# BIOCONTROL OF BACTERIAL WILT IN TOMATO USING ARBUSCULAR MYCORRHIZAL FUNGI

By

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**THESIS**

**Submitted in partial fulfilment of the requirement for the degree of**

**Master of Science in Agriculture**

**Faculty of Agriculture**

**Kerala Agricultural University**

**Department of Plant Pathology**

**COLLEGE OF HORTICULTURE**

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

**2003**

## DECLARATION

I hereby declare that the thesis entitled **"Biocontrol of bacterial wilt in tomato using arbuscular mycorrhizal fungi"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellanikkara
16/5/03

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CERTIFICATE

Certified that the thesis entitled **"Biocontrol of bacterial wilt in tomato using arbuscular mycorrhizal fungi"** is a record of research work done independently by Mr. Nandakumar A. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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*Dedicated to my Achan and Amma*

## ACKNOWLEDGEMENT

*I bow my head before the God Almighty, who enabled me to complete this venture successfully.*

*I express my profound sense of gratitude to my guide* **Dr. K. Surendra Gopal**, *Assistant Professor (Microbiology), Department of Plant Pathology for his able guidance, valuable comments, ever willing help and constructive criticism rendered throughout the period of investigation and in the preparation of this manuscript. I am deeply obliged to him for his constant encouragement, understanding and whole hearted co-operation received during the period under his guidance.*

*I thankfully acknowledge* **Dr. Koshy Abraham**, *Associate Professor and Head, Department of Plant Pathology and member of advisory committee for his valuable suggestions and support and critical analysis of the thesis.*

*I am grateful to* **Dr. D. Girija** *Assistant Professor (Plant Pathology), Centre for Plant Biotechnology and Molecular Biology and member of advisory committee for her guidance, warm and friendly nature and critical scrutiny of the manuscript of the thesis.*

*I accord my sincere thanks to* **Dr. S. Nirmala Devi**, *Associate Professor, AICVIP, Department of Olericulture and member of my advisory committee for her whole hearted co-operation, help and valuable suggestions rendered during various stages of study and preparation of the manuscript.*

*My profound sense of gratitude is due to* **Dr. Sally K. Mathew**, *Associate Professor, Department of Plant Pathology for her ever-willing help extended to me throughout the period of investigation and also in the critical evaluation of the manuscript.*

*I am deeply obliged to* **Dr. M.V. Rajendran Pillai**, *Associate Professor, Department of Plant Pathology for his help in the preparation of photographs.*

*I express my deep sense of gratitude to all the staff members of the Department of Plant Pathology for their help and encouragement during the entire course of study.*

*I am grateful to* ***Dr. K. C. Marykutty,*** *Associate Professor and Head, Department of Soil Science and Agrl.chemistry for allowing me to do the soil nutrient analysis in the soil science lab.*

*My special thanks to* ***Shajuettan, Vargheese, Prince*** *and* ***Binichechi, Shantachechi*** *and* ***Nabeezachechi*** *for their valuable help and co-operation rendered to me during the conduct of research work.*

*I am deeply indebted to my friends* ***Rajesh, Shinoj, Biju, Sanal, Anup, Sajnanath, Rajiv, Johnkutty, Sineesh, Sajeesh, Rajan, Suresh*** *and* ***Allan*** *for their sincere support, motivation and relentless help during the difficult times faced by me during the period of study.*

*My profound sense of gratitude to* ***Usha, Reshmy, Smitha, Sujatha, Minisankar*** *and* ***Manimala*** *for offering all possible help during the thesis work.*

*I express my heartfelt thanks to my friends* ***Amjath*** *and* ***Byju*** *(GKVK campus, Bangalore),* ***Harish*** *(IARI) and* ***Riaz*** *(TNAU, Coimbatore) for their help in getting the references and materials required for my study.*

*I am in dearth of words to express my deep sense of gratitude to my friend* ***Pratheesh*** *for his invaluable help and support without which the thesis would not have acquired the present form.*

*A special word of thanks to* ***Santhosh*** *and* ***Jeo*** *for their help in computer work.*

*The award of KAU Junior Research Fellowship is thankfully acknowledged.*

*Lastly but not the least, I owe my profound sense of gratitude to my dear father, mother, gopan and padmaja and all my near and dear ones for their boundless affection, constant prayers, moral support and unfailing inspiration all along my study. With respect and affection I dedicate my thesis to my loving parents.*

*Nandakumar A.*

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# INTRODUCTION

# 1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is the world's most important vegetable crop because of its wide spread production. It is also known as a protective food due to its special nutritive value. It is a rich source of minerals, vitamin A and C, organic acids, essential aminoacids and dietary fibres. The lycopene pigment in tomato is a powerful anti-oxidant and also helps in preventing the incidence of prostrate cancer.

At present, India ranks sixth in the world production of tomato with an area of 0.5 m.ha. and production of 8.4 m. t. in 1999-2000 (Indian Horticulture Database, 1999). The area under tomato in Kerala is very less due to various constraints and large-scale cultivation is mainly confined to the Palakkad district. Moreover, the tomato growing areas are facing a threat from several diseases such as bacterial wilt, damping-off, fusarium-wilt, early blight, late blight, mosaic, leaf -curl etc. which result in yield losses.

The bacterial wilt caused by *Ralstonia solanacearum* is one of the most important disease and cause extensive losses in tomatoes in Asia/South Pacific region (Persley, 1986). The pathogen is soil-borne and is known for its endemic persistence in the soil. The disease occurs in diverse soil types of acidic and alkaline nature. It affects solanaceous vegetable crops in most states of India including Kerala, which is having a tropical humid climate conducive for the incidence of bacterial wilt. The destructiveness of the disease is due to wide host range for the causal organism. The loss in yield may vary between 10.8 and 100 per cent depending on the varieties and stage at which infection occurs along with the environmental conditions (Kishun, 1987).

The control of bacterial wilt disease (*R. solanacearum*) with chemicals is not effective due to the variation in pathogen. Moreover, indiscriminate use of chemicals may result in development of resistant strains of the pathogen as well as affect the human health due to direct consumption of vegetables. In this context, biological control of plant diseases is an alternative due to its eco-friendly nature, cost-effectiveness and long lasting effects.

Arbuscular Mycorrhizal Fungi are ubiquitous in nature and are found in symbiotic association with majority of crop species. The role of AMF in relation to P uptake, and improving plant growth has been studied extensively, but its role as a biocontrol agent in suppression of soil-borne pathogens is not extensively studied. There are reports of AMF association with tomato reducing bacterial wilt caused by *Pseudomonas solanacearum* as early as 1979 (Halos and Zorilla, 1979). In India, many workers reported the beneficial effects of AMF as a biocontrol agent in inhibiting the growth of soil borne pathogens. Most of the work related to AMF as biocontrol agents have been carried out under pot culture conditions using commercial cultures. There are also reports that pre-inoculation of mycorrhiza before pathogen infection protected the plant from the disease. However, work related to the screening of native cultures of AMF and their role as biocontrol agents are very less in Kerala.

Hence, the present study on "Biocontrol of bacterial wilt in tomato using arbuscular mycorrhizal fungi" was undertaken with the following objectives.

- To identify promising AM fungi from tomato and maize rhizospheres of high and low wilt incidence
- To screen AMF cultures against *Ralstonia solanacearum*
- To determine the optimum inoculation time and inoculum density of AMF against bacterial wilt
- To test the efficacy of promising AMF cultures under field condition

# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE

*Pseudomonas solanacearum*, the bacterial wilt pathogen of solanaceous crops was first reported by Burril (1890) in potatoes as an unidentified bacterial pathogen from United States. The first definite description of *P. solanacearum* causing bacterial wilt in potato, tomato and eggplant was published by Smith (1896). *P. solanacearum* (Smith.) is the causal organism of bacterial wilt of solanaceous and also about 200 other plant species and is a widely prevalent soil-borne pathogen (Kelman, 1953). Review of the new records of bacterial wilt since 1956s showed that more than 20 additional families of plants are hosts of *P. solanacearum* (Hayward, 1994). The bacterium has been renamed as *Ralstonia solanacearum* (Yabuuchi *et al.*, 1995) based on sequencing of 16SrRNA which led to the proposal of genus *Ralstonia*.

The occurrence of bacterial wilt of tomato in India was first reported by Hedayathullah and Saha (1941) from West Bengal. Dass and Chattopadhyay (1955) studied in detail about bacterial wilt of brinjal and estimated an average reduction in yield of 54.6 to 62.3 per cent due to this disease.

The viability of the pathogen in the soil under laboratory conditions was 16 months (Dass and Chattopadhyay, 1955) and in some cases less than 1 year (Rangaswami and Thirunavikarasu, 1964). *R. solanacearum* (Smith) Yabuuchi *et al.* invades the roots of diverse plant hosts from the soil and aggressively colonizes the xylem vessels causing a lethal wilting known as bacterial wilt disease (Tans-Kersten *et al.*, 2001).

In Kerala, studies on the various aspects of bacterial wilt of solanaceous crops were carried out by many research workers. One of the major areas of study on bacterial wilt in solanaceous crops was the screening and selection of germplasm for varietal resistance to bacterial wilt in solanaceous vegetable crops (Rahim, 1972; Celine, 1981; Peter *et al.*, 1984; Rajan, 1985; Gopalakrishnan and Peter, 1991; Markose, 1996; Singh, 1996; Paul, 1998). The studies carried out by George (1973); Devi (1978); Nayar (1982) and Jyothi (1992)

pertained to the etiology, survival and control of bacterial wilt pathogen of solanaceous vegetable crops.

Vegetable growers are suffering from heavy losses every year due to this disease and cultivation of solanaceous vegetables is getting restricted. The disease affects solanaceous vegetable crops in most states of India and loss in yield due to the disease in tomato has been reported to be as high as 90 per cent (Rao, 1976; Kishun, 1986).

## 2.1 BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM*

The bacterial wilt caused by *R. solanacearum* has become a serious problem in the cultivation of crop plants, especially solanaceous vegetables. The pathogen has a wide host range and race differentiation, which makes it even more difficult to identify the pathogen and control the disease. Under this section the identification of the causal organism of bacterial wilt in various crops especially solanaceous crops with the host specific race and biovar causing the wilt disease has been reviewed.

