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GROWTH ENHANCEMENT AND MANAGEMENT OF *Phytophthora*-ROT IN VANILLA NURSERIES USING MICROBIAL INOCULANTS

By

SHAHIDA K.

THESIS

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Faculty of Agriculture
Kerala Agricultural University, Thrissur

Department of Plant Pathology

COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

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DECLARATION

I, Shahida, K (2002-11-43) hereby declare that this thesis entitled '**Growth enhancement and management of *Phytophthora*-rot in vanilla nurseries using microbial inoculants**' is a bonafied record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara
Date: 14-10-2006

Shahida,K

CERTIFICATE

Certified that this thesis, entitled **'Growth enhancement and management of *Phytophthora*-rot in vanilla nurseries using microbial inoculants'** is a record of research work done independently by **Ms. Shahida. K** 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.

Dr. Surendra Gopal,
(Major Advisor, Advisory Committee)
Assistant Professor(Microbiology),
Department of Plant Pathology,
College of Horticulture,
Kerala Agricultural University
Thrissur, Kerala.

Vellanikkara
Date: 14-10-2006

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Ms. K. Shahida**, a candidate for the degree of **Master of Science in Agriculture**, agree that this thesis entitled "**Growth enhancement and management of *Phytophthora*-rot in vanilla nurseries using microbial inoculants**" may be submitted by **Ms. K. Shahida,** in partial fulfillment of the requirement for the degree.

Dr. K.Surendra Gopal,
Major Advisor, Advisory committee
Assistant Professor (Microbiology),
Department of Plant Pathology,
College of Horticulture,
Vellanikkara.

Dr. Koshy Abraham,
Associate Professor and Head,
Department of Plant Pathology,
College of Horticulture,
Vellanikkara.
(Member)

Dr.Sally K. Mathew,
Associate Professor,
Department of Plant Pathology,
College of Horticulture,
Vellanikkara.
(Member)

Dr.Shylaja M.R.,
Associate Professor,
Department of Plantation Crops
and Spices,
College of Horticulture,
Vellanikkara.
(Member)

(EXTERNAL EXAMINER)
Dr. T. Makesh Kumar,
Scientist (Pl. Pathology)
CTCRI, Sreekaryam

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Shahida, K,

DEDICATED
TO MY
BELOVED FAMILY

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Introduction

INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews.) the "orchid of commerce" is a native of Mexico and Central America. It is mainly cultivated as a source of natural vanilla flavour, which is used in the preparation of ice creams, chocolates, bakery products, liquors and also in perfumery and pharmaceuticals. Since, most of the European countries banned the synthetic vanillin, there is high demand for natural vanilla. The organic vanilla has high preference in international market and fetches higher price. Vanilla is highly amenable to organic cultivation. Moreover, it is necessary to increase the production of natural vanilla by increasing the area under vanilla cultivation. To achieve this, large number of planting materials are required. However, the number of planting materials depends on the length of cuttings of plantlets. If shorter cuttings are used, the juvenile phase will be longer. Hence, the growth of vanilla plants in the nursery has to be enhanced for getting early flowering and to obtain more number of cuttings for planting.

In India, during initial years of vanilla cultivation, the fungal diseases were of minor concern. However, as intensive and widespread cultivation started, the occurrence of disease also became widespread both in plantations and nurseries. Among these, *Phytophthora* rot is one of the most important diseases affecting vanilla both in nurseries and plantations. It affects the vine, leaves and beans. It is more severe during southwest monsoon especially in shaded and poor drained soils. A very few studies have been conducted for efficient management of this disease.

The continuous use of agrochemicals destroyed the biological balance of nature. It adversely affects the environment and ecological processes. 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. Moreover, microbial inoculants are harmless, cheaper and ecofriendly which are used to promote plant vigour, improves yield, control or suppress various diseases caused by soil borne and seed borne pathogens and nematodes (Sivaprasad, 2002).

Considering the above facts, the present study was undertaken for the growth enhancement and management of *Phytophthora* rot in vanilla nurseries using microbial inoculants with the following objectives.

- Isolation of the pathogen causing *Phytophthora* rot in vanilla.
- Isolation of different microbial inoculants from vanilla growing areas of Thrissur and Ernakulam districts.
- Screening of antagonists against *Phytophthora* rot under *in vitro* conditions.
- Screening of AMF and *Azospirillum* isolates for growth enhancement of vanilla.
- Effect of efficient microbial inoculants for growth enhancement and management of *Phytophthora* rot in nursery.

Review of Literature

2. REVIEW OF LITERATURE

Vanilla (*Vanilla planifolia* Andrews.) is a tropical orchid, which is cultivated for its pleasant flavour. The number of planting materials depends on the length of cuttings of plantlets, which in turn is related to the flowering. If shorter cuttings are used, the juvenile phase will be longer. Hence, the growth of vanilla plants in the nursery has to be enhanced for getting early flowering and to obtain more number of cuttings. More over, vanilla is facing several diseases, which are reducing the yield. Among several diseases, *Phytophthora* rot is one of the most important fungal diseases affecting vanilla both in nurseries and plantations. It is most severe during southwest monsoon especially in shaded and poor drained soils.

2.1 THE PATHOGEN

Phytophthora rot affecting beans, leaves and stem of vanilla is a serious disease both in plantations and nurseries during southwest monsoon. Correll (1953) reported the fruit rot disease of vanilla caused by *Phytophthora parasitica* Dast. Blight or mildew attack in developing fruits of vanilla caused by *Phytophthora jatropha* Jens. was reported by Bouriquet (1954) from Malagasy Republic and was observed in all vanilla growing regions of the world. Tsao and Mu (1987) reported the incidence of root rot of vanilla along with leaf and stem blight and observed that *P. palmivora, P. capsici, and P. parasitica* were important causal agents of vanilla root rot in French Polynesia. The pathogenicity of these isolates on roots of excised vanilla cuttings has been established. In India, *Phytophthora* rot of vanilla was first reported by Bhai and Thomas (2000) from Perumbavoor and Koothattukulam areas.

Phytophthora also infected plantation crops like black pepper, arecanut, cocoa, cardamom, rubber, etc. Butler (1906) first reported fruit rot of arecanut caused by *Phytophthora* in South India and later he (1918) reported that *P. arecae*

was the causal organism. Mc Rac (1919) identified the pathogen causing abnormal leaf fall in rubber as *P. meadii*. In India, it was first reported by Thankamma *et al.* (1968). *Phytophthora* rot incidence in cardamom was first reported by Menon *et al.*(1972) and Thankamma and Pillai (1973) reported the causal organism as *P. nicotianae var nicotianae*. Intermediate pedicel length with caducous sporangia and swelling at the sporangiophore distinguished *P. meadii* from other species of *Phytophthora* (Thankamma and Pillai, 1973). Rebeiro (1978) reported that *P. meadii* did not produce chlamydospores in the medium and were considered as distinguished character of this fungus. Tsao and Alizadeh (1988) identified the pathogen causing foot rot in black pepper as *P.capsici*. Liyange and Wheeler (1991) found that mycelium and sporangia of *P. meadii* survived in soil for 3 weeks where as chlamydospores survived for 12 weeks. Bhai and Thomas (2000) identified the pathogen associated with *Phytophthora* rot of vanilla in Kerala as *Phytophthora meadii*. The fungus produced sporangia, which were ovoid to ellipsoidal, highly caducous, papillate and with intermediate pedicel length. Bhai and Sarma (2003) reported that pigmentation in casein hydrolysate tyrosine agar and growths at high temperature were ideal for differentiating *P. meadii* and *P. nicotianae* var *nicotianae* infecting cardamom. They found that *P. meadii* did not cause any pigmentation in casein hydrolysate tyrosine agar and the fungus did not grow at high temperature. Choudappa *et al.* (2003) found that the *P. meadii* isolates from arecanut, rubber and cardamom were uniform based on ITS-RFLP and AFLP patterns. He concluded that *P. meadii* was the main pathogen causing fruit rot of arecanut in India and there was no evidence of occurrence of *P. arecae*. Sdoodee (2004) reported that atleast six species of *Phytophthora* have been associated with diseases of rubber and most common species are *P. palmivora*, *P. meadii* and *P. botryosa*.

2.2 SYMPTOMATOLOGY

Phytophthora rot in vanilla produced symptoms on leaves, stem and beans. The symptom of the disease started as rotting in the form of brown patches on the petioles and lower portions of the youngest leaf, which was just unfolding (Correll, 1953). The affected portion gradually shrinked, the leaves became yellowish and vine dry off (Tsao and Mu, 1987). It also caused shoot tip rot. According to Bhai and Thomas (2000), rotting started from the bean tip and extended to the stalk. The infected portions of beans were water soaked, soft and dark brown. Small dull white coloured pinhead like sporangial clusters were seen on the infected beans. In advanced stages, the rotting extended to the leaves, stem, aerial roots and extended towards the basal portions. Infection on the stem was often observed in the form of brown coloured blighted portions sometimes extended several centimeters along the stem (Thomas *et al.*, 2003). It was often noticed in several vanilla gardens in India during summer months.

2.3 GROWTH ENHANCEMENT

2.3.1 Effect of Mycorrhiza

Mycorrhizas are highly evolved, symbiotic associations between soil fungi and plant roots. The mycorrhizal fungi have a unique role in the life cycle of plants in the Orchidaceae. Smith (1966) reported orchid mycorrhizal fungi like *Rhizoctonia repens* and *R.solani* which were able to utilize and translocate carbohydrates to the orchid plants and there by increasing the plant growth. Alexander and Hardley (1983) has shown that mycorrhizal infections in the orchid *Goodyera repens* bring about enhanced growth rate and increased phosphorus concentration within tissues. They showed improved resistance to drought and environmental stress (Allen and Boosalls, 1983). 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

(Abbot and Robson, 1984). 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). Anandaraj and Sharma (1994) reported the enhanced rooting and root growth on inoculation with *Glomus fasciculatum* on cuttings of pepper. Increased shoot dry weight and reduced mortality were observed in orchid plants inoculated with VAM (Wang and Gregg, 1994). The orchid mycorrhizal fungi such as *Epulorhiza repens* and *Ceratorhiza* fungi stimulated the growth of seedlings of *Dactylorhiza* and *Orchis morio* at low N (NH_4NO_3) availability in comparison to seedlings in non symbiotic culture (Dijk and Eck, 1995). Kandiannan *et al.* (2000) reported that plant height, leaf area biomass, dry matter and nutrient content were higher in blackpepper inoculated with biofertilizers like VAM, *Azospirillum* and phosphobacteria. When the rooted cuttings were inoculated with AMF, it improved the root as well as vegetative growth of pepper cuttings (Thanuja, 1999). Binisha (2003) found that girth of *Dendrobium* was increased by the application of AMF along with inorganic fertilizers.

Mycorrhiza in vanilla roots were first recorded by Decordenoy (1904) who observed the infection of roots adhering to their living supports and suggested that roots obtained their nutrients *via*; the fungus. Tonnier (1954) reproduced the mycorrhizal association in flasks by growing vanilla seedlings in agar with *Rhizoctonia*. The association of orchid mycorrhiza and arbuscular mycorrhizal fungi in vanilla was studied in detailed by Madhaiyan (1999). He observed that the 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 inoculation of orchid mycorrhizal fungi, AMF or combination of both over control. Madhaiyan *et al.* (2003) observed the infection of orchid mycorrhiza and peleton formation in the cortical cells, particularly in the outer cortical cells of vanilla seedlings inoculated with orchid mycorrhiza. Gopal (2005) found that the nutrient content in vanilla plant was

increased by the inoculation of AMF along with inorganic nutrients. She observed that N and P content was more in inoculated plants.

2.3.2 **Effect of *Azospirillum***

Azospirillum is an associative symbiotic diazotropic bacterium well known for its potential to fix atmospheric nitrogen. It can fix nitrogen about 20-25 kg per hectare under ideal conditions and there by reduces 25 per cent of the nitrogen fertilizer requirement. Treatment with *Azospirillum* also induced better root formation. It also produced phytohormones *via*; indol acetic acid, gibberellic acid, kinetin etc. that help the host plant by way of enhancing biomass production (Tien, *et al.*, 1979). Govindan and Chandy (1985) reported that inoculation of *Azospirillum* increased growth characteristics in black pepper. According to Hades and Okon (1987) there was a significant increase in root length, shoot and root dry weight and total leaf area in tomato inoculated with *Azospirillum* compared to non inoculated. Kumar *et al.* (1988) reported that inoculation of *Azospirillum* increased nut germination in cashew. It was also noted that it improved the growth characters such as seedling height and stem growth significantly. Raji (1995) reported that total fresh weight, dry weight, plant height and number of leaves were more in cowpea treated with *Azospirillum* along with fungicides and insecticides. Nair and Chandra (1997) found that the inoculation of *Azospirillum* resulted in the production of IAA, in different crops like pepper, nutmeg, clove, cashew etc. Inoculation of *Azospirillum* in nutmeg increased the seedling height, number of branches and number of leaves (Nair and Chandra, 2001). Madhaiyan (1999) reported that inoculation of *Azospirillum* improved the growth of vanilla and *Dendrobium*. He observed that the inoculation of *Azospirillum* alone and its combination with mycorrhiza increased the shoot and root dry weight and nutrient content in vanilla. Binisha (2003) also reported that inoculation of *Azospirillum* in combination with inorganic fertilizers and phosphobacteria produced higher dry matter in *Dendrobium*. She also observed

that combination of *Azospirillum* along with AMF, phosphobacteria and inorganic fertilizers increased number and length of roots in *Dendrobium*.

2.3.3 Effect of *Trichoderma*

In addition to their role as biocontrol agent *Trichoderma* spp. have been implicated to promote plant growth. Baker *et al.*(1984) first reported growth promotion of raddish 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 annuum*. 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* sp.can increase the rate of plant growth and development and also produced more robust roots. Lisha *et al.* (2002) reported that *Trichoderma* isolates obtained from black pepper rhizosphere showed increased growth promotion by 55-116% as compared to control. Vijayaraghavan (2003) also noted that inoculation of *T. viride* in solarized potting mixture increased the height and number of leaves of pepper cuttings in nursery.

2.3.4 **Effect of rhizobacterium**

The benefical rhizobacteria are termed as plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.*, 1981) due to their ability to improve plant growth through suppression of deleterious root colonizing microorganisms and by production of plant growth regulators (Suslow and Schroth, 1982) such as gibberellins, cytokinins and indole acetic acid (IAA) (Krause, 1992). Glick (1995) reported that phosphorus solubilization, biological nitrogen fixation, improvement of other plant nutrient uptake and phyto hormone production like IAA are some of the mechanism of PGPR that directly influence plant growth. Jubina and Girija (1998) found that inoculation of antagonistic rhizobacteria improved the growth characteristics of black pepper cutting in terms of shoot length, fresh weight and dry weight. The selected isolates of PGPR evaluated in green house for their efficiency for growth promotion and foot rot suppression in black pepper. Bacterial strain IISR-51 promoted growth of black pepper upto 55.15 per cent (Nisha *et al.*, 2002). Application of rhizobacterium (Pf IISR-51) resulted in enhanced growth of black pepper, which resulted in increased number of roots of rooted cuttings. Paul *et al.* (2003) reported a significant uptake of nitrogen and potassium in black pepper treated with PGPR. They also observed the enhanced nutrient mobilization resulted in enhanced plant vigour.

Madhaiyan (1999) reported that inoculation of *Pseudomonas striata* in vanilla increased the shoot length, root length, shoot and root dry weight. He found that *P.fluorescens* and *Bacillus subtilis* were also effective in improving shoot and root dry weight as well as the P content in vanilla. Joseph *et al.* (2003) reported the production of growth promoting substances like indol acetic acid and gibberellin by PGPR in *Hevea brasiliensis*. *Pseudomonas fluorescens* significantly increased the growth and biomass production of crops. Vijayan (2003) reported that seed coating with *Bacillus subtilis* and *P. fluorescens* had resulted in increased seed germination and enhanced growth and vigour as

expressed by increase in seedling height, root length and leaf area of different spice crops.

2.3.5 Effect of *Piriformospora indica*

Piriformospora indica is a newly described cultivable endophyte that colonizes the roots. Inoculation of fungus and application of fungal filtrates promote plant growth and biomass production (Verma *et al.*, 1998 and Varma *et al.*, 1999). They also observed that it mediated uptake of phosphorus from the medium and its translocation to the host in an energy dependent process and increased its content in shoot. The fungus promised to be a potential source for colonizing the orchids, their better growth and higher rate of survival of seeds. *P. indica* has a wide host range of monocots and dicots including legumes, terrestrial orchids (*Dactylorrhiza maculata*) and members of the bryophytes (*Aneura pinguis*) (Singh *et al.*, 2003). The combination of *P.indica*, *T. viride* and *P. fluorescens* resulted to maximum growth response in terms of height, number of leaves and length of root in *Withania* (Margode *et.al.*, 2003). Rai *et.al.* (2004) reported the antifungal activity of *Spilanthes calva* was enhanced due to the inoculation of *P. indica.* Scherameti *et.al.* (2005) reported that *P. indica* promoted growth of *Arabidopsis* and tobacco seedlings.

2.4 MANAGEMENT OF *Phytophthora* DISEASE

Management of *Phytophthora* disease should be based on a sound understanding of the biology of the pathogen, including its modes of survival and dissemination, host range and the role of environmental factors in disease. Management of *Phytophthora* disease is based on a number of principles such as avoiding infection through basic hygiene, limiting susceptibility through drainage and irrigation, improving soil health, use of disease resistant germplasm, and biological and chemical control (Drenth and Guest, 2004). Effective control was achieved through the integarated use of a number of these strategies.

