $T_{12}$ | 0.070 | 5.8 |
| $T_{13}$ | 0.031 | 11.2 |
| $T_{14}$ | 0.050 | 12.6 |
| $T_{15}$ | 0.064 | 13.4 |
| $T_{16}$ | 0.042 | 21.7 |
| $T_{17}$ | 0.047 | 11.6 |
| $T_{18}$ | 0.044 | 20.3 |
| PF (com) | 0.056 | 10.7 |
| *B.subtilis*(com) | 0.059 | 13.9 |
| TV (com) | 0.052 | 19.3 |

Mean of three replications

## 4.4 SALICYLIC ACID PRODUCTION BY PGPM

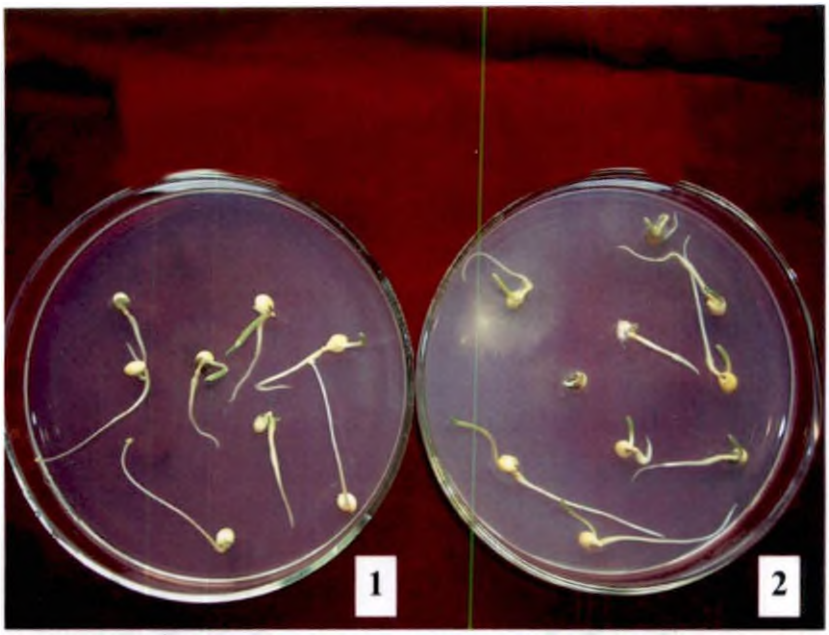
Production of salicylic acid by selected PGPM were tested and the results are given in the (Table 4., Fig.2).The treatments showed significant differences among treatments and maximum production of salicylic acid was recorded by $T_5$ (27.9 mg $ml^{-1}$) followed by $T_{16}$ (21.7 mg $ml^{-1}$).Among *Bacillus* sp., $T_{11}$ produced maximum salicylic acid (20.2 mg $ml^{-1}$).The lowest concentration of salicylic acid was recorded by $T_{12}$ (5.8 mg $ml^{-1}$).

## 4.5 EFFECT OF *Pseudomonas* sp. and *Bacillus* sp. ON SEED GERMINATION

Effect of *Pseudomonas* sp. and *Bacillus* sp. on seed germination and growth were tested with cowpea and sorghum. All the seeds attained 100 per cent germination with *Pseudomonas* sp. and *Bacillus* sp. In the case of cowpea seeds treated with *Pseudomonas* sp. under *in vitro* condition, $T_4$ recorded maximum shoot (7.6 cm) and root length (5.4 cm) (Table 5).Similarly, the maximum shoot length was recorded in the case of $T_6$ (6.2 cm) and root length by $T_1$ (6.4 cm) isolates for sorghum seeds( Table 6,Plate I ) .In the case of *Bacillus* sp. treated cowpea seeds, maximum shoot ( 7.4 cm) and root length (7.5 cm ) was recorded with $T_{11}$ isolate. Similarly, the maximum shoot length was recorded in the case of $T_8$ and $T_{12}$ (3.6 cm) for sorghum seeds. However, the maximum root length was recorded in the case of $T_{11}$(6.1 cm) isolate (Plate I )

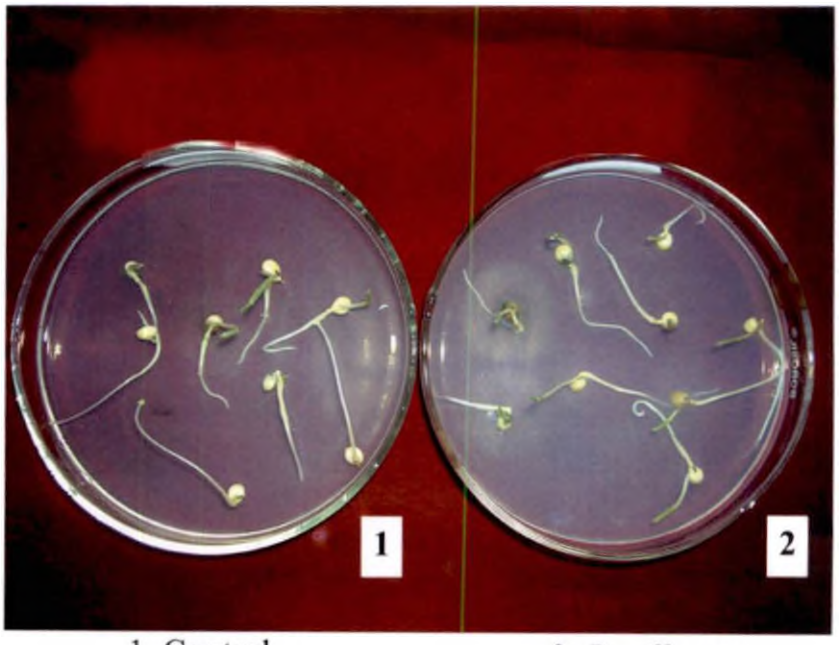
# Plate I

Effect of *Pseudomonas* sp on germination of sorghum seeds




1. Control 2. *Pseudomonas* sp

Effect of *Bacillus* sp on germination of sorghum seeds




1. Control 2. *Bacillus* sp

Table.5 Effect of *Pseudomonas* sp. and *Bacillus* sp. on germination of cowpea seeds

| Sl No. | Treatments | Shoot length (cm) | | Root length (cm) | |
|---|---|---|---|---|---|
| | | *Pseudomonas* sp. | *Bacillus* sp. | *Pseudomonas* sp. | *Bacillus* sp. |
| 1 | $T_1$ | 5.3 | 4.2 | 3.1 | 4.6 |
| 2 | $T_2$ | 4.8 | 4.1 | 4.7 | 4.4 |
| 3 | $T_3$ | 5.9 | 5.7 | 5.2 | 5.3 |
| 4 | $T_4$ | 7.6 | 5.9 | 5.4 | 6.8 |
| 5 | $T_5$ | 7.4 | 7.4 | 3.9 | 7.5 |
| 6 | $T_6$ | 5.8 | 5.1 | 4.1 | 7.3 |
| 7 | Co | 3.7 | 3.7 | 5.2 | 5.2 |
| Mean of three replications | | 5.8 | 5.2 | 4.5 | 5.9 |

All the treatments recorded cent per cent germination.

$T_1$ : CHL , $T_2$ : MDR, $T_3$ : VKA, $T_4$ : ABL, $T_5$ : KVL, $T_6$ : SBY , Co - Control

Table.6 Effect of *Pseudomonas* sp. and *Bacillus* sp. on germination of sorghum seeds

| Sl No. | Treatments | Shoot length (cm) | | Root length (cm) | |
|---|---|---|---|---|---|
| | | *Pseudomonas* sp. | *Bacillus* sp. | *Pseudomonas* sp. | *Bacillus* sp. |
| 1 | $T_1$ | 4 | 2.6 | 6.4 | 4.6 |
| 2 | $T_2$ | 3.2 | 3.6 | 6.2 | 4.8 |
| 3 | $T_3$ | 1.4 | 1.9 | 4.6 | 5.6 |
| 4 | $T_4$ | 3.5 | 2.5 | 4 | 4.7 |
| 5 | $T_5$ | 4.1 | 2.6 | 4.1 | 6.1 |
| 6 | $T_6$ | 6.2 | 3.6 | 6.2 | 5 |
| 7 | Co | 0.6 | 0.6 | 2.8 | 2.8 |
| Mean of three replications | | 3.3 | 2.5 | 4.9 | 4.8 |

All the treatments recorded cent per cent germination.

$T_1$ : CHL , $T_2$ : MDR, $T_3$ : VKA, $T_4$ : ABL, $T_5$ : KVL, $T_6$ : SBY , Co - Control

## 4.6 *IN VITRO* EVALUATION OF MUTUAL COMPATIBILITY AMONG SELECTED PGPM ISOLATES

### 4.6.1 *In vitro* evaluation of mutual compatibility of *Pseudomonas* sp and *Bacillus* sp

Mutual compatibility of *Pseudomonas* sp. and *Bacillus* sp. were carried out by cross streaking technique. Lysis was not observed at the juncture point of any of the two bacterial isolates for 72 h, which indicated that they are compatible to each other.(Plate II)

### 4.6.2 *In vitro* evaluation of mutual compatibility of *Pseudomonas* sp and *Trichoderma* sp.

Mutual compatibility of *Pseudomonas* sp. and *Trichoderma* sp. studied by dual culture technique on PDA medium. Both *Pseudomonas* sp and *Trichoderma* showed growth on mediated plates, which indicated that bacteria and fungus were compatible.

### 4.6.3 *In vitro* evaluation of compatibility of *Bacillus* sp. with *Trichoderma* sp.

Mutual compatibility of *Bacillus* sp. and *Trichoderma* sp. were studied by dual culture technique as mentioned above. Both *Bacillus* sp. and *Trichoderma* showed growth on mediated plates, which indicated that bacteria and fungus were compatible.

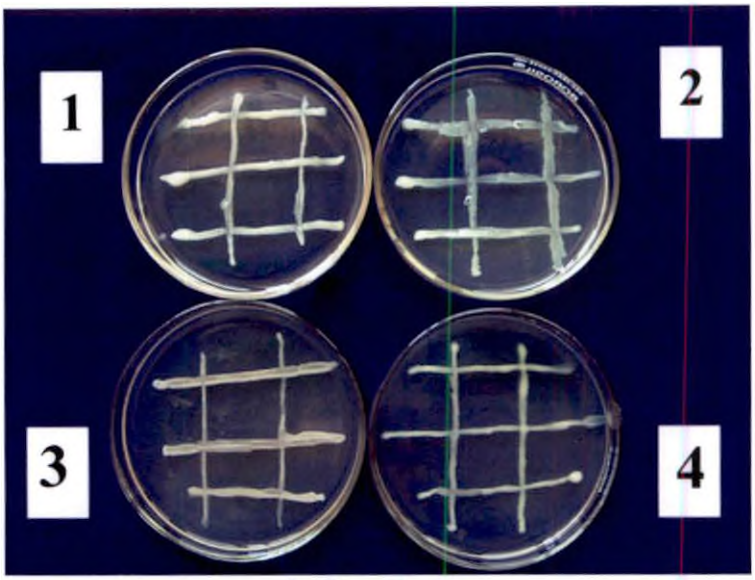
## 4.7 COMPATIBILITY OF PGPM WITH FUNGICIDES AND INSECTICIDES

Compatibility studies were carried out to find out whether the selected PGPM were compatible with commonly used fungicides and insecticides in vanilla

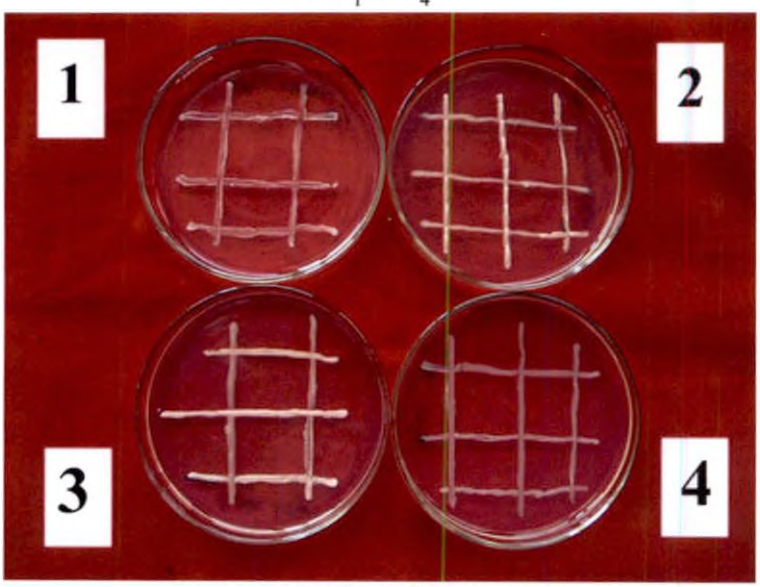
# Plate II

Compatibility study between bacterial isolates

Bacteria:Cross streaking method




1. $P_1$ x $B_1$
2. $P_1$ x $B_2$
3. $P_1$ x $B_3$
4. $P_1$ x $B_4$




1. $P_2$ x $B_1$
2. $P_2$ x $B_2$
3. $P_2$ x $B_3$
4. $P_2$ x $B_4$

### 4.7.1 Compatibility of selected fungicides and insecticides with *Pseudomonas* sp. and *Bacillus* sp.

Five fungicides viz., Bordeaux mixture, copper oxychloride, copper hydroxide, potassium phosphonate and mancozeb and three insecticides viz carbaryl, chlorpyriphos and lambda cyhalothrin were evaluated at three different concentrations to study the compatibility of these with the selected PGPM.

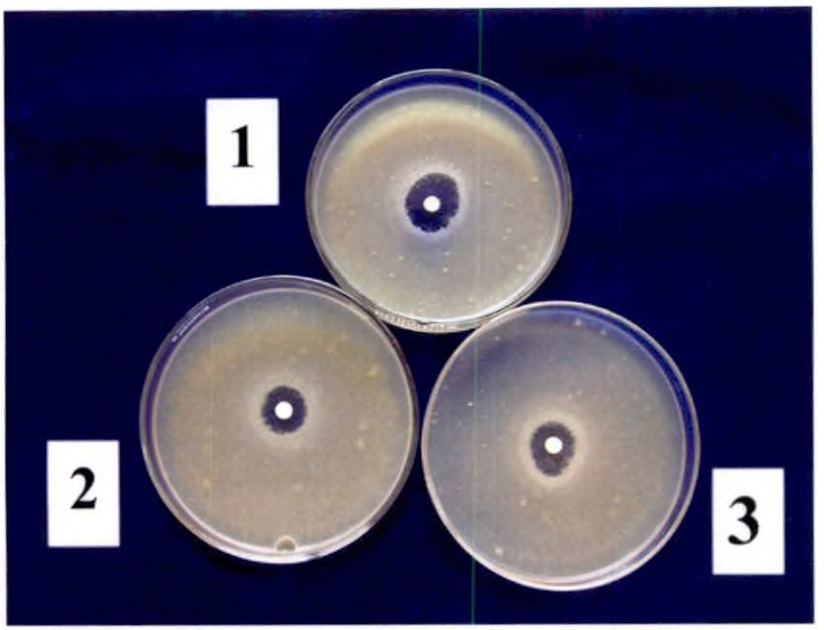
In the case of *Pseudomonas* sp., all the selected fungicides and insecticides at all concentrations completely inhibited their growth (Table7 and 8) (Plate III ) and in the case of *Bacillus* sp., all the selected fungicides and insecticides at all concentrations completely inhibited their growth except carbaryl which was compatible with 8isolates $T_7$ , $T_8$ and $T_9$ at all concentration (Table 9 and 10) (Plate III ).

### 4.7. 2 Compatibility of selected fungicides and insecticides with *Trichoderma* sp.

Five fungicides viz., Bordeaux mixture, copper oxychloride, copper hydroxide, potassium phosphonate and mancozeb and three insecticides viz carbaryl, chlorpyriphos and lambda cyhalothrin were evaluated at three different concentrations to study the compatibility of these with the selected PGPM. In the case of *Trichoderma* isolates, all the selected fungicides and insecticides at all concentrations completely inhibited their growth (Table 11 and 12).

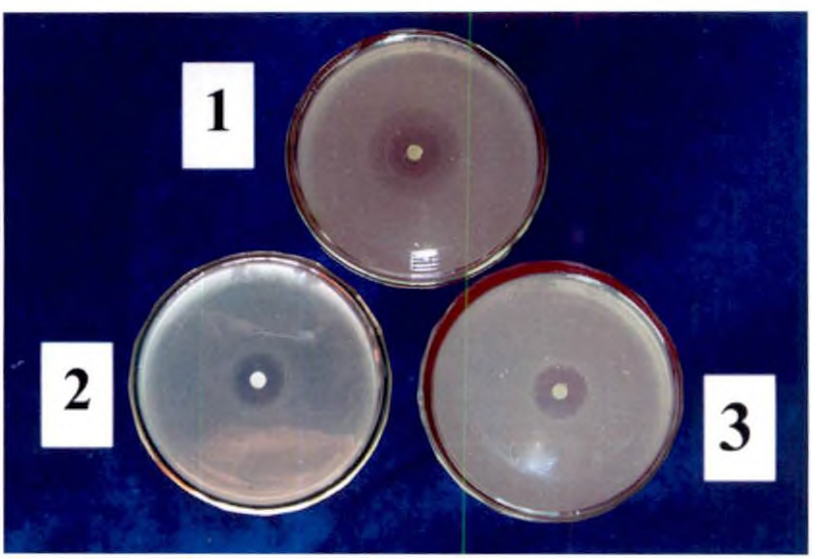
# Plate III

Compatibility between *Pseudomonas* sp and Bordeaux mixture




1. $P_1$ +BM(0.5per cent)
2. $P_1$ +BM(1.0per cent)
3. $P_1$ +BM(1.5per cent)

Compatibility between *Bacillus* sp and Bordeaux mixture




1. $B_1$ +BM(0.5per cent)
2. $B_1$ +BM(1.0per cent)
3. $B_1$ +BM(1.5per cent)

Table.7 *In vitro* evaluation of compatibility between *Pseudomonas* sp. and fungicides

| Isolates | Concentration (per cent) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bordeaux mixture | | | Copper oxychloride | | | Copper hydroxide | | | Potassium phosphonate | | | Mancozeb | | |
| | 0.5 | 1 | 1.5 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 |
| | Inhibition zone (mm) | | | | | | | | | | | | | | |
| $P_1$ | 6.0 | 7.0 | 10 | 15 | 20 | 30 | 6.1 | 7.0 | 15 | 6.0 | 8.0 | 9.0 | 6.0 | 9.0 | 15 |
| $P_2$ | 6.0 | 8.0 | 10 | 14 | 20 | 30 | 7.2 | 8.0 | 12 | 6.0 | 8.0 | 10 | 8.0 | 10 | 12 |
| $P_3$ | 8.0 | 9.0 | 12 | 16 | 24 | 32 | 8.2 | 9.0 | 14 | 7.0 | 9.0 | 13 | 8.0 | 13 | 15 |
| $P_4$ | 6.0 | 7.0 | 9.0 | 14 | 24 | 33 | 9 | 10 | 16 | 6.0 | 7.0 | 9.0 | 10 | 16 | 21 |
| $P_5$ | 6.0 | 8.0 | 12 | 15 | 25 | 34 | 9.2 | 10 | 15 | 0 | 6.0 | 9.0 | 9.0 | 12 | 18 |
| $P_6$ | 10 | 9.0 | 10 | 17 | 26 | 36 | 8.3 | 9.0 | 15 | 7.0 | 9.0 | 13 | 11 | 14 | 19 |