Gabr and Saleh (1998) isolated the bacterial pathogen from brown-rot infected potato tubers causing wilt of potato plants and compared for their pathogenicity in different hosts. Based on the results of physiological and biochemical tests, it was identified as race 2 of *Burkholderia* (*Pseudomonas*) *solanacearum* [*R. solanacearum*] Biovar III.

Gupta *et al.* (1998) reported the occurrence of bacterial wilt in tomato in Himachal Pradesh and the causal organism was identified as *P. solanacearum* [*R. solanacearum*] and its pathogenicity was confirmed. Abdalla *et al.* (1999) isolated *R. solanacearum* from wilted tomato plants and revealed that the isolated bacterium belonged to race 1 and biovar 4 of *R. solanacearum*. In a similar study, Garcia *et al.*, (1999) studied the bacterial disease of tomato (c.v. RioGrande) in different localities in Venezuela and reported that all the isolates were *R. solanacearum* belonging to biovar 2A.

Der and Hsien (1999) demonstrated that bacterial wilt in some, but not all ecotypes of *Arabidopsis thaliana*, were caused by strains of *R. solanacearum*. Khan *et al.*, (1999) identified the causal organism of bacterial wilt of tobacco as *R. solanacearum* by fatty acid profiles and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Physiological and biochemical test also confirmed this conclusion. Sharma and Rana (1999) observed that in 1994-95, bacterial wilt caused by *R. solanacearum* was present in 80 per cent of 310 *Zingiber officinale* fields surveyed in Himachal Pradesh. Huang *et al.*, (2000) observed the occurrence of bacterial wilt of jute in some fields in Central Taiwan. Based on cultural characteristics, physiological and biochemical properties, Biolog system and pathogenicity test, the causal organism was identified as *R. solanacearum*. The isolate obtained in this study was race 1 and biovar 4. The pathogen could induce wilting not only on jute but also on other solanaceous plants. Sumithra *et al.*, (2000) conducted a field survey in Karnataka during March-July, 1994 and observed that bacterial wilt of eggplant was widespread in the state. They isolated the pathogen and its pathogenicity test was carried out. The pathogen was identified as *R. solanacearum*.

Dookun *et al.*, (2001) determined the genetic diversity among *R. solanacearum* strains isolated from potato, tomato, beans and anthurium using RFLP technique. The bacterium belonging to race 1 biovar III has been reported in Mauritius on several crops and plant species. James (2001) observed biovar differentiation of the *Ralstonia* isolates of tomato, brinjal and chilli from 3 locations *viz.*, Vellanikkara, Kumarakom and Ambalavayal in Kerala. She identified the tomato isolate from Vellanikkara and chilli isolates from Kumarakom and Ambalavayal as belonging to biovar III A and the brinjal and chilli isolates of Vellanikkara, brinjal and tomato isolates of both Kumarakom and Ambalavayal as belonging to biovar III. She also reported that all the Vellanikkara and Kumarakom isolates belonged to race 1, whereas the Ambalavayal isolates belonged to race 3.

Osiru *et al.*, (2001) reported that bacterial wilt was a serious devastating disease limiting tomato production in Uganda and Worldwide. The causal organism was identified as *R. solanacearum*. The above mentioned reviews clearly points out that the pathogen *R.*

*solanacearum* has wide host range and it is the race 1 of the pathogen which cause wilting in tomato.

## 2.2 FACTORS INFLUENCING *RALSTONIA* POPULATION IN THE SOIL

The *Ralstonia* population is influenced by various environmental and soil factors. The environmental factors like rainfall, air temperature and season determines the pathogen population. The soil factors exert a much greater influence on the pathogen population. The type of soil, soil temperature, soil moisture, soil nutrients etc. are some of the soil factors influencing the pathogen population. The effect of these factors is reviewed under this section.

### 2.2.1. Environmental factors

Bora *et al.*, (1996) conducted a field experiment to investigate the effect of environmental factors on the incidence of bacterial wilt caused by *R. solanacearum* of tomatoes. They observed that bacterial wilt incidence was significantly correlated with soil temperature, air temperature and total rainfall. Small variations in an individual environmental parameter caused variations in wilt incidence and Relative Humidity had no correlation with wilt incidence. They were of the opinion that soil temperatures from 25-30 °C accompanied by a maximum air temperature of 26-30 °C and monthly rainfall ranges from 200-300mm favoured bacterial growth and multiplication resulting in severe wilt incidence.

Sunaina and Gupta (1998) studied the relationship between seasons and severity of bacterial wilt of potato under field conditions in the Kumaon hills of Uttar Pradesh. They observed that in summer the wilt incidence was high, presumably because high temperature and rainfall during these months promote bacterial wilt development, contrasting with the autumn season when no wilted plants were observed. The soil population of *Ralstonia* and

wilt incidence were maximum in July, the hottest and wettest summer month with mean minimum and maximum air temperature ranging between 20.5 and 28°C.

Hazarika and Das (1999) reported that wilt incidence was significantly correlated with mean temperature, rainfall and relative humidity during the crop growth period. The influence of environmental factors on the *Ralstonia* population has not been studied in detail. The available information in this aspect clearly denotes the role played by rainfall, season and air temperature on the *Ralstonia* population in the soil.

**2.2.2 Soil factors**

The soil factors are known to exert a much greater influence on the pathogen population in the soil. The influence of soil factors like soil type, soil temperature, soil moisture, soil pH and soil nutrient status on the pathogen population has been reviewed in this section. Keshwal (1976) noticed survival of *R. solanacearum* for six months at 35°C with 20-40 per cent moisture and for 24 months at 25°C with 60 per cent moisture under natural conditions. He also reported that *R. solanacearum* survived at 30cm depth for 6 months in cultivated fallow land and at a depth of 9cm in weed fallow land.

Shekhawat *et al.*, (1978) reported that *P. solanacearum* survived in the hills, deccan and central plateau and eastern plains in a variety of soil types having strongly acidic to alkaline reaction. Shekhawat *et al.*, (1979) noticed that *P. solanacearum* persisted in the soil in viable and virulent form at least for 2 years even in the absence of its host both in the hills and plateau. They also observed that the bacterium perpetuated, in the hills and in the plateau, in sandy-loam, medium black, red and yellow and brown hill soils and under soil pH ranging between 4.5 to 7.5.

Elsas *et al.*, (2000) studied the survival of a selected *R. solanacearum* biovar 2 isolate, strain 1609 in a loamy sand and silt loam soils to assess the effect of temperature and soil moisture content. At 12 or 15 and 20°C, a gradual decline of the population densities

was observed in both soils, from the established $10^5$ to $10^6$ cfu $g^{-1}$ of dry soil to significantly reduced levels, occasionally bordering the limit of detection ($10^2$ cfu $g^{-1}$ of dry soil) in period of approximately 90 to 210 days. Soil type affected the rate of population decline at 20°C with the greatest decline occurring in loamy sand soil. Moderate soil moisture fluctuations of approximately pF 2 did not affect the survival of *R. solanacearum* 1609 in soil. However, severe drought drastically reduced the population of strain 1609 in both the soils.

Keshwal *et al.*, (2000) revealed that all differences in pathogen population and disease incidence of wilt in different soils were statistically significant which indicated that the soil did affect the pathogen and the disease incidence significantly. They also observed that Rewa soil which was clay loam in texture exhibited highest degree of disease incidence (71.5 per cent) and the lowest disease incidence (25 per cent) was found in Balaghat soil which was silty clay. The silty clay soil also had the lowest pathogen population, whereas highest population of pathogen was observed in clay soil. High sand content and low silt or clay content of soils was found to be unfavourable for this pathogen and wilt incidence. Similarly, Keshwal *et al.*, (2000) studied the effect of water holding capacity (WHC) and field capacity (FC) in different soils of Madhya Pradesh on the pathogen population and disease index and observed that sandy-loam soil having 27.3 per cent FC and 34.6 per cent WHC harboured minimum number of *R. solanacearum* inciting 32.2 per cent wilt in tomato crop. The effect of WHC and FC of soil on the pathogen was more evident in clay soil. The clay soil having 66 per cent WHC and 36.2 per cent FC harboured more pathogen causing more disease (63 per cent) as compared to clay soil having lower FC and WHC.

Yamazaki (2001) investigated the relation between the development of bacterial wilt induced by *R. solanacearum* and calcium nutrition in tomato (*Lycopersicon esculentum* L.). Increased Ca concentrations in the nutrient solution reduced the disease severity in the seedlings of resistant cultivars, and decreased the population of the pathogen in stem. The resistance was affected by the Ca concentrations after infection with the pathogen, but not before infection, suggesting that the Ca concentration of the cell walls before infection

might not be directly involved in the Ca-dependent resistance. These results indicated that the resistance of tomato to bacterial wilt was markedly affected by Ca nutrition of the host.

The review work relating to influence of soil factors on pathogen population clearly indicated the role played by soil type, soil moisture, soil temperature and soil nutrition. Sandy soils in general recorded low *Ralstonia* population whereas clay soils increased the pathogen population. Among the soil nutrients, calcium was found to enhance the resistance of tomato to bacterial wilt.

## 2.3 DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF)

The AM fungi are ubiquitous in nature and are found in symbiotic association with the roots of higher plants. The AM fungi are influenced by various factors including host, environment and soil. So, there exists extreme diversity of AMF with changes in host, environment and soil factors. The influence of these factors on the AMF diversity is reviewed in this section. Lekha *et al.*, (1995) observed that *Glomus fasciculatum* was the most predominant arbuscular mycorrhizal fungus in organically rich forest soils of Kerala. The presence of *Sclerocystis coremioides* and *S. clavispora* has also been recorded. Similarly, *Glomus microcarpum* was the most predominant AMF spore found among the different AMF species identified in both south and north Kerala (Potty, 1990; Harikumar and Potty, 1999).

Mehrotra (1995) studied the mycorrhizal status of plants grown in an over burden soil at open cast coal mine sites and observed the predominance of several species of Glomus including *G. ambissporum, G. marquratum, Acaulospora scorbiculata* and *Scutellospora calospora*. Trimurtulu and Johri (1998) reported that *Glomus* was the most predominant genus and contributed more that 50 per cent of spore population in different tarai soils of Uttar Pradesh. They also observed that in general *Gigaspora* spp. were next to *Glomus* spp. in distribution with an exception in loam soils where *Acaulospora* spp. were next to *Glomus* spp.