2.4.1 **Chemical control**

Application of fungicides was found to be very effective against *Phytophthora* especially Bordeaux mixture and potassium phosphonate. Many studies were conducted to investigate the effectiveness of chemicals against *Phytophthora* diseases. Hishop (1963) reported that many of the fungicides like Bordeaux mixture, Ziram, captan, and Panolil were toxic to *P. palmivora* of cacao in laboratory test. It was also observed that Bordeaux mixture, copper oxychloride, cuprous oxide, cupric hydroxide, defolatan and organic tin compounds were effective in reducing the black pod rot in the field (Gorenz, 1970; Gregory, 1974 and Thorald, 1975).There are many reports on the chemical control of various other species of *Phytophthora* on different crops. Filani (1976) noticed the growth of *P. palmivora* was restricted by cuprous oxide, copper sulphate, copper hydroxide, and copper carbonate at all concentrations tested. Sarma *et al.* (1987) reported that spraying the cuttings in the black pepper nursery with Bordeaux mixture, copper oxychloride, or prophylatic spray with Ridomil and ziram at monthly intervals gave good control over *Phytophthora* rot in nursery. Sastry and Hegde (1988) found that the systemic fungicides Aliette and metalaxyl had some curative effects on fruit rot of *Areca catechu* caused by *P. meadii*. They also observed that Bordeaux mixture (one per cent) spray did not give complete control of the disease. According to Thomas *et al.* (1989), best control of *P. meadii* on *Elettaria cardamomum* was given by 0.3 per cent Aliette 80 WP or Bordeaux mixture (one per cent). Spraying of one per cent Bordeaux mixture, 0.3 per cent potassium phosphonate and drenching soil with 0.2 per cent copper oxychloride were effective against azhukal disease of cardamom by *P. meadii* (Spices Board, 1999). Spraying and soil drenching of potassium phosphonate (Akomin 40) gave maximum reduction of foliar and root infection (Veena and Sarma, 2000). They also observed the absence of phytotoxicity on black pepper even at higher concentration. Veena *et al.* (2000) observed that the sporulation of *P. capsici* of black pepper was the most sensitive stage to potassium phosphonate. The *in vitro* inhibitory effect of Bordeaux mixture,

copper oxychloride, copper hydroxide, mancozeb, metalaxyl and Antracol against foot rot pathogen of black pepper was reported by many workers (Turner, 1969., Mammootty, 1978., Ramachandran and Sarma, 1985 and KAU, 2000). Studies conducted at Kerala Agricultural University revealed that spraying and drenching of one per cent Bordeaux mixture, spraying of Dithane M-45 (0.25 per cent), Bayer 5072 drenching were effective in controlling the disease (KAU, 2000). Guest (2002) reported that fungicides such as phosphonate protect cocoa and coconut against *Phytophthora* infection, increased tree survival and yields and had an important role in integrated disease management strategies. Vijayaraghavan (2003) observed that Bordeaux mixture, Fytolan, Kocide, Captan, Ridomil Mz and Indofil M-45 at different concentrations inhibited the growth of *P. capsici*. She also noticed that the higher concentration on Akomin (0.2 and 0.3 per cent) inhibited the fungus. Bhai and Sarma (2003) reported that spraying of one per cent Bordeaux mixture and 0.35 per cent potassium phosphonate soon after the initiation of the monsoon showers resisted capsule rot of cardamom caused by P. *meadii* and reduced soil borne inoculam.

Bhai and Thomas (2000) observed that spraying of one per cent Bordeaux mixture twice and drenching with 0.25 per cent copper oxychloride were effective in controlling *Phytophthora* rot in vanilla. Application of one per cent Bordeaux mixture after the removal of disease affected portion will check the *Phytophthora* disease in vanilla (KAU, 2002). Thomas *et al.*(2003) reported that the spread of stem blight caused by *Phytophthora meadii* in vanilla could be controlled by spraying with one per cent Bordeaux mixture or potassium phosphonate (0.4 per cent).

2.4.2 Biological control

Baker and Cook (1974) defined biological control as the reduction of inoculum density or disease producing activities of a pathogen or a parasite in its active or dormant stage by one or more organisms accomplished naturally or

through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonists. Many fungus like *Gliocladium*, *Trichoderma*, *Latesaria*, *Sporodesmium*, *Aspergillus*, *Fusarium*, several bacteria and actinomycetes were known for their potential biocontrol activities against soil borne pathogens including several species of *Phytophthora* (Malajezuk, 1983., Adams, 1990., Naik and Sen,1992).

2.4.2.1. ***Trichoderma spp.***

Weindling (1932) first reported *Trichoderma* as mycoparasite .He (1934) also reported the toxic metabolic production by *Trichoderma* spp. It also produced exo and endo glucanases, cellobiase and chitinases (Papavizas, 1985). It caused coiling, penetration, production of haustoria and lysis of the pathogen hyphae. Elad *et al.* (1983) mentioned the mycoparasitism by enzymatic lysis of pathogenic fungal hyphae through the production of enzyme like b 1,3 glucanases, chitinases, cellulase and protease. Mycoparasitism by *T. harzianum* combined with the production of cell wall degrading enzymes and volatile alkyl pyrones and antibiotics has also been reported (Ridout *et al.*, 1986 and Claydon *et al*, 1987).

Chambers and Scott (1995) evaluated the antagonism of *Trichoderma pseudokoningi* and *T. hamatum* against *Phytophthora* spp. and found that both the species inhibited *Phytophthora* by mycoparasitism with evidence of parallel growth and coiling. Cruz and Cisterna (1998) found that the radial growth of *P. capsici* was inhibited in cultures with *T. harzianum*. Patel and Patel (1999) noticed *T. harzianum* engulfed the *P. parasitica* var. *nicotianae* in dual culture indicating it was highly antagonistic to the pathogen. It was also found that *T harzianum* was effective in inhibiting the growth of *P.meadii* (Spices Board, 1999). Stefanova *et al.*(1999) reported that *T. harzianum* and *T. viride* produced non volatile metabolites with antifungal activity which reduced the growth of *P. nicotianae* in *in vitro*. Vijayaraghavan (2003) noticed that *Trichoderma* spp. overgrown on *P.capsici* in dual culture. The mechanism was found to be mycoparasitism in the

form of hyphal coiling, penetration and desintegration of the host hyphae leading to the death of the pathogen.

Anandraj and Sarma (1994 and 1995) recorded potential biocontrol activity of *T.hamatum* and *Gliocladium virens* both under greenhouse and field conditions. Harman (2000) reported that *Trichoderma* spp.gave long term protection due to the mechanisms like rhizosphere competence, induced resitance and tolerance to stress through enhanced root and plant development.

Bhai *et al.* (1999) reported the effectiveness of *Trichoderma* spp. for control of the azhukal disease caused by *P. meadii* in cardamom. Ability of *T.harzianum* to control the rotting of pepper plant roots caused by *P. capsici* was given by Ahmed *et al.* (1999). Joe *et al.* (2000) reported that *Trichoderma* mixed with compost was effective in controlling root rot caused by *Phytophthora* sp. and wilt caused by *P.capsici* in cardamom and pepper. Kannan and Revathy (2002) found that inoculation of *Trichoderma viride* reduced the foot rot of pepper caused by *P.capsici*.They also observed that *T.viride* mixed with farmyard manure and Bordeaux mixture gave highest control of the disease. Rajan *et.al.* (2002) reported that the *Trichoderma* isolates (*T.virens*-12 and *T.harzianum*-26)were more effective to control the foot rot disease of black pepper and isolate *T.harzianum*-26 most adaptive to the rhizosphere. Jayasuriya *et al.* (2002) suggested a possibility of increasing the resistance towards leaf disease caused by *P. meadii* in susceptible clones by introducing *Trichoderma* as a biocontrol agent. Vijayaraghavan (2003) found that inoculation of *T.longibrachiatum* as well as *T.viride* along with soil solarisation and Ridomil MZ recorded more than 60 per cent efficiency in controlling *Phytophthora* rot of black pepper in nursery. Vijayan *et.al.* (2004) reported that *T.harzianum* had resulted in 100 per cent disease control inoculated with *P.meadii*.

2.4.2.2 Antagonistic Rhizobacteria.

Among the various approaches of biological control, the use of rhizobacteria as biocontrol agent is emerging as a popular trend due to its additional benefits of promoting growth and yield. Fluorescent pseudomonads were some of the effective agents for biological control of soil borne plant pathogens owing to their rhizosphere competence (Kloepper *et.al.*, 1980., 1981).

Kloepper *et.al.* (1980) have clearly demonstrated the role of siderophores in rhizobacteria mediated antagonism. Elad and Chet (1987) found that the competition for nutrients by the rhizobacteria suppressed disease caused by *Pythium aphanidermatum*. Paul *et.al.* (2001) also reported siderophore mediated antagonism in *P.capsici* and *P.fluorescens* antagonistic system.

Okamoto (1991) reported that *Serratia marcescens* was antagonistic to *P.capsici in vitro* and reduced damping off of cultivated cucumber seedlings. Stirling *et al.* (1992) found the *in vitro* antagonistic activity of three fluorescent pseudomonads and *Serratia* sp. against *P. cinnamomi*. Fernando and Linderman (1995) reported that *in vitro* inhibition of mycelial growth and sporangial production of *P. vignae* by producing agar diffusible and volatile inhibitors by rhizobacteria.

Jubina and Girija (1998) found that the rhizosphere bacteria suppressed the growth of *P. capsici* causing foot rot of black pepper both in *in vitro* and *in vivo* condition. They found that under *in vivo* condition, three bacterial isolates B5, B7, and B13 were effective in controlling the disease. Dual culture technique followed to test the antagonistic efficiency of rhiozobacteria against *P. capsici* in pepper showed upto 70 per cent inhibition on *P.capsici* (Paul *et al.*, 2001 and Minimol, 2002).

The two isolates of *P. fluorescens* (P_1 and P_{14}) developed by Kerala Agricultural university were found highly effective against *Phytophthora* infection in vanilla (KAU,2002). Anandaraj and Sarma (2003) reported that strains of *P. fluorescens* caused cytoplasmic coagulation in the mycelium of *P. capsici* when they were cultured together. They also found that these strains were efficient in inhibiting different species of *Phytophthora* inhabiting different crops viz., *P. meadii* of cardamom, *P.parasitica* of betel vine, *P. palmivora* of coconut and cocoa, *P. heveae* of rubber and *P meadii* of arecanut. Sarma *et al.* (2003) found that the rhizobacteria IISR 859 was *Phytophthora* suppressive both under *in vitro* and *in vivo* and the isolate IISR 147 and IISR 148 were suppressive to *P. meadii* and *F. oxysporum* in vanilla.

2.4.2.3 Mycorrhiza

Anandaraj *et al* reported that the suppressive effect of *Glomus fasciculatum* on three pathogens viz., *P. capsici*, *R. similis* and *M. incognita*. They observed that inoculation of *G. fasciculatum* resulted in reduction of root rot index by *P. capsici* from 3 to 0.75. Trotta *et al.* (1996) found that the presence of AM fungus decreased both weight reduction and root necrosis by *P. nicotianae* var *parasitica* in tomato and observed increased plant resistance to infection.

Sivaprasad (1997) reported enhanced crop protection against *P. capsici* from dual inoculation of VAM and *Trichoderma* spp. in black pepper. Robert (1998) observed that AMF, *Aspergillus* sp. and *Trichoderma viride* were effective in reducing symptom development in black pepper by *P. capsici*.

2.5 COMPATIBILITY STUDIES

2.5.1 **Compatibility of *Trichoderma* spp. with fungicides**

Papavizas and Lumsden (1980) reported that in integrated approach of disease management, biocontrol agents have been used with fungicides without

any toxic effect on antagonists. Use of some strains of *T. harzianum* tolerant to fungicides have also been reported for the integrated control of plant diseases (Henis,1978; Papavizas and Lewis, 1981; Papavizas *et al.*, 1982; Upadhyay and Mukhopadhey, 1986).

Shanmugham (1996) found that Bordeaux mixture completely inhibited the growth of *T. viride*. A complete inhibition of *Trichoderma* spp.was observed with Bordeaux mixture and higher percentage of inhibition was recorded with Kocide, Captaf, and Kvach 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.

2.5.2 Compatibility of *Trichoderma* with antagonistic rhizobacteria and *Azospirillum*

The advantage of using combination of antagonists for biocontrol 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.

2.5.3 Compatibility of rhizobacteria, *P. fluorescens* and *Azospirillum*

Compatibility of *Bacillus subtilis* with *Azospirillum* under *in vitro* and *in vivo* had been proved by Sankar and Jayarajan (1996). 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).

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

Materials and Methods

3. MATERIALS AND METHODS

The present study on growth enhancement and management of *Phytophthora* rot in vanilla nurseries using microbial inoculants was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara.

3.1 ISOLATION OF PATHOGEN

The pathogen causing *Phytophthora* rot disease of vanilla was isolated from naturally infected plants from Perumbavoor (Ernakulam district) using standard isolation technique (Riker and Riker, 1936). The infected plant parts were collected and brought to the laboratory. Samples were then washed under tap water and dried using blotting paper. Small bits of infected portions along with some healthy areas were surface sterilized with one per cent sodium hypochlorite and then washed in three changes of sterile water. These sterilized bits were then placed in sterile Petri dishes containing Oatmeal Agar medium (Appendix 1) and incubated at 28 ± 2 ^{0} C. When the fungal growth is visible, mycelial bits were transferred to sterile Petri dishes containing Carrot Agar (CA) medium (Appendix 2). Later, it was purified by hyphal tip method and transferred to Carrot Agar and Potato Dexrose Agar (PDA) slants and pure culture of the pathogen was maintained for further studies.

3.2 PATHOGENICITY TESTS

The pathogenicity of the organism associated with vanilla rot was proved by following Koch's postulate both *in vitro* and *in vivo* conditions.

3.2.1 ***In vitro*** **condition**

Healthy leaves collected from the field, were washed under tap water and then surface sterilized with 70 per cent ethyl alcohol. The leaves were inoculated

separately on both surface with mycelial growth of the isolate with and without pinpricks. Leaves inoculated with sterile water served as control. Inoculated leaves were kept in moistened polythene bags to maintain humidity and incubated at 28± 2 ^{0}C and observed daily for the symptom appearance. Pathogen was re-isolated from the infected leaves and compared with the original culture.

3.2.2 *In vivo* condition

Cuttings of vanilla were raised in polybags containing sterile potting mixture of soil: sand: cowdung mixture (1:1:1). When it attained sufficient growth, the cuttings were inoculated with pathogen suspension. Inoculated plants were kept under humid chamber and observed for the rotting symptom. The pathogen was re-isolated from the inoculated plants showing rotting symptom and compared with the original culture.

3.3 IDENTIFICATION OF PATHOGEN

The pathogen associated with vanilla rot was identified based on the cultural and morphological characters.

Cultural characters of the pathogen such as rate of growth, growth pattern etc. in the CA media were studied. Morphological characters of the pathogen like length of sporangia, L/B ratio, stalk length etc. were studied by slide culture technique. These characters were compared with description of CMI to identify the pathogen.

3.4 COLLECTION OF SOIL SAMPLES AND ROOTS

The rhizosphere soils of vanilla along with roots were collected from Vellanikkara (Thrissur district) and two other locations Perumbavoor and Mazhuvannur (Ernakulam district). This soil and roots were used for the isolation of AMF, *Azospirillum*, *Trichoderma* and rhizobacteria.

3.5 ISOLATION OF MICROFLORA

3. 5.1 **Isolation of orchid mycorrhiza and AMF**

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 three to five centimetre segments. Three root bits were placed into Petri dishes containing PDA as well as Czapak-Dox mineral medium (Raper and Thom,1949) (Appendix 3) and incubated in the dark for a period of seven days at 28± 2 ^{0}C.

AMF spores were isolated from rhizosphere soil by wet sieving and decanting method (Gerdemann and Nicolson, 1963). 250 g of rhizosphere soil was suspended in 1000 ml water and stirred well. After settling of the heavier particle, the supernatant was filtered through a set of sieves of size 1 mm, 425µm, 250µm, 105µm, and 45µ.m arranged in descending order. Finally, the soil suspension present in 105 and 45 were transferred to 100 ml beakers separately by gentle washing. The spore suspension was filtered through muslin cloth (100 mesh). The muslin cloth containing spores were placed in Petri dishes and observed under stereo microscope. The predominant spores from each location were picked and transferred to moistened filter paper.

3.5.1.1 *Mass multiplication of AMF*

Isolated spores were surface sterilized with two per cent Chloramin T for 20 minutes. It was washed with sterile water. Then again, surface sterilized with streptomycin sulphate (0.02 per cent) for 20 minutes and washed it thoroughly before the spores were transferred to a filter paper placed in a glass funnel. The glass funnels were filled with sterilized soil: sand mixture (1:1). The base of the funnels was plugged with cotton. Using glass rod, the spores were mixed with the soil: sand mixture. This funnel was kept in a bottle containing nutrient media

(Hoagland's solution). Then surface sterilized maize seeds were sown in the funnel. After four to six weeks, the shoot portions were cut and the roots mixed with the soil: sand mixture. It served as the mother inoculums. For mass multiplication, pots of 30 cm diameter were used after surface sterilizing with ethyl alcohol (70%). The pots were filled with sterilized soil: sand mixture (1:1). A hole was made at the center of the potting mixture and mother inoculum was placed and the surface sterilized maize seeds were sown. Pots were maintained for three to four months and inoculum of respective AMF were obtained for further studies.

3.5.2 **Isolation of *Azospirillum***

Azospirillum were isolated from the roots of vanilla collected from Thrissur and Ernakulam districts using Nitrogen Free bromothymol blue (NFb) semi solid medium (appendix 4) according to the procedure of Dobereiner *et al.* (1976). The roots were cut into small bits and they were surface sterilized using 70 per cent ethyl alcohol. After that, the bits were transferred into test tube containing NFb semisolid medium and incubated at 28± 2 ^{0}C for 48-72 h. White dense pellicle at the sub surface of medium indicated the presence of *Azospirillum* and the medium colour was changed from light green to blue. A loopful of culture was transferred to Malate medium and purified. Then purified culture was streaked on media and single colonies were transferred to NFb semisolid medium. Formation of white sub surface pellicle confirmed the presence of *Azospirillum*.

3.5.3 **Isolation of *Trichoderma***

Trichoderma was isolated by serial dilution plate technique (Johnson and Curl, 1972) using Martin's Rose Bengal Streptomycin Agar medium (Appendix 5). For this, 10^{-3} and 10^{-4} dilution of soil samples were used. The fungal colonies developed on media were transferred to Potato Dextrose Agar (PDA) medium (Appendix 6). Pure cultures of fungi were obtained by hyphal tip isolation method

and maintained in PDA slants for further studies.

3.5.4 **Isolation of rhizobacteria**

Rhizobacteria were isolated from vanilla rhizosphere soil using serial dilution plate technique (Johnson and Curl, 1972). The serial dilutions were prepared upto 10^{-6} dilution. From 10^{-4} and 10^{-6} dilution, 0.1 ml were transferred to Petri dishes containing Nutrient Agar (NA) (Appendix 7) as well as King's B media (Appendix 8). It was spread over the media using a sterilized glass spreader and kept for incubation at 28± 2 ^{0}C for 48 h. The predominant bacteria from each location were selected, purified and maintained for further studies.

3.6 SCREENING OF AMF and *Azospirillum* FOR GROWTH ENHANCEMENT IN NURSERY

Experiment was laid out in net house of the College of Horticulture, Vellanikkara. For this, three AMF (Vellanikkara, Perumbavoor and Mazhuvannur) and three *Azospirillum* (Vellanikkara, Perumbavoor and Mazhuvannur) isolates were used. Three noded vanilla cuttings were planted in sterilized soil: sand: cowdung mixture in 1: 1: 1 ratio. The sterilized potting mixture were filled in 6 x 8 inch polybags @ 1.5 kg / bag. The potting mixture without inoculation served as control. Twelve plants were kept for each treatments.