Table.8 *In vitro* evaluation of compatibility between *Pseudomonas* sp. and insecticides

| Isolates | Concentration (ai/lit) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Carbaryl | | | Chlorpyriphos | | | Lambda cyhalothrin | | |
| | 2 | 3 | 4 | 4 | 5 | 6 | 4 | 5 | 6 |
| | Inhibition zone (mm) | | | | | | | | |
| $P_1$ | 14 | 20 | 31 | 6.0 | 12 | 13 | 6.0 | 10 | 12 |
| $P_2$ | 19 | 30 | 8.0 | 6.0 | 16 | 18 | 7.0 | 11 | 16 |
| $P_3$ | 16 | 25 | 36 | 7.0 | 14 | 18 | 8.0 | 13 | 17 |
| $P_4$ | 17 | 26 | 32 | 8.0 | 16 | 19 | 8.0 | 12 | 17 |
| $P_5$ | 16 | 25 | 36 | 7.0 | 13 | 18 | 6.0 | 10 | 13 |
| $P_6$ | 15 | 25 | 38 | 7.0 | 13 | 19 | 7.0 | 11 | 16 |

$P_1$: CHL , $P_2$ : MDR , $P_3$ : VKA, $P_4$: ABL, $P_5$: KVL,, $P_6$: SBY

Table.9 *In vitro* evaluation of compatibility between *Bacillus* sp. and fungicides

| Isolates | Concentration (per cent) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bordeaux mixture | | | Copper oxychloride | | | Copper hydroxide | | | Potassium phosphonate | | | Mancozeb | | |
| | 0.5 | 1 | 1.5 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 |
| | Inhibition zone (mm) | | | | | | | | | | | | | | |
| $B_1$ | 6.0 | 7.0 | 10 | 15 | 20 | 30 | 7.0 | 9.0 | 15 | 7.0 | 8.0 | 9.0 | 13 | 15 | 20 |
| $B_2$ | 6.0 | 8.0 | 10 | 15 | 21 | 32 | 7.0 | 9.0 | 17 | 9.0 | 11 | 15 | 17 | 26 | 23 |
| $B_3$ | 6.0 | 8.0 | 11 | 16 | 24 | 32 | 8.0 | 10 | 12 | 6.0 | 7.0 | 8.0 | 15 | 22 | 26 |
| $B_4$ | 6.0 | 9.0 | 10 | 14 | 24 | 33 | 10 | 12 | 16 | 7.0 | 9.0 | 11 | 25 | 27 | 30 |
| $B_5$ | 7.0 | 9.0 | 11 | 15 | 25 | 34 | 9.0 | 10 | 12 | 7.0 | 8.0 | 11 | 20 | 25 | 30 |
| $B_6$ | 8.0 | 10 | 14 | 16 | 26 | 35 | 7.0 | 11 | 14 | 7.0 | 9.0 | 13 | 15 | 25 | 31 |

Table.10 *In vitro* evaluation of compatibility between *Bacillus* sp. and insecticides

| Isolates | Concentration (ai/lit) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Carbaryl | | | Chlorpyriphos | | | Lambdacyhalothrin | | |
| | 2 | 3 | 4 | 4 | 5 | 6 | 4 | 5 | 6 |
| | Inhibition zone (mm) | | | | | | | | |
| $B_1$ | 0 | 0 | 0 | 6.0 | 8.0 | 10 | 6.0 | 10 | 12 |
| $B_2$ | 0 | 0 | 0 | 8.0 | 10 | 13 | 7.0 | 11 | 16 |
| $B_3$ | 0 | 0 | 0 | 7.0 | 9.0 | 12 | 8.0 | 13 | 17 |
| $B_4$ | 19 | 19 | 19 | 8.0 | 10 | 12 | 6.0 | 10 | 13 |
| $B_5$ | 16 | 16 | 16 | 7.0 | 9.0 | 11 | 8.0 | 13 | 17 |
| $B_6$ | 15 | 25 | 38 | 7.0 | 10 | 12 | 8.0 | 13 | 15 |

$B_1$: CHL, $B_2$ : MDR , $B_3$ : VKA, $B_4$: ABL,$B_5$: KVL,, $B_6$: SBY

Table.11 *In vitro* evaluation of compatibility between *Trichoderma* sp. and fungicides

| Isolates | Concentration (per cent) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bordeaux mixture | | | Copper oxychloride | | | Copper hydroxide | | | Potassium phosphonate | | | Mancozeb | | |
| | 0.5 | 1 | 1.5 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 |
| | Growth after 4 days (cm) | | | | | | | | | | | | | | |
| $Tr_1$ | 1.0 | 2.2. | 3.0 | 4.0 | 4.5 | 5.0 | 1.0 | 2.3 | 3.2 | 0.9 | 1.2 | 2.3 | 2.3 | 1.5 | 2.0 |
| $Tr_2$ | 0.3 | 2.4 | 3.6 | 3.1 | 3.2 | 4.0 | 1.1 | 2.2 | 3.4 | 1.3 | 1.5 | 2.9 | 2.9 | 2.0 | 2.3 |
| $Tr_3$ | 1.3 | 2.5 | 3.6 | 3.1 | 3.3 | 3.6 | 1.2 | 2.2 | 3.4 | 1.0 | 2.3 | 3.5 | 3.5 | 1.9 | 2.3 |
| $Tr_4$ | 1.5 | 2.7 | 3.7 | 2.0 | 3.1 | 3.3 | 1.0 | 2.3 | 3.1 | 1.0 | 2.5 | 3.5 | 3.5 | 2.7 | 3.0 |
| $Tr_5$ | 1.2 | 2.4 | 3.2 | 2.4 | 3.2 | 4.0 | 0.9 | 2.0 | 2.6 | 1.2 | 2.4 | 3.7 | 3.7 | 2.5 | 3.2 |
| $Tr_6$ | 1.1 | 2.3 | 3.4 | 3.4 | 3.6 | 4.1 | 1.0 | 2.2 | 3.1 | 1.0 | 2.2 | 3.0 | 3 | 2.4 | 2.9 |
| Control | 5.0 | | | | | | | | | | | | | | |

Table.12 *In vitro* evaluation of compatibility between *Trichoderma* sp. and insecticides

| Isolates | Concentration (ai/lit) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Carbaryl | | | Chlorpyriphos | | | Lambdacyhalothrin | | |
| | 2 | 3 | 4 | 4 | 5 | 6 | 4 | 5 | 6 |
| | Growth after 4 days (cm) | | | | | | | | |
| $Tr_1$ | 1.4 | 1.7 | 2.0 | 0.6 | 1.1 | 2.2 | 0.7 | 1.1 | 1.3 |
| $Tr_2$ | 1.0 | 1.4 | 2.4 | 0.8 | 1.0 | 2.0 | 0.6 | 1.0 | 1.6 |
| $Tr_3$ | 2.5 | 2.7 | 3.2 | 0.7 | 0.9 | 1.1 | 0.6 | 1.3 | 1.4 |
| $Tr_4$ | 1.8 | 3.2 | 3.6 | 0.8 | 1.0 | 1.2 | 0.6 | 1.2 | 1.7 |
| $Tr_5$ | 2.4 | 3.0 | 3.5 | 0.6 | 0.8 | 1.1 | 0.7 | 1.2 | 1.5 |
| $Tr_6$ | 2.7 | 2.8 | 3.1 | 0.6 | 0.9 | 1.2 | 0.7 | 1.1 | 1.7 |
| Control | 5.0 | | | | | | | | |

$Tr_1$ :CHL, $Tr_2$ : MDR, $Tr_3$ : VKA, $Tr_4$: ABL, $Tr_5$: KVL, $Tr_6$: SBY

## 4.8 SCREENING OF PLANT GROWTH PROMOTING MICROORGANISMS CONSORTIA FOR ENCHANCING THE GROWTH OF VANILLA

A nursery experiment was carried out to find out the effect of individual *Pseudomonas*, *Bacillus* and *Trichoderma* isolates and their consortia for enhancing the growth of vanilla cuttings in the nursery. The experiment was carried out as described in Materials and Methods.

## 4.9 BIOMETRIC OBSERVATIONS

### 4.9.1 Number of days taken for sprouting

The number of days taken by plants in each treatment for sprouting of vanilla cuttings were recorded 30 days after planting till six months (Table 13, Fig.3). The results indicated that the treatments showed significant differences in the first two month after planting. Among all the treatments,$T_{25}$ , $T_{26}$ and $T_7$ sprouted early (30 DAP). Among the native isolates, $T_7$ sprouted early (30 DAP).From the table it was clear that $T_{22}$ (Ambalavayal consortia), $T_{23}$ (Kolvayal consortia), $T_{24}$ (Sulthan Bathery consortia) and $T_{19}$ (Chengaloor consortia) have taken less time for sprouting than control.

Among *Pseudomonas* treated plants, $T_1$ and $T_6$ sprouted early (44 DAP) and among *Bacillus,* $T_7$ sprouted early (30 DAP) followed $T_{10}$. Among *Trichoderma* isolates, $T_{17}$ sprouted early (40 DAP). In the case of consortia, $T_{22}$ (Ambalavayal consortia) sprouted early (40 DAP) followed by $T_{23}$ (Kolvayal consortia) (44DAP). Control plants sprouted 50 DAP.

### 4.9.2 Effect of different treatments on vine length of vanilla

The length of vine of vanilla was recorded at monthly interval up to six months (Table14, Fig. 4). Even though, the treatments did not show significant differences in

Table.13 Effect of PGPM and its consortia on number of days taken for sprouting in Vanilla

| Treatments | Number of days taken for sprouting | | |
|---|---|---|---|
| | 30 | 45 | 60 |
| $T_1$ | - | 45 | - |
| $T_2$ | - | 44 | - |
| $T_3$ | - | - | 48 |
| $T_4$ | - | 45 | - |
| $T_5$ | - | 45 | - |
| $T_6$ | - | 44 | - |
| $T_7$ | 30 | - | - |
| $T_8$ | - | 45 | - |
| $T_9$ | - | - | 55 |
| $T_{10}$ | - | 36 | - |
| $T_{11}$ | - | - | 65 |
| $T_{12}$ | - | - | 60 |
| $T_{13}$ | - | 45 | - |
| $T_{14}$ | - | - | 55 |
| $T_{15}$ | - | - | 54 |
| $T_{16}$ | - | - | 57 |
| $T_{17}$ | - | 40 | - |
| $T_{18}$ | - | - | 51 |
| $T_{19}$ | - | - | 48 |
| $T_{20}$ | - | - | 55 |
| $T_{21}$ | - | - | 59 |
| $T_{22}$ | - | 40 | - |
| $T_{23}$ | - | 44 | - |
| $T_{24}$ | - | 45 | - |
| $T_{25}$ | 30 | - | - |
| $T_{26}$ | 30 | - | - |
| $T_{27}$ | - | - | 50 |

*P.fluorescens* @10ml $kg^{-1}$ of soil *B.subtilis* @10ml $kg^{-1}$ of soil, *T.harzianum* @ 4g $kg^{-1}$ of soil

Table.14 Effect of PGPM and its consortia on the vine length of vanilla

| Treatments | Length of vine (cm) | | | | |
|---|---|---|---|---|---|
| | 2MAP | 3MAP | 4MAP | 5MAP | 6MAP |
| $T_1$ | $6.8^{bc}$ | $26.7^{abcd}$ | $46.7^{cd}$ | $72.7^{bcde}$ | $114.0^{abcde}$ |
| $T_2$ | $6.7^{bc}$ | $21.3^{abcd}$ | $44.7^{cd}$ | $79.3^{abcde}$ | $135.3^{abcd}$ |
| $T_3$ | $8.0^{bc}$ | $6.3^{abc}$ | $36.3^{cd}$ | $63.0^{cde}$ | $105^{abcde}$ |
| $T_4$ | $6.3^{bc}$ | $17.7^{cd}$ | $33.7^{cd}$ | $58.3^{cde}$ | $98.3^{bcde}$ |
| $T_5$ | $6.0^{bc}$ | $20.5^{bcd}$ | $34.0^{cd}$ | $57.0^{cde}$ | $83.0^{cde}$ |
| $T_6$ | $7.7^{bc}$ | $22.0^{abcd}$ | $43.3^{cd}$ | $73.7^{bcde}$ | $133.7^{abcd}$ |
| $T_7$ | $13^{bc}$ | $0.8^{ab}$ | $58.7^{bcd}$ | $83.3^{abcde}$ | $143.3^{abc}$ |
| $T_8$ | $6.0^{bc}$ | $21.7^{abcd}$ | $41.7^{cd}$ | $65.3^{bcde}$ | $100.3^{bcde}$ |
| $T_9$ | $4.7^{bc}$ | $23.0^{abcd}$ | $39.0^{cd}$ | $70.7^{bcde}$ | $95.3^{bcde}$ |
| $T_{10}$ | $12.3^{bc}$ | $34.7^{abc}$ | $68.3^{abc}$ | $94.3^{abc}$ | $143.7^{abc}$ |
| $T_{11}$ | $0.7^{c}$ | $14.7^{cd}$ | $25.0^{d}$ | $70.3^{e}$ | $64.3^{e}$ |
| $T_{12}$ | $1.3^{c}$ | $18.7^{cd}$ | $32.7^{cd}$ | $59.0^{cde}$ | $90.3^{bcde}$ |
| $T_{13}$ | $6.7^{bc}$ | $22.0^{abcd}$ | $36.7^{cd}$ | $54.7^{cde}$ | $76.7^{de}$ |
| $T_{14}$ | $3.3^{bc}$ | $23.0^{abcd}$ | $36.3^{cd}$ | $73.0^{bcde}$ | $118^{abcde}$ |
| $T_{15}$ | $3.7^{bc}$ | $21.7^{abcd}$ | $39.7^{cd}$ | $89.7^{abcd}$ | $134^{abcd}$ |
| $T_{16}$ | $3.0^{bc}$ | $8.3^{d}$ | $26.3^{d}$ | $43.3^{de}$ | $84.7^{cde}$ |
| $T_{17}$ | $9.0^{bc}$ | $28.7^{abcd}$ | $46.7^{cd}$ | $74.3^{bcde}$ | $140.0^{abcd}$ |
| $T_{18}$ | $4.5^{bc}$ | $25.3^{abcd}$ | $39.0^{cd}$ | $71.7^{bcde}$ | $107^{abcde}$ |
| $T_{19}$ | $5.7^{bc}$ | $21.0^{bcd}$ | $37.0^{cd}$ | $61.7^{cde}$ | $83.0^{cde}$ |
| $T_{20}$ | $3.3^{bc}$ | $25.0^{abcd}$ | $48.3^{bcd}$ | $97.7^{abc}$ | $136^{abcd}$ |
| $T_{21}$ | $4.3^{bc}$ | $26.0^{abcd}$ | $34.3^{cd}$ | $73.7^{bcde}$ | $115.7^{abcde}$ |
| $T_{22}$ | $9.7^{bc}$ | $22.3^{abcd}$ | $38.3^{cd}$ | $61.0^{cde}$ | $108.7^{abcde}$ |
| $T_{23}$ | $7.7^{bc}$ | $21.0b^{cd}$ | $35.3^{cd}$ | $57.0^{cde}$ | $88.3^{bcde}$ |
| $T_{24}$ | $7.0^{bc}$ | $25.7^{abcd}$ | $44.0^{cd}$ | $73.7^{bcde}$ | $106.3^{abcde}$ |
| $T_{25}$ | $16.3^{ab}$ | $42.3^{ab}$ | $82.3^{ab}$ | $111^{ab}$ | $166.0^{a}$ |
| $T_{26}$ | $27^{a}$ | $43.0^{a}$ | $95.0^{a}$ | $123.0^{a}$ | $151.3^{ab}$ |
| $T_{27}$ | $5.0^{bc}$ | $14.7^{cd}$ | $38.3^{cd}$ | $69.3^{bcde}$ | $119.3^{abcde}$ |

*Pseudomonas* sp and *Bacillus* sp @ 15 ml per polybags and *Trichoderma* @ 3g per bag

MAP- Month after planting

initial period, they showed significant differences on length of vine after three months of planting. From the table it was clear that among the different treatments $T_{25}$ (166cm) recorded maximum vine length.

Among all the native isolates selected, $T_{10}$ was the best which gave maximum height (143.7 cm).Among *Pseudomonas* treated plants, $T_2$ gave maximum height (135.3 cm) followed by $T_6$ (133.7 cm) and $T_{10}$ was the best *Bacillus* isolate which recorded maximum height (143.7 cm) followed by $T_7$ (143.3cm). Among *Trichoderma*, $T_{17}$ showed maximum height (140cm) followed by $T_{15}$ (134 cm). In the case of consortia, $T_{20}$ (Mundoor consortia) (136cm) followed by $T_{21}$ (Vellanikkara consortia) gave maximum height (115.7cm). The height of the control plant was lower than all the above mentioned results (119.3cm). The same trend was noticed up to six months after planting.

### 4.9.3 Effect of different treatments on number of leaves

The number of leaves of vanilla was recorded at monthly interval up to six months. Among all the treatments, $T_{25}$ gave maximum number of leaves (32). It was followed by $T_{26}$ which produced 27 leaves. Among all the native isolates selected, $T_6$ was the best which gave maximum number of leaves (23).

Among *Pseudomonas* treated plants, $T_2$ gave maximum number of leaves (21) followed by $T_1$ and $T_6$ (20) leaves and $T_7$ was the best *Bacillus* isolates which gave maximum number of leaves (23) followed by $T_{10}$ (22). Among *Trichoderma*, $T_{15}$ recorded maximum number of leaves (20) followed by $T_{14}$ (18). In the case of consortia, $T_{23}$ (Kolvayal consortia) gave maximum number of leaves (21) followed by $T_{22}$ (Ambalavayal consortia) (20). The lowest numbers of leaves were noticed in the case of $T_{12}$ which produced 27 leaves (Table 15., Fig.5). The control plants produced 21 leaves.