Harikumar and Potty (1999) reported the presence of AMF isolates belonging to the species of *Acaulospora, Gigaspora, Glomus, Scleorcystis* and *Scutellospora* from the sweet potato soils of Kerala. They also reported that the distribution pattern of AMF in soils of southern and northern Kerala varied considerably with southern Kerala soils harbouring 14 AMF species belonging to *Acaulospora, Gigaspora, Glomus, Sclerocystis and Scutellospora*, whereas soils of northern Kerala harboured 11 species belonging to these genera, except *Scutellospora.*

*Glomus* sp. was observed as the predominant AMF species from different cowpea growing areas of Thrissur district (Beena, 1999). Beena *et al.*, (2000) examined the diversity of arbuscular mycorrrhizal (AM) fungi associated with *Ipomoea pes-caprae* of the Moderately disturbed dunes (MDD) and Severely disturbed dune (SDD) of West Coast of India. The survey revealed the occurrence of 31 species in MDD and only 7 species in SDD. Members of family Glomaceae were predominant followed by Acaulosporaceae and Gigasporaceae. Pooled data indicated that *Glomus mosseae* was most dominant, followed by *G.dimorphicum, Gigaspora gigantea, Acualospora taiwania, Glomus fasciculatum and Glomus* sp. 27ss. *Glomus mosseae, G. dimorphicum* and *Gigaspora gigantea* were most common in both MDD and SDD. Raji (2002) reported *Glomus* sp. to be the predominant AMF spore type from different tomato growing areas of Kerala.

The review work carried out by research workers are in confirmation with the ubiquitous nature and diversity of AMF with respect to crop, location, environment and soil. It is also seen that *Glomus* spp. are the most predominant among AMF and are able to survive under diverse agroclimatic conditions.

## 2.4 BIOCONTROL OF BACTERIAL DISEASES USING AM FUNGI

Biocontrol potential of VAM fungi, which is a ubiquitous symbiont in the roots of higher plants in cultivated soil has been realized (Gerdemann, 1968). Biological protection

of the plants due to VAM association was first reported by Saffir (1968). Wilhelm (1973) pointed out that the interactions between VAM and plant pathogens in the rhizosphere have important implications in biological control and like most instances of biological control, mycorrhizae can never confer complete immunity against root diseases, but impart a degree of resistance or tolerance against soil borne plant pathogens. The role of AMF in the management of soil-borne diseases was well established (Schenck and Kellam, 1978; Schenck, 1981; Dehne, 1982; Bagyaraj, 1984; Rosendahl, 1985; Smith, 1988; Jalali and Chand, 1987; Azcon-Aguilar and Barea, 1997 and Kumar *et al.*, 2000). However, there are only few reports of biological suppression of bacterial diseases by VAM fungi.

Reduction in severity of bacterial wilt of tomato caused by *P. solanacearum* was reported by Halos and Zorilla (1979). They observed that prior inoculation of tomato roots with *Glomus mosseae* reduced the bacterial wilt incidence in both baked and non-baked soil to 9 per cent and 59.1 per cent respectively followed by dual inoculation (AMF + *P. solanacearum*), where the wilt percentage for baked and non-baked soil was 37.79 per cent and 56.32 per cent compared to 90 per cent wilt incidence in *P. solanacearum* inoculated plants.

Garcia-Garrido and Ocampo (1988) reported that tomato plants with AM fungi reduced rhizosphere population of *Erwinia caratovora*. Garcia-Garrido and Ocampo (1989) also studied the effect of inoculation of *Glomus mosseae* on *Pseudomonas syringae* in tomato. They reported that dual inoculation of *G. mosseae* and *P. syringae* reduced the population of the bacterium in the rhizosphere region.

Kobayashi (1991) observed that inoculation of VAM fungi along with application of charcoal compost was effective in reducing the level of bacterial wilt of tomato caused by *P. solanacearum* under green house conditions. Suresh and Rai (1991) noticed that mycorrhizal root extract significantly reduced the population of *P. solanacearum*. The population in control was 154.74 x $10^3$ cells $ml^{-1}$, while in treated it was 86.25 x $10^3$ cells $ml^{-1}$. Nasr

(1993) reported that mycorrhizal extracts from tomato roots infected with *Glomus fasciculatum* reduced populations of *P. solanacearum* in nutrient broth.

Sharma *et al.*, (1995) observed reduction in bacterial blight of mulberry caused by *Pseudomonas syringae* pv. *mori* due to inoculation of *G. mosseae* and *G. fasciculatum*. Sood *et al.*, (1997) evaluated the effect of five isolates of VAM fungi for the control of bacterial wilt of tomato c.v. Roma. *G. mosseae* was found to be highly effective in promoting germination, seedling vigour and completely controlling the disease till the termination of potted experiment 48 days after challenge inoculation with *R. solanacearum* (race I biovar III), followed by *G. fasciculatum*, in which disease appeared after 35 days of challenge inoculation. In a similar study, Akbar (2002) studied the effect of VAM application on the bacterial wilt incidence caused by *R. solanacearum* in the tomato varieties Pusa Ruby and Sakthi. He reported that the per cent wilt incidence of Pusa Ruby at 60 DAP was 44.44 per cent for control and for *G. fasciculatum* inoculated plants it was 25.93 per cent. However, for Sakthi variety the control plants recorded 33.3 per cent wilt incidence and the mycorrhizal treated plants recorded 7.4 per cent wilt incidence.

Raji (2002) reported a reduction in bacterial wilt incidence due to VAM inoculation. The bacterial wilt incidence recorded by the mycorrhizal plants of variety Sakthi was 17.72 per cent as against 44.25 per cent in uninoculated control. The VAM inoculated plants of variety BWR-1 recorded a five per cent reduction in wilt incidence compared to control. Kumar and Sood (2002) observed that enrichment of soil with VAM and application of bacterial antagonists *viz.*, *Pseudomonas fluorescens* and *Bacillus cereus* reduced the wilt incidence siginificantly as compared to control in tomato cvs. EC 191536 (moderately resistant) and Solan Gola (susceptible). The synergistic effect in the reduction of wilt incidence was observed, when *G. mosseae* was combined with antagonists.

## 2.5 INFLUENCE OF OPTIMUM TIME FOR AM FUNGI INOCULATION ON DISEASE SUPPRESSION

The plant pathogens and nematodes multiply rapidly and gain access into the root system of plants even before mycorrhiza are able to establish symbiotic association with root system of higher plants. The importance of optimum time for AMF inoculation is thus important. The importance of pre-inoculation of crop plants with mycorrhiza before the attack by the pathogen is reviewed in this section. Hussey and Roncadori (1982) observed that the sequence in which plants are inoculated with a pathogen relative to the time of VAM fungal inoculation might affect the nature of the interaction. Hussey and Roncadori (1982) reported that pre-colonization with VAM fungi is a realistic system for containerized or transplanted hosts that can be inoculated with VAM fungi before they are planted into field soil.

Linderman (1985) stated that root infection by fungal and nematode pathogens preceded mycorrhizal root colonization. He reported that the entry of these pathogens could be prevented if mycorrhiza were allowed to precolonize the root system before they are attacked by the pathogen. The inoculation method to pre-colonize the plants with the VAM fungus 2-4 weeks before pathogen inoculation was found to be the best time of VAM inoculation. This technique allowed VAM fungi time to colonize roots before they were challenged with the pathogen (Davis and Menge, 1979; Thompson *et al.*, 1983; MacGuidwin *et al.*, 1985; Cooper and Grandison, 1986; Grandison and Cooper, 1986; Smith *et al.*, 1986).

Iyer and Sundararaju (1993) investigated the interaction of AMF with *Pythium aphanidermatum* and *Meloidogyne incognita* and found that the AMF *Glomus multicaule* and *G. fasciculatum* significantly enhanced growth of ginger and reduced the disease incidence. They suggested that prior inoculation with AMF was effective in ameliorating the deleterious effect of the pathogen. Ansuya (1995) observed that pre-inoculation of AMF and

dual inoculation (AMF + *Fusarium*) enhanced plant survival and reduced wilt incidence of Chick pea. Sivaprasad (1995) studied the azhukkal disease (*Phytophthora meadii*) development in Cardamom seedlings pre-inoculated with different mycorrhizal fungi. On transplanting to the field of heavy pathogenic infection, complete disease suppression was recorded in seedlings inoculated with *G. mosseae* even after one year. Baby and Rao (1996) observed that prior inoculation of VAM fungi gave the best result for the control of Sheath Blight incidence of rice followed by simultaneous application. Inoculation of AM fungi after the disease establishment failed to protect the crop. The AMF inoculated black pepper cuttings when planted in a diseased field recorded significant reduction of disease incidence in mycorrhizal plants (16.5 per cent) as compared to control (28.5 per cent) (DARE, 1996).

Azcon-Aguilar and Barea (1997) reviewed the work of arbuscular mycorrhizal symbiont induced plant defense mechanisms in achieving disease suppression in various host plants. He suggested that primary access of mycorrhizal fungi to host root system resulted in utilization of carbon compounds necessary for the growth of the pathogen and thus provided protection against the invading pathogens. Vigo *et al.*, (2000) investigated the impact of colonization by arbuscular mycorrhizal fungus *G. mosseae* on tomato root necrosis caused by soil borne pathogen *Phytophthora parasitica*. Seven and Sixteen days after inoculation with zoospores of the pathogen, roots of plants colonized by VAM had 39 per cent and 30 per cent fewer infection loci respectively than those that were not inoculated. At harvest, 26 days after inoculation with the pathogen, 61 per cent of roots of non-colonized plants were necrotic compared with only 31 per cent in VAM colonized plants.

The importance of pre-inoculation of mycorrhiza in disease suppression has been very well emphasized by the research work carried out by many research workers. In addition, the pre-inoculation of mycorrhiza resulted in the development of mycorrhizal seedlings which could be transplanted to the main field. This is particularly suited for transplanted crops like tomato where we can develop mycorrhizal seedlings which has the potential to restrict the disease development when transplanted to the main field.