Treatment details

Design: CRD

Treatments: 7

Replication: 3

T_1: Soil application of AMF @ 10 g/ Kg soil (VKA) (15 spores/10g soil)

T_2: Soil application of AMF10 g/ Kg soil (PBR) (25 spores/10g soil)

T_3: Soil application of AMF10 g/ Kg soil (MVR) (20 spores/10g soil)

T_4: Soil application of *Azospirillum* 5 ml/ Kg soil (VKA) ($1.04x10^8$ cfu/ml)

T_5: Soil application of *Azospirillum* 5 ml/ Kg soil (PBR) ($0.6x10^8$ cfu/ml)

T_6: Soil application of *Azospirillum* 5 ml/ Kg soil (MVR) ($0.7x10^8$ cfu/ml)

T_7: Control

3.6.1 **Biometric observations**

3.6.1.1 ***Sprouting percentage***

Sprouting percentage of vanilla cuttings was observed after 30, 45 and 60 days after planting.

3.6.1.2 ***Length of vine***

Extension growth of sprout was first recorded one month after planting and there after at fortnightly intervals for a period of five and half months. Length of sprout was taken from the base to the tip of newly emerged leaf using a metre scale and expressed in centimeters.

3.6.1.3 ***Number of leaves***

Number of leaves in the sprout was first recorded 45 days of after planting and later at fortnightly intervals upto five and half months. The fully emerged leaves were counted.

3.6.1.4 ***Girth of vine***

Girth of sprout at the base was measured from two months of planting at fortnightly intervals upto five and half months.

3.6.1.5 ***Internodal length***

Internodal length was taken after the cuttings had attained sufficient length. It was taken as the average of three internodal length at (three internodes were marked randomly at bottom, middle and top portions of the vine) fortnightly interval upto five and half months.

3.6.1.6 ***Fresh weight of shoots***

Fresh weight of shoots were recorded five and half months after planting by uprooting the plants and separating the shoot portions.

3.6.1.7 ***Fresh weight of roots***

Fresh weight of roots was taken after uprooting the plants after five and half months of planting. The roots were separated, washed thoroughly, dried and weight was taken.

3.6.1.8 ***Dry weight of shoots***

Dry weight of shoots were taken after drying the sample in an oven at 60^0 C to constant weight.

3.6.1.9 ***Dry weight of roots***

Dry weight of roots were taken after drying the sample in an oven at 60^0 C to constant weight.

3.6.1.10 ***Number of roots***

Number of roots were counted after uprooting the plants after five and half

months of planting.

3.6.1.11 ***Root length***

Length of roots were taken from the base to the tip of longest root using a metre scale and expressed in centimeters.

3.6.2 **Enumeration of AMF and *Azospirillum***

Spore count of AMF and enumeration of *Azospirillum* were taken after three months and five and half months of planting. Spore count was taken by wet sieving and decanting method and enumeration of *Azospirillum* was done by most probable number technique (MPN technique) in NFb solid medium.

3.6.3. **Per cent root colonization of AMF**

The AMF per cent root colonization was assessed using the method described by Phillips and Hayman (1970). The per cent AMF colonization was determined using the formula:

$$\text{Per cent root colonization} = \frac{\text{Number of infected root segments}}{\text{Total number of root segments observed}} \times 100$$

3.6.4 **Nutrient analysis**

Nutrients like N, P, and K in plant samples were analysed after five and half months of planting. For this, the whole plant was uprooted, washed off soil particles and dried in an oven at 60 ^{0}C. After drying, samples were powdered and the fine powder was used for estimation of nutrient elements. N, P, K were analysed according to the procedure given by Jackson (1973).

3.7 SCREENING OF MICROBIAL ANTAGONISTS AGAINST *Phytophthora meadii*

3.7.1 **Screening of *Trichoderma* against *Phytophthora meadii***

Antagonistic effect of *Trichoderma* isolates against *Phytophthora* was tested by dual culture method outlined by Skidmore and Dickinson (1976). For this, three predominant isolates of *Trichoderma* selected from each location were used along with commercial culture of *T. viride*. Mycelial disc (6mm) of pathogen from seven day old culture grown on PDA was inoculated aseptically on one side of Petri dish containing PDA and incubated at 28 ± 2^0C for 24 h. After this, 6mm disc of *Trichoderma* isolates were inoculated in the same Petri dish 3.5 cm away from the pathogen and incubated for 5 days. Three replications were maintained for each isolate. Pathogen and *Trichoderma* isolates grown in monoculture served as control.

Growth measurements were taken at regular intervals after 24 h of inoculation of antagonist for four days. Nature of reaction of the antagonist on the pathogen were recorded. Per cent inhibition of mycelial growth of the pathogen was calculated using the formula suggested by Vincent (1927).

$$PI = \frac{C - T}{C} \times 100$$

C – Growth of pathogen in control

T – Growth of pathogen in dual culture

Antagonism index was also calculated as suggested by Kasinathan (1998)

AI = PI x CB x SOOP x IZ

PI : Per cent inhibition

CB : Colonization behaviour of antagonist on pathogen

SOOP: Speed of overgrowth on pathogen

IZ: Inhibition zone

3.7.2 Screening of rhizobacteria against *Phytophthora meadii*

Three predominant rhizobacteria selected from three locations along with commercial *P.fluorescens* were tested for their antagonistic effect against *Phytophthora* by dual culture method. For this, 6mm disc of seven day old culture of *Phytophthora* were placed on either side of Petri dish 3.5 cm apart. After 24 h of incubation, 24 h old culture of bacteria were streaked at the center of the Petri dish and kept for incubation at 28 ± 2 ^{0}C for 4 days. The pathogen alone served as control. Per cent inhibition was calculated as mentioned in 3.7.1.

3.8 IDENTIFICATION OF AMF, *Azospirillum*, *Trichoderma* and ANTAGONISTIC RHIZOBACTERIA

Effiecient AMF, *Azospirillum*, *Trichoderma* and antagonistic rhizobacteria selected from sceening tests were identified.

3.8.1 Identification of AMF

Efficient AMF was identified based on spore characters like colour, shape, size, surface configuration, wall layers, number of hyphae attached, form of hyphae and alighnment of hyphae with the spore axis were recorded under compound microscope (40x) and identified by comparing the spore characters with the synoptic keys (Trappe, 1982).

3.8.2 Identification of *Azospirillum*

Azospirillum was identified at genus level by morphological and cultural characters. Huckers' modification of Gram staining was employed to study the Gram reaction (Hucker and Conn, 1923) and shape of bacterium was identified under oil immersion objective of microscope. Cultural characters were studied by growing it on Okon's medium (Okon *et al.*, 1977) and rojo congo red medium.

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Growth on the salts of organic acids such as malate, succinate, lactate and pyruvate etc were noted.

3.8.3 Identification of *Trichoderma*

Cultural and morphological characters of the efficient *Trichoderma* was studied and the characters were compared with description for *Trichoderma*. Cultural characters like growth rate, growth pattern, colour of colony etc. were studied. Morphological characters like mycelium, shape and size of hyphae, conidiophore, phialide length, nature of phialide, phialospores etc. were studied by slide culture technique.

3.8.4 Identification of antagonistic rhizobacteria

For the identification of efficient antagonistic rhizobacteria, morphological, cultural and biochemical tests were done.

3.8.4.1 Morphological characters

Huckers' modification of Gram staining was carried out (Hucker and Conn, 1923) using 24 h old culture of bacterium to study morphological characters like size, shape, and arrangement of cells.

3.8.4.2 Cultural characters

Bacterial culture was streaked on Nutrient Agar medium in Petri dishes and after an incubation period of 24 h, colonies were observed for its shape, elevation and margin.

3.8.4.3 Biochemical test

3.8.4.3.1 KOH test

A loopful each of the bacterial culture was put on a clear glass slide. One drop of three per cent KOH solution was placed over it and thoroughly mixed with the help of a needle. The bacteria as thin threads indicated gram negative bacteria.

3.8.4.3.2 Pigment production

48 h old cultures of bacteria were streaked on both King's A and King's B medium and observed under UV light for fluorescent pigment production.

3.8.4.3.3 Oxidase test

48 h old bacterial cultures were spot inoculated on oxidase disc and change in colour of the disc from white to purple blue was observed.

3.8.4.3.4 Levan production from sucrose

The bacterial cultures were streaked on the sterilized peptone beef extract containing five per cent sucrose and growth characters were observed after 48 h. presence of large, white, domed, and mucoid colonies characterized the production of levan from sucrose (Hayward, 1964).

3.8.4.3.5 Starch Hydrolysis

Nutrient Agar containing 0.2 per cent soluble starch was used. 48 h old test culture was spotted on the Petri dish containing NA with 0.2% soluble starch. Starch hydrolysis was tested after 48 h of incubation by flooding the Agar surface

with Logroll's iodine solution (Cappucino and Sherman, 1992). A colourless zone around the bacterial growth in contrast to the blue background of the medium indicated positive reaction.

3.8.4.3.6 Denitrification test

48 h old bacterial cultures were streaked into the VMS medium and sealed with three ml of one per cent molten agar at 45 ^{0}C and examined daily for the production of gas under the seal (Hayward *et al.*, 1967).

3.8.4.3.7 Arginine dihydrolase reaction

48 h old bacterial cultures were stabbed into the semi solid medium of Thornley (1960) and the tubes were sealed with three ml of 1 per cent molten agar at 45^{0}C. The tubes were incubated at 28 ± 2^{0}C for seven days and the colour change was observed.

3.8.4.3.8 Catalase Test

Smears of 24 h old bacterial cultures were prepared on clean glass slide and covered with a few drops of three per cent hydrogen peroxide. Effervescence indicated the presence of catalase in the culture.

3.9 EFFICACY OF DIFFERENT FUNGICIDES AGAINST *Phytophthora meadii*

Five fungicides recommended against *Phytophthora* were used (Table 1). 100 ml PDA was prepared in conical flask and sterilized. After melting the media, required quantity of each fungicide were added to get desired concentration and poured into sterilized Petri dishes. When the media was solidified, 6mm disc of pathogen grown on CA media was placed at the centre of the Petri dish. The

Table 1. Fungicides used in *in vitro* evaluation against *Phytophthora meadii*

Sl. No.	Chemical name	Trade name	Concentration (Per cent)
1	$CuSO_4$ + Lime + H_2O	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	0.2, 0.3, 0.4
5	Mancozeb	Indofil M-45	0.1, 0.2, 0.3

pathogen alone served as control. Three replications were maintained for each concentration. Growth measurements were taken at regular intervals and per cent inhibition was calculated as mentioned in 3.7.1.

3.10 COMPATIBILITY STUDIES

Compatibility studies were carried out to find out whether the selected antagonists were compatible with the commonly used chemicals. Compatibility of *Trichoderma* isolates with antagonistic rhizobacteria and *Azospirillum* and the compatibility among these bacteria were also tested.

3.10.1 **Compatibility of different fungicides with *Trichoderma* spp.**

Five fungicides (Table 1) used against *Phytophthora* were used here also. Efficient *Trichoderma* isolate along with standard *Trichoderma viride* were tested. The quantity of fungicides needed to get the desired concentration was added to 100 ml sterilized, molten PDA medium. Then mixed well and poured into sterilized Petri dishes at the rate of 15-20 ml per plate. After solidification of the medium, mycelial disc of 6mm diameter from actively growing 72 h old fungal antagonists were cut and placed at the centre of each Petri dish. Control plates consisted of PDA medium alone inoculated with antagonists. Three replications were maintained for each concentration. The inoculated plates were incubated at 28 ± 2^0C and observations on mycelial growth of fungal antagonists were taken when control plates showed full growth. The per cent inhibition of growth of antagonists was calculated as given in the section 3.7.1.

3.10.2 **Compatibility of different fungicides with antagonistic rhizobacteria**

Sterile filter paper discs of 6mm diameter were soaked in different concentration of fungicides. The discs were placed at the centre of Petri dishes containing King's B medium seeded with 48 h old culture of efficient antagonistic rhizobacteria and *P. fluorescens* (commercial). Control plates consisted of filter

paper soaked in sterile water. Three replications were maintained for each concentration. The inoculated plates were incubated at 28± 2 °C and the observations on inhibition zone were recorded after 48 h.

3.10.3 **Compatibility of *Trichoderma* isolates with antagonistic bacteria and *Azospirillum***

For this, 6mm disc of *Trichoderma* was placed at one side of the Petri plate containing PDA. After 24 h incubation, antagonistic bacteria and *Azospirillum* were streaked separately on the other side of the Petri dish 3.5 cm away from the *Trichoderma* and kept for incubation at 28±2 ^{0}C for 4-5 days.

3.10.4 **Compatibility among antagonistic rhizobacteria, *Azospirillum* and *P.fluorescens***

Compatibility among antagonistic rhizobacterium and *Azospirillum* were tested. For this, *Azospirillum* was seeded with NA media and the antagonistic rhizobacterium and *P. fluorescens* were spot inoculated. For testing the compatibility between antagonistic rhizobacterium and *P. fluorescens,* rhizobacterium was seeded over the media and *P fluorescens* was spot inoculated and incubated for 48 h at 28±2 ^{0}C.

3.11 EVALUATION OF THE MOST EFFECTIVE MICROBIAL ISOLATES FOR GROWTH ENHANCEMENT AND *Phytophthora* ROT IN VANILLA NURSERIES

One effective isolate each of AMF, *Azospirillum*, *Trichoderma* and antagonistic rhizobacteria obtained under screening trial for efficiency were tested in the nursery for growth enhancement and management of *Phytophthora* rot in vanilla nurseries. For this, three noded vanilla cuttings were planted in polybags containing soil: sand: cowdung mixture in 1:1:1 ratio. In each treatment, 12 plants

were maintained. The different treatments were given at the time of planting. After the plants were established, the pathogen was artificially inoculated in half number of plants in each treatment. In each treatment six plants were inoculated with pathogen @ 20g inoculam/ 6' x 8' bag. Details of the treatments are given below:

Design : CRD

Treatments : 10

Replication : 3

No. of plants per treatment : 12

Treatments

T_1- Soil application of AMF (PBR) (@ 10 g/kg of soil, 20 spore/10g soil)

T_2- Soil application of *Azospirillum* sp. (MVR) (5 ml @10^8cells/ml, $1.3x10^8$ cfu/ml)

T_3- Soil application of *Trichoderma* sp. (VKA) (2 g/kg of soil, $1.4x10^6$ cfu)

T_4- Soil application of Antagonistic rhizobacterium (MVR) (3 g/kg of soil, $1.3x10^6$ cfu)

T_5- Soil application of *Piriformospora indica* (Pi) (10 ml/kg of soil)
(Commercial culture)

T_6 – Soil application of *Trichoderma viride* (2 g/kg soil)
(Commercial culture)

T_7- Soil application of *Pseudomonas fluorescens* (3 g/kg of soil)
(Commercial culture)

T_8-Soil drenching of Bordeaux mixture (1%)

T_9- Soil drenching of potassium phosphonate (0.3%)

T_{10}- Control.

3.11.2 **Sprouting**

Per cent sprouting of the cuttings were recorded after 30, 45 and 60 days of planting.

3.11.3 **Length of vine**

Extension growth of sprout was first recorded one month after planting and there after at fortnightly intervals. Length of sprout was taken from the base to the tip of newly emerged leaf using a metre scale and expressed in centimeters.

3.11.4 ***Number of leaves***

Number of leaves in the sprout was first recorded 45 days after planting and later at fortnightly intervals. The fully emerged leaves were counted.

3.11.4 **Per cent disease incidence**

Per cent disease incidence was taken

3.11.5 **Evaluation of *T.harzianum*, *T.viride* (commercial), *Pseudomonas* spp. and *P.fluorescens* (commercial) against *Phtophthora* rot in vanilla**

A trial was conducted to study the evaluation different antagonists (*T.harzianum*, *T.viride* (commercial), *Pseudomonas* spp. and *P.fluorescens* (commercial)) used in the experiment against *Phytophthora* rot in vanilla. For this same quantity of antagonists and pathogen (2g /kg soil) was inoculated. After one month of application of antagonists, pathogen was inoculated. Observations were taken on disease incidence.

Results

4. RESULTS

Results of the study on growth enhancement and *Phytophthora* rot management in vanilla nurseries using microbial inoculants are presented in this chapter.

4.1 ISOLATION OF THE PATHOGEN

Pathogen causing *Phytophthora* rot in vanilla nursery was isolated from the naturally infected cuttings collected from Perumbavoor. The fungus was purified by hyphal tip method and maintained on Carrot Agar (CA) slants as well as on potato dextrose agar (PDA) slants by periodic sub culturing (Plate 1)

4.1.1 **Pathogenicity**

Pathogenicity of the isolate was tested on detached leaves as well as on whole plants. On artificial inoculation, pale water soaked lesions appeared on leaves after three days (Plate 2). The lesions gradually enlarged and resulted in complete rotting of leaves. The pathogen was reisolated from artificially inoculated leaves and yielded the same organism there by proving the pathogenicity. On whole plant also symptom appered on leaves after three days of inoculation. Then the rotting started from the base of vine and complete rotting was observed within one week.

4.1.2 **Cultural and morphological characters of the pathogen**

The isolated pathogen was subjected to cultural and morphological studies.

In CA medium, the colonies were white with uniformly dense cotton wool like aerial mycelium over entire colony. Hyphae aseptate and 2.8μ m wide. Hyphae became septate when old. Sporangia were ovoid to ellipsoid, highly

Plate 1: Culture of *P. meadii*

A. Artificial inoculation

B. Natural Infection

Plate 2: Symptoms of Phytophthora rot on vanilla

caducous and papillate with intermediate stalk length. Sporangia measured 35-50 x 19-35μ m with L/B ratio 1.6 some upto 1.9. Based on these characters and pathogenicity test, the pathogen was identified as *Phytophthora meadii* Mc Rac (Mc Rac,1919).

4.2 ISOLATION OF MICROFLORA

Among the several isolates obtained from each location, the most predominant AMF, *Azospirillum, Trichoderma* and rhizobacteria were selected from Thrissur (Vellanikkara) and Ernakulam (Perumbavoor and Mazhuvannur) districts as mentioned in chapter 3.5.A total of three AMF isolates (VKA, PBR and MVR), three *Azospirillum* (VKA,PBR and MVR), three *Trichoderma* (VKA,PBR and MVR) and three rhizobacteria (VKA,PBR and MVR) were selected for this study. They were purified and mass multiplied for further studies.