Table.15 Effect of PGPM and its consortia on the number of leaves

| Treatments | Number of leaves | | | | |
|---|---|---|---|---|---|
| | 2MAP | 3MAP | 4MAP | 5MAP | 6MAP |
| $T_1$ | $2^{ab}$ | $5.0^{abc}$ | $9.7^{b}$ | $13.7^{cde}$ | $20.0^{bcde}$ |
| $T_2$ | $2.3^{ab}$ | $5.7^{abc}$ | $10.3^{b}$ | $14.0^{cde}$ | $21.7^{bcd}$ |
| $T_3$ | $2.7^{ab}$ | $6.3^{abc}$ | $9.0^{b}$ | $13.7^{cde}$ | $18.3^{cde}$ |
| $T_4$ | $3.0^{ab}$ | $6.0^{abc}$ | $9.3^{b}$ | $13.7^{cde}$ | $19.7^{bcde}$ |
| $T_5$ | $2.0^{ab}$ | $5.0^{abc}$ | $8.3^{b}$ | $12.0^{cde}$ | $16.3^{cde}$ |
| $T_6$ | $3.0^{ab}$ | $5.7^{abc}$ | $10^{b}$ | $15.0^{cde}$ | $20.0^{bcde}$ |
| $T_7$ | $4.3^{ab}$ | $8.0^{ab}$ | $11.7^{b}$ | $18.0^{abc}$ | $22.7^{bc}$ |
| $T_8$ | $1.3^{b}$ | $6.3^{abc}$ | $8.7^{b}$ | 13.3cde | $18.0^{cde}$ |
| $T_9$ | $1.7^{b}$ | $5.7^{abc}$ | $9.7^{b}$ | $14.3^{cde}$ | $17.3^{cde}$ |
| $T_{10}$ | $3.7^{ab}$ | $7.7^{abc}$ | $11.7^{b}$ | $17.3^{abc}$ | $21.7^{bcd}$ |
| $T_{11}$ | $0.7^{b}$ | $3.7^{bc}$ | $6.0^{b}$ | $9.0^{e}$ | $12.0^{e}$ |
| $T_{12}$ | $0.7^{b}$ | $4.3^{bc}$ | $8.7^{b}$ | $12.7^{cde}$ | $15.7^{cde}$ |
| $T_{13}$ | $2.7^{ab}$ | $5.7^{abc}$ | $9.3^{b}$ | $12.3^{cde}$ | $17.0^{cde}$ |
| $T_{14}$ | $1.0^{b}$ | $5.0^{abc}$ | $7.7^{b}$ | $13.0^{cde}$ | $17.7^{cde}$ |
| $T_{15}$ | $1.3^{b}$ | $5.7^{abc}$ | $9.0^{b}$ | $14.0^{cde}$ | $20.3^{bcd}$ |
| $T_{16}$ | $1.0^{b}$ | $3.0^{c}$ | $7.0^{b}$ | $10.0^{de}$ | $17.3^{cde}$ |
| $T_{17}$ | $3.0^{ab}$ | $6.7^{abc}$ | $49.7^{a}$ | $13.7^{cde}$ | $13.7^{de}$ |
| $T_{18}$ | $1.3^{b}$ | $5.3^{abc}$ | $9.3^{b}$ | $14.7^{cde}$ | $14.7^{cde}$ |
| $T_{19}$ | $1.7^{b}$ | $5.7^{abc}$ | $9.7^{b}$ | $14.0^{cde}$ | $17.7^{cde}$ |
| $T_{20}$ | $2.3^{ab}$ | $5.7^{abc}$ | $11.0^{b}$ | $15.7^{cd}$ | $19.6^{bcde}$ |
| $T_{21}$ | $1.3^{b}$ | $4.3^{bc}$ | $7.7^{b}$ | $13.3^{cde}$ | $19.3^{cde}$ |
| $T_{22}$ | $3.7^{ab}$ | $6.3^{abc}$ | $10.0^{b}$ | $12.7^{cde}$ | $20.3^{bcd}$ |
| $T_{23}$ | $2.3^{ab}$ | $6.3^{abc}$ | $9.7^{b}$ | $13.3^{cde}$ | $20.7^{bcd}$ |
| $T_{24}$ | $2.3^{ab}$ | $6.7^{abc}$ | $10.7^{b}$ | $14.7^{cde}$ | $17.0^{cde}$ |
| $T_{25}$ | $4.0^{ab}$ | $8.0^{ab}$ | $16.0^{b}$ | $23.0^{a}$ | $31.7^{a}$ |
| $T_{26}$ | $6.0^{a}$ | $9.7^{a}$ | $16.3^{b}$ | $21.7^{ab}$ | $27.3^{ab}$ |
| $T_{27}$ | $1.0^{b}$ | $5.3^{abc}$ | $10.0^{b}$ | $16.0^{bcd}$ | $21.3^{bcd}$ |

MAP- Month after planting
*Pseudomonas* sp and *Bacillus* sp @ 15 ml per polybags and *Trichoderma* @ 3g per bag

### 4.9.4 Effect of different treatments on vine girth

The girth of vine of vanilla was recorded at monthly interval up to six months (Table 16). The treatments did not show significant differences in the girth of vine through out the planting period. After six months of planting, $T_{25}$ recorded maximum girth (4.5cm) and the lowest girth recorded was that of $T_{11}$(2.5cm).

Among *Pseudomonas* treatments, $T_2$recorded maximum girth (3.2cm). Among *Bacillus* isolates, most treatments had the similar girth (3cm). It is also true in the case of consortia and control plants. In the case of *Trichoderma*, $T_{15}$ recorded maximum girth (3.2cm).

### 4.9.5 Effect of different treatments on the internodal length

Internodal length was taken after the plant had attained sufficient length. It was taken as an average of three internodal length at monthly interval up to six months. Here also, the treatments did not differ significantly (Table 17).

After six months of planting, $T_{15}$ and $T_{16}$ isolates showed maximum internodal length (8 cm). Among *Pseudomonas* treatments, $T_6$ had recorded maximum internodal length ( 7.7cm) followed by $T_1$ (7.3cm) .While in the case of *Bacillus* isolates, maximum internodal length was produced by $T_7$(7.7 cm) followed by $T_{12}$ (6.3cm) and among consortia, $T_{20}$ (Mundoor consortia containing *P.fluorescens, B.subtilis* and *T.harzianum*) which produced an internodal length of (7.3 cm) followed by $T_{21}$ (Vellanikkara consortia containing *P.fluorescens, B.subtilis* and *T.harzianum* ) (6.7cm). $T_{25}$ recorded an internodal length of (7cm) and $T_{26}$ (6.3cm).The control plants recorded internodal length of 7cm.

Table.16 Effect of PGPM and its consortia on the girth of vine in vanilla

| Treatments | Girth of vine (cm) | | | | |
|---|---|---|---|---|---|
| | 2MAP | 3MAP | 4MAP | 5MAP | 6MAP |
| $T_1$ | $1.3^{a}$ | $1.7^{a}$ | $2.0^{ab}$ | $2.7^{abc}$ | $3.0^{bc}$ |
| $T_2$ | $1.3^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.2^{ab}$ | $3.2^{b}$ |
| $T_3$ | $1.4^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_4$ | $1.3^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.2^{ab}$ | $3.0^{bc}$ |
| $T_5$ | $1.3^{a}$ | $1.7^{a}$ | $1.8^{ab}$ | $2.7^{abc}$ | $2.7^{cd}$ |
| $T_6$ | $1.3^{a}$ | $1.8^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_7$ | $1.8^{a}$ | $2^{a}$ | $2.0^{ab}$ | $2.7^{abc}$ | $3.0^{bc}$ |
| $T_8$ | $0.8^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_9$ | $0.5^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{10}$ | $2.0^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{11}$ | $0.2^{a}$ | $1.2^{ab}$ | $1.3^{b}$ | $2.0^{c}$ | $2.5^{d}$ |
| $T_{12}$ | $0.7^{a}$ | $1.8^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{13}$ | $1.3^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{14}$ | $1.3^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{15}$ | $0.7^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.2^{ab}$ | $3.2^{b}$ |
| $T_{16}$ | $0.5^{a}$ | $0.5^{b}$ | $1.8^{ab}$ | $2.3^{bc}$ | $3.0^{bc}$ |
| $T_{17}$ | $1.3^{a}$ | $1.8^{a}$ | $2.0^{ab}$ | $3.2^{ab}$ | $3.0^{b}$ |
| $T_{18}$ | $1.3^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{19}$ | $0.8^{a}$ | $1.8^{a}$ | $1.8^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{20}$ | $1.5^{a}$ | $2^{a}$ | $2.0^{ab}$ | $3.2^{ab}$ | $3.0^{bc}$ |
| $T_{21}$ | $1.8^{a}$ | $1.7^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{22}$ | $1.3^{a}$ | $1.7^{a}$ | $2.0^{ab}$ | $2.7^{abc}$ | $3.0^{bc}$ |
| $T_{23}$ | $1.3^{a}$ | $1.8^{a}$ | $2.0^{ab}$ | $2.7^{abc}$ | $3.0^{bc}$ |
| $T_{24}$ | $1.2^{a}$ | $2.0^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{25}$ | $2.0^{a}$ | $2.0^{a}$ | $2.3^{a}$ | $3.0^{ab}$ | $4.5^{a}$ |
| $T_{26}$ | $2.0^{a}$ | $2.0^{a}$ | $2.0^{ab}$ | $3.0^{ab}$ | $3.0^{bc}$ |
| $T_{27}$ | $0.7^{a}$ $(1)^{a}$ | $1.3^{ab}$ $(1.2)^{ab}$ | $2^{ab}$ $(1.5)^{ab}$ | $3.3^{a}$ $(2)^{a}$ | $3^{bc}$ $(1.9)^{bc}$ |

MAP- Month after planting

*Pseudomonas* sp and *Bacillus* sp @ 15 ml per polybags and *Trichoderma* @ 3g per bag

Table.17 Effect of PGPM and its consortia on the internodal length of vanilla

| Treatment | Internodal length (cm) | | | | |
|---|---|---|---|---|---|
| | 2 MAP | 3 MAP | 4 MAP | 5 MAP | 6 MAP |
| $T_1$ | $0.3^{a}$ | $3.3^{ab}$ | $4.3^{abcd}$ | $5.0^{abcd}$ | $7.3^{ab}$ |
| $T_2$ | $0.8^{cd}$ | $2.2^{ab}$ | $4.0^{abcd}$ | $5.7^{abc}$ | $7.0^{abc}$ |
| $T_3$ | $1.3^{cd}$ | $4.3^{ab}$ | $4.7^{abcd}$ | $5.3^{abcd}$ | $7.0^{abc}$ |
| $T_4$ | $0.8^{cd}$ | $2.3^{ab}$ | $2.7^{d}$ | $5.0^{abcd}$ | $7.0^{abc}$ |
| $T_5$ | $1.0^{cd}$ | $3.2^{ab}$ | $3.7^{bcd}$ | $4.0^{cd}$ | $5.0^{cd}$ |
| $T_6$ | $0.8^{cd}$ | $3.8^{ab}$ | $4.0^{abcd}$ | $6.0^{abc}$ | $7.7^{ab}$ |
| $T_7$ | $2.7^{abc}$ | $5.0^{a}$ | $5.0^{abcd}$ | $7.0^{ab}$ | $7.7^{ab}$ |
| $T_8$ | $0.7^{cd}$ | $4.0^{ab}$ | $4.0^{abcd}$ | $6.3^{abc}$ | $7.0^{abc}$ |
| $T_9$ | $0.7^{cd}$ | $4.0^{ab}$ | $4.0^{abcd}$ | $5.3^{abcd}$ | $7.0^{abc}$ |
| $T_{10}$ | $1.0^{bcd}$ | $5.0^{a}$ | $5.3^{abc}$ | $5.7^{abc}$ | $7.3^{ab}$ |
| $T_{11}$ | $0.3^{a}$ | $3.0^{ab}$ | $3.0^{cd}$ | $3^{d}$ | $4.7^{d}$ |
| $T_{12}$ | $0.3^{a}$ | $3.0^{ab}$ | $3.3^{bcd}$ | $5.3^{abcd}$ | $6.3^{abcd}$ |
| $T_{13}$ | $1.0^{cd}$ | $4.3^{ab}$ | $4..3^{abcd}$ | $5.7^{abc}$ | $7.0^{abc}$ |
| $T_{14}$ | $0.5^{cd}$ | $3.0^{ab}$ | $4.0^{abcd}$ | $5.3^{abcd}$ | $7.0^{abc}$ |
| $T_{15}$ | $0.7^{cd}$ | $3.0^{ab}$ | $3.7^{bcd}$ | $7.7^{a}$ | $8.0^{a}$ |
| $T_{16}$ | $0.2^{d}$ | $1.3^{b}$ | $3.3^{bcd}$ | $4.7^{bcd}$ | $8.0^{a}$ |
| $T_{17}$ | $1.2^{cd}$ | $4.0^{ab}$ | $4.0^{abcd}$ | $4.2^{cd}$ | $6.3^{abcd}$ |
| $T_{18}$ | $0.2^{d}$ | $4.0^{ab}$ | $4.0^{abcd}$ | $5.3^{abcd}$ | $7.0^{abc}$ |
| $T_{19}$ | $0.7^{cd}$ | $3.3^{ab}$ | $3.3^{bcd}$ | $4.5^{bcd}$ | $5.0^{cd}$ |
| $T_{20}$ | $0.8^{cd}$ | $4.0^{ab}$ | $4.0^{abcd}$ | $5.7^{abc}$ | $7.3^{ab}$ |
| $T_{21}$ | $0.3^{a}$ | $3.7^{ab}$ | $5.0^{abcd}$ | $5.7^{abc}$ | $6.7^{abcd}$ |
| $T_{22}$ | $1.5^{cd}$ | $4.3^{ab}$ | $4.3^{abcd}$ | $4.3^{bcd}$ | $6.0^{abcd}$ |
| $T_{23}$ | $0.8^{cd}$ | $3.7^{ab}$ | $3.7^{bcd}$ | $4.7^{bcd}$ | $5.7^{bcd}$ |
| $T_{24}$ | $1.0^{cd}$ | $4.0^{ab}$ | $4.0^{abcd}$ | $6.7^{abc}$ | $6.3^{abcd}$ |
| $T_{25}$ | $4.3^{a}$ | $5.0^{a}$ | $6.3^{a}$ | $7.0^{ab}$ | $7.0^{abc}$ |
| $T_{26}$ | $3.7^{ab}$ | $4.3^{ab}$ | $5.7^{ab}$ | $6.3^{abc}$ | $6.3^{abcd}$ |
| $T_{27}$ | $1.7^{cd}$ | $2.3^{ab}$ | $3.3^{bcd}$ | $6.0^{abc}$ | $6.3^{abc}$ |

MAP- Month after planting
*Pseudomonas* sp and *Bacillus* sp @ 15 ml per polybags and *Trichoderma* @ 3g per bag

### 4.9.6 Effect of different treatments on fresh weight of vanilla

The fresh and dry weight of shoot, root, and whole plant are given in (Table 18., Fig.6 ). In the case of shoot fresh weight, significant differences were noticed among the treatments. $T_{25}$ registered the highest shoot fresh weight (360 g) followed by $T_{26}$ (235 g).

Among all the native isolates, $T_7$ gave maximum shoot fresh weight (226.7g). Among *Pseudomonas* treatments, $T_2$ (225g) followed by $T_6$ (200g) and $T_7$ (226.7g) among *Bacillus* followed by $T_8$ (221.7g) gave maximum shoot fresh weight. In the case of *Trichoderma* isolates, $T_{18}$ (221.7g) followed by $T_{14}$ (220g) gave maximum shoot fresh weight. Among consortia, $T_{24}$ (Sulthan Bathery consortia) produced maximum fresh shoot weight (71.9g) followed by $T_{21}$ (Vellanikkara consortia) (210g).The lowest weight was registered in $T_{22}$ (Ambalavayal consortia) (103.3 g) which was on par with $T_{16}$. The control plants registered shoot weight of 210g.

The treatments differed significantly with respect to root fresh weight However, maximum root fresh weight was recorded in $T_{25}$ (16.3g) followed by $T_{15}$ and $T_{21}$ (Vellanikkara consortia) (12.7g). Among *Pseudomonas* treatments, $T_5$ recorded root fresh weight of 10.9 g followed by $T_3$ (10.5g). In the case of *Bacillus* isolates, maximum root weight recorded by $T_8$ (12.3g) followed by $T_9$ (11.4g). In the case of *Trichoderma* isolates, $T_{15}$ recorded maximum root fresh weight (12.7g) followed by $T_{18}$ (11.4g). In the case consortia, $T_{21}$ (Vellanikkara consortia)(12.7g)followed by $T_{24}$ (Sulthan Bathery consortia) (11.4g) recorded maximum root fresh weight. The control plants had root fresh weight of 11.5g.

With respect to fresh weight of whole plant, the treatments varied significantly. $T_{25}$ showed highest value of fresh weight (376.3 g) followed by $T_{26}$ (246.5 g). Among all the native isolates, $T_7$ gave maximum fresh weight of whole plant (234.8g) Among *Pseudomonas* treatments, $T_2$ (234.7g) followed by $T_6$ (208.6g) and $T_7$

Table.18 Effect of PGPM and its consortia on the fresh weight (shoot & root), dry weight (shoot & root) and root length of vanilla

| Treatment | Fresh weight (g) | | | Dry weight (g) | | | Root length (cm) |
|---|---|---|---|---|---|---|---|
| | Shoot | Root | Plant | shoot | root | Plant | |
| $T_1$ | 166.7$^{b}$ | 8.03$^{bc}$ | 174.73 | 51.1$^{abc}$ | 1.5$^{de}$ | 52.6 | 43$^{c}$ |
| $T_2$ | 225$^{b}$ | 9.7$^{bc}$ | 234.7 | 59.1$^{abc}$ | 2.5$^{abcde}$ | 61.6 | 62.3$^{bc}$ |
| $T_3$ | 153.3$^{b}$ | 10.5$^{abc}$ | 163.8 | 43.4$^{bc}$ | 2.5$^{abcde}$ | 45.9 | 68$^{bc}$ |
| $T_4$ | 133.3$^{b}$ | 9.1$^{bc}$ | 142.4 | 38.5$^{bc}$ | 2.3$^{bcde}$ | 40.8 | 49.2$^{bc}$ |
| $T_5$ | 158.3$^{b}$ | 10.9$^{ab}$ | 169.2 | 47.4$^{bc}$ | 2.9$^{abcd}$ | 50.3 | 52.7$^{bc}$ |
| $T_6$ | 200$^{b}$ | 8.6b$^{c}$ | 208.6 | 54.8$^{abc}$ | 2.6$^{abcde}$ | 57.4 | 52.5$^{bc}$ |
| $T_7$ | 226.7$^{b}$ | 8.1$^{bc}$ | 234.8 | 71.9$^{abc}$ | 2.2$^{bcde}$ | 74.1 | 58.2$^{bc}$ |
| $T_8$ | 221.7$^{b}$ | 12.3$^{ab}$ | ,234 | 68.7$^{abc}$ | 2.7$^{abcde}$ | 71.4 | 62$^{bc}$ |
| $T_9$ | 163.3$^{b}$ | 11.4$^{ab}$ | 174.7 | 46.4$^{bc}$ | 2.8$^{abcd}$ | 49.2 | 552$^{bc}$ |
| $T_{10}$ | 178.3$^{b}$ | 9.7$^{bc}$ | 188 | 57.1$^{abc}$ | 2.6$^{abcde}$ | 59.7 | 54$^{bc}$ |
| $T_{11}$ | 111.7$^{b}$ | 7.4$^{bc}$ | 119 | 33.2$^{b}$ | 1.7$^{cde}$ | 34.9 | 107.3$^{ab}$ |
| $T_{12}$ | 193.3$^{b}$ | 7.1$^{bc}$ | 200.4 | 55.5$^{abc}$ | 2.6$^{abcde}$ | 58.1 | 56.7$^{bc}$ |
| $T_{13}$ | 146.7$^{b}$ | 6.7$^{bc}$ | 151.4 | 48$^{bc}$ | 1.8$^{cde}$ | 49.8 | 44.5$^{c}$ |
| $T_{14}$ | 220$^{b}$ | 10.6$^{abc}$ | 230.6 | 64$^{abc}$ | 2.7$^{abcde}$ | 66.7 | 53$^{bc}$ |
| $T_{15}$ | 210$^{b}$ | 12.7$^{ab}$ | 222.7 | 60.4$^{abc}$ | 3.2$^{abc}$ | 63.6 | 76$^{bc}$ |
| $T_{16}$ | 103.3$^{b}$ | 4.5$^{c}$ | 107.8 | 31.1$^{b}$ | 1.3$^{e}$ | 32.4 | 36.8$^{c}$ |
| $T_{17}$ | 173.3$^{b}$ | 9.2$^{bc}$ | 182.5 | 54.9$^{abc}$ | 2.5$^{abcde}$ | 57.4 | 44.5$^{c}$ |
| $T_{18}$ | 221.7$^{b}$ | 11.4$^{ab}$ | 233.1 | 69$^{ab}$ | 2.6$^{abcde}$ | 71.6 | 66$^{bc}$ |
| $T_{19}$ | 146.7$^{b}$ | 6.7$^{bc}$ | 153.4 | 48$^{bc}$ | 1.8$^{cde}$ | 49.8 | 44.5$^{c}$ |
| $T_{20}$ | 196.7$^{b}$ | 9.5$^{bc}$ | 206.2 | 60.4$^{abc}$ | 2.5$^{abcde}$ | 62.9 | 49.5$^{bc}$ |
| $T_{21}$ | 210$^{b}$ | 12.7$^{ab}$ | 222.7 | 66.4$^{abc}$ | 3.2$^{abc}$ | 67.6 | 76$^{bc}$ |
| $T_{22}$ | 103.3$^{b}$ | 4.5$^{c}$ | 107.8 | 31.1$^{b}$ | 1.3$^{e}$ | 32.4 | 36.8$^{c}$ |
| $T_{23}$ | 173.3$^{b}$ | 9.2$^{bc}$ | 182.5 | 54.9$^{abc}$ | 2.5$^{abcde}$ | 57.4 | 44.5$^{c}$ |
| $T_{24}$ | 221.7$^{b}$ | 11.4$^{ab}$ | 233.1 | 69bc | 2.6$^{abcde}$ | 71.6 | 66$^{bc}$ |
| $T_{25}$ | 360$^{a}$ | 16.3$^{a}$ | 376.3 | 92$^{a}$ | 3.9$^{a}$ | 95.9 | 138$^{a}$ |
| $T_{26}$ | 235$^{b}$ | 11.5$^{ab}$ | 246.5 | 77.4$^{ab}$ | 3$^{ab}$ | 81.4 | 64$^{bc}$ |
| $T_{27}$ | 210$^{b}$ | 11.5$^{ab}$ | 221.5 | 52.7$^{abc}$ | 2.9$^{abcd}$ | 55.6 | 73.5$^{abc}$ |

*Pseudomonas* sp and *Bacillus* sp @ 15 ml per polybags and *Trichoderma* @ 3g per bag

(234.8g) among *Bacillus* followed by $T_8$ (234g) gave maximum fresh weight of whole plant. In the case of *Trichoderma* isolates, $T_{18}$ (233.1g) followed by $T_{14}$ (230.6g) gave maximum fresh weight of whole plant. Among consortia, $T_{24}$ (Sulthan Bathery consortia) produced maximum fresh weight of whole plant (233.1g) followed by $T_{21}$ (Vellanikkara consortia) (222.7g).The lowest weight was registered in $T_{22}$ (Ambalavayal consortia) (107.8 g) which was on par with $T_{16}$. The control plants recorded (221.5g) as fresh weight of whole plant. The lowest value was observed with $T_{22}$ and $T_{16}$, both were on par (107.8g).