## 2.6 INFLUENCE OF AM FUNGAL INOCULUM DENSITY

Researchers seeking to increase plant growth stimulation or inoculum production have carefully studied the influence of inoculum density on root colonization and subsequent sporulation. Baylis (1969) reported that greater inoculum dosages would result in no further increase in root colonization once an upper limit of root colonization was reached. Daft and Nicolson (1968) demonstrated lower initial colonization levels in tomatoes inoculated with low inoculum numbers than in plants which received large inoculum dosage. Regardless of inoculum level, similar colonization levels were achieved by the end of the experiment. However, in annual crops with short growing seasons high inoculum dosages may be required if maximum plant growth stimulation is to be achieved. The studies conducted by many researchers (Johnson, 1977; Carling *et al.*, 1979 and Ferguson, 1981) have also shown that increased inoculum dosage resulted in increased percentage root colonization.

Ortas (1996) inoculated Sorghum cv. $SSV_2$ and leek plants with four mycorrhizal fungi and recorded maximum spore population with *G. mosseae* in sorghum and with *G. etunicatum* in leek plants. He observed maximum dry weight, phosphorus uptake and per cent VAM infection with inoculum rate of 18g $pot^{-1}$ in the soil with low phosphorus.

Lovato *et al.* (1999) reported that theoretically, one good propagule is sufficient to assure arbuscular mycorrhizal infection, but in this case the process of colonization may be too long to be of agronomic interest. In practical terms, however a good application rate is about 1-2 kg of bulk soil inoculum $m^{-2}$ of seedbed (which means approximately 5000 to 10000 propagules). In the case of higher seedling densities the inoculum density can be lowered as the plantlets have a greater probability of encountering a propagule. Akbar (2002) studied the percent wilt incidence for the tomato varieties Pusa Ruby and Sakthi at 20DAP, 40DAP and 60DAP under pot culture conditions. He inoculated *Glomus fasciculatum* @ 50g $35cm^{-1}$ pots. The AM infection 60 DAP for Pusa Ruby was 12.42 per

cent and 11.11 per cent for Sakthi. The wilt incidence 60 DAP was 25.93 per cent and 7.4 per cent for Pusa Ruby and Sakthi respectively, when compared to 44.44 per cent and 33.33 per cent in control for the two varieties.

Raji (2002) investigated the pre-inoculation effect of Eruthimapthi isolate (*Glomus* sp.) on the per cent wilt incidence of three tomato varieties Pusa Ruby, Sakthi and BWR-1 under field conditions in comparison with uninoculated Pusa Ruby, Sakthi and BWR-1. The nursery was inoculated with Eruthiampathy isolate @ 500g $m^{-2}$ and it was observed that the per cent wilt incidence of VAM inoculated Sakthi variety was 17.62 per cent compared to its control (48.25 per cent) after six weeks. Similarly, VAM inoculated BWR-1 recorded 38.33 per cent wilt incidence compared to 43.70 per cent for its control and variety, Pusa Ruby recorded per cent wilt incidence of 98.75 per cent and 100 per cent for VAM inoculated and uninoculated control respectively. Studies with inoculation of rice cv. Ranjit with 20-200g $pot^{-1}$ of *Acualospora* sp. showed that a level of 100g $pot^{-1}$ was best for plant growth and root colonization. Dry weight of plants and root colonization was significantly higher in mycorrhizal plants than non-mycorrhizal plants, but root proliferation was lower (Singha *et al.*, 2000).

The inoculum density was found to vary with the type of AMF, host, soil factors etc. Moreover, it was observed that higher initial inoculum level particularly in annual crops with short growing season might be required if maximum plant growth stimulation is to be achieved. Thus, the optimum inoculum density of AMF cannot be standardized, but has to be formulated based on specific agroclimatic conditions.

## 2.7 FACTORS INFLUENCING THE EFFICIENCY OF AM FUNGI

The AMF population in soil is influenced by many factors that are broadly classified under two headings *viz.*, abiotic and biotic factors. The major abiotic factor influencing AMF population is the soil factor. In addition, the effect of light intensity, seasons and fertilizer application are also reviewed under this heading. The host plants are the most

important biotic factor influencing AMF population in soil. In addition, the influence of other soil microflora is also reviewed in this section.

### 2.7.1 Abiotic factors

#### 2.7.1.1 *Soil factors*

The arbuscular mycorrhizal fungi popularly known as AMF are symbiotic fungi associated with the roots of higher plants. The soil factors therefore exert maximum influence on the AMF. The influence of various soil factors such as soil type and tillage, soil pH, soil moisture, soil temperature, soil salinity, heavy metal accumulation and soil fertility on AMF have been reviewed in this section.

##### 2.7.1.1.1 *Soil type and tillage*

Bakersphicegal (1953) observed that light textured soil supported the endomycorrhizal fungi to sporulate heavily, but their survival was generally more in loamy soils than in sandy soils. Potty (1990) observed highest spore load in alluvial soils followed by sandy and sandy loam soils. Trimurtulu and Johri (1998) reported that silty clay loam soil recorded the highest population of VA mycorrhizal spores and the least was recorded by loam soils.

McGonigle *et al.*, (1990) proposed the hypothesis that there is a hyphal network in the soil which can be reactivated by new fungal development and that disturbance of the soil damages this network and consequently its functioning. They also observed that the P uptake in undisturbed soil even when there was no difference in the level of infection in the plant roots. Jasper *et al.*, (1991) observed a lowering of AMF infectivity in disturbed soil. Miller and Jastrow (1992) reported that there was an array of interactions between the symbiotic association and modifications in soil conditions and tillage deeply affect the performance of AMF.

#### 2.7.1.1.2 *Soil pH*

Green *et al.*, (1976) observed that the pH optimum for spore germination would probably differ with each VAM species and the environment to which each is indigenous. They observed that *Gigaspora coralloidea* and *G. heterogama* from more acidic soils germinated best at pH from 4 to 6. It was also noticed that *G. epigaeum* occurred over a wide range of soil pH with optimum germination occurring between 6 and 8. Gerdemann and Trappe (1974) observed that *G. mosseae* common in alkaline flatland soils germinated well on water or soil extract agar at pH 6 to 9. Thus, it appears that pH can influence the germination of VAM fungal spores, but germination seems to occur within a range which is still acceptable for plant growth.

AMF species have distinct behaviors at different levels of pH as demonstrated by Graw (1979). In this work, it was shown that plant growth was more affected by arbuscular myrorrhizal symbiotic efficacy than by the level of root colonization which means that it was not the extent of development of the fungus itself that was impaired but the functioning of the symbiosis. This reinforces the idea that AMF must be chosen according to the soil characteristics. Young *et al.* (1985) reported that VAM spores were highly influenced by pH of the soil than any other factor.

#### 2.7.1.1.3 *Soil moisture*

The influence of soil water potential on VAM fungal spores has been studied by Daniels and Trappe (1980) using *G. epigaeum* added to silt loam of varied moisture contents. *G. epigaeum* spores germinated best at moisture contents between field capacity and soil saturation. Below field capacity, germination declined with no germination occurring below –31 bars. Koske (1981) studied the effect of soil water potential on germination of *Gigaspora gigantea* placed on sand to which concentrations of polyethylene glycol were added. *G. gigantea* germination was strongly inhibited at – 10 bars but higher

levels of germination could eventually be obtained at low water potential, if spores were incubated longer. He further observed that germ tube length was reduced at low water potential.

Nelsen and Safir (1982) observed that greater root colonization occurs in drought-stressed plants than in plants receiving adequate water. They suggested that low moisture levels could reduce the diffusion rate of nutrients such as phosphorus and decrease the availability of these nutrients to the plants. Graham and Timmer (1984) and Fitter (1988) have reported that the higher resistance of mycorrhizal plants to drought may in many cases be explained by a better nutrition of the plant. Experiments with *Vigna* and Soybeans led Auge *et al.*, (1992) to conclude that mycorrhizal plants present greater resistance to drought and /or faster recovery after a stress period due to either a better performance of the mycorrhizal roots in extracting water from the soil or to a different hydraulic relation between roots and shoots caused by the presence of AMF. However, Kaushal (2000) observed that spore population of VAM was negatively correlated with soil moisture.

**2.7.1.1.4** ***Soil temperature***

Furlan and Fortin (1973) and Hayman (1974) reported that higher temperatures generally resulted in greater root colonization and increased sporulation. Schenck and Schroder (1974) studying the effects of temperature on VAM establishment, observed that maximum arbuscle development occurred near 30°C but the mycelial colonization of the root surface was greatest between 28 and 34°C. However, sporulation and vesicle development was greatest at 35°C.

Schenck *et al.*, (1975) suggested that the temperature range over which germination occurs also depend on the species of VAM fungi and environments to which they are ecologically adapated. They observed that Florida isolates of *Gigaspora* sp. germinated best on soil extract agar incubated at 25 to 35°C, while *Glomus mosseae* from a cooler Washington state environment germinated best when incubated at 18 to 20°C. Daniels and

Trappe (1980) observed that *Glomus epigaeum* from Oregon germinated best at 22°C. Moreover, Koske (1981) found optimum germination for *Gigaspora gigantea* from Rhode island to be 30°C. Ferguson (1981) noticed that periods of cold stress followed by maintenance of high soil temperature increased colonization and sporulation. Smith and Bowen (1979) and Graham *et al.*, (1982) hypothesized that high temperature increased both VAM fungus growth and root exudation, which could lead to increased VAM colonization. It must be remembered that soil temperature are far more important in the production of VAM inoculum than air temperature.

#### 2.7.1.1.5 *Soil salinity*

Salinity is a common problem in arid areas and it may affect mycorrhizae. AMF associated with citrus roots or soils had chlamydospores positively correlated with pH and Sodium which are indicators of soil salinity (Nemec *et al.*, 1981).

Ojala *et al.*, (1983) reported that arbuscular mycorrhizae may increase plant tolerance to salinity either by improved P nutrition or by other mechanisms which are not clear. Improved growth and productivity of *Sesbania grandiflora* under salinity stress through *Glomus macrocarpum* inoculation was reported by Giri and Mukerji (1999). The percentage VAM colonization of roots, production of VAM fungal spores in the rhizosphere and number of root nodules were significantly higher.