4.3 SCREENING OF AMF AND *Azospirillum* FOR GROWTH ENHANCEMENT OF VANILLA UNDER STERILE CONDITION IN NURSERY

A nursery experiment was carried out to select the efficient AMF and *Azospirillum* isolate to improve growth of vanilla. The experiment was carried out as described in Materials and Methods. Observations were taken on various parameters (Plate 3)

4.3.1 Effect of different treatments on sprouting of vanilla

Numbers of vanilla cuttings sprouted in each treatment were recorded at 30, 40 and 60 days after planting and the sprouting percentage calculated. The results are shown in Table 2.

From the table, it was clear that the treatments showed significant

T_7 T_6 T_5 T_4 T_7 T_6 T_5 T_4 T_3 T_2 T_1

Plate 3: Effect of different treatments on vanilla cuttings

Table. 2. Effect of different AMF and *Azospirillum* isolates on the sprouting percentage of vanilla cuttings

Treatment No.	Treatments	Percentage sprouting		
		Days after planting		
		30	45	60
T_1	AMF (VKA)	75.00[ab]	91.60	91.67
T_2	AMF (PBR)	91.60[a]	100.00	100.00
T_3	AMF(MVR)	75.00[ab]	100.00	100.00
T_4	*Azospirillum* (VKA)	33.00[b]	66.67	100.00
T_5	*Azospirillum* (PBR)	66.67[ab]	75.00	100.00
T_6	*Azospirillum* (MVR)	50.00[ab]	91.67	100.00
T_7	Control	58.30[ab]	83.33	100.00
			NS	NS

Mean of three replications

NS: Non significant

Figures followed by same letter do not differ significantly according to DMRT

VKA: Vellanikkara

PBR: Perumbavoor

MVR: Mazhuvannur

differences at one month after planting. While after that, there were no significant differences among treatments on the sprouting percentage.

Among AMF treatments, T_2 showed maximum sprouting per cent and among *Azospirillum* isolates T_5 gave better sprouting per cent (91.6 and 66.67 per cent respectively) after one month of planting. After 45 days of planting, T_2 and T_3 showed cent per cent sprouting followed by T_1 and while T_6 (91.67 per cent) gave maximum sprouting per cent among *Azospirillum* treatments. Lowest sprouting per cent was noticed in T_4. While after two months of planting, all the treatments gave cent percent sprouting except T_1 (91.67 per cent). It was found that the mycorrhizal isolates gave early sprouting.

4.3.2 Effect of different treatments on length of vine of vanilla

Length of vine of vanilla was recorded at fortnightly interval upto five and half months (Table 3). Although, there was no significant differences, among mycorrhizal isolates T_2 (AMF PBR) gave maximum height (82.17 cm) and T_6 (*Azospirillum* MVR) was the best *Azospirillum* isolate (81.54 cm). It was 16 per cent and 15.3 per cent more than that of control (70.71cm).

Among AMF, T_2 showed maximum height after one month of planting (1.46 cm) which is 82.5 per cent more than control. Among *Azospirillum* isolates, T_6 (1.29 cm) performed well . The same trend was noticed upto five and half months after planting.

4.3.4 Effect of different treatments on number of leaves

Number of leaves were not significantly influenced by different treatments. However, T_2 and T_6 gave maximum number of leaves (15) among AMF and *Azospirillum* isolates respectively. It was followed by T_3 (14). The lowest number of leaves were noticed in the case of T_4 (Table 4).

Table. 3. Effect of different AMF and *Azospirillum* isolates on the length of vine in vanilla cuttings

Treatment No.	Treatments	Length of vine (cm)									
		Days after planting									
		30	45	60	75	90	105	120	135	150	165
T_1	AMF (VKA)	1.18	6.12	10.86	12.72	17.96	24.25	29.63	36.43	46.17	56.75
T_2	AMF (PBR)	1.46	8.61	15.99	21.83	29.13	36.92	45.88	55.14	66.89	82.17
T_3	AMF(MVR)	1.22	7.34	13.70	20.62	26.67	38.53	39.50	48.58	59.88	73.29
T_4	*Azospirillum* (VKA)	0.51	3.74	8.70	14.71	17.46	25.17	31.04	39.63	50.63	62.33
T_5	*Azospirillum* (PBR)	0.80	5.92	10.34	16.29	19.93	27.29	32.63	41.65	52.54	65.50
T_6	*Azospirillum* (MVR)	1.29	7.41	14.53	22.32	27.21	34.59	41.88	53.64	67.15	81.54
T_7	Control	0.80	6.02	13.02	19.23	27.34	33.79	39.88	48.93	59.59	70.71
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Mean of three replications

NS: Non significant

VKA: Vellanikkara

PBR: Perumbavoor

MVR: Mazhuvannur

Table. 4. Effect of different AMF and *Azospirillum* isolates on number of leaves in vanilla cuttings

Treatment No.	Treatments	Number of leaves								
		Days after planting								
		45	60	75	90	105	120	135	150	165
T_1	AMF (VKA)	1	2	4	4	6	8	9	11	12
T_2	AMF (PBR)	2	4	5	6	8	10	12	12	15
T_3	AMF(MVR)	2	3	5	6	7	9	11	12	14
T_4	*Azospirillum* (VKA)	1	2	3	4	6	7	9	10	11
T_5	*Azospirillum* (PBR)	2	2	4	5	6	8	9	11	12
T_6	*Azospirillum* (MVR)	2	3	5	7	8	9	11	13	15
T_7	Control	2	3	4	6	8	9	11	12	14
		NS	NS	NS	NS	NS	NS	NS	NS	NS

Mean of three replications

NS : Non significant

Values are rounded to nearest number

PBR: Perumbavoor

MVR: Mazhuvannur

4.3.5 Effect of different treatments on girth of vine

Girth of vine was taken at fortnightly interval after two months of planting (Table 5). Even though, the treatments did not show significant differences in initial period, after three months of planting they showed significant differences on girth of vine. After five and half months of planting, AMF isolate T_2 (PBR) recorded maximum girth (2.02 cm), which is on par with T_1 (1.95 cm) and T_3 (1.93 cm). Among the *Azospirillum* isolates, T_6 (MVR) recorded maximum girth (1.87 cm), which is on par with treatments T_4 and T_5. Among the AMF isolates, T_1 recorded maximum girth (1.71 cm) upto four months of planting, which is on par with the treatments T_2 and T_3. While among *Azospirillum* isolates, T_5 showed maximum girth upto three months of planting.

4.3.6 Effect of different treatments on the internodal length

Internodal length was taken after four months of planting at fortnightly intervals. Here also, the treatments did not differ significantly (Table 6). After five and half months of planting, T_5 is the best *Azospirillum* isolates showed maximum internodal length (6.24 cm) and T_2 is the best mycorrhizal isolate (6.05 cm). Control plants showed a length of 5.17 cm. While in the initial period, T_3 showed maximum internodal length (5.19 cm) followed by T_2 (5.13 cm) among AMF isolates and T_6 showed maximum internodal length among *Azospirillum* isolates.

4.3.7 Effect of different treatments on fresh weight of vanilla

The fresh and dry weight of shoot, root, and whole plant are given in Table 7. In the case of shoot fresh weight, significant differences were noticed among the treatments. T_2 (AMF PBR) registered the highest value of shoot fresh weight (64.81 g) followed by T_3 (63.72 g). Among *Azospirillum* treatments, T_6 (*Azospirillum* MVR) gave maximum fresh weight (61.38 g). The lowest weight

Table. 5. Effect of different AMF and *Azospirillum* isolates on the girth of vanilla cuttings

Treatment No.	Treatments	Girth of sprout (cm)							
		Days after planting							
		60	75	90	105	120	135	150	165
T_1	AMF (VKA)	0.45	1.52	1.71^{a}	1.76^{a}	1.83^{a}	1.85^{a}	1.90^{a}	1.95^{a}
T_2	AMF (PBR)	1.23	1.51	1.7^{ab}	1.74^{a}	1.78^{ab}	1.86^{a}	1.94^{a}	2.02^{a}
T_3	AMF(MVR)	0.87	1.32	1.63^{ab}	1.67^{ab}	1.75^{ab}	1.83^{ab}	1.88^{ab}	1.93^{ab}
T_4	*Azospirillum* (VKA)	0.56	1.02	1.30^{b}	1.47^{ab}	1.64^{ab}	1.69^{ab}	1.76^{ab}	1.82^{ab}
T_5	*Azospirillum* (PBR)	0.95	1.04	1.47^{ab}	1.42^{b}	1.61^{b}	1.66^{ab}	1.71^{ab}	1.79^{ab}
T_6	*Azospirillum* (MVR)	0.87	1.02	1.57^{ab}	1.57^{ab}	1.72^{ab}	1.77^{ab}	1.83^{ab}	1.87^{ab}
T_7	Control	0.69	1.16	1.49^{ab}	1.58^{ab}	1.58^{b}	1.62^{b}	1.66^{b}	1.73^{b}
		NS	NS						

Mean of three replications

NS: Non significant

In each column figures followed by same letter do not differ significantly according to DMRT

VKA: Vellanikkara

PBR: Perumbavoor

MVR: Mazhuvannur

Table. 6. Effect of different AMF and *Azospirillum* isolates on the internodal length of vanilla cuttings

Treatment No.	Treatments	Internodal length (cm)			
		Days after planting			
		120	135	150	165
T_1	AMF(VKA)	4.00	4.25	4.44	4.78
T_2	AMF (PBR)	5.13	5.39	5.60	6.05
T_3	AMF(MVR)	5.19	5.48	5.60	5.98
T_4	*Azospirillum* (VKA)	3.87	4.74	5.09	5.74
T_5	*Azospirillum* (PBR)	4.81	5.15	5.53	6.24
T_6	*Azospirillum* (MVR)	5.12	5.33	5.53	5.89
T_7	Control	4.30	4.74	4.90	5.17
		NS	NS	NS	NS

Mean of three replications

NS: Non significant

VKA: Vellanikkara

PBR: Perumbavoor

MVR: Mazhuvannur

was registered in T_1 (41.64 g).

The treatments did not differ significantly with respect to root fresh weight. However, maximum root fresh weight was recorded in T_3 (4.57 g) followed by T_2 (4.54 g). Among *Azospirillum* isolates, T6 performed well and recorded a weight of 3.98 g. The lowest weight was observed with control (3.86 g).

With respect to fresh weight of whole plant, the treatments did not vary significantly. T_2 showed highest value of fresh weight (69.35 g) followed by T_3 (68.29 g). The lowest value was observed with T_1 (45.56 g). The treatment T_6 showed maximum whole plant weight (65.36 g) among *Azospirillum* isolates.

4.3.8 Effect of different treatments on dry weight of vanilla

In the case of shoot dry weight, the treatments showed significant differences (Table 7). The T_6 (*Azospirillum* MVR) yielded maximum dry weight (4.98 g) among *Azospirillum* isolates, while T_2 (AMF PBR) recorded maximum weight among AMF isolates (4.87 g). This was on par with T_3 and T_4 (4.56 g and 4.50 g respectively). While T_3 recorded maximum root dry weight among AMF isolates and T_4 gave maximum among *Azospirillum* isolates. The control showed a dry weight of 1.03 g. The treatments did not show significant variations with respect to the dry weight of whole plant. T_2 showed maximum dry weight (6.27 g) followed by T_3 (6.02 g). Among *Azospirillum* isolates T_6 yielded maximum dry weight. The lowest weight was observed in the case of T_1 (3.94 g).

4.3.9 Effect of different treatments on number and length of roots

Treatments varied significantly with respect to root number (Table 8). Higher number of roots was given by T_2 (17.33) followed by T_3 (17). Treatment

Table. 7. Effect of different AMF and *Azospirillum* isolates on the fresh and dry weight of vanilla cuttings

Treatment No.	Treatments	Fresh weight (g)			Dry weight (g)		
		Shoot	Root	Whole Plant	Shoot	Root	Whole Plant
T_1	AMF (VKA)	41.64^{b}	3.92	45.56	3.24^{b}	0.70^{b}	3.94
T_2	AMF (PBR)	64.81^{a}	4.54	69.35	4.87^{a}	1.40^{a}	6.27
T_3	AMF(MVR)	63.72^{a}	4.57	68.29	4.56^{a}	1.461^{a}	6.02
T_4	*Azospirillum* (VKA)	50.45^{ab}	4.15	54.60	4.50^{a}	0.98^{ab}	5.48
T_5	*Azospirillum* (PBR)	55.53^{ab}	3.95	59.48	4.35^{ab}	0.87^{ab}	5.22
T_6	*Azospirillum* (MVR)	61.38^{a}	3.98	65.36	4.98^{a}	0.90^{ab}	5.88
T_7	Control	60.20^{a}	3.86	64.06	4.58^{a}	1.03^{ab}	5.61
			NS	NS			NS

Mean of three replications

NS : Non significant

In each column figures followed by same letter do not differ significantly according to DMRT

VKA: Vellanikkara

PBR: Perumbavoor

MVR: Mazhuvannur

Table. 8. Effect of different AMF and *Azospirillum* isolates on the number and length of roots in vanilla cuttings

Treatment No.	Treatments	Number of roots	Root length (cm)
T_1	AMF (VKA)	10.67^{c}	20.92^{a}
T_2	AMF (PBR)	17.33^{a}	23.08^{a}
T_3	AMF(MVR)	17.00^{ab}	25.33^{a}
T_4	*Azospirillum* (VKA)	16.00^{ab}	18.46^{ab}
T_5	*Azospirillum* (PBR)	12.00^{bc}	21.79^{a}
T_6	*Azospirillum* (MVR)	17.00^{ab}	22.17^{a}
T_7	Control	15.67^{ab}	12.75^{b}

Mean of three replications

NS: Non significant

In each column figures followed by same letter do not differ significantly according to DMRT

VKA: Vellanikkara

PBR: Perumbavoor

MVR: Mazhuvannur

T_6 gave maximum number of roots among *Azospirillum* isolates. Root length also varied significantly with respect to different treatments (Table 8). Highest length was observed with T_3 (25.33 cm) followed by T_2 (23.08 cm). Among the *Azospirillum* isolates, T_6 yielded highest length (22.17cm), which is on par with T_5. The control plants showed a length of 12.75 cm.

4.3.10 Enumeration of AMF and *Azospirillum*

Spore count of AMF and enumeration of *Azospirillum* were done after three months and five and half months of planting (Table 9). Population of T_3 was maximum after 3 months while population of T_1 was maximum after five and half months of planting in *Azospirillum* isolates. In the case of AMF, T_2 recorded maximum spore count after three and five and half months of planting. Per cent root colonization of AMF was taken after five and half months of planting. None of the root segments were infected by AMF.

4.3.11 Effect of different treatments on plant nutrients

4.3.11.1 Nitrogen

From the table 10, it was evident that the treatments showed significant differences in the case of nitrogen content. The treatment T_2 (AMF PBR) gave maximum nitrogen content (0.93 per cent). It was followed by T_1, which is on par with T_3. Among *Azospirillum* isolates, T_6 yielded maximum N content (0.83 per cent) followed by T_5 (0.83 per cent). The lowest value was registered by T_4 (0.63 per cent). Control plants recorded 0.47 per cent.

4.3.11.2 Phosphorus content

Although, the treatments did not differ significantly, the mycorrhiza inoculated plants gave maximum P content. T_3 recorded highest value (0.44 per cent) followed by T_1 and T_2 (0.39 and 0.38 per cent respectively). Among *Azospirillum* isolates, T_6 yielded highest value (0.34per cent) followed by T_5 (0.30

Table. 9. Effect of different treatments on AMF and *Azospirillum* population in vanilla rhizosphere

Treatment No.	*Azospirillum*	x10^5 cfu g^{-1} soil		AMF	No. of spores 10 g^{-1} soil	
		3 MAP	5 ½ MAP		3 MAP	5 ½ MAP
T_1	*Azospirillum* (VKA)	0.12	1.7	AMF(VKA)	24	32
T_2	*Azospirillum* (PBR)	0.17	0.14	AMF (PBR)	36	62
T_3	*Azospirillum* (MVR)	0.20	1.40	AMF(MVR)	28	40
T_4	Control	0.04	0.07	Control	4	6

Mean of three replications

MAP : Months after planting

VKA: Vellanikkara

PBR: Perumbavoor

MVR: Mazhuvannur

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Table. 10. Effect of different AMF and *Azospirillum* isolates on nutrient content in vanilla cuttings

Treatment No.	Treatments	Plant nutrients (per cent)			Per cent increase/ decrease over control		
		N	P	K	N	P	K
T_1	AMF (VKA)	0.74^{ab}	0.39	0.32	57.45	39.29	6.67
T_2	AMF (PBR)	0.93^{a}	0.38	0.32	97.8	35.71	6.67
T_3	AMF(MVR)	0.66^{ab}	0.44	0.28	40.43	57.14	-6.67
T_4	*Azospirillum* (VKA)	0.63^{ab}	0.29	0.17	34.04	3.57	-43.33
T_5	*Azospirillum* (PBR)	0.80^{ab}	0.30	0.20	70.21	7.14	-33.33
T_6	*Azospirillum* (MVR)	0.83^{ab}	0.34	0.18	76.59	21.42	-40.0
T_7	Control	0.47^{b}	0.28	0.30			
			NS	NS			

Mean of three replications

NS: Non significant

In each column figures followed by same letter do not differ significantly according to DMRT

VKA: Vellanikkara

PBR: Perumbavoor

MVR: Mazhuvannur

per cent). Control plants showed lowest value (0.28 per cent).

4.3.11.3 Potassium content

Here also, the treatments showed no significant differences. Maximum K content was given by T_1 and T_2 (0.32 per cent) followed by control (0.30 per cent). Among *Azospirillum* isolates, T_5 recorded maximum K content (0.20 per cent). The lowest value was noticed with T_4 (0.17 per cent).

Analysing the above results on biometric characters and nutrient contents, treatments T_2 (AMF PBR) and T_6 (*Azospirillum* MVR) were selected as best AMF and *Azospirillum* isolates respectively and were used for further studies.

4.4 SCREENING OF MICROBIAL ANTAGONIST AGAINST *Phytophthora meadii*

4.4.1 *In vitro* evaluation of *Trichoderma* spp. against *P. meadii*

Three isolates of *Trichoderma* selected from each locations were tested against *Phytophthora meadii* for their antagonistic activity under *in vitro* condition. The *Trichoderma viride* (commercial culture) was also evaluated. All the isolates showed overgrowth on the pathogen (Table 11). Among the different isolates, most efficient isolate was selected by employing the method described in Materials and Methods. It was noticed that isolate *Trichoderma* (VKA) showed maximum antagonism index (1500). It was same as that of commercial culture. The lowest antagonism index was noticed in the case of *Trichoderma* (MVR) (1375) (Plate 4).