### 4.9.7 Effect of different treatments on dry weight of vanilla

In the case of shoot dry weight, the treatments showed significant differences (Table 18.,Fig. 7 ). $T_{25}$ recorded the highest value of shoot dry weight (92 g) followed by $T_{26}$ (77.4 g). Among all the native isolates, $T_6$ gave maximum shoot dry weight (71.9g). Among *Pseudomonas* treatments, $T_2$ (59.1g) followed by $T_6$ (51.1g) and $T_7$ (71.9g) among *Bacillus* followed by $T_8$ (67.7g) gave maximum shoot dry weight. In the case of *Trichoderma* isolates, $T_{18}$ (69g) followed by $T_{14}$ (64g) gave maximum shoot dry weight. Among consortia, $T_{24}$ (Sulthan Bathery consortia) produced maximum dry shoot weight (69g) followed by $T_{21}$ (Vellanikkara consortia) (66.4g).The lowest weight was registered in $T_{22}$ (Ambalavayal consortia) (31.1 g) which was on par with $T_{16}$. The control plants registered shoot weight of 52.7g.

The treatments differed significantly with respect to root dry weight. However, maximum root dry weight was recorded in $T_{25}$ (3.9g) followed by $T_{15}$ and $T_{21}$ (Vellanikkara consortia) with a root dry weight of (3.2g). Among *Pseudomonas* isolates, $T_5$ recorded root dry weight of 2.9 g followed by $T_6$ (2.6g). In the case of *Bacillus* isolates, maximum root weight recorded by $T_9$ (2.8g) followed by $T_8$ (2.7g). In the case of *Trichoderma* isolates, $T_{15}$ recorded maximum root dry weight (3.2g) followed by $T_{14}$ (2.7g). In the case consortia, $T_{21}$ (Vellanikkara consortia) (3.2g) followed by $T_{24}$

(Sulthan Bathery consortia) (2.6g) recorded maximum root dry weight. The control plants had root fresh weight of 2.9g.

With respect to dry weight of whole plant, the treatments varied significantly. $T_{25}$ showed highest value of dry weight (95.9 g) followed by $T_{26}$ (81.4 g).Among all the native isolates , $T_7$ gave maximum dry weight of whole plant (74.1g) Among *Pseudomonas* treatments, $T_2$ (61.6g) followed by $T_6$ (57.4g) and $T_7$ (74.1g) among *Bacillus* isolates followed by $T_8$ (71.4g) gave maximum dry weight of whole plant. In the case of *Trichoderma* isolates, $T_{18}$ (71.6g) followed by $T_{14}$ (66.7g) recorded maximum dry weight of whole plant. Among consortia $T_{24}$ (Sulthan Bathery consortia) produced maximum dry weight of whole plant (71.6g) followed by $T_{21}$ (Vellanikkara consortia)(67.6g).The lowest weight was registered in $T_{22}$ (Ambalavayal consortia) (32.4 g) which was on par with $T_{16}$. The control plants recorded 55.6g as dry weight of whole plant.

### 4.9.8 Effect of different treatments on length of roots

The root length varied significantly with respect to different treatments (Table 18., Fig.8). Highest length was observed with $T_{25}$ (138cm) followed by $T_{11}$ (107.3cm).

Among all the native isolates, highest root length was observed with $T_{11}$ (107.3cm) followed by $T_{15}$ and $T_{21}$ (Vellanikkara consortia) (76cm). Among *Pseudomonas* treatments, $T_3$ recorded maximum root length (68cm) followed by $T_2$ (62.3cm). Among *Bacillus* isolates, $T_{11}$ (107.3cm) recorded maximum. Among *Trichoderma* isolates, $T_{15}$ produced roots having a length of (76cm) followed by $T_{18}$ (66cm), while in consortia maximum root length was noticed in $T_{21}$ (Vellanikkara consortia) 76 cm followed by $T_{24}$ (Sulthan Bathery consortia) (66cm). The control plants showed a root length of 73.5 cm.

## 4.10 ESTIMATION OF TOTAL PHENOL IN PLANT

Total phenol in vanilla was estimated and the results are presented in (Table 19., Fig.9). The treatments showed significant differences with maximum production of total phenol in the case of $T_{18}$ (0.043 mg $g^{-1}$) followed by $T_{24}$ (Sulthan Bathery consortia) (0.024 mg $g^{-1}$). Among *Bacillus* isolates, $T_7$ produced maximum total phenol (0.022 mg $g^{-1}$).$T_4$ produced maximum total phenol among *Pseudomonas* treatments (0.151 mg $g^{-1}$). The lowest rate of production of total phenol was by $T_{26}$ and control plants (0.004 mg $g^{-1}$).

## 4.11 ENUMERATION OF PGPM

The microbial count of inoculated PGPM was taken by serial dilution and plate count technique at monthly intervals. The result of enumeration of *Pseudomonas* sp. are presented (Table 20).The population of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. was maximum in the first two months, then it reduced. Highest population count was noticed in *Pseudomonas* sp. treated plants. In the case of *Bacillus* sp. (Table 21) and *Trichoderma* sp. (Table 22), population was maximum in the first two months and then it reduced. Microbial count of inoculated PGPM was lower in consortia treatments compared to individual treatments.

Table.19 Effect of PGPM and its consortia on the total phenol content of vanilla plant

| Treatments | Total phenol (mg $g^{-1}$ of sample) |
|---|---|
| $T_1$ | 0.019 |
| $T_2$ | 0.110 |
| $T_3$ | 0.140 |
| $T_4$ | 0.151 |
| $T_5$ | 0.016 |
| $T_6$ | 0.137 |
| $T_7$ | 0.022 |
| $T_8$ | 0.014 |
| $T_9$ | 0.020 |
| $T_{10}$ | 0.012 |
| $T_{11}$ | 0.017 |
| $T_{12}$ | 0.019 |
| $T_{13}$ | 0.022 |
| $T_{14}$ | 0.030 |
| $T_{15}$ | 0.034 |
| $T_{16}$ | 0.028 |
| $T_{17}$ | 0.026 |
| $T_{18}$ | 0.043 |
| $T_{19}$ | 0.028 |
| $T_{20}$ | 0.021 |
| $T_{21}$ | 0.016 |
| $T_{22}$ | 0.029 |
| $T_{23}$ | 0.025 |
| $T_{24}$ | 0.024 |
| $T_{25}$ | 0.016 |
| $T_{26}$ | 0.004 |
| $T_{27}$ | 0.004 |

Mean of three replications

Table. 20 Population of *Pseudomonas* sp. in vanilla rhizosphere at monthly interval

| Treatment | cfu $g^{-1}$ of soil ($\times 10^4$) | | | | | |
|---|---|---|---|---|---|---|
| | 1 MAP | 2 MAP | 3 MAP | 4 MAP | 5 MAP | 6 MAP |
| $P_1$ | $22.7^{d}$ $(4.8)^{d}$ | $13.3^{c}$ $(3.7)^{c}$ | $1.3^{e}$ $(1.3)^{e}$ | $9.7^{a}$ $(3.1)^{a}$ | $9.0^{a}$ $(3.1)^{a}$ | $4.3^{ab}$ $(2.2)^{ab}$ |
| $P_2$ | $31.0^{ab}$ $(5.6)^{ab}$ | $14.7^{bc}$ $(3.9)^{bc}$ | $3.3^{cd}$ $(1.9)^{cd}$ | $3.3^{b}$ $(1.9)^{b}$ | $5.3^{bc}$ $(2.3)^{bc}$ | $2.7^{bcd}$ $(1.7)^{bcd}$ |
| $P_3$ | $26.7^{bcd}$ $(5.2)^{bcd}$ | $17.0^{ab}$ $(4.2)^{ab}$ | $6.3^{ab}$ $(2.6)^{ab}$ | $8.3^{a}$ $(3.0)^{a}$ | $7.0^{ab}$ $(2.7)^{ab}$ | $5.0^{a}$ $(2.3)^{a}$ |
| $P_4$ | $29.0^{abc}$ $(5.4)^{abc}$ | $17.7^{a}$ $(4.3)^{a}$ | $4.0^{cd}$ $(2.1)^{cd}$ | $8.0^{a}$ $(2.9)^{a}$ | $7.0^{ab}$ $(2.7)^{ab}$ | $4.0^{abc}$ $(2.1)^{abc}$ |
| $P_5$ | $33.7^{a}$ $(5.8)^{a}$ | $15.3^{abc}$ $(4.0)^{abc}$ | $7.7^{a}$ $(2.8)^{a}$ | $7.7^{a}$ $(2.8)^{a}$ | $8.3^{a}$ $(3.0)^{a}$ | $5.0^{a}$ $(2.3)^{a}$ |
| $P_6$ | $24.7^{cd}$ $(5.0)^{cd}$ | $14.3^{bc}$ $(3.8)^{bc}$ | $4.0^{cd}$ $(2.1)^{cd}$ | $9.0^{a}$ $(3.1)^{a}$ | $8.3^{a}$ $(3.0)^{a}$ | $5.0^{a}$ $(2.3)^{a}$ |
| $C_1$ | $7.0^{ef}$ $(2.7)^{ef}$ | $6.0^{f}$ $(2.5)^{f}$ | $6.0^{ab}$ $(2.5)^{ab}$ | $3.0^{b}$ $(1.9)^{b}$ | $2.0^{de}$ $(1.6)^{de}$ | $2.0^{cd}$ $(1.6)^{cd}$ |
| $C_2$ | $5.3^{e}$ $(2.4)^{e}$ | $8.0^{def}$ $(2.9)^{def}$ | $4.0^{cd}$ $(2.1)^{cd}$ | $4.0^{b}$ $(2.1)^{b}$ | $1.0^{e}$ $(1.2)^{e}$ | $1.0^{d}$ $(1.2)^{d}$ |
| $C_3$ | $5.3^{e}$ $(2.4)^{e}$ | $10.0^{d}$ $(3.2)^{d}$ | $3.0^{d}$ $(1.9)^{d}$ | $6.0^{ab}$ $(2.5)^{ab}$ | $3.0^{cde}$ $(1.9)^{cde}$ | $3.0^{abcd}$ $(1.9)^{abcd}$ |
| $C_4$ | $7.7^{ef}$ $(2.9)^{ef}$ | $9.0^{de}$ $(3.1)^{de}$ | $4.0^{cd}$ $(2.1)^{cd}$ | $4.0^{b}$ $(2.1)^{b}$ | $4.0^{cd}$ $(2.1)^{cd}$ | $4.0^{abc}$ $(2.1)^{abc}$ |
| $C_5$ | $5.7^{e}$ $(2.5)^{e}$ | $6.0^{f}$ $(2.5)^{f}$ | $3.0^{d}$ $(1.9)^{d}$ | $3.0^{b}$ $(1.9)^{b}$ | $2.0^{de}$ $(1.6)^{de}$ | $2.0^{cd}$ $(1.6)^{cd}$ |
| $C_6$ | $9.0^{e}$ $(3.1)^{e}$ | $7.0^{ef}$ $(2.7)^{ef}$ | $5.0^{bc}$ $(2.3)^{bc}$ | $4.0^{b}$ $(2.1)^{b}$ | $3.0^{cde}$ $(1.9)^{cde}$ | $3.0^{abcd}$ $(1.9)^{abcd}$ |
| Control | $2.0^{cd}$ $1.6^{cd}$ | $2.0^{cd}$ $1.6^{cd}$ | $3.0^{cd}$ $1.8^{cd}$ | $2.0^{cd}$ $1.6^{cd}$ | $3.0^{cd}$ $1.8^{cd}$ | $3.0^{cd}$ $1.8^{cd}$ |

MAP- Month after planting
Each value represents mean of three replications.
Figures in parenthesis shows $\sqrt{x}+0.5$ transformed values

$P_1$: CHL , $P_2$ : MDR , $P_3$ : VKA, $P_4$: ABL, $P_5$: KVL,, $P_6$: SBY,$C_1$: CHL , $C_2$ : MDR , $C_3$ : VKA, $C_4$: ABL, $C_5$: KVL,, $C_6$: SBY

Table.21 Population of *Bacillus* sp. in vanilla rhizosphere at monthly interval

| Treatment | cfu $g^{-1}$ of soil ($\times 10^4$) | | | | | |
|---|---|---|---|---|---|---|
| | 1 MAP | 2 MAP | 3 MAP | 4 MAP | 5 MAP | 6 MAP |
| $B_1$ | $18.7^{abc}$ $(4.4)^{abc}$ | $10.3^{a}$ $(3.3)^{a}$ | $10.3^{a}$ $(3.3)^{a}$ | $8.0^{a}$ $(2.9)^{a}$ | $6.3^{ab}$ $(2.6)^{ab}$ | $4.3^{ab}$ $(2.1)^{ab}$ |
| $B_2$ | $21.3^{ab}$ $(4.7)^{ab}$ | $9.7^{a}$ $(3.2)^{a}$ | $8.0^{abc}$ $(2.9)^{abc}$ | $3.7^{cde}$ $(2.0)^{cd}$ | $6.3^{ab}$ $(2.6)^{ab}$ | $3.0^{b}$ $(1.9)^{b}$ |
| $B_3$ | $25.7^{a}$ $(5.1)^{a}$ | $9.3^{a}$ $(3.1)^{a}$ | $9.7^{ab}$ $(3.2)^{ab}$ | $4.0^{cde}$ $(2.1)^{cde}$ | $8.0^{a}$ $(2.9)^{a}$ | $4.0^{ab}$ $(2.0)^{ab}$ |
| $B_4$ | $21.7^{ab}$ $(4.7)^{ab}$ | $7.7^{ab}$ $(2.8)^{ab}$ | $7.7^{bcd}$ $(2.8)^{bcd}$ | $5.3^{bc}$ $(2.4)^{bc}$ | $6.7^{ab}$ $(2.6)^{ab}$ | $4.7^{ab}$ $(2.2)^{ab}$ |
| $B_5$ | $24.3^{a}$ $(4.9)^{a}$ | $10^{a}$ $(3.2)^{a}$ | $7.0^{cde}$ $(2.7)^{cde}$ | $4.7^{cd}$ $(2.3)^{cd}$ | $3.0^{c}$ $(1.8)^{c}$ | $2.0^{b}$ $(1.6)^{b}$ |
| $B_6$ | $21.7^{ab}$ $(4.7)^{ab}$ | $8.3^{ab}$ $(3)^{ab}$ | $5.3^{def}$ $(2.4)^{def}$ | $7.0^{ab}$ $(2.7^{ab}$ | $4.0^{bc}$ $(2.1)^{bc}$ | $3.0^{b}$ $(1.8)^{b}$ |
| $C_1$ | $11.3^{cde}$ $(3.4)^{cde}$ | $9.0^{a}$ $(3.1)^{a}$ | $3.0^{f}$ $(1.9)^{f}$ | $2.0^{e}$ $(1.6)^{e}$ | $4.0^{bc}$ $(2.1)^{bc}$ | $4.0^{ab}$ $(2.1)^{ab}$ |
| $C_2$ | $7.0^{e}$ $(2.7)^{e}$ | $3.0^{c}$ $(1.9)^{c}$ | $4.0^{f}$ $(2.1)^{ef}$ | $3.0^{de}$ $(1.9)^{de}$ | $3.0^{c}$ $(1.8)^{c}$ | $3.0^{b}$ $(1.9)^{b}$ |
| $C_3$ | $16.0^{bcd}$ $(4.1)^{bcd}$ | $8.0^{ab}$ $(2.9)^{ab}$ | $3.0^{f}$ $(1.9)^{f}$ | $8.0^{a}$ $(2.9)^{a}$ | $7.0^{ab}$ $(2.7)^{ab}$ | $7.0^{a}$ $(2.7)^{a}$ |
| $C_4$ | $9.0^{ef}$ $(3.1)^{ef}$ | $7.0^{ab}$ $(2.7)^{ab}$ | $5^{ef}$ $(2.3)^{ef}$ | $3.0^{de}$ $(1.9)^{de}$ | $5.0^{abc}$ $(2.3)^{abc}$ | $5.0^{ab}$ $(2.3)^{ab}$ |
| $C_5$ | $14.0^{cde}$ $(3.8)^{cde}$ | $10.0^{a}$ $(3.2)^{a}$ | $4.0^{f}$ $(2.1)^{f}$ | $3.0^{de}$ $(1.9)^{de}$ | $4.0^{bc}$ $(2.1)^{bc}$ | $4.0^{ab}$ $(2.1)^{ab}$ |
| $C_6$ | $10.0^{de}$ $(3.2)^{de}$ | $5.0^{bc}$ $(2.3)^{bc}$ | $3.0^{f}$ $(1.9)^{f}$ | $5.0^{cd}$ $(2.3)^{cd}$ | $5.0^{abc}$ $(2.3)^{abc}$ | $5.0^{ab}$ $(2.3)^{ab}$ |
| Control | $3.0^{b}$ $(1.8)^{b}$ | $3.0^{b}$ $(1.8)^{b}$ | $4.0^{ab}$ $(2.0)^{ab}$ | $2.0^{b}$ $(1.6)^{b}$ | $4.0^{ab}$ $(2.0)^{ab}$ | $3.0^{b}$ $(1.8)^{b}$ |

MAP- Month after planting
Each value represents mean of three replications.
Figures in parenthesis shows $\sqrt{x}+0.5$ transformed values

$B_1$: CHL, $B_2$ : MDR, $B_3$ : VKA, $B_4$: ABL, $B_5$: KVL, $B_6$: SBY, $C_1$ :CHL, $C_2$ : MDR, $C_3$ : VKA, $C_4$: ABL, $C_5$: KVL, $C_6$: SBY

Table. 22 Population of *Trichoderma* sp. in vanilla rhizosphere at monthly interval.