#### *2.7.1.1.6 Accumulation of heavy metals*

The major limiting factor to plant growth in acid soils is often related to high levels of exchangeable aluminium which seriously impair root development and affect P absorption. However, arbuscular mycorrhizae may help to overcome this problem. Maluf *et al.*, (1988) reported the role of arbuscular mycorrhizas as an important factor of adaptation of plants to soils with high levels of aluminium. They worked with two varieties of *Laeucaena leucocephala*, one tolerant and another intolerant to acidity and soil aluminium and observed that the mycorrhizal effect was more pronounced on the intolerant

variety. Mikanova *et al.*, (2001) determined the effect of heavy metal pollutants (Cd, Pb, Zn and As) on the soil microflora and their activities. Increased content of heavy metals in the soil - plant system resulted in a decrease in VAM colonization paramcters.

#### 2.7.1.1.7 *Soil fertility*

The effect of high soil fertility on root colonization depends on the host plant grown. Strezemska (1975) observed that root colonization of rye, wheat, barley and oats was reduced after years of cropping in highly fertilized soils, but colonization of bean roots was not similarly reduced under these conditions. Menge *et al.*, (1978) reported that much of the influence of soil fertility on root colonization is plant mediated. The VAM root colonization is inhibited at high phosphorus levels because of the decreased root exudation. Thus, the content of phosphorus in the roots can mediate root colonization by VAM fungi (Ratnayake *et al.*, 1978; Sieverding, 1979; Graham *et al.*, 1981; Nelsen and Safir, 1982).

### 2.7.1.2 *Light intensity*

Many workers studied the effect of light on the AMF sporulation and root colonization. All these studies have been done under green house conditions and more research is necessary to see whether the results apply to the field as well. It was generally observed that increased light intensity increased percentage colonization (Peyronel, 1940; Hayman, 1974; Furlan and Fortin, 1977 and Ferguson, 1981) and Boullard (1957, 1959); Tolle (1958); McCool (1981) and Johnson *et al.*, (1982) noticed that daylengths also increased root colonization.

Hayman (1974) and McCool (1981) reported that a photoperiod of more than 12 hr was important than light intensity in providing high levels of root colonization. Daft and El-Giahmi (1978) reported that if suitable daylength was provided, increased light intensity might still increase colonization. Ferguson (1981) observed that low light intensity can significantly reduce root colonization, but its effect on sporulation may be less pronounced.

*2.7.1.3 Effect of fertilizers*

It is widely accepted that maximum root colonization and sporulation occur in soils of low fertility. It is observed that phosphorus at high levels significantly reduce root colonization (Kruschcheva, 1960; Daft and Nicolson, 1968; Hayman, 1970; Ross, 1971 and Khan, 1972). Similarly, application of nitrogen fertilizer not only reduced root colonization but suppressed spore germination as well (Hayman, 1970, 1975); Porter and Beute, 1972 and Redhead, 1975).

Bevege (1972) found that root colonization increased as nitrogen content increased, if phosphorus levels were moderate. However, at higher levels of phosphorus nitrogen applications were inhibitory.

Daniels and Trappe (1980) observed that addition of nitrogen and potassium did not apparently stimulate or inhibit germination. Koske (1981) observed no difference in germination of *Gigaspora gigantea* spores regardless of phosphorus concentrations. Siqueria *et al*., (1982) however observed that a phosphorus amendment increased spore germination on water agar, but nitrogen and potassium amendments had no effect. Jalali and Thareja (1985) reported that in phosphate rich soils mycorrhizal density was poor, while root samples from soils of low phosphorus status had extensive mycorrhizal colonization.

Manjunath and Bagyaraj (1986) reported that the application of phosphorus as Superphosphate did not reduce the percentage root colonization by VAM fungi *G. fasciculatum*, but increased the extramatricial chlamydospores in the soil. O'Keefe and Sylvia (1991) observed that heavy P fertilization cause reduction in root colonization by AMF and this may cause a decrease in the concentration of Cu and Zn in plant tissue. Sreenivasa (1994) studied the response of chilli to inoculation of VAM fungi at different phosphorus levels in the field. Root colonization and sporulation by the VAM fungi *G. macrocarpum* and *G. fasciculatum* increased with the addition of P upto 56.2 kg ha$^{-1}$, but

decreased with further increase in P. Majjigudda and Sreenivasa (1996) reported that per cent root colonization and spore count of *G. fasciculatum* in wheat were increased with increase in P level upto 75 per cent of the recommended dose.

Devi and Sitaramaiah (1998) observed increased root colonization of black gram by *G. fasciculatum* and *G. constrictum* in superphosphate treatment when compared to rock phosphate treatment. Fathima *et al.*, (2000) studied the effect of different levels and sources of phosphorus on VAM mycorrhizal root colonization and spore load in mulberry cv. Kanva- 2 seedlings. In saplings inoculated with *G. fasciculatum* root colonization was highest with P at 30 kg $ha^{-1}$ $yr^{-1}$ in the form of DAP. In the case of *G. mosseae* inoculated seedlings, root colonization was highest with application of P at 30 kg $ha^{-1}$ $yr^{-1}$ in the form of MRP (Mussoric Rock Phosphate). Spore load in the mulberry rhizosphere was siginificantly higher with p applied at 30 kg $ha^{-1}$ $yr^{-1}$ as MRP than with at the recommended rate of 120 kg $ha^{-1}$ $yr^{-1}$ as SSP.

Alloush *et al.*, (2000) reported that the percentage root colonization of VAM (*G. clarum*) increased two fold or more when mycorrhizal plants were grown with organic matter (12.25 g $kg^{-1}$) + soluble phosphorus (50 mg $K H_2 PO_4$ $kg^{-1}$) and organic matter + rock phosphate (200 mg P $kg^{-1}$). Kaushal (2000) observed that spore population was negatively correlated with organic carbon and soil phosphorus. However, there was no correlation in spore population and percent of root colonization with regards to changes in soil nitrogen.

Sharma and Adholeya (2000) reported that a positive response to mycorrhizal inoculation was evident at lower levels of P only. The percentage of root length colonized by AM fungi decreased from 31 percent to three percent, as the concentration of P increased beyond 10 ppm soil P.

Valentine *et al.*, (2001) studied the interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization. Plants grown at low P with high concentrations of other nutrients had the highest VAM infection.

*2.7.1.4 Seasons*

VAM fungal population have been reported to show seasonal variation based on the spore numbers isolated (Mason, 1964; Hayman, 1970; Sutton and Barron, 1972 and Black and Tinker, 1979). Saif and Khan (1975) have reported increased VAM spore population in winter months. Singh *et al.*, (1992) correlated the variation in spore density and VA mycorrhizal colonization of kinnow and rough lemon seedlings with change of season. The infection of VA mycorrhizal fungi was observed maximum in June and minimum in November. The maximum and minimum spore populations of endophytes in soil were recorded in June and October respectively.

Mago and Mukerji (1994) observed seasonal variation in the percent root colonization with VAM fungi. They noticed that the lowest colonization was during winter and highest during late summer and autumn. Shamin *et al.* (1994) also recorded seasonal variation in VAM colonization on perennial plant species and found maximum colonization in spring and then it gradually decreased in the following season reaching minimum in winter.

However, Requena *et al.*, (1996) reported that climatic variation influenced the selection of AMF or regulated the incidence of certain specific strains in the soil. Bhaskaran and Selvaraj (1997) observed a relatively high fungal spore density during the summer season at all four different coastal locations of Tamil Nadu. Harikumar and Potty (1999) noticed species variation of AMF in soils of southern and northern parts of Kerala. The marked difference observed in the species diversity can be attributed to the influence of agroclimatic differences with the northern part of Kerala experiencing heavy-rainfall (4000 mm) than the southern part (1500 mm).

Kaushal (2000) reported seasonal variations in the spore population. The number of spores was highest in the rhizosphere soil of *Acacia nilotica* during the months of July and August and lowest during summer (May and June).

The importance of soil factors on the AMF population has been clearly demonstrated by the studies conducted by many research workers. The undisturbed soil greatly favoured the AMF population and diversity. The soil pH acceptable for plant growth was also suited for the germination of AMF spores. It was also observed that low soil moisture potential favoured the AMF population in soil. A soil temperature between 28 – 34$^0$C favoured AMF root colonization and it was found that soil temperature was far more important for the production of VAM inoculum than air temperature. Heavy metal accumulation and high soil fertility reduced the AMF population and root colonization. The increase in light intensity and photoperiod had a positive influence on the growth of AMF and root colonization. Among the fertilizers, heavy P fertilization was found to decrease root colonization. The AMF spore population and diversity were also found to be influenced by seasonal variation. Thus, it was observed that the various abiotic factors individually and in combination play an important role in determining the AMF spore population and the extent of root colonization.

### 2.7.2 Biotic factors

The AM fungi are found in symbiotic association with roots of higher plants. Therefore, in addition to abiotic factors the biotic factors like host, genotypic variation among the host, cropping sequence, rhizosphere effect and root exudates also exert an equal influence in determining the AMF population in soil. In addition to host factors the soil microflora also influence the AMF population in soil.

#### *2.7.2.1 Host factors*

Baylis (1969) suggested that no evolutionary stimulus for spore production existed, if root growth was not intermittent. He found that in temperate climate where root growth by

perennial plants is more or less continuous, few spores were produced despite relatively high levels of root colonization.

Mosse (1973) reported that certain VAM species may be more efficient in stimulating the growth of certain plant species, but each VAM fungus is generally able to colonize every VAM host species. The incidence of VAM fungal species (determined as spore numbers in soil) depended upon the plant species which was colonized. It appeared that the host plant could affect sporulation and possibly survival of VAM fungi (Kruckelmann, 1975; Schenck and Kinloch, 1980). Daniels and Trappe (1980) observed no additional stimulation of germination in the presence of host roots and germination occurred equally well in the presence of non-host or ectomycorrhizal plant roots which could lead to reduced populations of VAM fungi in soils.

Cultivation in general and monoculture in particular reduced the spectrum of species found in a soil and relatively few species were present after several years of continuous cultivation (Allen and Boosalis, 1983; Daniels and Bloom, 1983). Brundrett *et al.*, (1996) suggested that factors like cultural practices and vegetation in the locality may also be contributing in deciding the dominance of a particular AMF species.