4.4.2 *In vitro* evaluation of rhizobacteria against *P. meadii*

Bacterial isolates were also evaluated for their antagonistic effect against

Table. 11. *In vitro* evaluation of *Trichoderma* isolates against *P. meadii*

Sl.No	Antagonists		Days after inoculation (growth cm)										Antagonism Index
			1		2		3		4		5		
1	Trichoderma (VKA)		P	A	P	A	P	A	P	A	P	A	1500
		D	2.5	2.2	3.1	5.5	1.4	7.1	0.0	9.0	0.0	9.0	
		M	2.9	2.7	5.5	5.7	7.0	9.0	8.4	9.0	9.0	9.0	
2	Trichoderma (MVR)	D	2.6	2.2	4.1	4.9	2.9	6.1	0.7	8.3	0.0	9.0	1375
		M	2.9	2.4	5.5	5.4	7.0	7.5	8.4	9.0	9.0	9.0	
3	Trichoderma (PBR)	D	2.6	2.1	4.2	4.8	2.7	6.3	0.6	8.4	0.0	9.0	1395
		M	2.9	2.5	5.5	4.7	7.0	7.4	8.4	9.0	9.0	9.0	
4	*T.viride* (Com)	D	2.5	2.4	3.3	5.4	1.3	7.4	0.0	9.0	0.0	9.0	1500
		M	2.9	2.5	5.5	5.8	7.0	9.0	8.4	9.0	9.0	9.0	

Mean of three replications

Pathogen inoculated one day prior to the inoculation of antagonist

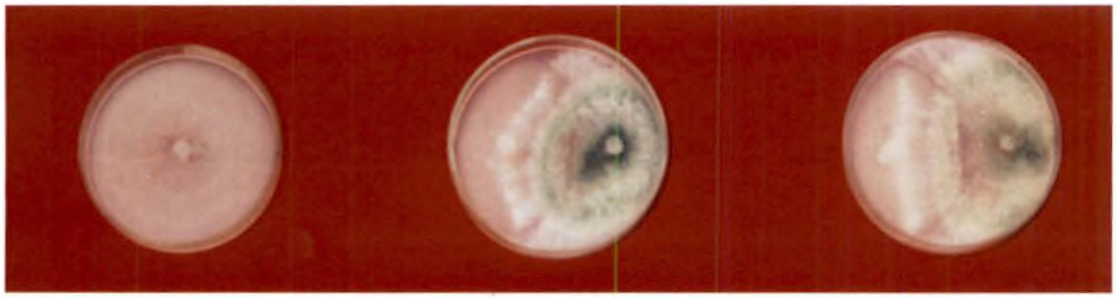
P: Pathogen A: Antagonist

D: Dual culture M: Monoculture

VKA: Vellanikkara

PBR: Perumbavoor

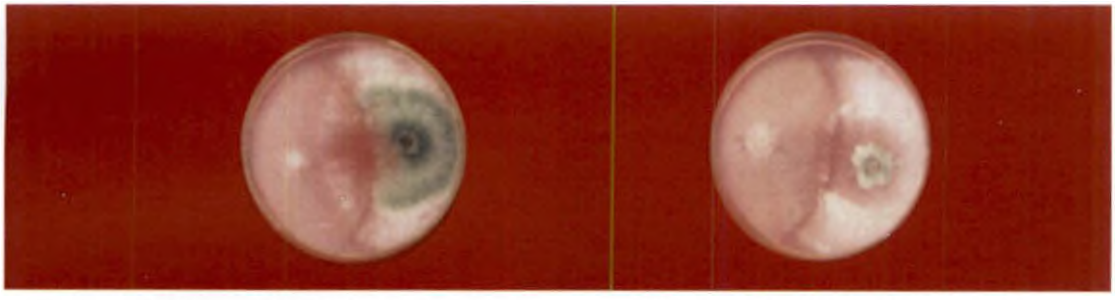
MVR: Mazhuvannur



a. Control

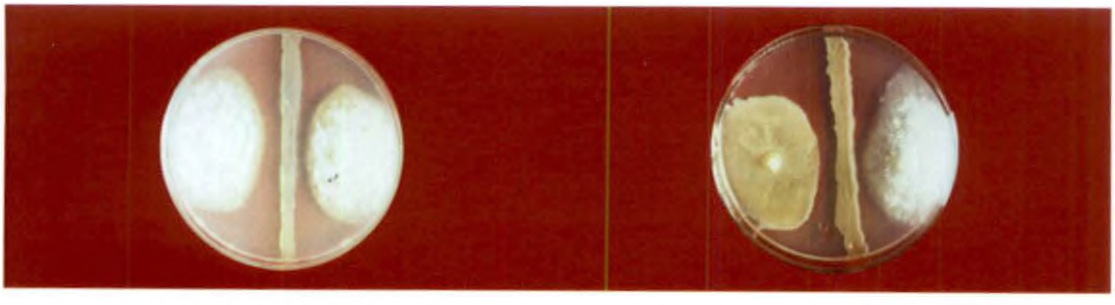
b. *P. meadii x Trichoderma* sp (VKA)

c. *P. meadii x T.viride* (Commercial)



d. *P. meadii x Trichoderma* sp (PBR)

e. *P. meadii x Trichoderma* sp (MVR)



a. *P. meadii x P. fluorescens* (commercial)

b. *P. meadii x Rhizobacteria* (MVR)

Plate 4. Antagonistic effect of *Trichoderma* spp. and Rhizobacteria on *P. meadii* in dual culture.

Phytophthora meadii. Maximum inhibition was noticed in the case of rhizobacterium (MVR) (24.4 per cent) followed by standard culture of *Pseudomonas fluorescens* (20 per cent). Lowest antagonistic activity was noticed with rhizobacterium (VKA) (Table 12). Rhizobacterium (PBR) showed no antagonistic activity on *Phytophthora meadii* (Plate 4).

4.5 IDENTIFICATION OF EFFICIENT MICROBIAL ISOLATES

4.5.1 Identification of AMF

Effective AMF isolate was identified based on the synoptic key (Trappe, 1982). The spore was honey coloured (light brown) globose, 30-40 µm., smooth single wall layer and straight single aseptate hyphea was attached. Based on these characters the isolate was identified as *Glomus* spp.

4.5.2 Identification of *Azospirillum*

Azospirillum was identified as prescribed in Materials and Methods. The bacterium was Gram negative and rod shaped. Growth of bacterium changed the Okon's media colour from light green to blue. In rojo congo red media, colonies formed scarlet red colonies. It grew well on the salts of organic acids. Based on these characters the bacterium was identified as *Azospirillum*.

4.5.3 Identification of *Trichoderma* sp.

Efficient *Trichoderma* (VKA) was identified based on the cultural and morphological characters (Plate 5). Growth was rapid (7-9 cm in 3 days) and are light green to dark green. Mycelium hyaline, smooth and septate. Conidiophores were regular and highly branched. Phialides consisted of whorls of 2-3, skittle shaped, they are bulged at the middle, narrower at the base and attenuated abruptly into sharp pointed neck. They were 10-12µm long and 3- 3.5µm width. Phialospores were single, accumulate at the tip of each phialide. They were sub globose or short ovoid, smooth, pale green, and it measured about 3- 4 µm. Based

Table. 12. *In vitro* evaluation of rhizobacteria against *P. meadii*

Sl.No.	Antagonists	Growth of pathogen (cm)		Per cent inhibition
		1 DAI	2 DAI	
1	Rhizobacterium (VKA)	2.5	3.7	17.8
2	Rhizobacterium (MVR)	2.5	3.4	24.4
3	*P.fluorescens* (Com)	2.4	3.6	20.0
4	Control	2.6	4.5	

Mean of three replications

DAI : Days after inoculation

VKA: Vellanikkara

MVR: (Mazhuvannur)

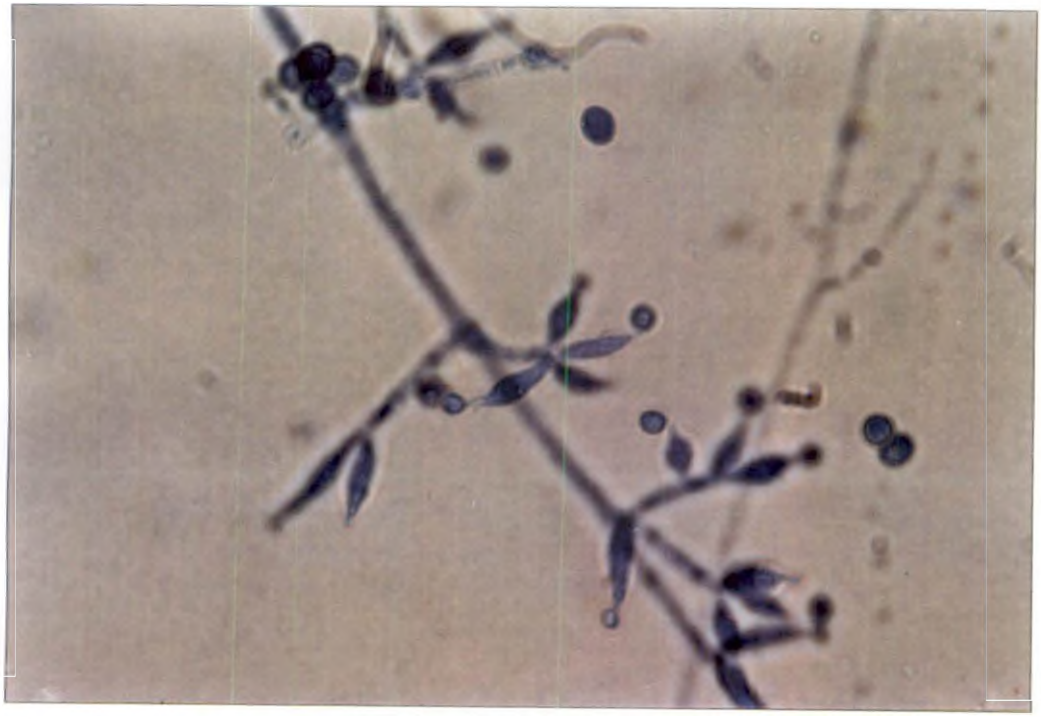


Plate 5: Photo micrograph of *T. harzianum*

on these characters, the isolate was identified as *Trichoderma harzianum* Rifai. (Samuel,J.G.,2004).It was also confirmed from Agharkar Research Institute,Pune.

4.5.4 **Identification of antagonistic rhizobacteria**

Based on the cultural, morphological and biochemical characters. Bacterium was gram negative and rod shaped. Colony appeared as smooth and grew well on both Nutrient Agar and King's B medium. Colony appeared as smooth at first and became rough as old. No pigmentation was observed on King's A and B media. It gave positive reaction for catalase test, 3%KOH test, starch hydrolysis and arginine hydrolysis. Levan production from sucrose was also observed. It gave negative result for denitrification test. Based on the above characters the rhizobacterium (MVR) was tentatively identified as *Pseudomonas* sp.

4.6 *IN VITRO* EVALUATION OF DIFFERENT FUNGICIDES AGAINST *Phytophthora meadii*

The data on *in vitro* evaluation of fungicides against *Phytophthora* are presented in Table 13. Out of the five fungicides tested, Bordeaux mixture, copper hydroxide, and copper oxychloride at all concentrations completely inhibited growth of the pathogen. The potassium phosphonate completely inhibited the growth at 0.3 and 0.4 per cent concentration while at 0.2 per cent, it was 90.37 per cent inhibition. Mancozeb gave lowest inhibition (27, 44 and 65.92 per cent at 0.2, 0.3 and 0.4 per cent concentration respectively).

4.7 COMPATIBILITY STUDIES

4.7.1 ***In vitro* compatibility of fungicides with *Trichoderma* spp.**

Five fungicides viz., Bordeaux mixture, copper oxychloride, copper

Table. 13. *In vitro* evaluation of different fungicides against *P. meadii*

Sl. No.	Fungicides	Concentration Per cent	2 DAI		6 DAI	
			Growth in diameter (mm)	Per cent inhibition	Growth in diameter (mm)	Per cent inhibition
1	Bordeaux Mixture	0.5	0 (0.71)	100	0 (0.71)	100
		1.0	0(0.71)	100	0(0.71)	100
		1.5	0 (0.71)	100	0 (0.71)	100
2	Copper oxychloride	0.1	0(0.71)	100	0 (0.71)	100
		0.2	0(0.71)	100	0(0.71)	100
		0.3	0 (0.71)	100	0 (0.71)	100
3	Copper hydroxide	0.1	0 (0.71)	100	0 (0.71)	100
		0.2	0(0.71)	100	0 (0.71)	100
		0.3	0 (0.71)	100	0(0.71)	100
4	Potassium Phosphonate	0.2	0(0.71)	100	8.67(3.02)	90.37
		0.3	0 (0.71)	100	0 (0.71)	100
		0.4	0(0.71)	100	0(0.71)	100
5	Mancozeb	0.1	14 (3.8)	42.46	65.67(8.3)	27.00
		0.2	2.33(1.38)	90.86	50.33(7.1)	44.00
		0.3	0 (0.71)	100	30.67(5.58)	65.92
6	Control		25 (5.05)		90(9.51)	

Mean of three replications

DAI : Days after inoculation

In each column figures followed by same letter do not differ significantly according to DMRT

Figures in parenthesis shows $\sqrt{x+0.5}$ transformed values

hydroxide, potassium phosphonate and Mancozeb each at three concentrations were evaluated to study the compatibility of these fungicides to the selected antagonist *T. harzianum* and *T. viride* (commercial) .The results are presented in Table 14.

Among different fungicides, Bordeaux mixture at all concentrations completely inhibited the growth of both isolates. While other three fungicides, (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. However, results showed that response of different fungicides varied significantly. In the case of *T. viride* (commercial), maximum inhibition was noticed with copper hydroxide at 0.3 per cent (87.4 per cent). At 0.2 per cent, it was 85.56 and 83.7 per cent at 0.1 per cent. Copper oxychloride showed 67.4 per cent inhibition at 0.3 per cent, 66.67 per cent at 0.2 per cent and 38.14 at 0.1 per cent concentration. Lowest inhibition was noticed in the case of mancozeb at 0.1 per cent (16.3 per cent), followed by 0.2 per cent (18.52 per cent).

The isolate *T. harzianum* also showed a same trend of response to different fungicides. Maximum inhibition was observed with copper hydroxide 0.3 and 0.2 per cent followed by 0.1 per cent (85.92 per cent and 81.86 per cent respectively). Lowest inhibition was noticed in the case of mancozeb at 0.1 per cent (16.3 per cent).

4.7.2 *In vitro* compatibility of fungicides with antagonistic rhizobacteria

The potassium phosphonate at all three concentrations were compatible with both the antagonistic bacteria (rhizobacterium (MVR) and *P.fluorescens* (commercial) (Table 15). In the case of rhizobacterium (MVR), Bordeaux mixture at 1.5 per cent gave maximum inhibition (4.0 mm) followed by Bordeaux mixture

Table. 14. *In vitro* compatibility of different fungicides with promising *Trichoderma* sp.

Treatment No.	Treatment details	Concentration	*Trichoderma harzianum*		*Trchoderma viride (Com)*	
			Growth in mm	Per cent inhibition	Growth in mm	Per cent inhibition
1	Bordeaux mixture	0.5	0.00 (0.71)^{c}	100	0.00 (0.71)^{e}	100
		1.0	0.00 (0.71)^{c}	100	0.00 (0.71)^{e}	100
		1.5	0.00 (0.71)^{c}	100	0.00 (0.71)^{e}	100
2	Copper oxychloride	0.1	22.67 (4.81)^{b}	74.81	55.67 (7.49)^{b}	38.14
		0.2	20.33 (4.53)^{b}	77.41	30.00 (5.52)^{c}	66.67
		0.3	18.67 (4.38)^{b}	79.26	29.33 (5.46)^{cd}	67.4
3	Copper hydroxide	0.1	16.33 (4.04)^{b}	81.86	14.67 (3.87)^{cd}	83.7
		0.2	12.67 (3.62)^{b}	85.92	13 (3.67)^{cd}	85.56
		0.3	12.67 (3.62)^{b}	85.92	11.33 (3.44)^{d}	87.4
4	Potassium Phosphonate	0.2	90.00 (9.51)^{a}	0.00	90.00 (9.51)^{a}	0.00
		0.3	90.00 (9.51)^{a}	0.00	90.00 (9.51)^{a}	0.00
		0.4	90.00 (9.51)^{a}	0.00	90.00 (9.51)^{a}	0.00
5	Mancozeb	0.1	75.33 (8.77)^{a}	16.3	75.33 (8.71)^{a}	16.3
		0.2	71.33 (8.48)^{a}	20.74	73.33 (8.59)^{a}	18.52
		0.3	67.00 (8.22)^{b}	25.55	60.00 (7.78)^{b}	33.33
6	Control		90.00 (9.51)^{a}		90.00 (9.51)^{a}	

Mean of three replications In each column figures followed by same letter do not differ significantly according to DMRT Figures in parenthesis shows $\sqrt{x+0.5}$ transformed values

Table. 15 *In vitro* compatibitlity of different fungicides with antagonistic rhizobacterium

Treatment No.	Treatment details	Concentration (per cent)	Inhibition Zone (mm)	
			Rhizobacterium (MVR)	*P.fluorescens* (Com)
1	Bordeaux mixture	0.5	3.0	1.67
		1.0	3.67	2.67
		1.5	4.0	3.67
2	Copper oxychloride	0.1	2.0	1.67
		0.2	3.0	2.67
		0.3	3.0	2.67
3	Copper hydroxide	0.1	2.33	1.0
		0.2	3.0	2.0
		0.3	3.0	2.33
4	Potassium Phosphonate	0.2	0.0	0.0
		0.3	0.0	0.0
		0.4	0.0	0.0
5	Mancozeb	0.1	1.0	1.0
		0.2	2.67	2.0
		0.3	3.0	3.0
6	Control		0	0

Mean of three replications

MVR: Mazhuvannur

at one per cent (3.67), where as lowest inhibition was noticed with mancozeb at 0.1 per cent (1.0 mm).

Effect of fungicides to *P.fluorescens* was also similar to that of rhizobacterium (MVR). Maximum inhibition was noticed with Bordeaux mixture 1.5 per cent (3.67 mm) followed by mancozeb 0.3 per cent (3.0 mm). An inhibition zone of 2.67 mm was noticed in the case of Bordeaux mixture 1.0 per cent and also with copper oxychloride 0.2 per cent and 0.3 per cent. The lowest inhibition was observed in copper hydroxide 0.1 per cent and mancozeb 0.1 per cent.