| Treatment | cfu $g^{-1}$ of soil ($\times 10^2$) | | | | | |
|---|---|---|---|---|---|---|
| | 1 MAP | 2 MAP | 3 MAP | 4 MAP | 5 MAP | 6 MAP |
| $Tr_1$ | $3.0^{cd}$ $(1.8)^{cd}$ | $5.3^{bc}$ $(2.4)^{bc}$ | $4.3^{ab}$ $(2.2)^{ab}$ | $3.7^{abc}$ $(2.0)^{abc}$ | $4.7^{ab}$ $(2.3)^{ab}$ | $3.0^{a}$ $(1.9)^{a}$ |
| $Tr_2$ | $4.3^{bcd}$ $(2.2)^{bcd}$ | $5.3^{bc}$ $(2.4)^{bc}$ | $5.0^{a}$ $(2.3)^{a}$ | $5.3^{a}$ $(2.4)^{a}$ | $5.0^{a}$ $(2.3)^{a}$ | $1.3^{bc}$ $(1.3)^{bc}$ |
| $Tr_3$ | $4.7^{bc}$ $(2.3)^{bc}$ | $5.0^{bc}$ $(2.3)^{bc}$ | $5.3^{a}$ $(2.4)^{a}$ | $5.3^{a}$ $(2.4)^{a}$ | $4.3^{ab}$ $(2.2)^{ab}$ | $3.3^{a}$ $(1.9)^{a}$ |
| $Tr_4$ | $3.7^{cd}$ $(2.0)^{bcd}$ | $3.3^{c}$ $(1.9)^{c}$ | $3.0^{bc}$ $(1.9)^{bc}$ | $3.0^{bc}$ $(1.9)^{bc}$ | $4.7^{ab}$ $(2.3)^{ab}$ | $2.0^{abc}$ $(1.6)^{abc}$ |
| $Tr_5$ | $2.3^{d}$ $(1.6)^{d}$ | $6.0^{abc}$ $(2.5)^{abc}$ | $5.0^{a}$ $(2.3)^{a}$ | $3.3^{bc}$ $(1.9)^{bc}$ | $3.7^{abc}$ $(2.0)^{abc}$ | $2.0^{abc}$ $(1.6^{abc}$ |
| $Tr_6$ | $4.0^{bcd}$ $(2.1)^{bcd}$ | $7.7^{ab}$ $(2.8)^{ab}$ | $5.0^{a}$ $(2.3)^{a}$ | $4.3^{ab}$ $(2.2)^{ab}$ | $4.7^{ab}$ $(2.3)^{ab}$ | $2.7^{ab}$ $(1.8)^{ab}$ |
| $C_1$ | $5.0^{abc}$ $(2.3)^{abc}$ | $3.0^{c}$ $(1.9)^{c}$ | $2.0^{cd}$ $(1.6)^{cd}$ | $3.0^{bc}$ $(1.9)^{bc}$ | $1.0^{d}$ $(1.2)^{d}$ | $1.0^{c}$ $(1.2)^{c}$ |
| $C_2$ | $4.0^{bcd}$ $(2.1)^{bcd}$ | $6.0^{abc}$ $(2.5)^{abc}$ | $3.0^{bc}$ $(1.9)^{bc}$ | $3.0^{bc}$ $(1.9)^{bc}$ | $2.0^{cd}$ $(1.6)^{cd}$ | $2.0^{abc}$ $(1.6)^{abc}$ |
| $C_3$ | $6.0^{ab}$ $(2.5)^{ab}$ | $5.0^{bc}$ $(2.3)^{bc}$ | $1.0^{d}$ $(1.2)^{d}$ | $2.0^{c}$ $(1.6)^{c}$ | $2.0^{cd}$ $(1.6)^{cd}$ | $2.0^{abc}$ $(1.6)^{abc}$ |
| $C_4$ | $8.0^{a}$ $(2.9)^{a}$ | $3.0^{c}$ $(1.9)^{c}$ | $3.0^{bc}$ $(1.9)^{bc}$ | $2.0^{c}$ $(1.6)^{c}$ | $3.0^{bc}$ $(1.9)^{bc}$ | $3.0^{a}$ $(1.9)^{a}$ |
| $C_5$ | $5.0^{bc}$ $(2.3)^{bc}$ | $9.0^{a}$ $(3.1)^{a}$ | $4.0^{ab}$ $(2.1)^{ab}$ | $4.0^{ab}$ $(2.1)^{ab}$ | $2.0^{cd}$ $(1.6)^{cd}$ | $2.0^{abc}$ $(1.6)^{abc}$ |
| $C_6$ | $8.0^{a}$ $(2.9)^{a}$ | $6.0^{abc}$ $(2.5)^{abc}$ | $3.0^{bc}$ $(1.9)^{bc}$ | $4.0^{ab}$ $(2.1)^{ab}$ | $2.0^{cd}$ $(1.6)^{cd}$ | $2.0^{abc}$ $(1.6)^{abc}$ |
| Control | $1.0^{c}$ $(1.2)^{c}$ | $2.0^{abc}$ $(1.6)^{abc}$ | $2.0^{abc}$ $(1.6)^{abc}$ | $1.0^{c}$ $(1.2)^{c}$ | $2.0^{abc}$ $(1.6)^{abc}$ | $1.0^{c}$ $(1.2)^{c}$ |

MAP- Month after planting
Each value represents mean of three replications.
Figures in parenthesis shows $\sqrt{x}+0.5$ transformed values

$Tr_1$ :CHL, $Tr_2$ : MDR, $Tr_3$ : VKA, $Tr_4$: ABL, $Tr_5$: KVL, $Tr_6$: SBY, $C_1$ :CHL, $C_2$ : MDR, $C_3$ : VKA, $C_4$: ABL, $C_5$: KVL, $C_6$: SBY

4. 12 N, P AND K ANALYSIS

### 4.12.1 Nitrogen

N, P and K in the plant samples were analysed after six months of planting. The nitrogen content of plant was estimated by Micro-kjeldhal method. While phosphorous and potassium was estimated by spectrophotometry and flame photometry respectively.

From the (Table 23., Fig.10) it was evident that the treatments showed significant differences in the case of nitrogen content. The treatment $T_{25}$ recorded maximum $N_2$ content (4.9 per cent) followed by $T_6$, $T_{15}$ and $T_{21}$ (Vellanikkara consortia) (4.0 per cent). Among *Bacillus* isolates, $T_8$ recorded maximum N content (3.9 per cent) followed by $T_7$ (3.8 per cent) and lowest value was recorded by $T_{17}$, (2.3 per cent). The control plants recorded only 3 per cent.

### 4.12.2 Phosphorus

From (Table 23., Fig.11), it was evident that the treatments didn't show significant differences in the case of phosphorous content. Although, the treatments did not differ significantly, $T_{25}$ inoculated plants gave maximum P content (0.5 per cent). Among *Bacillus* isolates, $T_7$ and $T_8$ recorded maximum P content (0.4 per cent) which was on par with $T_5$, $T_6$, $T_{14}$ ,$T_{20}$(Mundoor consortia) , $T_{21}$ (Vellanikkara consortia) , $T_{23}$ (Kolvayal consortia) and $T_{24}$(Sulthan Bathery consortia). The lowest value was recorded by $T_{11}$ (0.34 per cent).The control plants recorded 0.3 per cent.

Table.23 Effect of PGPM and its consortia on nitrogen, phosphorous and potassium content of vanilla

| Treatment | N(%) | P(%) | K(%) |
|---|---|---|---|
| $T_1$ | $3.4^{ab}$ | $0.32^{ab}$ | $1.6^{b}$ |
| $T_2$ | $3.5^{ab}$ | $0.34^{ab}$ | $1.6^{b}$ |
| $T_3$ | $3.0^{b}$ | $0.27^{b}$ | $1.5^{b}$ |
| $T_4$ | $2.5^{b}$ | $0.21^{b}$ | $1.1^{b}$ |
| $T_5$ | $3.8^{ab}$ | $0.4^{ab}$ | $1.9^{ab}$ |
| $T_6$ | $4.0^{ab}$ | $0.4^{ab}$ | $2.0^{ab}$ |
| $T_7$ | $3.8^{ab}$ | $0.4^{ab}$ | $1.8^{b}$ |
| $T_8$ | $3.9^{ab}$ | $0.4^{ab}$ | $1.6^{b}$ |
| $T_9$ | $3.0^{b}$ | $0.3^{b}$ | $1.1^{b}$ |
| $T_{10}$ | $3.3^{ab}$ | $0.24^{b}$ | $1.4^{b}$ |
| $T_{11}$ | $2.5^{b}$ | $0.18^{b}$ | $1.1^{b}$ |
| $T_{12}$ | $3.4^{ab}$ | $0.36^{b}$ | $1.7^{b}$ |
| $T_{13}$ | $2.8^{b}$ | $0.25^{b}$ | $1.3^{b}$ |
| $T_{14}$ | $3.9^{ab}$ | $0.4^{ab}$ | $1.7^{b}$ |
| $T_{15}$ | $4.0^{ab}$ | $0.5^{ab}$ | $1.8^{b}$ |
| $T_{16}$ | $2.3^{b}$ | $0.2^{b}$ | $1.3^{b}$ |
| $T_{17}$ | $3.4^{ab}$ | $0.3^{ab}$ | $1.5^{b}$ |
| $T_{18}$ | $3.7^{ab}$ | $0.4^{ab}$ | $1.6^{b}$ |
| $T_{19}$ | $2.8^{b}$ | $0.3^{ab}$ | $1.1^{b}$ |
| $T_{20}$ | $3.6^{ab}$ | $0.4^{ab}$ | $1.5^{b}$ |
| $T_{21}$ | $4.0^{ab}$ | $0.4^{ab}$ | $1.9^{ab}$ |
| $T_{22}$ | $2.4^{b}$ | $0.2^{b}$ | $1.1^{b}$ |
| $T_{23}$ | $3.4^{ab}$ | $0.4^{ab}$ | $1.4^{b}$ |
| $T_{24}$ | $3.6^{ab}$ | $0.4^{ab}$ | $1.6^{b}$ |
| $T_{25}$ | $4.9^{a}$ | $0.5^{a}$ | $2.8^{a}$ |
| $T_{26}$ | $3.4^{ab}$ | $0.3^{b}$ | $1.5^{b}$ |
| $T_{27}$ | $3.0^{4ab}$ | $0.3^{b}$ | $1.4^{b}$ |

*Pseudomonas* sp and *Bacillus* sp @ 15 ml per polybags and *Trichoderma* @ 3g per bag

### 4.12.3 Potassium

The treatments showed no significant differences (Table 23., Fig.12). Maximum K content was recorded by $T_{25}$ (2.8 per cent) followed by $T_6$ (2 per cent). Among *Bacillus* isolates, $T_7$ recorded maximum K content (1.8 per cent) which was on par with $T_{15}$. The control plants recorded 1.4 per cent where as the lowest value was noticed with $T_4$ , $T_9$ , $T_{11}$, $T_{19}$ (Chengaloor consortia) and $T_{22}$ (Ambalavayal consortia) (1.1 per cent).

## 4.13 IDENTIFICATION OF MOST EFFECTIVE NATIVE FROM VANILLA RHIZOSPHERE

Based on the results of the experiment, the most promising native isolates were identified based on the biochemical characters.

### 4.13.1 Biochemical Tests

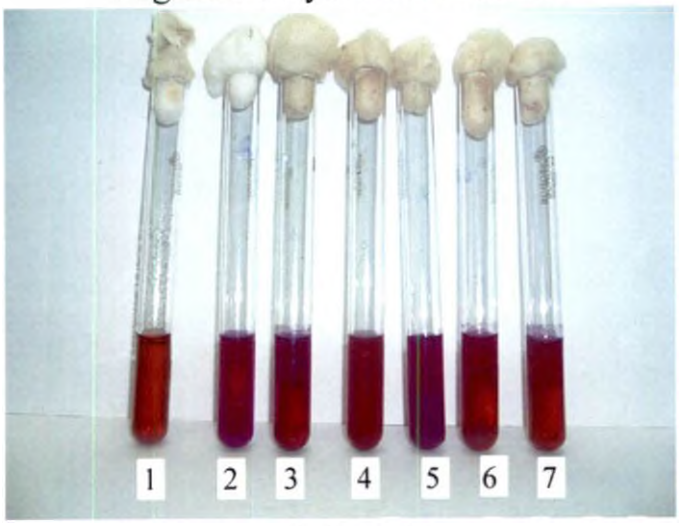
#### *4.13.1.1 Identification of bacterial isolates*

For biochemical characterization of *Pseudomonas* sp., Potassium hydroxide test, Catalase activity, Starch hydrolysis, Arginine dihydrolase reaction, Nitrate reduction test and Urease test were carried out while for *Bacillus* sp., Potassium hydroxide test, Catalase activity, Starch hydrolysis, Voges-Proskauer test and Glucose- Nutrient agar (one per cent) were done.

For *Pseudomonas* sp., Catalase activity, Urease test, Arginine dihydrolase reaction and Potassium hydroxide test were positive and hydrolysis of starch and reduction of nitrate were negative (Plate IV ). These characters were compared with characters described in Bergey's Manual of Systematic Bacteriology and *Pseudomonas* sp. was tentatively identified as *P.fluorescens*. For *Bacillus* sp., Catalase activity, growth

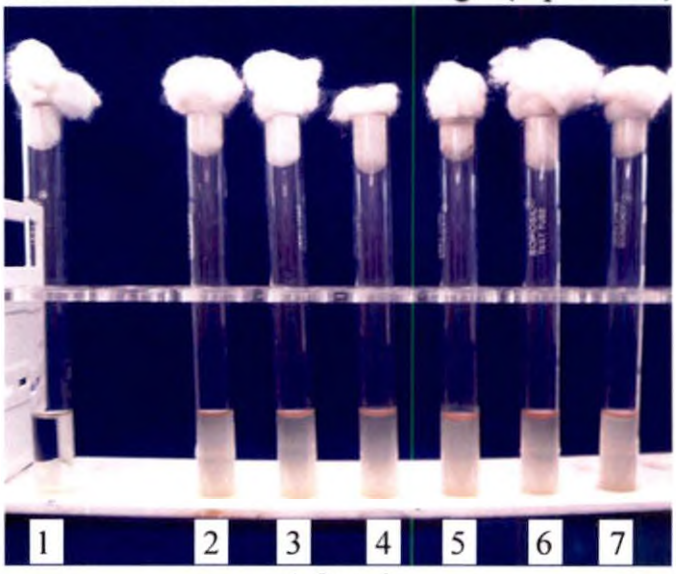
# Plate IV

Arginine dihydrolase reaction




1. Control
2. $P_1$
3. $P_2$
4. $P_3$
5. $P_4$
6. $P_5$
7. $P_6$

Growth on Glucose-nutrient agar(1 per cent)




1. Control
2. $B_1$
3. $B_2$
4. $B_3$
5. $B_4$
6. $B_5$
7. $B_6$

Table.24 Cultural and morphological characters of *Pseudomonas* sp. isolated from vanilla rhizosphere

| Sl No. | Isolates | Shape of cells | Colour of colony | Gram reaction | Catalase activity | Urease test | Arginine Dihydrolase test | Starch hydrolysis | Pigment production | KOH test | Nitrate reduction test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $P_1$ | Rod | Pale yellow | -ve | + | + | + | - | + | + | - |
| 2 | $P_2$ | Rod | Pale yellow | - ve | + | + | + | - | + | + | - |
| 3 | $P_3$ | Rod | Pale yellow | - ve | + | + | + | - | + | + | - |
| 4 | $P_4$ | Rod | Pale yellow | - ve | + | + | + | - | + | + | - |
| 5 | $P_5$ | Rod | Pale yellow | - ve | + | + | + | - | + | + | - |
| 6 | $P_6$ | Rod | Pale yellow | - ve | + | + | + | - | + | + | - |

+ : Positive
- : Negative

$P_1$: CHL , $P_2$ : MDR , $P_3$ : VKA, $P_4$: ABL, $P_5$: KVL., $P_6$: SBY

Table.25 Cultural and morphological characters of *Bacillus* sp. isolated from vanilla rhizosphere soil

| Sl No. | Isolates | Shape of cells | Colour of colony | Gram reaction | Catalase activity | pH in V-P broth | | 1% Glucose-Nutrient Agar | Starch hydrolysis | KOH test | Endospore staining |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | <6 | >7 | | | | |
| 1 | $B_1$ | Rod | Cream | + ve | + | - | ++ | + | + | - | + |
| 2 | $B_2$ | Rod | Cream | + ve | + | - | ++ | + | + | - | + |
| 3 | $B_3$ | Rod | Cream | + ve | + | - | ++ | + | + | - | + |
| 4 | $B_4$ | Rod | Cream | + ve | + | - | ++ | + | + | - | + |
| 5 | $B_5$ | Rod | Cream | + ve | + | - | + | + | + | - | + |
| 6 | $B_6$ | Rod | Cream | + ve | + | - | + | + | + | - | + |

+ : Positive
- : Negative
++: High growth

$B_1$: CHL, $B_2$ : MDR , $B_3$ : VKA, $B_4$: ABL,$B_5$: KVL., $B_6$: SBY

at pH >7( V-P test), growth in Glucose- Nutrient agar (one per cent)( (Plate IV ) and hydrolysis of starch were positive and growth at pH <6 ( V-P test) and Potassium hydroxide test were negative. These characters were compared with characters described in Bergey's Manual of Systematic Bacteriology and *Bacillus* sp. was tentatively identified as *B.subtilis*.

### *4.13.1.2* Identification *of Trichoderma* sp.

Cultural and morphological characters of the efficient *Trichoderma* were studied and the characters were compared with description for *Trichoderma*. The mycelial growth of fungi was rapid, light green to bright green, hyaline, smooth and septate. Chlamydospores were mostly globose, smooth and 6-12μm in diameter. Conidiophores were loose tuft and produced numerous side branches especially at the lower portion. Phialides consisted of whorls up to five, short, skittle shaped, narrower at the base and bulged at the middle and attenuated abruptly into sharp pointed neck. Phialides were 10-12 μm long and 3- 4 μm width. Phialospores were single and accumulate. They were sub globose or short ovoid, smooth, pale green, much darker in mass. Based on these characters the isolate was identified as *T. harzianum* .

# *Discussion*

# 5. DISCUSSION

Vanilla (*Vanilla planifolia* Andrews.), a native of Mexico belongs to Orchidaceae family and is mainly cultivated as a source of natural vanillin and this vanillin is widely used in the preparation of ice creams, chocolates, bakery products, liquors and also in perfumery and pharmaceuticals. At present, synthetic products such as ethyl vanillin and synthetic vanillin are used instead of natural vanillin. Due to shortage of natural vanillin, manufactures of confectionary and others are forced to use synthetic vanillin as a substitute for natural one. But, such products are being rejected by consumers world over, which led to increased demand for natural vanillin. Most of the countries have banned the synthetic vanillin, especially in Europe. In this context, there is high demand for natural vanillin in the international market which fetches higher price. Under these circumstances, the roles of rhizosphere microorganisms assume greater importance.

Vanilla is known to be highly amenable to organic cultivation. Therefore, it is necessary to increase the production of natural vanillin by increasing the area under vanilla cultivation. In order to increase the area, more number of planting materials are required. The number of planting materials depends on the length of cuttings. If shorter cuttings are used, the juvenile phase will be longer. Hence, the growth of vanilla in the nursery has to be enhanced for getting early flowering and to obtain more number of cuttings as planting materials.

Plant growth promoting microorganisms (PGPM) are abundant in the rhizosphere region. These PGPM produce various growth promoting substances, which promote root growth and helps in absorbing sufficient nutrients leading to vigorous plant growth and also increase the activity of beneficial organisms in the rhizosphere. The use of rhizosphere microorganisms are gaining much importance in recent years because of its cost effectiveness and ecofriendly nature which maintains the biological balance of

nature (Sivaprasad, 2002).Even though, combination of different PGPM are known to have a better effect on plants than single, it is essential to test its efficacy in comparison with individual PGPM as sometimes, individual PGPM performs better in certain situations of plant microbe interaction. Hence, the present study was taken up to induce growth promotion in vanilla seedlings in nursery through consortial application of PGPM.

Beneficial rhizobacteria and fungus are important because of their ability to improve plant growth through production of plant growth regulators as well as suppression of deleterious root colonizing microorganisms ( Kloepper *et. al.,* 1980 and Suslow,1982). Even though, there are several beneficial rhizobacteria and fungus in the rhizosphere, their efficiency varies depending on its competitive ability, population and density as well as its ability to survive under stress conditions. Therefore, it is essential to evaluate their efficiency in improving the growth of plants .Hence, different isolates of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were screened under *in vitro* conditions and then tested their consortial effect on growth promotion under *in planta* conditions.