#### 2.7.2.1 *Cropping sequence*

The number of growing seasons in the absence of a host plant apparently influence VAM survival whether the soil is fallowed or cropped to a non-host. Hayman (1970) in sampling wheat plants through a growing season, observed that root colonization and spore production increased through the season, peaking just prior to harvest.

Black and Tinker (1979) reported that the spore levels generally decreased by the end of a barley growing season, but a greater rate of decrease was evident in fallowed or non-host cropped soil. They also noted that a year of fallow or non-host cropping reduced by half the colonization level in the subsequent barley crop. In the case of monoculture of non-

host plants, germination in the absence of host roots could be detrimental and resulted in reduced populations of VAM fungi in the soil (Powell, 1979).

Ocampo (1980) however observed no difference in colonization levels whether plants were grown in soil kept fallow for 10 weeks prior to planting or in soil amended with inoculum stored under refrigeration. These results suggest that the length of time a host is absent may influence the amount of inoculum that survives. Ocampo *et al.*, (1980) also suggested that VAM fungi may be able to derive some benefit from non-host plants and that non-host cropping may favour the development of VAM fungi more than fallowing. The greatest initial root colonization occurred in soil pre-cropped with a host plant, more root colonization occurred in soil pre-cropped with a non host plant than in soil previously fallow. After 8 weeks, however, the colonization level was similar in all plants.

### 2.7.2.2 *Genotypic variation of the host*

St. John (1980) observed that plants might vary in their dependence on the symbiotic association with AMF due to their physiological and/or anatomical differences. Trouvelot *et al.*, (1982) reported differences among old and more recent wheat varieties in their receptivity to AMF. Potty (1990) conducted a detailed survey on AMF association in tuber crops grown in different soil types and a observed wide variation in spore population and AMF colonization with host genotype. The dependence of citrus on arbuscular mycorrhizae was related to differences among root stock properties such as root anatomy and plant growth rate, and that plants dependent on mycorrhiza have metabolic characteristics that stimulated infection, which did not occur in less dependent root stocks (Graham and Sylvertsen, 1985; Graham *et al.*, 1991).

Guillemin *et al.*, (1992) in their work with pineapple observed that there was specificity among plant varieties and fungal isolates regarding stimulation of plant growth. Plants of the Queen and Smooth Cayenne varieties grew better when inoculated with *Glomus* sp. (isolate LPA 21) than with *Glomus clarum*, *Scutellospora pellucida* or *Glomus*

sp. (isolate LPA 25), while plants of Spanish variety showed highest growth increase for *Glomus* sp. (isolated LPA 25).

Sivaprasad (1995) reported the influence of host genotype and soil types on AMF association in cardamom, pepper, ginger and turmeric. All these works point out the necessity of taking into consideration the existence of arbuscular mycorrhizal symbiosis in the selection processes, since greater yields at lower costs can only be obtained when better fitness of plant species or varieties to this association is exploited.

#### 2.7.2.3 *Rhizosphere effect and root exudates*

The presence of plant roots causes a rapid and intense stimulation of the microbial population in the rhizosphere region (Hiltner, 1904). Hepper and Mosse (1975) observed that only about 50 per cent (on average) of the inoculated spores readily germinate indicating a certain degree of soil fungistasis towards AMF spores. They also observed that the proximity of roots to spores could overcome such fungistasis. Mosse and Hepper (1975) and Powell (1976) showed that the initial direction of germ tubes produced by germinating spores of AMF was not influenced by the presence of host roots.

Smith and Walker (1981) observed that mycorrhizal colonization was initiated at the zone of elongation from where root exudation was the greatest. Graham (1982) observed that germination of *Glomus epigaeum* spores was increased and that germ tube length was greater when spores were exposed to root exudates. The germ tubes of these treated spores also branched more frequently than non-treated spore germ tubes. Volatile exudates released from roots were responsible for a chemotropic response by aerial germ tubes of *Gigaspora gigantea* (Koske, 1982). There was indirect evidence that volatile organic compounds act as messengers affecting direction and /or growth rate of the germ tubes of AMF (St. John *et al.*, 1983).

Barea (1986) observed that the contribution of autotrophic higher plants to the mutualistic relationships of the arbuscular mycorrhiza (AM) appears to begin before the AMF have been established in the root cortex. Becard and Piche (1989a) observed that the presence of a growing root significantly stimulated the growth of AMF fungi even when there was no root fungal contact and active fungal growth ceased up on root removal. In another experiment, Becard and Piche (1989b) used transformed roots of carrot to ascertain the effects of some root metabolites on the axenic growth of AMF. They demonstrated that the hyphal growth of *Gigaspora margarita* was stimulated greatly by a synergistic interaction between volatile compounds from the root and other root exudates. Root volatiles alone provided little stimulation and root exudates alone had no effect. It was also apparent that $CO_2$ was a critical root volatile involved in the stimulation of hyphal growth from spores of AMF. Becard and Piche (1989b) and Gianinazzi-Pearson *et al.*, (1989) suggested the presence of flavanoids as active components in the root exudates which could actively stimulate growth of AMF.

Koske and Gemma (1992) reported that volatile compounds released by roots acted as a common form of chemical communication between the microbial components in soil by their ability to move over greater distances and this is especially important to AMF as they can be stimulated by plant signal far from the root surface. Root exudation affected the saprophytic growth and development of AM fungi by acting on the rate of the hyphal growth, frequency of branching and direction of growth (Koske and Gemma, 1992; Giovannetti *et al.*, 1996).

### 2.7.2.5 *Interaction between soil microorganisms and AM fungi*

Griffin (1972) observed that the activity of soil microflora influenced by soil temperature may affect the rate of VAM hyphal lysis or hyper-parasitism of spores. It was noticed that bacteria depleted the nutrients around spores, thus increasing the leaching of nutrients from spores and induced autolysis of fungal spores. Old and Wong (1976) suggested that bacteria can penetrate spores but more commonly remain on the spore

surface, while producing fungal cell wall degrading enzymes as well as enzymes to disintegrate the fungal protoplasm. The presence of the nematode *Meloidogyne arenaria* completely negated the beneficial effect of the mycorrhizal fungus *Glomus fasciculatum* in grape vine and reduced mycorrhizal root colonization.

Ross and Ruttencutter (1977) suggested that the comparative susceptibility of VAM fungal species to hyperparasites probably influenced their survival in soil and may also influence the competitive ability of these fungi. The fungi most frequently found to hyperparasitize VAM fungal spores belong to the Mastigomycotina with a zoosporic stage in their life cycle which included *Rhizidiomycopsis* sp. (Schenk and Nicolson, 1977; Sparrow, 1977), *Phlyctochytrium* sp. (Ross and Ruttencutter, 1977; Daniels and Menge, 1980) and a *Pythium* like fungus (Ross and Ruttencutter, 1977). Anderson and Patrick (1978) discovered that the perforations and depressions in spores were caused by vampyrid amoebae which could constrict their bodies sufficiently to pass through the nucleopore filters.

Krishna *et al.*, (1982) studied the interaction between the VAM fungus *G. fasciculatum* and the actinomycete *Streptomyces cinnamomeus* introduced into the rhizosphere of finger millet. Individual inoculation of either organism resulted in enhanced plant growth, whereas simultaneous inoculation had an antagonistic effect on each other each suppressing the growth and multiplication of the other in the rhizosphere.

Warnock *et al.*, (1982) observed that leek plants colonized by *Glomus fasciculatum* failed to show a growth response, if Collembola or Springtails were present. They suggested that in the presence of Collembola, the VAM fungus might not be active or that Collembola might have grazed on external VAM hyphae. Sylvia and Schenck (1983) recorded inhibitory effect of certain fungi including *Trichoderma* sp. on spore germination of *Glomus* spp.

Linderman (1985) correlated response of mycorrhizal plants to mycorrhizosphere and observed the microbial community stimulated the development of arbuscular mycorrhizal fungal hyphae and rhizomorphs and suppressed the growth of soil borne

pathogens. Mycelial growth and spore germination of *G. mosseae* was stimulated by the presence of *Trichoderma* spp. under axenic conditions (Calvet *et al.*, 1992). The effect was due to fungal exudates in the presence of moderate concentrations of carbondioxide. Wyss *et al.*, (1992) reported the inhibition of mycorrhizal colonization by saprophytic fungi

According to Calvet *et al.*, (1993) the combined inoculation of *Trichoderma aureoviride* and *G. mosseae* resulted in synergistic effect on the growth of marigold (*Tegetes erecta*). The synergism between the fungi imparted host protection against *Pythium ultimum*.

Datnoff *et al.*, (1995) reported that root rot disease severity was significantly reduced when a combination of *T. harzianum* and *G. intraradices* were used in tomato.

The host factors was found to influence the AMF population in soil as these fungi are found in symbiotic association with the plant roots. Moreover, it was also observed that monoculture reduced the spectrum of AMF species found in soil. The length of time a host was absent and variation in host genotype influenced the amount of inoculum that survived. The importance of root exudates has also been emphasized in the review with the root exudates, especially volatile organic compounds acting as messengers affecting growth rate and direction of growth of germ tube. It was also observed that soil microorganisms stimulated the growth of AMF hyphae and rhizomorphs and suppressed the growth of soil-borne pathogens. However, soil microorganism inhibiting the AMF have also been reported. Thus, the selection of AMF should take into consideration the symbiotic association the fungus has with the root system of higher plants together with their interaction with soil microflora.

## 2.8 PERFORMANCE OF AM FUNGI UNDER FIELD CONDITIONS

Khan (1972 and 1975) carried out the trials on VAM inoculation of maize and wheat and found very large growth responses in shoot dry matter and grain yield to mycorrhizal inoculation. The mycorrhizal seedlings were pre-inoculated before transplant and the field soils were deliberately chosen to have low populations of indigenous mycorrhizal fungi.

Saif and Khan (1977) reported a 290 per cent response in grain yield to VAM inoculation with a similarly large response in vegetative growth. The mycorrhizal plants were inoculated as seedlings in the glasshouse and transplanted to the field at 4 weeks old and approximately 85 per cent of the root system were already mycorrhizal. Build up of mycorrhizal infection in the control plants was very slow with only 20 per cent of the root system mycorrhizal with the indigenous fungi after 3 months. They concluded that a large part of the apparent response to inoculation of the barley crop was due to the pre-inoculation of the seedlings. Menge *et al.*, (1978) observed decreased transplant shock in mycorrhizal avocado plants rather than in non-mycorrhizal.