4.7.3 *In vitro* compatibility of *Trichoderma* isolates with antagonistic rhizobacteria and *Azospirillum*

Compatibility among efficient *T.harzianum*, efficient rhizobacterium and *Azospirillum* were tested along with standard cultures of *T.viride* and *P.fluorescens.* Results revealed that all the isolates were compatible with each other (Table 16). Growth of both *Trichoderma* isolates were slow when they were grown with *Azospirillum* (MVR). While along with rhizobacterium (MVR), growth of *T viride* was normal but growth of *T.harzianum* was slow.

4.7.4 *In vitro* compatibility among antagonistic rhizobacteria and *Azospirillum*

Antagonistic rhizobacterium and *Azospirillum* were tested along with *P. fluorescens* to test their compatibility. It was observed that *Azospirillum* and *antagonistic* rhizobacterium were compatible with *P. fluorescens* . While a small inhibition zone was observed in the case of rhizobacterium and *Azospirillum* (3.0 mm).

Table. 16 *In vitro* compatibility of *Trichoderma* spp. with antagonistic rhizobacteria and *Azospirillum*

Treatnment No.	Treatment details	*Trichoderma harzianum*				*Trichoderma viride* (Com)			
		Growth (mm)				Growth (mm)			
		1DAI	2DAI	3DAI	4DAI	1DAI	2DAI	3DAI	4DAI
1	*Azospirillum* (MVR)	19.5	37.5	50.0	90.0	18.5	35.0	47.0	90.0
2	Rhizobacterium (MVR)	22.0	37.5	47.5	90.0	21.0	38.0	90.0	90.0
3	*P.fluorescens* (Com)	19.0	43.0	90.0	90.0	21.0	43.0	90.0	90.0
4	Control	24.0	47.5	90.0	90.0	26.0	48.0	90.0	90.0

Mean of three replications

DAI : days after inoculation

MVR: Mazhuvannur

4.8 EVALUATION OF MOST EFFECTIVE MICROBIAL ISOLATES FOR GROWTH ENHANCEMENT AND *Phytophthora* ROT MANAGEMENT IN VANILLA NURSERIES

A nursery experiment was carried out to select the efficient microbial isolates to enhance the growth as well as to control the *Phytophthora* rot disease.

4.8.1 Effect of different treatments on sprouting of vanilla in nursery

Per cent sprouting was recorded at 30, 45 and 60 days after planting. At 30 days after planting, the treatments showed significant differences on sprouting of vanilla. While, after 45 and 60 days of planting, there was no significant difference (Table 17).

The treatment T_5 showed maximum sprouting per cent (83.33 per cent) followed by T_3 (75.0 per cent). The treatments T_1, T_2, T_4 gave 58.33 per cent sprouting and was on par with the treatment T_7 (50 per cent). Lowest sprouting per cent was observed with control. After 45 days of planting, T_2, T_3 and T_5 gave cent per cent sprouting, while the other six treatments showed 91.67 per cent sprouting. The T_6 showed the lowest sprouting percentage (83.3 per cent). After 60 days of planting all the treatments except T_1 and control, showed cent per cent sprouting. While the other two treatments, showed 91.67 per cent sprouting.

4.8.2 Effect of different treatments on the length of vine in vanilla cuttings

Length of vine of vanilla was recorded at fortnightly interval upto two months (Table 18). Although, the treatments did not vary significantly T_5 (*P. indica*) exerted highest length followed by T_2 (*Azospirillum* MVR). They gave a length of 21.55 cm and 18.43 cm respectively after two months. Control plants gave a length of 14.96 cm.

Table. 17. Effect of different treatments on sprouting of vanilla cuttings

Treatment No.	Treatment details	Per cent sprouting (DAP)		
		30	45	60
T_1	AMF (PBR)	58.33[abc]	91.67	91.67
T_2	*Azospirillum* (MVR)	58.33[abc]	100.00	100.00
T_3	*T.harzianum*	75.00[ab]	100.00	100.00
T_4	Rhizobacterium (MVR)	58.33[abc]	91.67	100.00
T_5	*P. indica* (Com)	83.33[a]	100.00	100.00
T_6	*T.viride* (Com)	41.67[bc]	83.33	100.00
T_7	*P.fluorescens* (Com)	50.00[abc]	91.67	100.00
T_8	Bordeaux mixture (1%)	33.00[c]	91.67	100.00
T_9	Potassium phosphonate (0.3%)	33.00[c]	91.67	100.00
T_{10}	Control	25.00[c]	91.67	91.67
			NS	NS

Mean of three replications

DAP : Days after planting

NS : Non significant

In each column figures followed by same letter do not differ significantly according to DMRT

PBR: Perumbavoor

MVR: Mazhuvannur

Table. 18. Effect of different treatments on the length of vine in vanilla cuttings

Treatment No.	Treatment details	Length of sprout (DAP)		
		30	45	60
T_1	AMF (PBR)	3.53	8.75	15.28
T_2	*Azospirillum* (MVR)	6.23	12.05	18.43
T_3	*T.harzianum*	3.68	8.78	15.33
T_4	Rhizobacterium (MVR)	3.93	9.54	17.12
T_5	*P. indica* (Com)	5.71	13.79	21.55
T_6	*T.viride* (Com)	2.68	7.38	14.13
T_7	*P.fluorescens* (Com)	3.70	9.08	16.84
T_8	Bordeaux mixture (1%)	3.73	9.85	17.03
T_9	Potassium phosphonate (0.3%)	3.28	8.11	14.96
T_{10}	Control	2.78	7.78	14.96
		NS	NS	NS

4.8.3 Effect of different treatments on number of leaves in vanilla cuttings

Number of leaves were not significantly influenced by the treatments (Table 19). However, T_1 (AMF PBR) recorded more number of leaves (4.68) followed by T_2 (*Azospirillum* MVR, 4.0). The control plants recorded 3.33 leaves.

4.8.4 Effect of different treatments on *Phytophthora* disease incidence in vanilla nursery

The treatments were evaluated for *Phytophthora* rot disease incidence by artificial inoculation of pathogen and also natural incidence of disease (Table 20)

In the case of artificially inoculated plants, treatments showed significant variation in disease incidence. T_9 (potassium phosphonate) showed best control against pathogen (33.33 per cent incidence). It was 66.67 per cent less than that of control. It was followed by T_8 (Bordeaux mixture-50 per cent incidence). T_1 and T_7 gave 66.67 per cent disease incidence, which is on par with T_3, T_4, T_5, and T_6 (83.33 per cent incidence). Cent per cent disease incidence was observed in the case of control and T_2.

The treatment T_2 gave better control of disease in naturally infected plants (33.33 per cent disease incidence). There were no significant differences among the treatments on natural infection of diseases. T_7 and T_9 gave 50 per cent control followed by T_1 and T_8 (33.33 per cent control). Control showed 83.33 per cent disease incidence.

4.8.5 Evaluation of *T.harzianum*, *T.viride* (commercial), *Pseudomonas* spp. and *P.fluorescens* (commercial) against *Phtophthora* rot in vanilla

Results on experiment with the antagonists against *Phytophthora* rot revealed that, antagonistic rhizobacteria and *P. fluorescens* were highly effective

Table. 19. Effect of different treatments on number of leaves in vanilla cuttings

Treatment No.	Treatment details	Number of Leaves (DAP)	
		45	60
T_1	AMF (PBR)	2.67	4.67
T_2	*Azospirillum* (MVR)	2.67	4.0
T_3	*T.harzianum*	1.67	3.67
T_4	Rhizobacterium (MVR)	1.67	3.33
T_5	*P. indica* (Com)	1.67	3.67
T_6	*T.viride* (Com)	1.67	3.33
T_7	*P.fluorescens* (Com)	1.67	3.67
T_8	Bordeaux mixture (1%)	1.67	3.33
T_9	Potassium phosphonate (0.3%)	2.0	3.33
T_{10}	Control	1.33	3.33
		NS	NS

Table. 20. Effect of different treatments on *Phytophthora* disease incidence in vanilla cuttings

Treatment No.	Treatment details	Disease incidence (%)	
		Artificial inoculation	Natural infection
T_1	AMF (PBR)	66.67[ab]	66.67
T_2	*Azospirillum* (MVR)	100.00[a]	33.33
T_3	*T.harzianum*	83.33[ab]	100.00
T_4	Rhizobacterium (MVR)	83.33[ab]	83.33
T_5	*P. indica* (Com)	83.33[ab]	83.33
T_6	*T.viride* (Com)	83.33[ab]	50.00
T_7	*P.fluorescens* (Com)	66.67[ab]	50.00
T_8	Bordeaux mixture (1%)	50.00[ab]	66.67
T_9	Potassium phosphonate (0.3%)	33.33[b]	50.00
T_{10}	Control	100.00[a]	83.33
			NS

Mean of three replications

NS: Non significant

In each column figures followed by same letter do not differ significantly according to DMRT

PBR: Perumbavoor

MVR: Mazhuvannur

against *Phytophthora*. No symptom was observed in the case of plants inoculated by these organisms. Eventhough, both *T. harzianum* and *T. viride* failed to control disease, they also exerted some control against pathogen. The symptom development in these plants were slow compared to control plants.

Discussion

5. DISCUSSION

Vanilla (*Vanilla planifolia* Andrews.), the "orchid of commerce" is cultivated for its pleasant flavour. The number of planting materials depends on the length of cuttings of plantlets, which in turn is related to the flowering. If shorter cuttings are used, the juvenile phase will be longer. Hence, the growth of vanilla plants in the nursery has to be enhanced for getting early flowering and to obtain more number of cuttings. More over, the vanilla is also facing several diseases, which are reducing the yield. Among several diseases, the *Phytophthora* rot is one of the most important fungal diseases affecting vanilla both in nurseries and plantations. It is most severe during southwest monsoon especially in shaded and poor drained soils. A very few studies have been conducted for efficient control of this disease. Now a days chemical means are adopted for efficient management of *Phytophthora* rot. But, continuous use of chemicals have destroyed the biological balance of nature. It adversely affects the environment and ecological processes. 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 isolates for growth enhancement and control of diseases assumes greater role than resorting to chemical means. The use of microbial inoculants, which are cheap, eco friendly and also maintains the biological balance are gaining lots of importance in sustainable agriculture. Hence, the present study was taken up to enhance the growth of vanilla and manage the *Phytophthora* rot disease of vanilla in nurseries using selected microbial isolates.

For the present study, different microbial isolates namely AMF, *Azospirillum*, *Trichoderma* and antagonistic rhizobacteria were isolated from Thrissur (Vellanikkara) and Ernakulam (Perumbavoor and Mazhuvannur) districts. The most predominant isolates were selected from each location. Orchid mycorrhiza was isolated from vanilla roots. The entire study was conducted as

two experiments. In the first experiment, different isolates of AMF and *Azospirillum* were screened to find out the most efficient isolate for enhancing the growth in nursery under sterile conditions. Different *Trichoderma* spp. and rhizobacteria isolates were screened under *in vitro* conditions to find out most effective isolate against *Phytophthora* by dual culture method. In the second experiment, the most efficient isolates of AMF, *Azospirillum*, *Trichoderma* and antagonistic rhizobacteria obtained were tested along with chemicals as well as with standard cultures of *Trichoderma viride*, *Pseudomonas fluorescens*, *Piriformospora indica* for growth enhancement and *Phytophthora* rot management in vanilla nurseries.

The pathogen causing *Phytophthora* rot in vanilla was isolated from infected plants of vanilla nurseries and pathogenicity of the isolate was established. The cultural and morphological characters of the pathogen were studied and identified the pathogen. The fungus produced uniformly dense cotton wool like aerial mycelium over entire colony. Hyphae were aseptate and 2.8 µm wide. Hyphae became septate when old. Sporangia were ovoid to ellipsoid highly caduceus and papillate with intermediate stalk length. Sporangia measured 35-40µm x19-35 µm with L/B ratio between 1.6 to 1.9. The characters studied were in conformity with those reported by Bhai and Thomas (2000) who reported that *Phytophthora* rot was caused by *P. meadii* and hence, the identity of the fungal culture obtained in the present study was identified as *Phytophthora meadii* Mc Rac.

Different isolates of AMF and *Azospirillum* were tested to screen the efficient isolate. Among different isolates of AMF and *Azospirillum*, AMF isolates gave early sprouting. Maximum sprouting per cent was noticed in the case of T_2 (91.60 per cent). In a similar study, Anandaraj and Sarma (1994) reported the enhanced rooting and root growth on inoculation with *Glomus fasciculatum* on cuttings of pepper. T_6 (91.67 per cent) gave maximum sprouting per cent among

Azospirillum treatments. Kumar *et al.* (1988) reported that inoculation of *Azospirillum* increased nut germination in cashew. It was also noted that it improved the growth characters such as seedling height and stem growth significantly.

Result on biometric characters like length of sprout, number of leaves and internodal length revealed that AMF and *Azospirillum* isolates did not show significant differences. However, T_2 gave highest length among AMF isolates and T_6 exerted highest length among A*zospirillum* isolates (Table 3., Fig1). They exerted 16 and 15.6 per cent more length respectively over control. In the case of internodal length, all the treatments except T_1 resulted more internodal length than control (Table 6., Fig 4). Though, the leaf number did not show significant differences, T_2 and T_6 isolates showed maximum leaf number (Table 4., Fig 2). Girth of vine varied significantly with different isolates. All the treatments gave more girth than control. AMF isolate T_2 (AMF Perumbavoor isolate) gave more girth and among *Azospirillum* isolates T_6 (*Azospirillum* Mazhuvannur isolate) gave more girth (Table 5., Fig3). These results are in accordance with the observations of Madhaiyan (1999) who observed increase in the shoot length and the growth of vanilla due to the inoculation of orchid mycorrhizal fungus, VA mycorrhizal fungus and along with *Azospirillum*. Thanuja (1999) also reported improved vegetative and root growth in black pepper inoculated with AMF.

Fresh weight of shoot, root and dry weight of shoot did not vary significantly (Table 7 Fig 5,6,7,8). T_2 registered highest value of shoot fresh weight (64.81 g) and among *Azosprillum* treatments, T_6 gave maximum fresh weight (61.38 g). Maximum root fresh weight was recorded in T_3 (4.57 g) while, T_6 performed well among *Azospirillum* isolates and recorded a weight of 3.98 g. The T_6 yielded maximum shoot dry weight (4.98 g) among *Azospirillum* isolates while T_2 (4.87 g) recorded maximum shoot dry weight among AMF isolates. While T_3 recorded maximum root dry weight among AMF isolates and T_4 gave maximum among *Azospirillum* isolates. Root length as well as root number were