For the present study, six isolates each of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. isolated from three locations each of Thrissur (Chengaloor, Mundoor and Vellanikkara) and Wynad (Ambalavayal, Kolvayal and Sulthan Bathery) districts were used singly and in combinations along with commercial cultures of *P.fluorescens* and *T.viride.* In the present study, several attempts made on the isolation of orchid mycorrhiza, was a failure. The probable reasons for this might be due to the fact that the vanilla is an introduced crop. Moreover, establishment of orchid mycorrhiza in the rhizosphere might take a long time before it establishes a symbiotic association with vanilla or the orchid mycorrhiza fungus could have lost its viability due to digestion by host (Bernard ,1909). This is beyond the scope of present investigation to go deeper into these aspects.

The selected PGPM like *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were identified based on cultural and morphological characters and these characters were compared with description documented by various workers. Cultural characters of bacteria like sliminess of the colony and yellowish green fluorescence under UV light and morphological character like negative gram reaction and shape of cells were compared with the key characters described in Bergey's Manual of Systematic Bacteriology and the bacterial isolate was identified as fluorescent *Pseudomonas* sp. The bacterial isolates having dry, wrinkled and cream coloured colonies which are gram positive and central endospore forming was compared with the key characters mentioned in Bergey's Manual of Systematic Bacteriology and the bacterial isolate was identified as *Bacillus* sp. The fungus was identified based on the cultural and morphological characters. The mycelial growth of fungi was rapid, light green to bright green, hyaline, smooth and septate. Chlamydospores were mostly globose, smooth and 6-12µm in diameter. Conidiophores were loose tuft and produced numerous side branches especially at the lower portion. Phialides consisted of whorls up to five, short, skittle shaped, narrower at the base and bulged at the middle and attenuated abruptly into sharp pointed neck. Phialides were 10-12 µm long and 3- 4 µm width. Phialospores were single and accumulate. They were sub globose or short ovoid, smooth, pale green, much darker in mass. Based on these characters the isolate was identified as *Trichoderma* sp.

*Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. are known to produce growth promoting substances and there by enhance the growth of plants. Hence, in the present study *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were evaluated for its efficiency in production of growth promoting substances like IAA under *in vitro* conditions. Among all the isolates tested, fluorescent *Pseudomonas* (0.099 mg $ml^{-1}$) isolated from Sulthan Bathery ($T_6$) recorded higher IAA production (Fig.1). This result was in accordance with study of Paul *et. al.* (2005), who reported that *P. fluorescencs* strains could significantly increase the root biomass and root length in treated black pepper, which was due to the production of plant growth hormones like IAA, gibberllic

Fig. 1 Effect of PGPM on the IAA production in vanilla plants

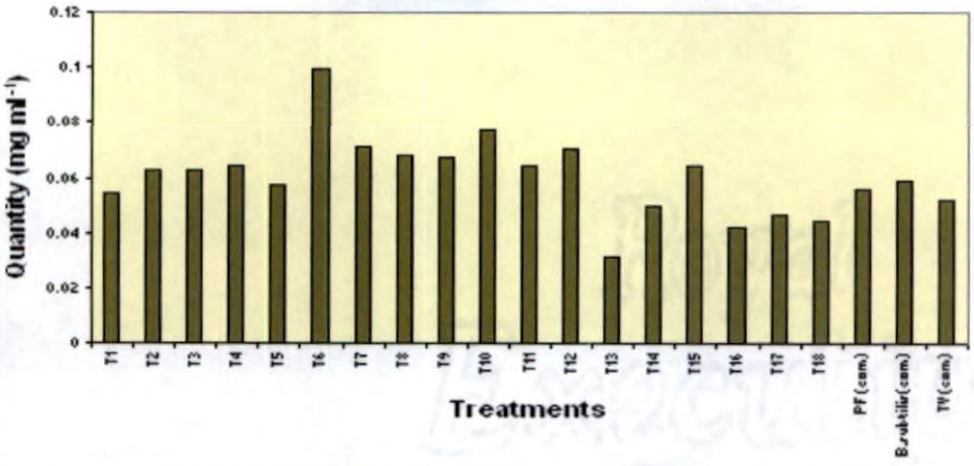
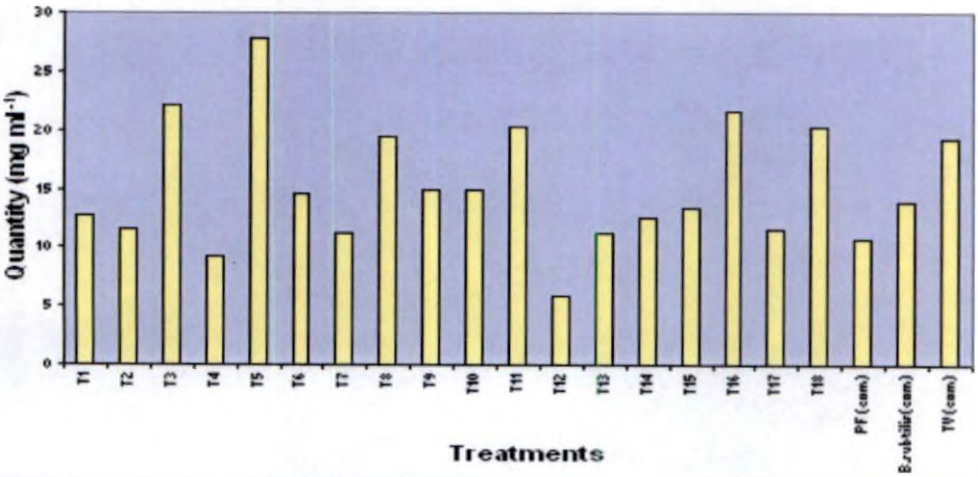



Fig .2 Effect of PGPM on the salicylic acid production in vanilla plants



acid by these bacterial strains and their phosphate solubilization potential. Gill *et. al.* (1989) also reported that beneficial fluorescent pseudomonads and other microorganisms can promote plant growth and induce disease suppressiveness by mechanisms other than siderophore production namely through competition for carbon or through production of hormones, antibiotics or bacteriocins. In the case of *Bacillus* sp. maximum IAA was produced by the isolate from Ambalavayal ($T_{10}$) (0.077 mg $ml^{-1}$). In a similar experiment, Bochow *et. al.* (1999) studied the mode of action of root colonizing *B.subtilis* and showed an increased level of phytohormones, which significantly increased activity of $\beta$1,3-glucanase.However, a search on literature revealed many reports indicating the efficiency of *Trichoderma* spp. in growth promotion of various crops, which might be due to the effect of phytohormones produced by *Trichoderma* spp. Even though, no reports are available regarding the production of IAA by *Trichoderma* spp. in the present study all *Trichoderma* isolates tested produced IAA ranging from 0.031- 0.064 mg $ml^{-1}$, of which 0.064 mg $ml^{-1}$ was recorded in *Trichoderma* isolate of Vellanikkara ($T_{15}$).

The isolates were also studied for its effect on germination as well as seedling vigour using cowpea and sorghum seeds. All the seeds attained 100 per cent germination with *Pseudomonas* sp. and *Bacillus* sp. In the case of cowpea seeds treated with bacteria under *in vitro* condition, *Pseudomonas* sp. from Ambalavayal ($T_4$) recorded maximum shoot length (7.6 cm) and root length (5.4 cm).Similarly, the maximum shoot length was recorded by *Pseudomonas* sp. of Sulthan Bathery($T_6$) (6.2 cm) and root length by Chengaloor *Pseudomonas* ($T_1$) (6.4 cm) isolates for sorghum seeds .In the case of *Bacillus* sp. treated cowpea seeds, maximum shoot length (7.4 cm) and root length (7.5 cm) was recorded with Kolvayal isolate($T_{11}$). But, the maximum shoot length was recorded in the case of Mundoor ($T_8$) and Sulthan Bathery ($T_{12}$) *Bacillus* sp. (3.6 cm) for sorghum seeds. However, the maximum root length was (6.1 cm) recorded by Kolvayal *Bacillus* ($T_{11}$). The studies on IAA production and seed germination test using different isolates indicated the potentiality of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. as the plant growth promoter. In a similar study, Thomas and Vijayan (2003) reported

that seed coating with *B. subtilis* and *P. fluorescens* had resulted in increased seed germination and enhanced growth and vigor due to increase in seedling height, root length and leaf area of cardamom. Niranjan *et. al.*(2003) reported that *B. subtilis* IN937b and other *Bacillus* spp., when used as fresh suspension or powdered formulation, significantly enhanced the germination rate of pearl millet seeds compared with untreated controls. Srivastava *et. al.* (1999) reported that *Pseudomonas* strains promoted wheat growth in terms of root and shoot length and weight. This test indicated that bacterial isolates, *Pseudomonas* and *Bacillus* enhance the germination per cent of seeds and growth of seedlings.

Salicylic acid is an important factor for the defense mechanism in plants against pathogens. Application of PGPM help in inducing systemic resistance in plants, of which salicylic acid play an important role. The next point of investigation was to study the salicylic acid production of different PGPM under *in vitro* condition. Among the microorganisms used, *Pseudomonas* sp. recorded maximum salicylic acid production as compared to *Bacillus* sp. and *Trichoderma* sp. (Fig.2). Among the bacteria, Kolvayal isolates of *Pseudomonas* ($T_5$) and *Bacillus* ($T_{11}$) recorded maximum salicylic acid production of 27.9 $\mu g\ ml^{-1}$ and 20.2 $\mu g\ ml^{-1}$ respectively. However, among *Trichoderma* sp. Ambalavayal isolate ($T_{16}$) recorded maximum concentration (21.7$\mu g\ ml^{-1}$). Meyer *et al.*(1997), Maurhofer *et. al.*(1998) and Muthukumar and Bhaskaran (2007), reported that *P. fluorescens* showed higher production of salicylic acid under *in vitro* conditions.

As consortia development involves the combination of different isolates to have broad spectrum of biological activity and synergistic effect, it is necessary to study the compatibility between the isolates before the microorganisms are mixed. Therefore, in the present study, all the six isolates of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were evaluated for mutual compatibility between the isolates under *in vitro* condition. The results indicated that all the *Pseudomonas* sp. and *Bacillus* sp. combinations were mutually compatible with each other. Similar to this study, the compatibility of *B. subtilis* with *Azospirillum* under *in vitro* and *in vivo* has been

reported by Sankar and Jayarajan (1996).Anandaraj and Sarma (2003) reported the same that *T. harzianum* and fluorescent *Pseudomonas* are mutually compatible and successfully colonized black pepper rhizosphere. In the annual report of DBT (2007), they also reported that all *Trichoderma* and bacterial antagonists tested were compatible, except *T.Virens* from ginger and *Bacillus* sp from chilli. In the annual report of KSCSTE project (2007), they showed that *P. fluorescens* and *Bacillus* sp. and *P. fluorescens* and *Trichoderma* sp. isolated from vanilla rhizosphere are mutually compatible. Shahida (2007) also reported that *Trichoderma* and *P. fluorescens* were compatible with each other. The present studies on mutual compatibility indicated that all the isolates obtained were compatible with each other.

In recent years lots of fungicides and insecticides are being used to control various diseases and pests in several crops. Moreover, farmers use various agrochemicals along with microbial inoculants, particularly when the diseases and pest occurrence is rapid and serious. Hence, a study was conducted to evaluate for compatibility between *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. with selected fungicides and insecticides. In the case of *Pseudomonas* isolates, all the selected fungicides like Bordeaux mixture, copper oxychloride, copper hydroxide, potassium phosphonate and mancozeb and insecticides like carbaryl, chlorpyriphos and lambda cyhalothrin at different concentrations completely inhibited their growth The results were similar to Bhavani (2004), who observed that Bordeaux mixture and Fytolan at all concentrations inhibited the growth of *P.fluorescens*. Priya (2005) also observed that Bordeaux mixture, Kocide and Fytolan were inhibitory to the *P. fluorescens*. Shahida (2007) reported that Bordeaux mixture, Fytolan, copper hydroxide and mancozeb exerted varying level of inhibition on growth of *P. fluorescens*.

In the case of *Bacillus* isolates, all the selected fungicides and insecticides at all concentrations completely inhibited their growth, except carbaryl which was compatible with isolates of Chengaloor ($T_7$), Mundoor ($T_8$) and Vellanikkara ($T_9$) at all concentration. Guven *et. al.* (2003) reported that Mancozeb at as low as 0.1 ppm

inhibited *B.subtilis*. Bhattacharya *et. al.*(2004) reported that Carbaryl was compatible with *B. subtilis*. Similarly, in the case of *Trichoderma* isolates, all the selected fungicides and insecticides at all concentrations completely inhibited their growth. Bordeaux mixture at all concentrations, completely inhibited the growth of *Trichoderma* sp.The result is in agreement with the findings of many workers (Shanmugham, 1996., Paciulyte, (2000)., Vijayaraghavan, 2003., Bhavani,2004., Priya,2005., Shahida, 2007), where they observed that Bordeaux mixture at all concentrations tested completely inhibited the growth of *T. harzianum* and *T. viride*. Maximum per cent of inhibition was noticed with copper hydroxide and copper oxychloride at all the three concentrations tested. Bhavani (2004) reported that carbaryl inhibits *T. harzianum* and *T. viride* at all concentrations.

Present studies on compatibility between PGPM isolates and selected fungicides and insecticides indicated that all the *Pseudomonas* isolates were incompatible with all the selected fungicides and insecticides at different concentrations tested. However, all *Bacillus* isolates were incompatible with all fungicides and insecticides at all concentrations tested. Where as, carbaryl was compatible with Chengaloor ($T_7$) ,Mundoor ($T_8$) and Vellanikkara ($T_9$) isolates at all concentrations. Similarly, in the case of *Trichoderma* isolates, all the selected fungicides and insecticides at all concentrations completely inhibited their growth. From the compatibility studies carried out to find out whether the selected PGPM were compatible with commonly used fungicides and insecticides, it was clear that most of the chemicals were inhibitory to the rhizosphere microflora. So, the use of chemicals has to be decreased for the effectiveness of the PGPM.

Different isolates of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were screened for efficiency in enhancing the growth of vanilla in nursery. The *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were used singly and in combinations in the study. Among the native isolates treated plants, *Bacillus* sp of Chengaloor ($T_7$) sprouted early (30 DAP). From the (Fig.3), it was clear that different consortia of Chengaloor ($T_{19}$),

Fig.3 Effect of PGPM and its consortia on the no. of days taken for sprouting of vanilla

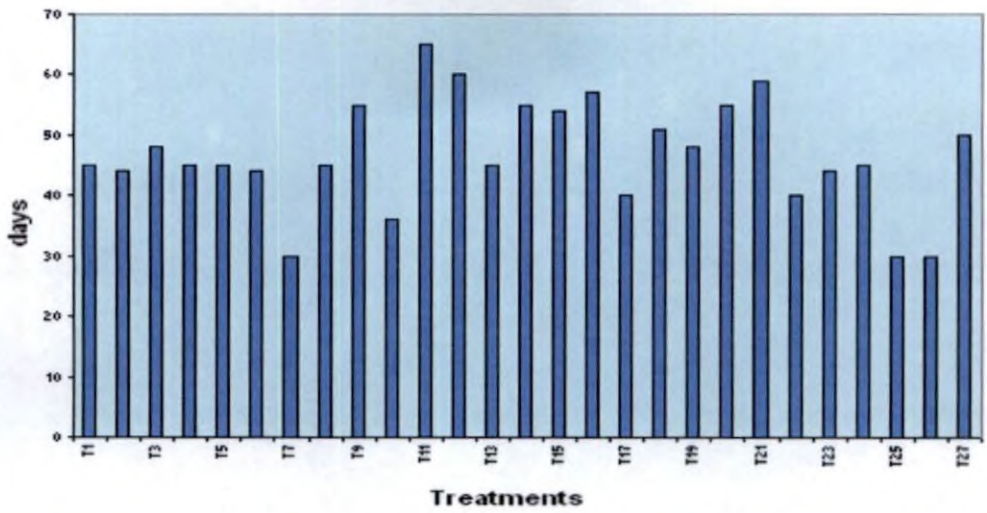
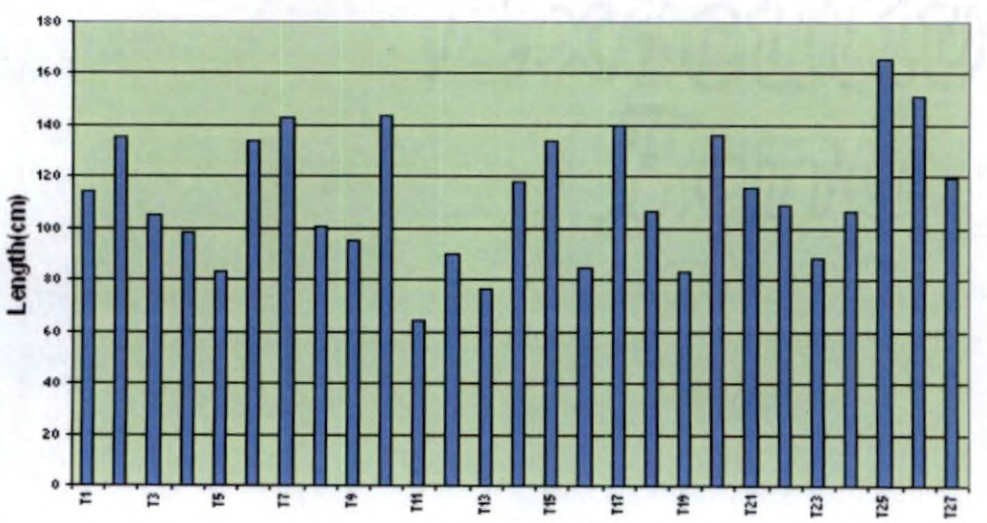



Fig.4 Effect of PGPM and its consortia on the vine length of vanilla



Ambalavayal ($T_{22}$), Kolvayal ($T_{23}$) and Sulthan Bathery ($T_{24}$) have taken less time for sprouting than control. Based on the results in the present study, most of the individual as well as consortia treated vanilla cuttings sprouted early as compared to control. It was clear that selected PGPM have good effect on the number of days taken for sprouting in vanilla. Gulati (1999) reported that application of fluorescent Pseudomonads significantly increased the growth of crop plants. It also caused increase in shoot length, root length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight and plant biomass. In a similar study, the extracts of the volatiles produced by PGPR strains *B. subtilis* (GBO3) and *B. amyloliquefaciens* (IN937a) induced plant growth promotion at a level similar to that induced by other PGPR strains (Ryu *et. al.,* 2003). According to Binimol (2000) and Harman (2000), *Trichoderma* spp. can increase the rate of growth and development of pepper plant and also produced more robust roots. In the present study among *Trichoderma*, Kolvayal isolate ($T_{17}$) sprouted early (40 DAP). In the case of consortial applications, Ambalavayal consortia ($T_{22}$) sprouted early (40 DAP). Sidorenko *et. al.* (1996) reported that combined inoculation of *Azotobacter, Bacillus* and *Pseudomonas* increased plant height, biomass and tuber yield in potato. Field trials with formulations of several PGPR *viz.* LS213 (*B.subtilis* strain GBO3+*B.amyloliquifaciens* strain IN937a), LS254 (*B.subtilis* strain GBO3+*B.pumilus* strainSE34), LS255 (*B.subtilis* strain GBO3+ *B.subtilis* strain IN937b), LS256 (*B.subtilis* strain GBO3+*B.pumilus* strainINR7) and LS261 (*B.subtilis* strain GBO3+*B.cereus* strain C4) showed significant increase in tomato and pepper growth and yield (Burelle *et al.*, 2002). In a similar study, Karunakaran *et. al.* (2003) reported that under glass house conditions, the treatment combinations *P. fluorescens*+ *B. subtilis* and *P. fluorescens* + *T. viride* were found to be the best. Shahida (2007) reported that *T.harzianum* and *Pseudomonas* sp exerted higher per cent of sprouting of vanilla cuttings over control. The result on biometric characters like length of sprout, number of leaves and girth of vine revealed that the selected isolates showed significant differences among them. Among all the isolates studied, *Bacillus* of Ambalavayal ($T_{10}$) was the best which gave maximum length (143.7 cm). When we compare the individual isolates with consortia, the isolates particularly *Bacillus* sp gave maximum vine length when compared to

consortia treatments (Fig. 4). However, Mundoor consortia ($T_{20}$) (136cm) was found to be better than control treatment. It was clear that all the isolates tested had good effect on the vine length of vanilla. This result is in accordance with the earlier observations of Srivastava *et. al.* (1999), who also reported that *Pseudomonas* strains promoted growth of crop plants in terms of root and shoot length and weight. In one of the experiments, Ramamoorthy *et. al.* (2002) found that *P.fluorescens* caused increased plant growth in tomato and hot pepper compared to *P. putida*. Paul *et. al.* (2003) reported that the treatment with *P.fluorescens* resulted in enhanced plant vigor due to the increased nutrient mobilization in the rhizosphere of black pepper. Fluorescent *Pseudomonas* has significant effect on growth and root development of black pepper (Sivaprasad *et. al.*, 2003).In the case of number of leaves, Chengaloor *Bacillus* ($T_7$) was the best which gave maximum number of leaves (23) (Fig.5). When compared with the individual isolates and consortia, it was clear that individual isolates particularly *Bacillus* sp. gave maximum number of leaves when compared to consortia treatments. Sattar *et. al.* (2003) reported that the *B. subtilis* treatment increased the survival of geranium cuttings, initiated early root production and produced higher growth and herb yield. Among all the native isolates treated plants selected, *Pseudomonas* isolated from Mundoor ($T_2$) recorded maximum girth (3.2cm). All other isolates produced almost same girth of vine (3 cm). The girth was almost similar in individual isolates treated plants, consortia treated plants and control plants. Internodal length was taken after the cutting had attained sufficient length. It was taken as an average of three internodal length at monthly interval up to six months after planting. After six months of planting, *Trichoderma* from Vellanikkara ($T_{15}$) and Ambalavayal ($T_{16}$) isolates showed maximum internodal length (8 cm). Here also, the treatments did not differ significantly .When compared among treatments as a whole, *Trichoderma* from Vellanikkara ($T_{15}$) and Ambalavayal ($T_{16}$) isolates recorded maximum internodal length of 8 cm. The internodal length of plants treated with different consortia was lower than that of individual isolates. When we compare individual isolates with commercial isolates, individual isolates are found to be better, especially *Trichoderma* sp. But, when we compare consortia treatments with control treatment, consortia was found to be the best. In a