Johnson and Crews (1979) transplanted *Azalea* with or without prior VAM inoculation into an unsterilized field site and found much better survival and growth of the inoculated plants at 4 months after transplant. This demonstrated the long-term responses of VAM inoculation in unsterilized field soils. Islam and Ayanaba (1981) carried out a field experiment to compare pre-inoculation (and subsequent transplanting) with seed inoculation directly into the field. Both inoculation techniques increased plant shoot dry matter and nodule matter exactly over control plants. Grain yield data, in which VAM inoculation in the field seed bed carried out, was increased by 50 per cent and that of pre-inoculation treatment by 26 per cent.

Powell and Bagyaraj (1982) reported that VAM inoculation in the seed furrow lead to highest mycorrhizal infection levels and best growth responses. Sreeramulu and Bagyaraj (1986) inoculated chilli nursery beds with four different VAM fungi, *G. fasciculatum*, *G. albidum*, *G. macrocarpum* and isolate $I_4$ and mycorrhizal seedlings were transplanted to the field. Inoculation of *G. fasciculatum* resulted in maximum increase in plant height, number of flowers, shoot dry weight and yield. Akbar (2002) conducted a field experiment to study the effect of *G. fasciculatum* in controlling the bacterial wilt incidence of the tomato variety ,Sakthi. Mycorrhiza was inoculated at the time of sowing @ 50g $pot^{-1}$ to raise seedlings for transplanting to the field. The *G. fasciculatum* treated tomato plants recorded increase in plant height, wet weight, dry weight, root length and yield over control. The wilt incidence

of the VAM inoculated plants were 65.78 per cent when compared to 73.84 per cent in control.

Raji (2002) investigated the effect of the Eruthiampathy isolate (*Glomus* sp.) on the percent wilt incidence of three tomato varieties Pusa Ruby, Sakthi and BWR-1 under field conditions. The tomato seedlings were pre-inoculated with the native isolate @500g $m^{-2}$ in nursery beds. The percent wilt incidence of the variety Sakthi and BWR-1 were considerably less, when compared to control, which recorded 100 per cent wilt. She also observed a marked increase in fresh weight, dry weight, root weight and fruit yield over the uninoculated plants. The percentage root colonization and VAM spore count also recorded an increase in mycorrhiza inoculated plants over that of control.

The pathogen, *R. solanacearum* was found to be associated with the bacterial wilt disease of many crop plants including solanaceous vegetables. The bacterium belonging to race 1 was found to incite the bacterial wilt in tomato. Both environmental and soil factors influence the pathogen population in the soil. Sandy soils generally recorded low *Ralstonia* population and the pathogen was found to survive over a wide range of pH. Higher calcium content in soil however imparted resistance to the plants against the bacterial wilt pathogen.

The AM fungi are ubiquitous in nature and found in symbiotic association with the roots of higher plants. Among the diverse types of AMF, *Glomus* spp. was found to be the most predominant genus. The role played by AMF as a biocontrol agent has been proved beyond doubt. It is also observed that pre-inoculation of plants with AMF provides much greater protection against soil-borne pathogens. The optimum dosage of AMF varied with the host type and soil factors. It was observed that higher initial inoculum density particularly in annual crops with short growing season may be required, if maximum plant growth stimulation is to be achieved. In addition, various abiotic and biotic factors exert their influence on the AMF spore germination, population and root colonization. The performance of AMF in the field has not been studied extensively, but the available information suggests that AMF is able to enhance the growth and yield of crops in the field.

# MATERIALS AND METHODS

# 3. MATERIALS AND METHODS

The studies on 'Biocontrol of bacterial wilt in tomato using arbuscular mycorrhizal fungi' was conducted at the College of Horticulture, Vellanikkara, Thrissur during the period 2000-2002. The isolation and identification of AM fungi from tomato and maize rhizosphere of high and low wilt incidence, screening of AMF cultures against *Ralstonia solanacearum* under pot culture conditions, determination of the optimum time of inoculation and inoculum density for the control of bacterial wilt under pot culture as well as field experiment were conducted for the study. The details of the materials used and methods followed are presented below.

## 3.1 COLLECTION OF SOIL SAMPLES

The rhizosphere soils were collected from tomato and maize plants of high wilt (Vellanikkara, Thrissur dt.) and low wilt incidence (Ozhalapathy and Eruthiampathy, Palakkad dt.) areas. A total of six soil samples were collected (Table 3.1 and Fig.1).

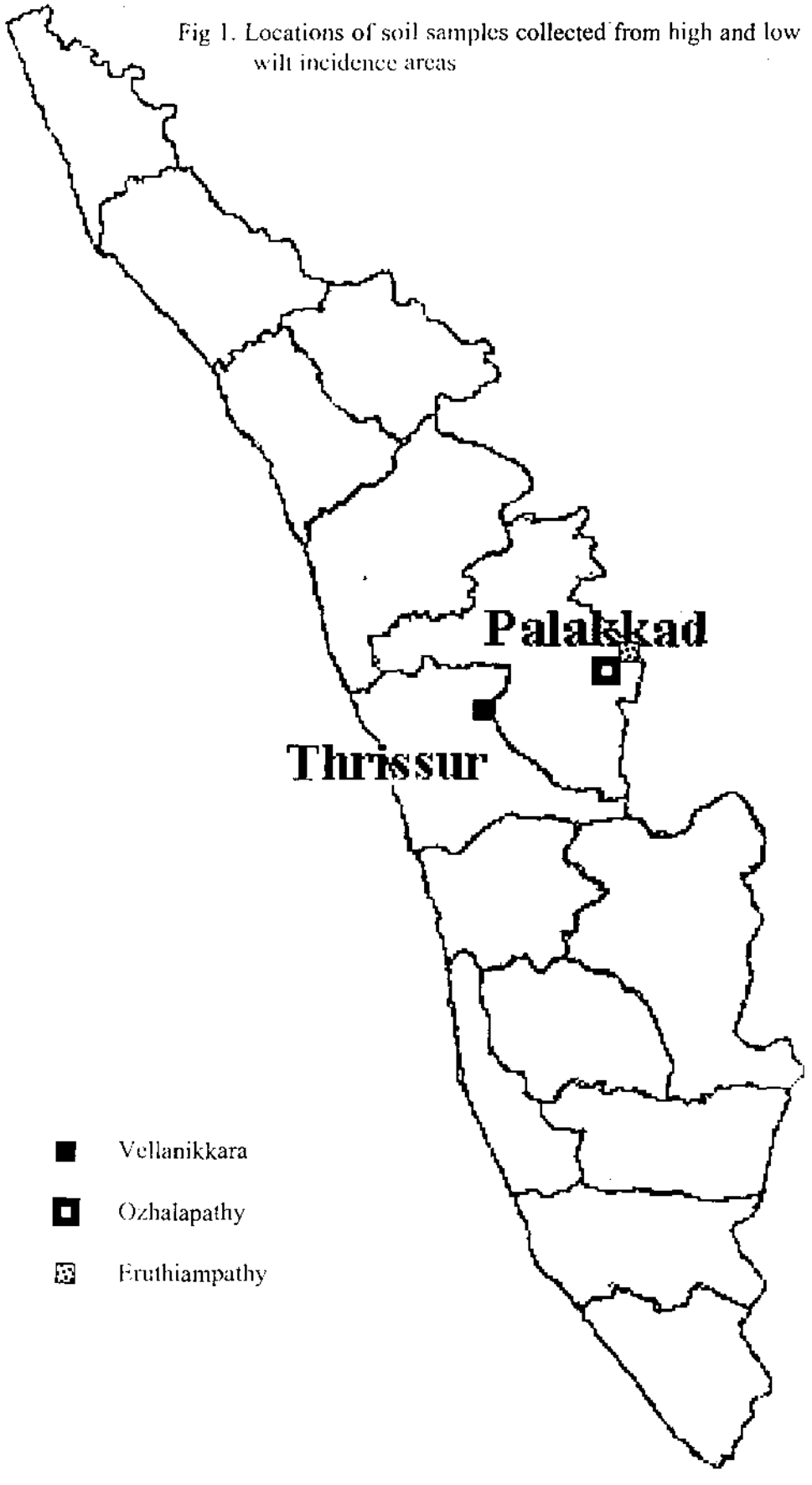
## 3.2 ISOLATION OF AM FUNGAL SPORES

The AM fungal spores were isolated from the rhizosphere soil by wet sieving and decanting method (Gerdemann and Nicolson, 1963). About 250g of rhizosphere soil was suspended in 1000ml water and stirred well. After settling of the heavier particles, the supernatant was filtered through a set of sieves of size 425, 250, 105 and 45 microns (Jayant test sieves, Jayant Scientific Ind. Mumbai, 400 002). Finally, the soil suspension present in 45,105 and 250 micron sieves were transferred to 100ml beakers separately by gentle washing. The spore suspension was filtered through Whatmann No: 1 filter paper. The filter paper containing spores were placed in a petridish and observed under stereomicroscope. The number of similar spores were picked and counted separately based on the shape and colour of spores. The isolated spores were transferred to moistened filter paper for further studies.

**Table 3.1 Locations of Soil Samples Collected from High and Low Wilt Incidence Areas**

| Sl.No. | Location | Host | Degree of wilt incidence |
|---|---|---|---|
| 1 | Ozhalapathy (OT) (Palakkad district) | Tomato (local variety) | Low wilt |
| 2 | Eruthiampathy (ER) (Palakkad district) | Tomato (Sakthi-resistant variety) | Low wilt |
| 3 | Ozhalapathy (OM) (Palakkad district) | Maize | Low wilt |
| 4 | Vellanikkara (VTM) (Thrissur district) | Tomato (Mukthi-moderately resistant variety) | High wilt |
| 5 | Vellanikkara (VBT) (Thrissur district) | Tomato (BT-1 – Susceptible Variety) | High wilt |
| 6 | Vellanikkara (VM) (Thrissur district) | Maize | High wilt |

Fig 1. Locations of soil samples collected from high and low wilt incidence areas



### 3.2.1 Identification of AM fungi

The spore characters *viz.*, colour, shape, size, surface configuration, wall layers, number of hyphae, form of hyphae and alignment of hyphae with the spore axis were recorded. They were identified by comparing the spore characters with the synoptic keys (Trappe, 1982). The predominant spores from each soil sample alone were selected for further studies.