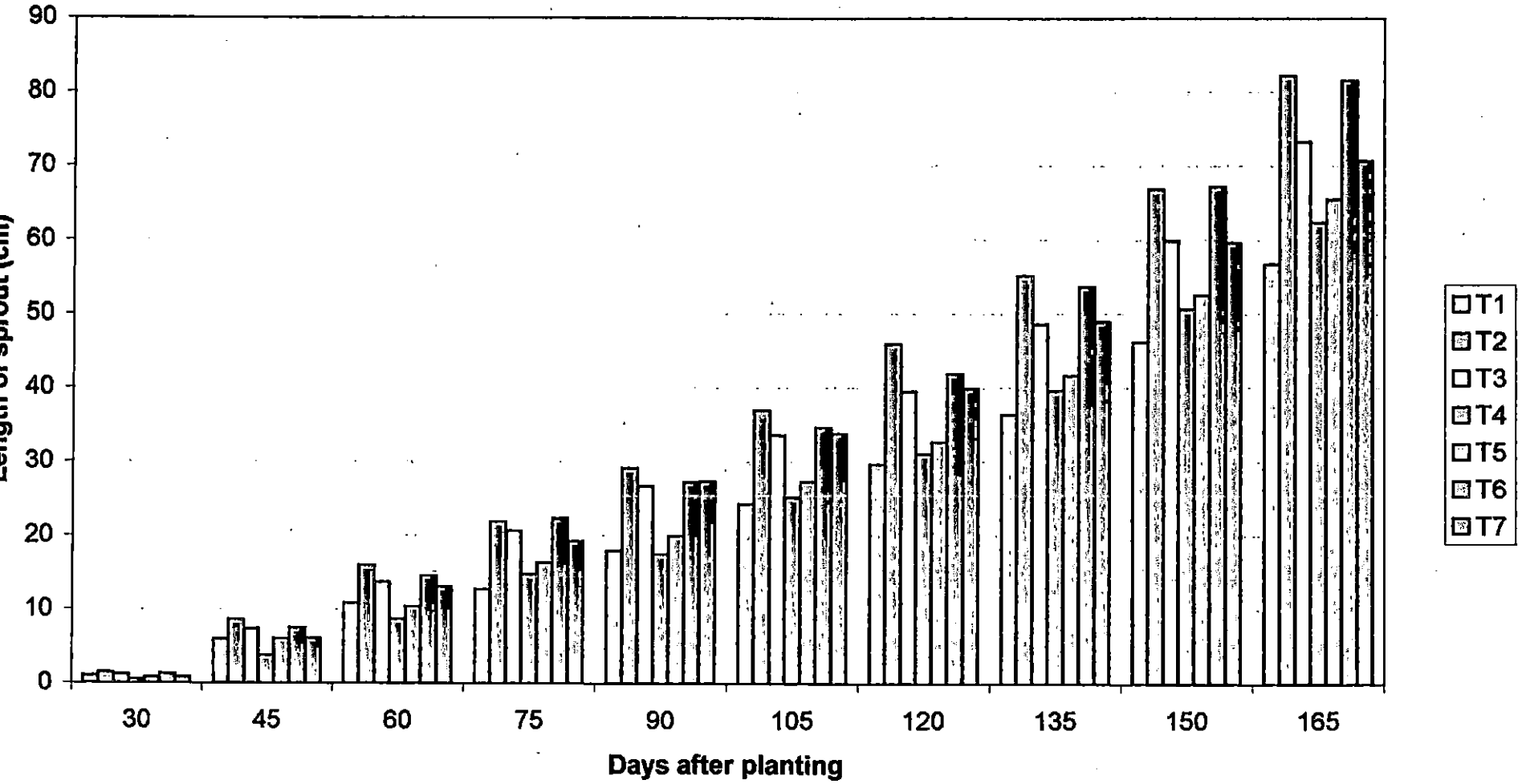


Fig 1. Effect of different treatments on length of sprout of vanilla

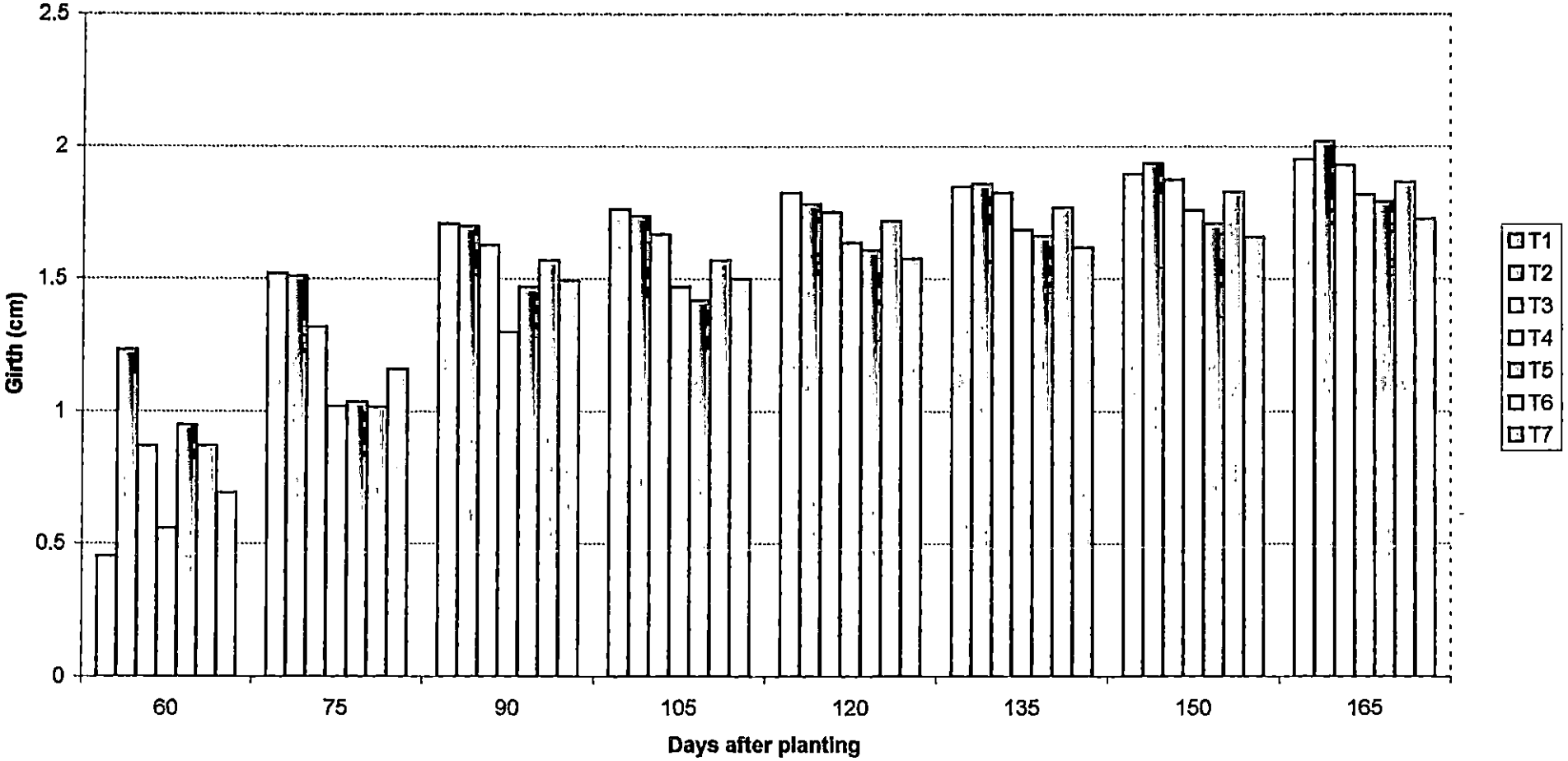


Fig 3. Effect of different treatments on girth of vanilla

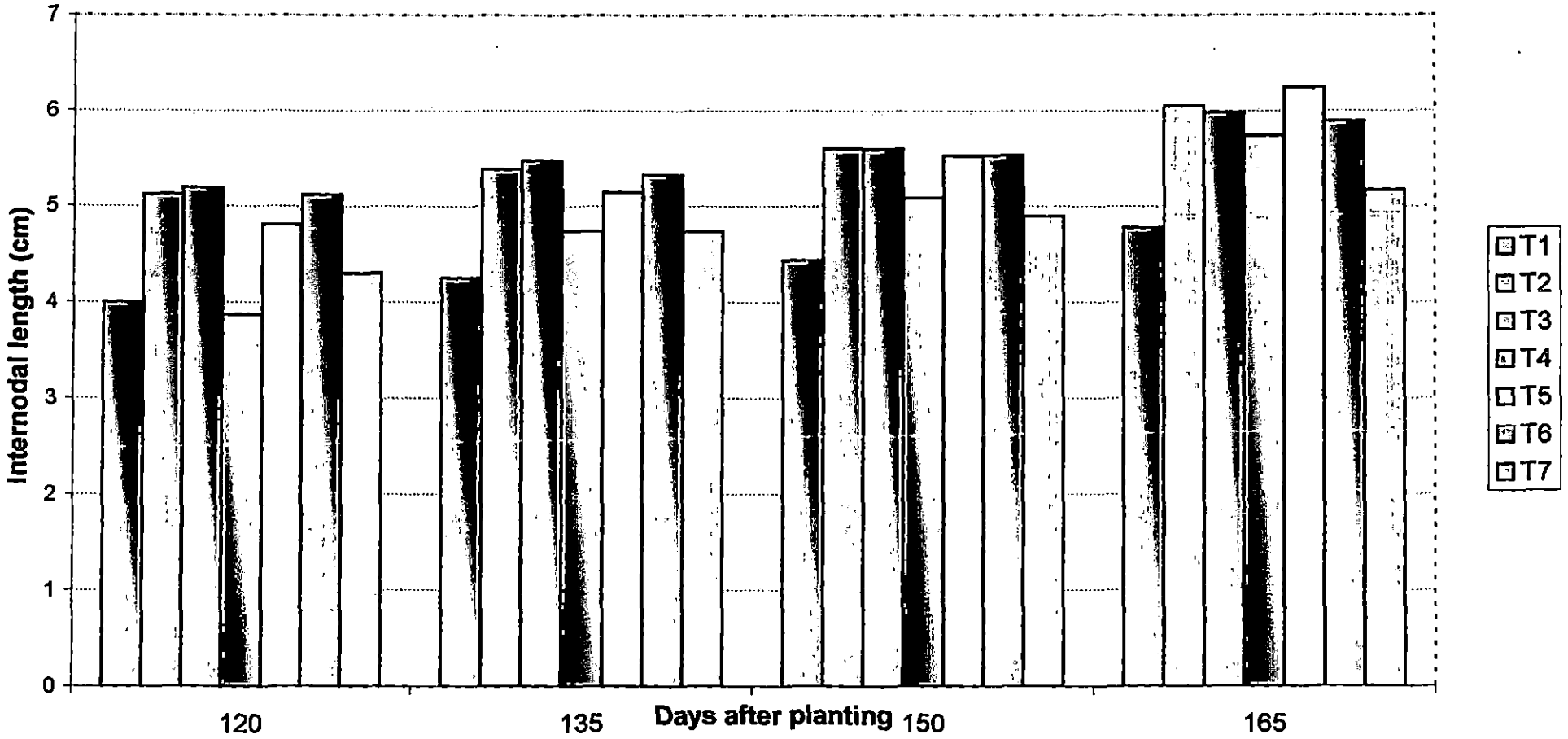


Fig 4: Effect of different treatments on internodal length of vanilla

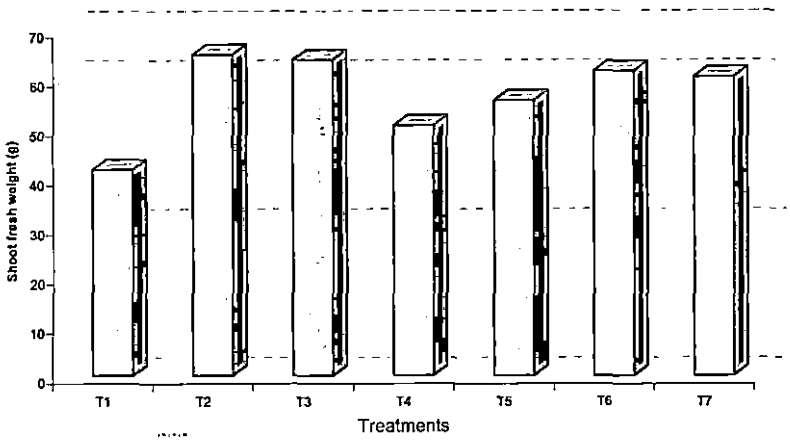


Fig 5. Effect of different treatments on shoot freshweight of vanilla

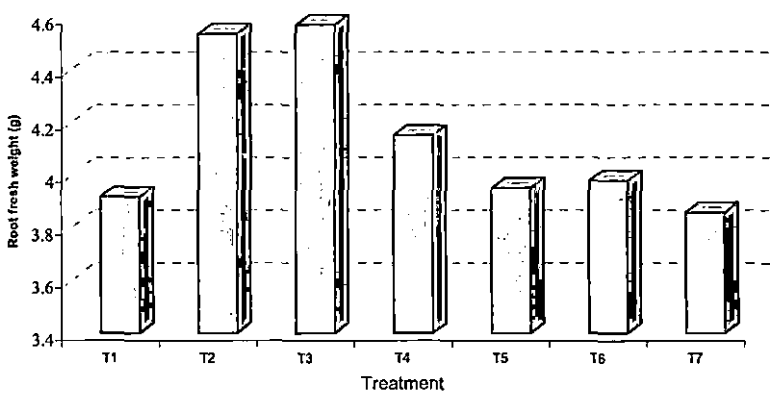


Fig 6. Effect of different treatments on root freshweight of vanilla

also more in the case of AMF and *Azospirillum* inoculated plants. Higher numbers of roots were recorded by T_2 (17.33) followed by T_3. T_6 gave maximum number of roots among *Azospirillum* isolates. Highest root length was observed with T_3 (25.33 cm). Among *Azospirillum* isolates, T_6 yielded highest length (22.17cm). (Table 8., Fig 9, 10). This was also in agreement with that reported by Madhaiyan (1999). He observed that, inoculation of AMF and *Azospirillum* increased fresh and dry weight, root length and root number of vanilla plants. Increased shoot dry weight and reduced mortality was observed in orchid plants inoculated with VAM (Wang and Gregg, 1994). *Azospirillum* produced phytohormones viz., indol acetic acid, gibberellic acid, kinetin etc. that help the host plant by way of enhancing biomass production (Tien *et. al.*, 1979).

Nutrient status of the plants were also tested in the experiment. Significant differences were observed in the case of nitrogen content. The T_2 (AMF Perumbavoor iaolate) gave maximum nitrogen content, which is 97.87 per cent more over control (Table 9., Fig 11). In the case of phosphorus content, AMF isolates yielded maximum. Potassium content was maximum in the case of T_1 and T_2. Among *Azospirillum* isolates, T_6 yielded maximum nitrogen and phosphorus content (0.83 per cent and 0.34per cent respectively) while, T_5 recorded maximum potassium content. 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 obtained by Madhaiyan (1999) where it was found that the mycorrhizal inoculated plants gave 20.56, 56.25, 77.22 per cent N, P, and K respectively over control. Gopal (2005) also reported increased nutrient content (N and P) in AMF inoculated vanilla plants. Increased nutrient content by the inoculation of AMF and *Azospirillum* in black pepper was reported by Kandiannan *et al.* (2000). Binisha (2003) also found that inoculation of *Azospirillum* along with inorganic fertilizer increased the nutrient content in *Dendrobium*. The present study also indicated the beneficial effects of AMF and *Azospirillum* spp. in improving the growth of vanilla.

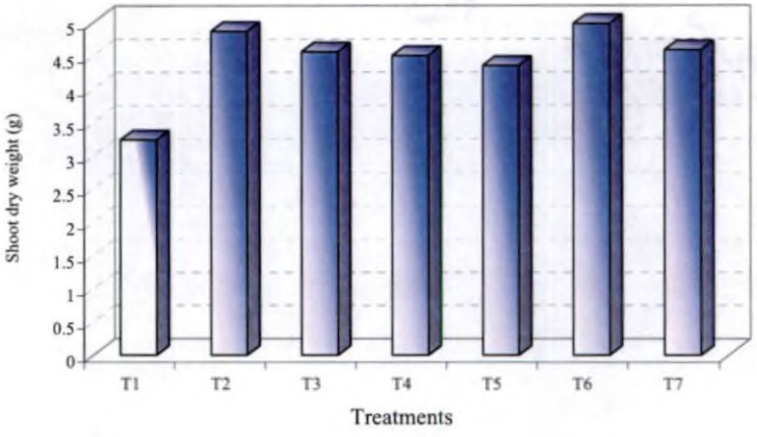


Fig 7. Effect of different treatments on shoot dryweight of vanilla

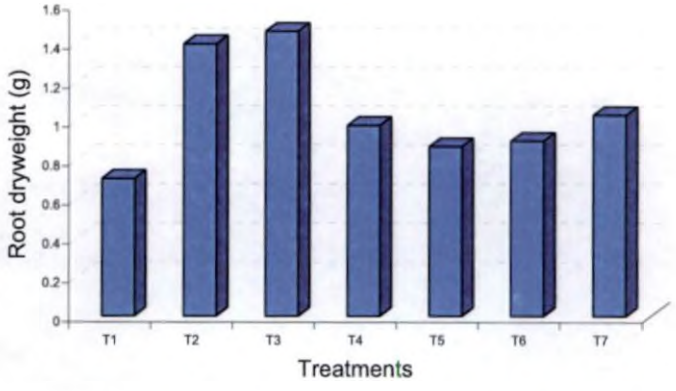


Fig 8. Effect of different treatments on root dryweight of vanilla

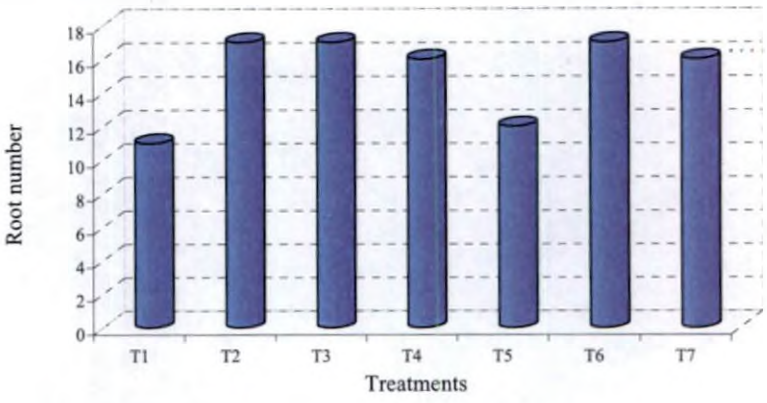


Fig 9. Effect of different treatments on number of roots of vanilla

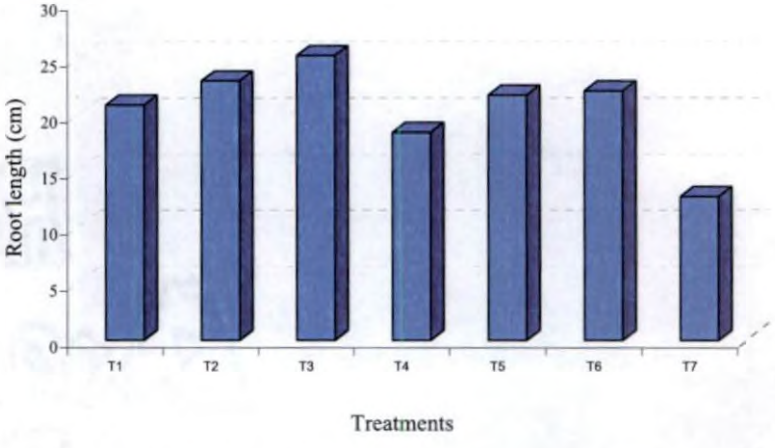


Fig10. Effect of different treatments on root length of vanilla

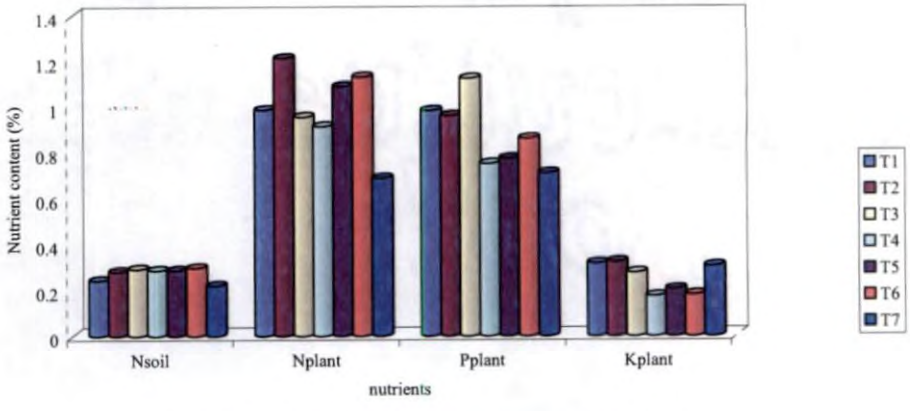


Fig 11. Effect of different treatments on nutrient content of vanilla

The improved growth characters in AMF and *Azospirillum* inoculated plants may be due to better nutrient uptake and production of growth hormones. It was found that 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). Growth promotion by *Azospirillum* isolates was not only due to nitrogen fixation but also by the production of IAA and other growth regulators in association with plants. This additive hypothesis was suggested by Bashan and Holguin (1997) to explain growth and yield promotion with *Azospirillum.* Although, most of the parameters did not show significant differences in the present study as the observations were taken only upto five and half months of planting. Microbial isolates may take more time for better colonization and for their better action. That may be the reason for less differences among treatments. Binisha (2003) found that during the initial four months of plant growth, not much influence of biofertilizers was noticed in *Dendrobium*. While, after six months biofertilizers were found to have significant influence on plant height, internodal length, leaf number etc. therefore, the benefits of AMF and *Azospirillum* on vanilla plants will be more pronounced after the initial period of plant growth. In the present study, T_2 (AMF Perumbavoor) and T_6 (*Azospirillum* Mazhuvannur) were selected as best AMF and *Azospirillum* isolates and were used for further studies.

In another study, predominant *Trichoderma* and rhizobacteria from each location were tested for their antagonistic activity against *P. meadii* by dual culture method (Skidmore and Dickinson, 1976., Utkhade and Rahe, 1983) and the most efficient *Trichoderma* and rhizobacteria were selected.. The efficiency of these isolates was also compared with standard cultures of *T. viride* and *P. fluorescens* also.