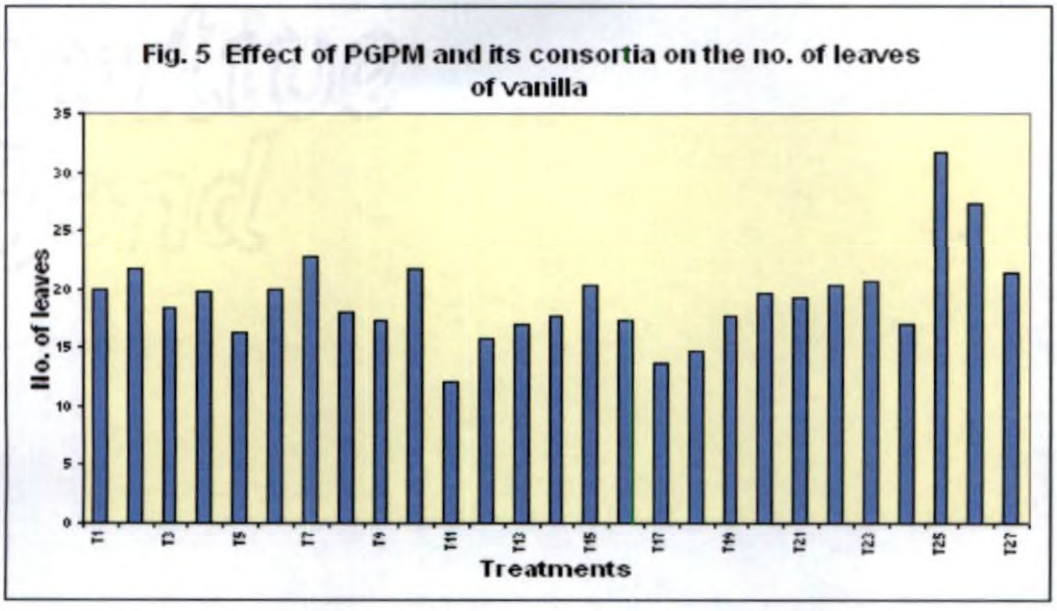


Fig. 5 Effect of PGPM and its consortia on the no. of leaves of vanilla
No. of leaves
0
5
10
15
20
25
30
35
T1
T3
T5
T7
T9
T11
T13
T15
T17
T19
T21
T23
T25
T27
Treatments

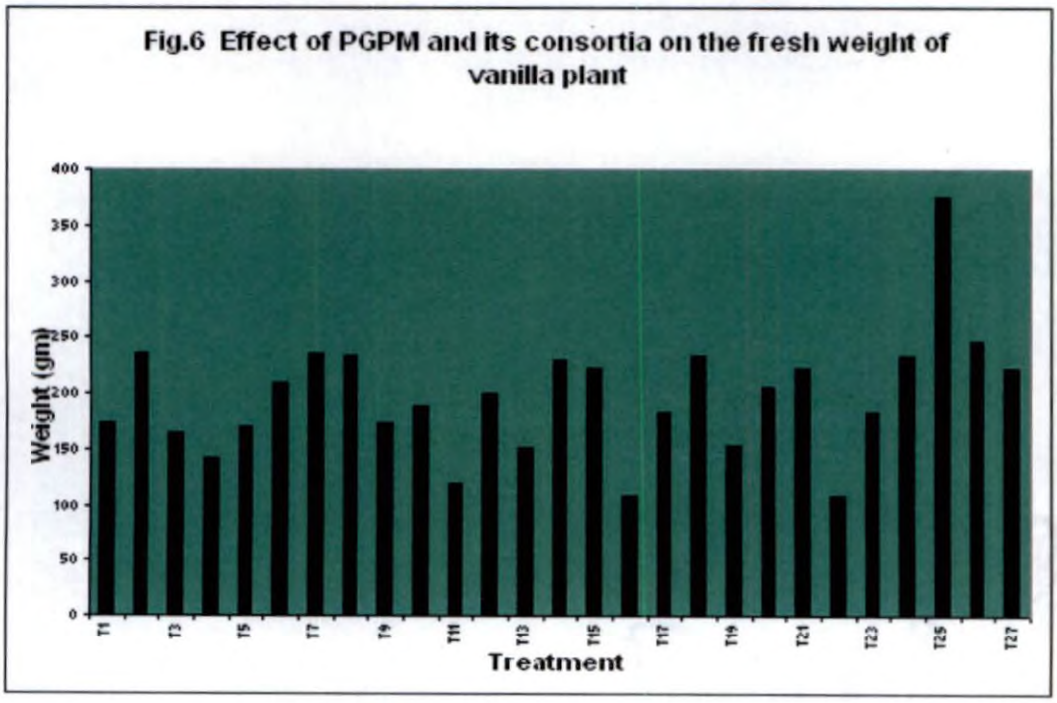


Fig.6 Effect of PGPM and its consortia on the fresh weight of vanilla plant
Weight (gm)
0
50
100
150
200
250
300
350
400
T1
T3
T5
T7
T9
T11
T13
T15
T17
T19
T21
T23
T25
T27
Treatment

similar study, Shahida (2007) reported that inoculation of PGPM like *Pseudomonas* and *Trichoderma* in vanilla nursery increased girth, number of leaves, internodal length, length of sprout and also gave early sprouting.

One of the important parameter for evaluation of efficacy of PGPM is to inoculate the plants and assess the plant biomass. In the present study, inoculated isolates showed that fresh weight of shoot, root and dry weight of shoot, root and root length varied significantly .Among all the treatments, Chengaloor *Bacillus* ($T_7$) gave maximum shoot fresh weight of 226.7g and fresh weight of whole plant 234.8g (Fig.6 ). In the case of root fresh weight, *Trichoderma* of Vellanikkara ($T_{15}$) and Vellanikkara consortia ($T_{21}$) recorded maximum (12.7g). Among all the treatments, Chengaloor *Bacillus* ($T_7$) recorded maximum shoot dry weight (71.9g) and dry weight of whole plant (74.1g) (Fig.7). In the case of root dry weight, *Trichoderma* of Vellanikkara ($T_{15}$) and Vellanikkara consortia ($T_{21}$) recorded maximum root dry weight (3.2g). When compared fresh weight and dry weight of vanilla as a whole, individual treatment of *Bacillus* sp. was found to be best consortia treatments and control. But, when compared consortia treatments with control treatment, consortia was found to be the best. So, the over all result indicated that individual treatment were better than consortia treatments. This was also in agreement with Madhaiyan (1999), who found that *P. fluorescens* and *B. subtilis* were effective in improving shoot and root dry weight as well as the P content in vanilla. *Pseudomonas* strains promoted crop growth in terms of root and shoot length and weight (Srivastava *et. al.,* 1999) and dry weight, root length and root number of vanilla plants. Manimala (2003) recorded that treatment of chilli with *T.viride* increased all biometric characters by recording maximum shoot and root length, number of leaves, fresh and dry weight of plant and fruit weight. In a similar study, Shahida (2007) reported that inoculation of PGPM like *Pseudomonas* and *Trichoderma* in vanilla nursery increased the plant biomass. Root length was also higher in the case of commercial *P.fluorescens* ($T_{25}$) (138cm). In a similar study, Paul *et. al.* (2005) reported that *P. fluorescencs* strains could significantly increase the root biomass and root length in treated black pepper. The root length varied significantly with respect to different treatments (Fig.8). Among all

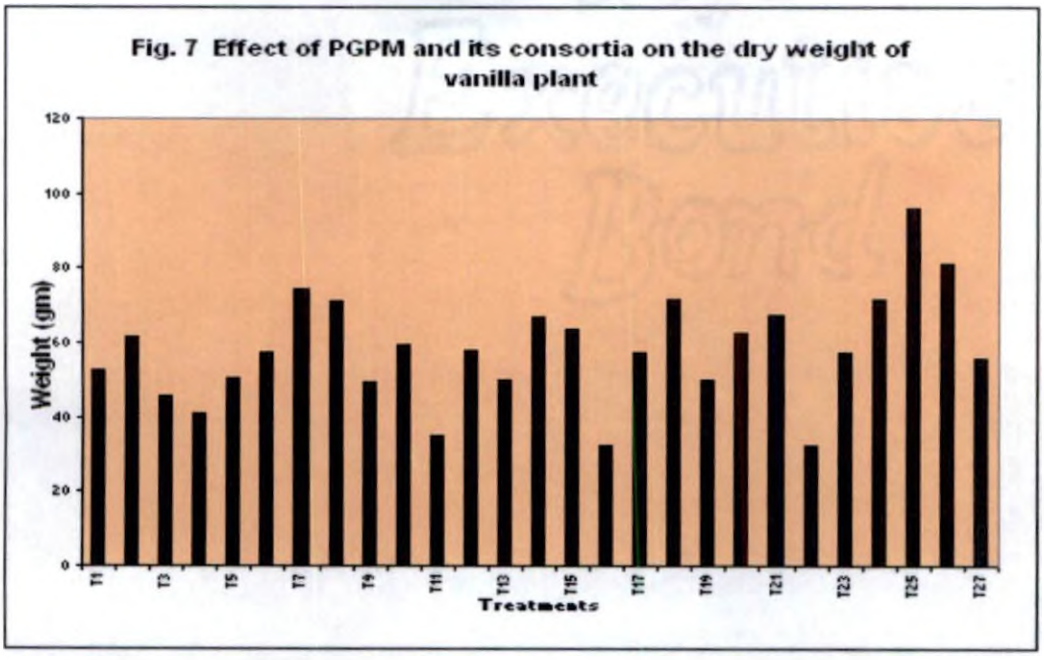


Fig. 7 Effect of PGPM and its consortia on the dry weight of vanilla plant
Weight (gm)
120
100
80
60
40
20
0
T1
T3
T5
T7
T9
T11
T13
T15
T17
T19
T21
T23
T25
T27
Treatments

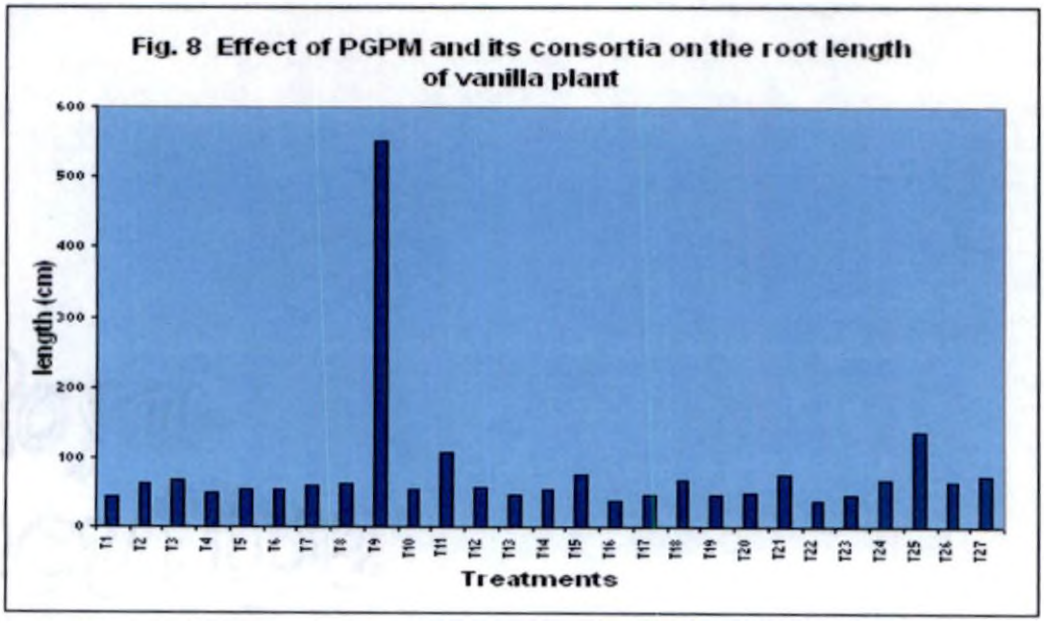


Fig. 8 Effect of PGPM and its consortia on the root length of vanilla plant
length (cm)
600
500
400
300
200
100
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
T13
T14
T15
T16
T17
T18
T19
T20
T21
T22
T23
T24
T25
T26
T27
Treatments

the treatments, highest root length was observed with *Bacillus* from Kolvayal ($T_{11}$) (107.3cm) followed by Vellanikkara *Trichoderma* ($T_{15}$) and consortia ($T_{21}$) (76cm). In the case of root length of vanilla, when compared individual treatment with consortia treatments, individual treatment of *Bacillus* sp was found to be best. But, when compared consortia treatments with control treatment, consortia was found to be the best. Similar finding are reported by Manimala (2003) in chilli and Shahida (2007) in vanilla. Sunaina and Ajay (2005) reported a large and heavily branched root system in potato plants arising from PGPR (*B. subtilis*) treated experiment due to improved uptake of water and nutrients. The results of the present studies on growth enhancement of vanilla using individual isolates and its consortia are contradictory to the earlier reports where the combinations of microorganisms were having better effect on the growth of plants (Burelle *et. al.*,2002., Anandaraj and Sarma,2003., Karunakaran *et. al.*,2003).

The result on enumeration of *Pseudomonas* sp. indicated that population increased in the first two months and then reduced. This was on par with population of *Bacillus* sp. and *Trichoderma* sp. This may due to the lack of nutrients in the soil, as the present study was conducted under sterile conditions. Schroth and Hancock (1982) found that enhanced plant growth caused by *P. fluorescens* was often accompanied by reduction in root zone populations of fungi and bacteria. However, among all the isolates, *Bacillus* population was maximum after six months of planting, which might be due to the tolerance of *Bacillus* against stress conditions.

Total phenol in vanilla was estimated (Fig.9). The treatments showed significant differences with maximum production of total phenol in the case of Sulthan Bathery *Trichoderma* ($T_{18}$) (0.043 mg $g^{-1}$). Total phenol, which provides resistance to plants against diseases, was high in PGPM treated plants when compared to control.

Nitrogen, phosphorous and potassium status of the plants were also tested in the experiment. From (Fig.10), it was evident that the treatments showed significant differences in the case of nitrogen content. Among all the treatments, *Pseudomonas*

Fig . 9 Effect of PGPM and its consortia on total phenol production in vanilla plants

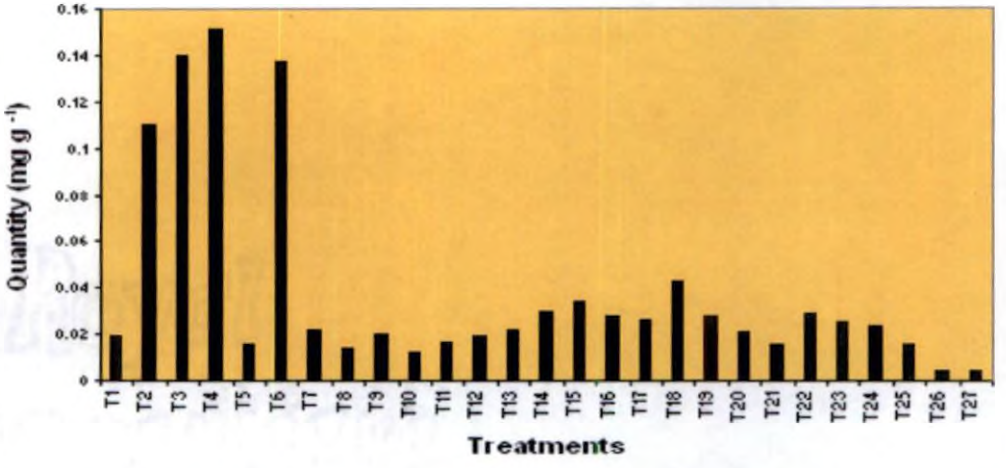
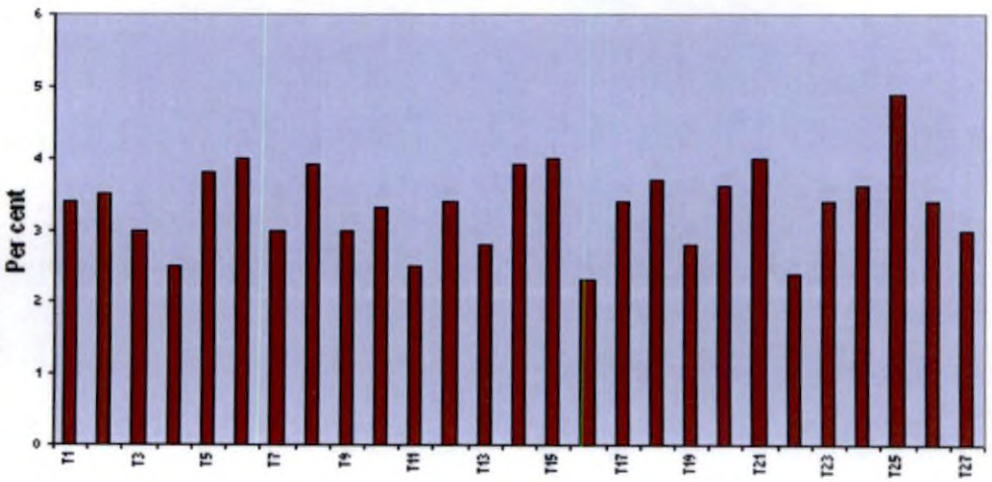



Fig .10 Effect of PGPM and its consortia on the nitrogen content of vanilla plants



isolate of Sulthan Bathery ($T_6$), Vellanikkara *Trichoderma* ($T_{15}$) and consortia ($T_{21}$) recorded maximum N content (4.0 per cent). Paul (2003) reported that the treatment with *P.fluorescens* resulted in enhanced plant vigor due to the increased nutrient mobilization in the rhizosphere of black pepper. In the case of phosphorus, *Bacillus* from Chengaloor ($T_7$) and Mundoor ($T_8$) recorded maximum P content (0.4 per cent) which was on par with *Pseudomonas* of Kolvayal ($T_5$) and Sulthan Bathery ($T_6$), Mundoor *Trichoderma* ($T_{14}$) ,Mundoor consortia ($T_{20}$) , Vellanikkara consortia ($T_{21}$), Kolvayal consortia ($T_{23}$) and Sulthan Bathery consortia containing ($T_{24}$) (Fig.11). From the above result it can be concluded that there was not much difference in phosphorous content of the plants in individual and consortia treatments. But, when compared consortia treatments with control plants, consortia was found to be good. The maximum K content was recorded by *Pseudomonas* of Sulthan Bathery ($T_6$), 2 per cent (Fig.12). From the above result it can be conclude that there was not much difference in potassium content of the plants in individual and consortia treatments. The increased nutrient status of inoculated plants may be due to the increased uptake of nutrients by the inoculated plants from the soil. Similar results were reported by Madhaiyan (1999) who found that *P. fluorescens* and *B. subtilis* were effective in improving shoot and root dry weight as well as the P content in vanilla. Sunaina and Ajay (2005) reported a large and heavily branched root system in crop plants arising from PGPR (*B.subtilis*) treated experiment. Similar reports are given by Shahida (2007) in vanilla. However, in the present study, *P.fluorescens* which was used for comparison with individual isolates was recorded maximum growth and nutrient status in plant except for root growth and total phenol.

The results on the screening of individual native isolates as well as their combinations indicated that among native isolates, *Bacillus* sp. were more effective PGPM when compared with *Pseudomonas* sp., *Trichoderma* sp. and their combinations. Among *Bacillus* sp., Chengaloor isolate ($T_7$) was found to be most effective based on the number of days taken for sprouting, number of leaves, shoot and plant fresh weight, plant dry weight and phosphorous content in vanilla plants. However, among consortia treated plants, Mundoor ($T_{21}$) and Sulthan Bathery (T24) were more effective in

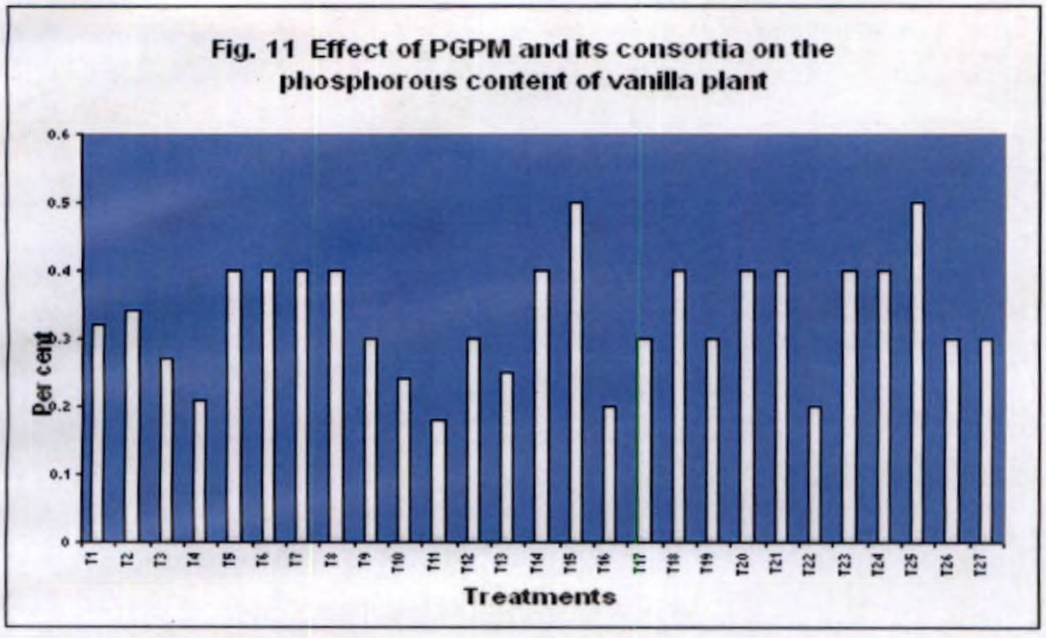


Fig. 11 Effect of PGPM and its consortia on the phosphorous content of vanilla plant
Per cent
0.6
0.5
0.4
0.3
0.2
0.1
0
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12 T13 T14 T15 T16 T17 T18 T19 T20 T21 T22 T23 T24 T25 T26 T27
Treatments

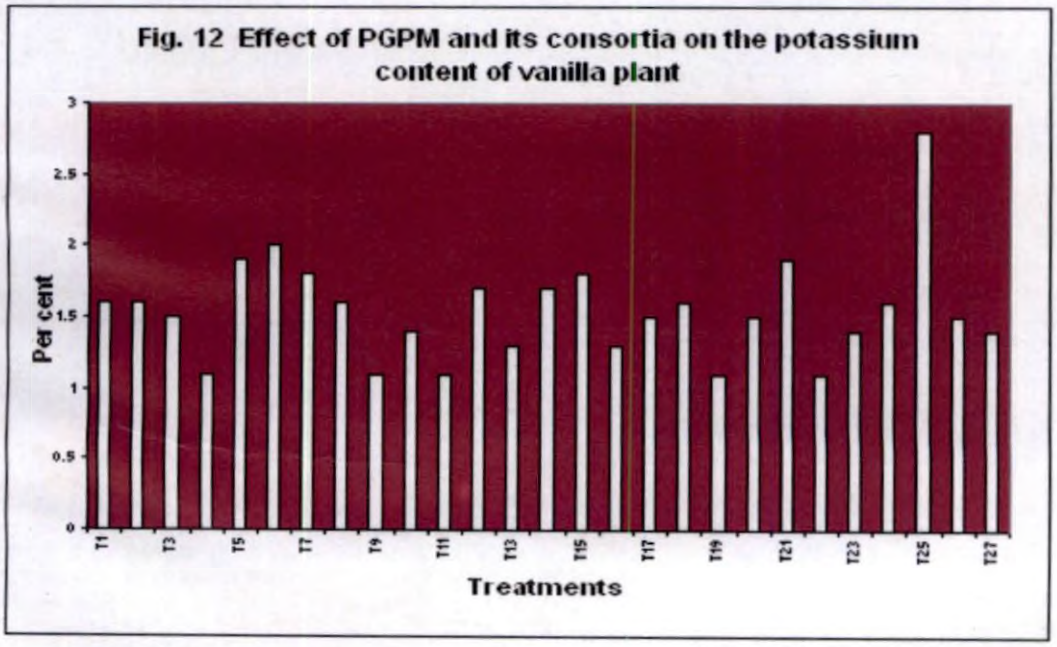


Fig. 12 Effect of PGPM and its consortia on the potassium content of vanilla plant
Per cent
3
2.5
2
1.5
1
0.5
0
T1 T3 T5 T7 T9 T11 T13 T15 T17 T19 T21 T23 T25 T27
Treatments

enhancing the plant biomass than other consortia. In the present study, most of the plant growth parameters recorded was maximum with individual treatments, especially *Bacillus* sp., which performed better than consortia treatments. The use of individual PGPM namely *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. and their consortia exerted better growth characteristics in vanilla than untreated control. This result was in accordance with study of Chiarini *et. al.*(1998), who reported that dual strain inoculation (*Burkholderia cepacia* and *P.fluorescens*) on crop plants did not have any significant effect on plant growth in contrast to the separate inoculation of both strains. He also reported that establishment of large populations of bacterial inoculants on root did not appear to be essential for plant growth promotion. Kamble *e.t al.* (2000) also found that individual inoculation of seeds with *Azospirillum, P.fluorescens* and phosphobacteria were more effective than combined inoculation. In a similar study, it was observed that combined application of two antagonists (*T. harzianum* and *P. fluorescence*) had good effect on increasing the yield of chilli. While, combination of more than two antagonists had no effect on yield as the single treatments and control recorded higher yield than these treatments (KSCSTE project report , 2007).

The most effective Chengaloor *Bacillus* isolate ($T_7$), Mundoor ($T_{21}$) and Sulthan Bathery (T24) consortial isolates were identified based on the key characters as described in Bergey's Manual of Systematic Bacteriology. The *Bacillus* sp was found to be Gram positive, catalase positive, growth in V-P broth at >7 pH, growth on one per cent Glucose- Nutrient agar, positive hydrolysis of starch and presence of central endospore. These characters were compared with characters as described by Cohn (1872).Accordingly, the *Bacillus* sp was tentatively identified as *B. subtilis*. Consortia consisting of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were also identified. *Pseudomonas* sp. was found to be Gram negative, catalase positive, urease positive, Arginine dihydrolase positive, negative hydrolysis of starch and nitrate production and yellowish green pigment production. These characters were compared with characters as described by Palleroni (1984) and tentatively identified as *P.fluorescens*. The fungus was identified based on the cultural and morphological characters. The mycelial growth

of fungi was rapid, light green to bright green, hyaline, smooth and septate. Chlamydospores were mostly globose, smooth and 6-12µm in diameter. Conidiophores were loose tuft and produced numerous side branches especially at the lower portion. Phialides consisted of whorls up to five, short, skittle shaped, narrower at the base and bulged at the middle and attenuated abruptly into sharp pointed neck. Phialides were 10-12 µm long and 3- 4 µm width. Phialospores were single and accumulate. They were sub globose or short ovoid, smooth, pale green, much darker in mass .These characters were compared with characters described by Rifai (1964) and tentatively identified as *T. harzianum.*

Even though, the isolates were found to be very compatible under *in vitro* condition, they failed to perform in nursery condition. So, more investigation needs be done using large number of microbial isolate from different locations and its consortia in order to get a better influence on the growth of plant. Moreover, the microbial isolates have to be extensively tested under field conditions so as to find suitable consortia for growth enhancement of vanilla.

# *Summary*

# 6. SUMMARY

The present study on "Induction of growth promotion in vanilla through plant growth promoting microorganisms consortia" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2004-2006.The entire study was conducted as two experiments. In the first experiment, different *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were isolated and screened under *in vitro* conditions to find out the compatible isolates for enhancing the growth of vanilla in nursery. In the second experiment, compatible combinations of *Pseudomonas*, *Bacillus* and *Trichoderma* isolates were tested for growth enhancement in vanilla along with its individual cultures. Commercial cultures of *P. fluorescens* and *T. viride* were also used for comparison.

For the present study, six isolates each of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. along with their consortia and commercial cultures of *P.fluorescens* and *T.viride* were used. Selected PGPM were isolated from three locations each of Thrissur (Chengaloor, Mundoor and Vellanikkara) and Wynad (Ambalavayal, Kolvayal and Sulthan Bathery) districts. In the present study, several attempts were made to isolate orchid mycorrhiza. But, it was a failure. Cultural and morphological characters of the efficient isolates of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were studied and were identified.

*Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were evaluated for its efficiency in production of growth promoting substances like IAA and salicylic acid under *in vitro* condition. IAA production was maximum in the case of $P_6$ isolate (0.099 mg $ml^{-1}$) and salicylic acid production was maximum in the case of *Pseudomonas* sp., $P_5$ ( 27.9 µg ml1). The isolates were also studied for its effect on seed germination as well as seedling vigour using cowpea and sorghum. All the seeds attained 100 per cent germination with *Pseudomonas* sp. and *Bacillus* sp. In the case of cowpea seeds treated

with *Pseudomonas* sp. under *in vitro* condition, $P_4$ recorded maximum shoot length ( 7.6 cm) and root length (5.4 cm).Similarly, the maximum shoot length was recorded in the case of $P_6$ ( 6.2 cm) and root length by $P_1$ ( 6.4 cm) isolates for sorghum seeds .In the case of *Bacillus* sp treated cowpea seeds, maximum shoot length ( 7.4 cm) and root length ( 7.5 cm ) was recorded with $B_5$ isolate. Similarly, the maximum shoot length was recorded in the case of $B_2$ and $B_6$ (3.6 cm) for sorghum seeds. However, the maximum root length was recorded in the case of $B_5$ (6.1 cm) isolate. The studies on IAA production and seed germination test using different isolates indicated the potentiality of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. as the plant growth promoter.

All the six isolates of *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. were evaluated for mutual compatibility between the isolates under *in vitro* condition and all were found compatible. A study was conducted to evaluate for compatibility between *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. with selected fungicides like Bordeaux mixture, copper oxychloride, copper hydroxide, potassium phosphonate and mancozeb and insecticides like carbaryl, chlorpyriphos and lambda cyhalothrin and the results indicated that all the *Pseudomonas* sp. were incompatible with the fungicides and insecticides at all concentrations tested. However, all *Bacillus* sp. were incompatible with all fungicides and insecticides at all concentrations tested. Where as, carbaryl was compatible with $B_1$, $B_2$ and $B_3$ isolates at all concentrations. Similarly, in the case of *Trichoderma* sp., all the selected fungicides and insecticides at all concentrations completely inhibited their growth.

All the six isolates of *Pseudomonas* sp., *Bacillus* sp., *Trichoderma* sp. and their consortia obtained were screened for their effectiveness in growth enhancement of vanilla in nurseries under sterile condition. Biometric observations as well as nutrient analysis were done. The results on the screening of individual isolates as well as their combinations indicated that among the native isolates *Bacillus* sp. were more effective PGPM when compared with *Pseudomonas* sp., *Trichoderma* sp. and their combinations. Among *Bacillus* sp., $B_1$ ($T_7$) isolate was found to be most effective based on the number

of days taken for sprouting, number of leaves, shoot and plant fresh weight, plant dry weight and phosphorous content in vanilla plants. However, among consortia treated plants, $C_3$ ($T_{21}$) and $C_6$ (T24) were more effective in enhancing the plant biomass than other consortia. Most of the growth parameters recorded was maximum with individual treatments especially *Bacillus* sp., which performed better than consortia treatments. The use of individual PGPM namely *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. and their consortia exerted better growth characteristics in vanilla than untreated control.

The most effective $B_1$, $C_3$ and $C_6$ isolates were identified based on the key characters as described in Bergey's Manual of Systematic Bacteriology. Accordingly, the *Pseudomonas* sp. was tentatively identified as *P.fluorescens* and *Bacillus* sp. as *B. subtilis*. *Trichoderma* sp. was identified based on the cultural and morphological characters as *T. harzianum*.

The results of the present studies on growth enhancement of vanilla using individual isolates and its consortia are contradictory to the earlier reports where the combinations of microorganisms were having better effect on the growth of plants. Even though, the isolates were found to be very compatible under *in vitro* condition, they failed to perform in nursery condition.

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# Appendix

## APPENDIX I

## MEDIA COMPOSITION

(Ingredients per litre)

### I. KING'S B MEDIUM

| | |
|---|---|
| Peptone | : 20.0 g |
| Glycerol | : 10.0 ml |
| $K_2HPO_4$ | : 10.0 g |
| $MgSO_4.7H_2O$ | : 1.5 g |
| Agar | : 20.0 g |
| pH | : 7.2 – 7.4 |

### II. NUTRIENT AGAR MEDIUM

| | |
|---|---|
| Glucose | : 5.0g |
| Peptone | : 5.0g |
| Beef extract | :3.0g |
| NaCl | : 5.0g |
| Agar | : 20.0g |
| pH | : 6.5 to 7.5 |

## III . MARTIN'S ROSE BENGAL STREPTOMYCIN AGAR

| | |
|---|---|
| Dextrose | : 10.0g |
| Peptone | : 5.0 g |
| $KH_2PO_4$ | : 1.0 g |
| $MgSO_4$ | : 0.5 g |
| Agar | : 20.0 g |
| Rose Bengal | : 0.03 g |
| Streptomycin | : 30 |

## IV . POTATO DEXTROSE AGAR

| | |
|---|---|
| Potato | : 200.0 g |
| Dextrose | : 20.0 g |
| Agar | : 20.0 g |

## V. FUNGAL ISOLATING MEDIA (FIM)

| | |
|---|---|
| $NaNO_3$ | : 0.3 g |
| $KH_2PO_4$ | : 0.2 g |
| $MgSO_4$ 7 $H_2O$ | : 0.1 g |
| KCl | : 0.1 g |
| $CaNO_3.4H_2O$ | : 0.1 g |
| Agar | : 8 g |
| Sucrose | : 5 g |

## VI. LURIA BERTANI

| | |
|---|---|
| Tryptone | : 10 g |
| Yeast extract | : 5 g |
| NaCl | :10 g |
| Agar | : 20 g |
| pH | : 7 |

## VII. CASAMINOACID BROTH

| | |
|---|---|
| Peptone | : 10 g |
| Dextrose | : 5 g |
| Casein hydrolate | : 1 g |
| pH | : 7.2 – 7.4 |

## VIII. THORNLEY'S SEMI SOLID MEDIUM

| | |
|---|---|
| Peptone | : 1.0g |
| $K_2HPO_4$ | : 0.3 g |
| NaCl | : 5.0g |
| Agar | : 3 g |

## IX. NITRATE REDUCTION MEDIUM

| | |
|---|---|
| $KNO_3$ (Nitrate free) | : 1.0 g |
| Peptone | : 10.0 g |
| Beef extract | : 5.0g |
| Agar | : 15 g |
| pH | : 7 |

## X. CHRISTENSEN'S UREA AGAR MEDIUM

| | |
|---|---|
| Peptone | : 1.0g |
| $KH_2PO_4$ | : 2 g |
| NaCl | : 5.0g |
| Glucose | : 1.0 g |
| Phenol red (0.2 per cent ) | : 6 ml |
| Agar | : 20 g |
| pH | : 6.8 |

## XI . ONE PER CENT GLUCOSE- NUTRIENT AGAR

| | |
|---|---|
| Glucose | : 15.0g |
| Peptone | : 5.0g |
| Beef extract | : 3.0g |
| NaCl | : 5.0g |
| Agar | : 20.0g |
| pH | : 6.5 to 7.5 |

# APPENDIX II

## STAINS USED IN MICROBIOLOGICAL STUDIES

### I. CRYSTAL VIOLET

One volume saturated alcohol solution of crystal violet in four volumes of 1 per cent aqueous ammonium oxalate.

### II. GRAM'S IODINE

| | | |
|---|---|---|
| Iodine crystals | - | 1.0 g |
| Potassium iodide | - | 2.0 g |
| Distilled water | – | 300 ml |

### III. SAFRANIN

| | | |
|---|---|---|
| Safranin O | - | 0.25 g |
| Ethanol (95%) | - | 10.0 ml |
| Distilled water | - | 100 ml |

Dissolve safranin in ethanol and then in water and filter.

### IV. MALACHITE GREEN

| | | |
|---|---|---|
| Malachite green | - | 5.0 g |
| Distilled water | – | 100 ml |

# INDUCTION OF GROWTH PROMOTION IN VANILLA THROUGH PLANT GROWTH PROMOTING MICROORGANISMS CONSORTIA

By

**DHANYA, V**

## ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

**Master of Science in Agriculture**

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# ABSTRACT

A study on "Induction of growth promotion in vanilla through plant growth promoting microorganisms consortia" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara. The salient findings are abstracted below:

Isolates of *Pseudomonas* spp., *Bacillus* spp. and *Trichoderma* spp.were evaluated for its efficiency in production of growth promoting substances like IAA and salicylic acid and observed that *Pseudomonas* sp. was most efficient. Results on compatibility between selected PGPM like *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. showed that all isolates were compatible with each other under *in vitro* condition.

In the case of *Pseudomonas* sp, all the selected fungicides and insecticides at all concentrations completely inhibited their growth and among *Bacillus* isolates, all the selected fungicides and insecticides at all concentrations completely inhibited their growth except carbaryl which was compatible with isolates $B_1$ , $B_2$ and $B_3$ at all concentration .Similarly, in the case of *Trichoderma* isolates, all the selected fungicides and insecticides at all concentrations completely inhibited their growth.

In the present study, most of the growth parameters of vanilla in the nursery experiment was maximum with individual treatments especially *Bacillus* sp., which performed better than consortia treatments. The use of individual PGPM namely; *Pseudomonas* sp., *Bacillus* sp. and *Trichoderma* sp. and their consortia exerted better growth characteristics in vanilla than untreated control.