All the *Trichoderma* isolates showed antagonistic effect against *Phytophthora meadii* (Table 11). For the selection of efficient antagonists, the method suggested by Kasinathan (1998) was employed. For this, antagonism

index was worked out. The isolate *Trichoderma* (Vellanikkara isolate) showed maximum antagonism index (1500). It was same as that of commercial *T. viride*. Potential role of *Trichoderma* as biocontrol agent has been established by many workers (Harman, 2000; Rajan *et* al., 2002; Vijayaraghavan, 2003). The effectiveness of *T. harzianum* against *P. meadii* was also reported by Spices Board (1999). Vijayaraghavan (2003) found that *T. harzianum* exerted cent per cent inhibition on the growth of *P. capsici*. Hence, *Trichoderma* has a great potential to be used for biocontrol of *Phytophthora* spp. as indicated by the present study. Rhizobacteria also showed varying level of inhibition against *P. meadii* (Table 12). Rhizobacteria (Mazhuvannur isolate) registered maximum per cent of inhibition. Recently, rhizobacteria are gaining more importance in disease management due to its additional benefits in improving plant growth and yield. They act against pathogens by different mechanisms such as competition, antibiosis, parasitism and lysis and induced systemic resistance. Similar results were reported by Anandaraj and Sarma (2003) where, it was observed that *P. fluorescens* inhibited different species of *Phytophthora*. Sarma *et al*., (2003) also observed that IISR 147 and IISR 148 (*Pseudomonas* sp.) isolates were suppressive to *P. meadii* in vanilla. Bhavani (2004) also studied the effectiveness of *P. fluorescens* against *Phytophthora* causing black pod rot in cocoa. Based on the antagonistic activity of *Trichoderma* spp. and rhizobacteria in the present study, it was found that *Trichoderma* sp. (Vellanikkara) and rhizobacterium (Mazhuvannur) were most effective isolates in inhibiting the growth of *P. meadii*.

Most efficient isolates of AMF, *Azospirillum, Trichoderma* and antagonistic rhizobacteria obtained under screening trials were identified. The promising isolate of AMF (Perumbavoor) was identified as *Glomus* spp. *Trichoderma* (Vellanikkara) was identified as *Trichoderma harzianum* by studying cultural and morphological characters. Efficient antagonistic rhizobacterium (Mazhuvannur isolate) was also identified based on the cultural,

morphological and biochemical characters and tentatively identified as *Pseudomonas* spp.

Now a days chemical means are adopted for efficient management of *Phytophthora* rot. So, an *in vitro* evaluation was conducted to study the sensitivity of *P. meadii* against different fungicides (Table 13). Different concentrations of Bordeaux mixture, copper oxychloride, copper hydroxide, potassium phosphonate and mancozeb were used for this study. Results showed that Bordeaux mixture, copper oxychloride and copper hydroxide at all concentrations tested and potassium phosphonate at higher concentrations completely inhibited the growth of pathogen. Mancozeb showed less inhibition against *P. meadii*. At higher concentration (0.3 per cent), it recorded 65.92 per cent inhibition. In a similar study, Vijayaraghavan (2003) and Bhavani (2004) also observed that Bordeaux mixture, copper oxychloride, copper hydroxide at all concentrations and potassium phosphonate at higher concentrations completely inhibited the growth of *P. capsici* and *P. palmivora*. Similar findings were reported by many workers against *P. capsici* (Turner, 1969., Filani, 1976., Mammootty, 1978., Ramachandran and Sarma, 1985., KAU, 2000) which were in accordance with the present study.

In integrated disease management, for getting better production, chemicals are used with biocontrol agents. So, the fungicides used must be compatible with the biocontrol agents for the effective management. So, in the present study most effective *Trichoderma* spp. and antagonistic rhizobacteria were tested to evaluate compatibility with commonly used fungicides in vanilla cultivation as mentioned earlier. Results on compatibility of *Trichoderma* spp.to different fungicides revealed that fungicides showed varying level of inhibition (Table 14). Bordeaux mixture at all concentrations tested completely inhibited the growth of *T. harzianum* as well as commercial culture of *T. viride*. This finding is in agreement with the findings of many workers (Shanmugham, 1996., Vijayaraghavan, 2003., Bhavani,2004., Priya,2005) where they observed that Bordeaux mixture at all

concentrations tested completely inhibited the growth of *T. harzianum* and *T. viride*. Higher per. cent of inhibition was noticed with copper hydroxide and copper oxychloride at all the three concentrations tested (85.92 and 79.26 respectively). Commercial culture of *T.viride* was more sensitive to copper hydroxide while, *T. harzianum* was more sensitive to copper oxychloride. However, it was observed that potassium phosphonate at all concentrations were compatible with *T. harzianum* and *T. viride*. No inhibition was observed. This finding was in agreement with the report of Rajan and Sarma (1997). They reported compatibility of eight species of *Trichoderma* with potassium phosphonate even at higher concentration. Mancozeb was also found to be compatible with both *Trichoderma* spp. as it exerted only less inhibition. Compatibility of mancozeb and potassium phosphonate with *Trichoderma* spp.were reported by many workers (Moeity *et al.*, 1982., Wongwathanarat and Sivasithamparam, 1991., Shanmugham, 1996., Rajan and Sarma, 1997., May and Kimat, 2000., Akbari and Parakhia, 2001., Vijayaraghavan, 2003., Bhavani,2004). So the selected *T. harzianum* obtained in the present studies can be safely used along with mancozeb and potassium phosphonate but not with copper fungicides.

Results on study of compatibility of antagonistic rhizobacteria (Mazhuvannur) with different fungicides revealed that all the fungicides except potassium phosphonate exerted varying level of inhibition on growth of bacteria (Table 15). The selected *Pseudomonas* sp.was more sensitive to fungicides compared to commercial culture. Bordeaux mixture exerted maximum inhibition. Almost same level of inhibition was observed with other three fungicides. These findings were in agreement with the results of Bhavani (2004). He reported that potassium phosphonate was compatible with *P.fluorescens*. Priya (2005) observed that Bordeaux mixture, Kocide and Fytolan were inhibitory to the *P.fluorescens*. In the present study, antagonistic rhizobacteria was found to be compatible with potassium phosphonate.

Biocontrol consortium approach for disease management in plantation and spice crops was suggested earlier (Sarma and Anandaraj, 1998). It is better to use several antagonists rather than one because of variation in the soil condition and nature of root exudates during crop growth stages. So a greater thrust should be given for development of biological consortia with multiple modes of action. Hence, in the present study, compatibility of selected *Trichoderma* spp. with antagonistic rhizobacterium and *Azospirillum* (Table 16) were studied along with commercial *T.viride* and *P. fluorescens*. Results showed that *T.harzianum and T. viride (commercial)* were compatible with both selected antagonistic rhizobacterium and *P. fluorescens* (commercial). Compatibility of *Trichoderma* spp. and rhizobacteria were reported by Sarma *et al.*, (2000) and Jisha *et al.* (2002). They found that *P. fluorescens* and *T. harzianum* were compatible and can be used as efficient consortium for the management of foot rot of black pepper caused by *P. capsici*. It was also observed that both *Trichoderma* spp. were compatible with efficient *Azospirillum* tested. Compatibility of *T.viride* with *Azospirillum* under *in vitro* has been proved by Sankar and Jayarajan (1996). The present study was in agreement with above findings.

Another investigation was done to find out compatibility among antagonistic rhizobacterium, *Azospirillum* and *P. fluorescens* (commercial). It was observed that commercial culture of *P. fluorescens* was compatible with selected *Pseudomonas* sp. and *Azospirillum*. Compatibility of antagonistic rhizobacteria with *Azospirillum* (Sankar and Jayarajan, 1996) and also compatibility among different rhizobacteria (Anandaraj and Sarma, 2003) were reported earlier. However, in the present study selected *Azospirillum* and *Pseudomonas* sp. were found to be incompatible. It may be due to the competition among these isolates, Kamble *et al.* (2000) found that *Azospirillum* and *P.fluorescens* were incompatible when they were tested together in paddy field condition, which was in agreement with the present findings.

An experiment was conducted to find out most efficient microbial isolates for growth enhancement and *Phytophthora* rot management in vanilla nurseries. Most promising isolates of AMF, *Azospirillum*, *Trichoderma* and antagonistic rhizobacteria were used for this study along with commercial cultures of *P. fluorescens*, *T. viride*, *Piriformospora indica*, and potassium phosphonate and Bordeaux mixture as recommended plant protection chemicals.

Result on per cent sprouting revealed that all the treatments resulted in early sprouting of vanilla cuttings (Table 17). *Piriformospora indica* gave highest per cent of sprouting followed by *T. harzianum*. All other isolates exerted higher per cent of sprouting over control. Length and leaves vines were recorded and T_5 (*P.indica*) excerted maximum height followed by T_2 (*Azospirillum* MVR). Number of leaves were higher in the case of T_1 (AMF PBR) followed bty T_2 (*Azospirillum* MVR). Verma *et al.* (1998) and Varma *et al* (1999) reported that inoculation of *P. indica* and application of its filtrates promote plant growth and biomass production They also observed that it mediated uptake of phosphorus from the medium and its translocation to the host in an energy dependent process and increased its content in shoot. Improved growth characteristics by the inoculation of *Azospirillum* was explained by many workers (Kumar *et al.*, 1988., Madhaiyan,1999., Binisha, 2003)

Different isolates were tested along with fungicides for their effectiveness against *Phytophthora* rot (Table 20). Potassium phosphonate gave maximum control of the disease followed by Bordeaux mixture. Among the native microbial isolates, T_2 (AMF Perumbavoor isolate) and *P.fluorescens* (commercial) also gave some extent of control over *Phytophthora* rot. Effectiveness of Bordeaux mixture and potassium phosphonate against *P. meadii* was reported by Bhai and Thomas (2000) and Thomas *et al.*, (2003). Efficiency of microbial isolates on *Phytophthora meadii* was less, which may be due to their less colonization. They may require more time for better colonization and for their better action. More over, inoculum density of applied *Phytophthora meadii* was more than that of

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microbial isolates applied. The heavy rains at the time of experiment might have favoured the disease development. Due to the above reasons, an experiment was conducted to study the effectiveness of microbial inoculants against *Phytophthora* rot. For this native antagonistic rhizobacteria and *Trichoderma* spp.along with commercial cultures of *P.fluorescens* and *T. viride* were used. Same quantity of antagonists and pathogen were applied. Results revealed that, rhizobacteria and *P.fluorescens* tested were very effective against *Phytophthora* rot. They gave control upto four months. While *Trichoderma* spp. were not much effective. They also exerted some extend of control over rot than control plants.

In the present study it was observed that, use of microbial inoculants exerted better growth characteristics. Even though, the antagonists were found to be very effective under *in vitro* condition, they failed to perform in field condition. It may be due to the less colonization of microbial isolates on roots and competition among microorganisms. Sometimes more inoculation density should be there for better colonization. So more investigation should be done using microbial isolates using different inoculum density and using better delivery methods inorder to get a better influence on the growth of plant and disease management. Moreover, the microbial isolates have to be tested under field conditions so as to find a suitable isolate for growth enhancement and *Phytophthora* rot management.

Summary

6. SUMMARY

The present study on growth enhancement and management of *Phytophthora* rot in vanilla nurseries using microbial inoculants was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2002-2004. The experiment included isolation of different microbial inoculants like AMF, *Azospirillum*, *Trichoderma* and rhizobacteria from vanilla rhizosphere, isolation and characterization of pathogen causing *Phytophthora* rot in vanilla nurseries, screening of AMF and *Azospirillum* for growth enhancement in vanilla nurseries and screening of *Trichoderma* and rhizobacteria against *Phytophthora*. In the second experiment, the most efficient isolates of AMF, *Azospirillum*, *Trichoderma* and antagonistic rhizobacteria were tested along with chemicals as well as with commercial cultures of *Trichoderma viride*, *Pseudomonas fluorescens*, *Piriformospora indica* for growth enhancement and *Phytophthora* rot management in vanilla nurseries

The rhizosphere soil of vanilla along with roots were collected from Vellanikkara (Thrissur district) and from two locations namely Perumbavoor and Mazhuvannur (Ernakulam district) and was used for the isolation of microflora. Most predominant isolates were selected from each location and used for further studies. The pathogen causing *Phytophthora* rot disease of vanilla was isolated from naturally infected plants from Perumbavoor (Ernakulam district) and the pathogenicity was established. Cultural and morphological characters of the pathogen were studied and was identified as *Phytophthora meadii* Mc Rac.

Different AMF and *Azospirillum* isolates 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. Maximum sprouting per cent was noticed in the case of AMF (Perumbavoor). *Azospirillum* (Mazhuvannur) gave maximum sprouting per cent among *Azospirillum* treatments.

Though, in most of the cases the treatments did not show significant differences, AMF and *Azospirillum* inoculated plants showed better growth characteristics over control. Among the AMF, T_2 (AMF Perumbavoor) was the best isolate while among *Azospirillum* isolates, T_6 (*Azospirillum* Mazhuvannur) performed well and these isolates were selected for next experiment. *Trichoderma* and rhizobacteria were screened for their antagonistic activity against *P.meadii*. It was done by dual culture method. *Trichoderma* isolate Vellanikkara recorded maximum antagonism index of 1500 and selected for further studies. Among rhizobacteria, mazhuvannur isolate showed maximum antagonistic activity against the pathogen.

Cultural and morphological characters of the efficient isolates of AMF,*Azospirillum*, *Trichoderma* and antagonistic rhizobacteria were studied and were identified.

An *in vitro* evaluation was conducted to study the effect of different fungicides against *P. meadii*. Bordeaux mixture, copper hydroxide and copper oxychloride at all concentrations tested and potassium phosphonate at higher concentrations completely inhibited the growth of the pathogen while, mancozeb exerted less inhibition.

Compatibility of selected antagonists with different fungicides as well as compatibility among the microbial isolates were tested. Bordeaux mixture at all concentrations tested completely inhibited the growth of *Trichoderma*. The other copper fungicides also exerted higher per cent of inhibition while, potassium phosphonate was found to be compatible at all concentrations tested. In the case of antagonistic rhizobacteria, fungicides except potassium phosphonate exerted varying level of inhibition on growth of bacteria. Results on compatibility between *Trichoderma* and antagonistic bacteria showed that *Trichoderma* was compatible with antagonistic bacteria as well as *Azospirillum*.

In the second experiment, selected isolates of AMF,*Azospirillum*, *Trichoderma* and antagonistic rhizobacteria were evaluated for their efficiency in growth enhancement and *Phytophthora* rot management along with commercial cultures of *T. viride*, *P.fluorescens*, *P. indica* and chemicals. Per cent sprouting of cuttings was recorded. *Piriformospora indica* gave higher per cent of germination followed by *T. harzianum*. All other isolates exerted higher per cent of sprouting over control. The disesase incidence were recorded both with artificial and natural condition. Potassium phosphonate gave maximum control of the disease followed by Bordeaux mixture. Among the microbial isolates, T_1 (AMF Perumbavoor) and *P.fluorescens* (commercial) also gave some extend of control over *Phytophthora*. The treatment T_2 (*Azospirillum* Mazhuvannur) gave better control of disease in naturally infected plants (33.33 per cent disease incidence).

Even though, the microbial isolates failed to perform well in field conditions, they were found to be efficient under *in vitro* condition for growth enhancement and disease management.

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* Originals not seen

Appendix

APPENDIX

MEDIA COMPOSITION

(Ingredients per litre)

1. OAT MEAL AGAR

Rolled oats : 75 g

(600ml water)

Agar : 20 g

(400 ml water)

2. CARROT AGAR

Carrot : 200.0 g

Agar : 20.0 g

3. CZEPEK-DOX MINERAL MEDIUM

Glucose : 0.5 g

Yeast extract : 0.1 g

Sodium nitrate : 3.0 g

Magnesium sulphate : 0.5 g

Potassium chloride : 0.5 g

Ferrous sulphide : 0.01 g

Di- potassium hydrogen phosphate: 1.0 g

Distilled water : 1000 ml

PH : 6.0-6.5

Agar : 12 g

4. NITROGEN FREE BROMOTHYMOL BLUE

Malic acid : 5.0g

KOH : 4.0g

K_2HPO_4 : 0.5 g

$FeSO_4\ 7H_2O$: 0.5g

$MnSO_4\ H_2O$: 0.01g

$MgSO_4\ 7H_2O$: 0.10g

NaCl : 0.02g

$CaCl_2$: 0.01g

Na_2MoO_4 : 0.002g

Agar : 1.75g

Distilled water : 1000 ml

Bromothymol blue : 2ml

(0.5 per cent alcoholic solution)

pH 6.6-7

5. 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 mg (added aseptically to the sterilized medium)

6.POTATO DEXTROSE AGAR

Potato	: 200.0 g
Dextrose	: 20.0 g
Agar	: 20.0 g

7. 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

8. 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

GROWTH ENHANCEMENT AND MANAGEMENT OF *Phytophthora*-ROT IN VANILLA NURSERIES USING MICROBIAL INOCULANTS

By

SHAHIDA K.

ABSTRACT OF THE THESIS

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COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

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ABSTRACT

A study on "Growth enhancement and management of *Phytophthora* rot in vanilla nurseries using microbial inoculants" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara. The salient findings are abstracted below:

The study revealed that *Phytophthora meadii* Mc. Rac.was the pathogen causing *Phytophthora* rot in vanilla nurseries. Even though, the treatments did not influence growth characters significantly, AMF (Perumbavoor) and *Azospirillum* (Mazhuvannur) were the most effective AMF and *Azospirillum* isolates for enhancement of growth and nutrient content in vanilla. *In vitro* screening of antagonists against *P. meadii* revealed that *Trichoderma* (Vellanikkara) and rhizobacteria (Mazhuvannur) were very effective against the pathogen. The effective microbial isolates obtained were identified.

Among the fungicides tested, Bordeaux mixture, copper hydroxide and copper oxychloride (at all concentrations) and potassium phosphonate (at higher concentrations) completely inhibited the growth of the pathogen. Compatibility studies revealed that potassium phosphonate was compatible with antagonists, while copper fungicides were not compatible with antagonists. Results on compatibility between *Trichoderma* and antagonistic bacteria showed that *Trichoderma* was compatible with antagonistic rhizobacteria as well as *Azospirillum*.

The experiment on growth enhancement and *Phytophthora* rot management in vanilla revealed that all the microbial isolates gave early sprouting. *Azospirillum* (MVR) gave maximum length. AMF (PBR) and *P. fluorescens* (commercial) were found to be effective against *Phytophthora* rot in artificially inoculated vanilla cuttings. Among chemicals tested, potassium phosphonate gave best control against *Phytophthora* rot.