### 3.2.2 Size of AM fungal spores

The spores transferred to moistened filter paper were measured using ocular micrometer pre-calibrated with stage micrometer under 40x magnification.

### 3.2.3 Enumeration of AM fungal spores in the rhizosphere soil

The number of AMF spores in the rhizosphere soil was recorded as described in section 3.2.

### 3.2.4 Enumeration of *Ralstonia solanacearum* in the rhizosphere soil

The rhizosphere population of *R. solanacearum* in the six soil samples collected from high and low wilt incidence areas were estimated by serial-dilution technique using Triphenyl Tetrazolium Chloride (TZC) medium (Kelman, 1954) (Appendix.1).

### 3.2.5 Soil nutrient analysis

The six soil samples were analysed for the nutrients N, P, K, Ca and Mg by the standard methods (Table 2) and the pH of the six soil samples were also determined.

### 3.2.6 Commercial AMF culture

The commercial AMF culture was obtained from Dept. of Agrl. Microbiology, TNAU, Coimbatore for comparison and testing with native cultures.

**Table 3. 2 Method used for soil nutrient analysis**

| Sl. No. | Nutrient | Method of estimation | Reference |
|---|---|---|---|
| 1 | N (Available) | Alkaline Potassium Permanganate method | Subbiah and Asija (1956) |
| 2 | P (Available) | Ascorbic acid blue colour method (Bray-1 extraction) | Watanabe and Olsen (1965) |
| 3 | K (Available) | Flame Photometer | Jackson (1958) |
| 4 | Ca and Mg (Available) | EDTA method | Hesse (1971) |

### 3.2.7 Mass multiplication of AM fungal inoculum

The predominant spores from each soil sample were surface sterilised in Chloramine T (0.2 per cent) solution followed by streptomycin (0.02 per cent) and washed with sterile water 3-4 times. The seeds of sorghum were surface sterilised with Sodium hypochlorite (0.1 per cent) for 10 minutes and washed with sterile water. The sterilised soil : sand (1: 1) was added to earthern pots (capacity 5 kg). A small quantity of soil mixture was taken out at the centre with a surface sterilized glass rod and the surface sterilised spores were washed down from the filter paper using sterile water and surface sterilised sorghum seeds were sown and covered with soil. The TNAU commercial culture was also mass multiplied in sterile soil : sand (1:1) with sorghum as host. The plants were watered daily using sterile distilled water. Ruakara nutrient solution (Smith *et al.*, 1983) (Appendix 2) was applied @ 50ml $pot^{-1}$ at 10 days interval for a period of 60 days. The plants were maintained for 90 days. The shoot portion of the sorghum plants were cut and removed 90 days after sowing. The roots were also cut into small pieces and mixed thoroughly with the soil. The infected root bits, hyphae and rhizosphere soil from the pots were used as inoculum for further studies.

## 3.3 SCREENING OF AMF CULTURES AGAINST *R. SOLANACEARUM* UNDER STERILE CONDITIONS

A pot culture experiment was conducted for screening the AMF cultures against *R. solanacearum*. The inoculum was obtained by collecting the ooze from wilted tomato plants in the field. The predominant six native isolates of AMF obtained were screened along with one commercial culture individually and its combinations (Table 3.3). The experiment was conducted in polybags (18 cm x 13 cm) containing 650 g of sterile potting mixture (soil : sand : cowdung) in the ratio of 2:1:1. The experiment was laid out in Completely Randomised Design (CRD) with three replications and each replication had two plants.

### 3.3.1 Tomato variety used

The highly susceptible variety Pusa Ruby was used for the experiment and the seeds were obtained from IARI, New Delhi

**Table 3. 3 AMF culturesused against *Ralstonia solanacearum***

| Sl No. | Treatments | AMF culture |
|---|---|---|
| 1 | $T_1$ | *Glomus* sp. (OT) |
| 2 | $T_2$ | *Glomus* sp. (OT) + *Glomus* sp. (ER) |
| 3 | $T_3$ | *Glomus* sp. (OT) + *Glomus* sp. (OM) |
| 4 | $T_4$ | *Glomus* sp. (OT) + *Glomus* sp. (VTM) |
| 5 | $T_5$ | *Glomus* sp. (OT) + *Glomus* sp. (VBT) |
| 6 | $T_6$ | *Glomus* sp. (OT) + *Glomus* sp. (VM) |
| 7 | $T_7$ | *Glomus* sp. (OT) + Commercial culture (TN) |
| 8 | $T_8$ | *Glomus* sp. (ER) |
| 9 | $T_9$ | *Glomus* sp. (ER) + *Glomus* sp. (OM) |
| 10 | $T_{10}$ | *Glomus* sp. (ER) + *Glomus* sp. (VTM) |
| 11 | $T_{11}$ | *Glomus* sp. (ER) + *Glomus* sp. (VBT) |
| 12 | $T_{12}$ | *Glomus* sp. (ER) + *Glomus* sp. (VM) |
| 13 | $T_{13}$ | *Glomus* sp. (ER) + Commercial culture (TN) |
| 14 | $T_{14}$ | *Glomus* sp. (OM) |
| 15 | $T_{15}$ | *Glomus* sp. (OM) + *Glomus* sp. (VTM) |
| 16 | $T_{16}$ | *Glomus* sp. (OM) + *Glomus* sp. (VBT) |
| 17 | $T_{17}$ | *Glomus* sp. (OM) + *Glomus* sp. (VM) |
| 18 | $T_{18}$ | *Glomus* sp. (OM) + Commercial culture (TN) |
| 19 | $T_{19}$ | *Glomus* sp. (VTM) |
| 20 | $T_{20}$ | *Glomus* sp. (VTM) + *Glomus* sp. (VBT) |
| 21 | $T_{21}$ | *Glomus* sp. (VTM) + *Glomus* sp. (VM) |
| 22 | $T_{22}$ | *Glomus* sp. (VTM) + Commercial culture (TN) |
| 23 | $T_{23}$ | *Glomus* sp. (VBT) |
| 24 | $T_{24}$ | *Glomus* sp. (VBT) + *Glomus* sp. (VM) |
| 25 | $T_{25}$ | *Glomus* sp. (VBT) + Commercial culture (TN) |
| 26 | $T_{26}$ | *Glomus* sp. (VM) |
| 27 | $T_{27}$ | *Glomus* sp. (VM) + Commercial culture (TN) |
| 28 | $T_{28}$ | Commercial culture (TN) |
| 29 | $T_{29}$ | Uninoculated control |

OT – Ozhalapathy tomato,
ER – Eruthiampathy tomato
OM – Ozhalapathy maize
VTM – Vellanikkara tomato (Mukthi)
VBT – Vellanikkara tomato (BT-1)
VM – Vellanikkara maize
TN – Commercial culture from TNAU

### 3.3.2 Raising of tomato seedlings

Sterilised soil : sand : cowdung mixture (1:1:1) were filled in the plastic pots (20.32 cm size of capacity 3 kg) and surface sterilized Pusa Ruby seeds (0.1 % sodium hypochlorite for 10 minutes) were sown.

### 3.3.3 AMF inoculation and transplanting

At the time of transplanting, the AMF inoculums in different combinations (Table.3) were added to the tomato plants. Sixteen grams of AMF inoculum (@25g $kg^{-1}$ soil) were added to each polybag in the case of individual treatments and in the case of combinations a total of 32 g AMF culture were taken and mixed before adding to the polybags. In the control, no AMF was added. The 30-day-old seedlings were transplanted to polybags (@ 2 $bag^{-1}$). The plants were watered daily using sterile water.

### 3.3.4 Artificial inoculation of *R. solanacearum* to mycorrhizal infected tomato plants

The wilted tomato plants were collected from the wilt-sick field of the Dept. of Olericulture, College of Horticulture, Vellanikkara. The basal portion of the wilted plants was washed thoroughly and a slant cut was given using a sharp knife. The cut portion was then kept immersed in a beaker containing water for 45 minutes without disturbing to collect the bacterial ooze. A horizontal cut was made in the rhizosphere region of mycorrhizal infected tomato plants 30 days after AMF inoculation with a sharp knife to injure the roots (James, 2001). The freshly collected bacterial ooze of 10 ml (O.D. = 0.3 at 600 nm) was poured in the rhizosphere region of the tomato plants in each polybag.

### 3.3.5 Observations

The AMF per cent root colonization, the rhizosphere spore count, the percent wilt incidence, number of days of plant survival and biometric characters of the plants were recorded.

*3.3.5.1 Per cent root colonization*

The AMF per cent root colonization was assessed using the method described by Phillips and Hayman (1970). The roots were washed in tap water to remove the adhering soil particles and were then cut into bits of one cm length and fixed in formalin: acetic acid: alcohol mixture (FAA) (Appendix 3). The root bits fixed in FAA were washed thoroughly in water to remove the fixative. The washed root bits were softened by simmering in 10 per cent KOH at $90^0$C for one hour. After cooling, the excess KOH was washed - off in tap water and then neutralized with two per cent HCl. The root bits were then stained with 0.05 per cent trypan blue in lactophenol (Appendix 4) for three minutes. The excess stain from the root tissue was removed by cleaning in lactophenol. The root bits were examined under microscope (40x) for AMF colonization. The per cent AMF colonization was determined using the following formula.

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

*3.3.5.2 AMF spore count*

The AMF spores in the rhizosphere region were determined as described in the section 3.2.

*3.3.5.3 Percent wilt incidence*

The number of plants showing wilt symptoms were observed daily upto 90 days after transplanting and percent wilt incidence was calculated as follows:

$$\text{Per cent wilt incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

*3.3.5.4 Number of days the plants survived*

The observation for number of days of plant survival was recorded daily upto flowering.

*3.3.5.5 Dry weight of the plant*

The dry weight of the wilted plants was determined after drying the plant samples to a constant weight at $60^0$C.

*3.3.5.6 Root length*

The root length of the wilted plants was taken from the collar region to the tip of the longest root using a measuring scale and expressed as centimetre.

## 3.4 DETERMINATION OF OPTIMUM TIME FOR AMF INOCULATION AND THE OPTIMUM INOCULUM DENSITY FOR THE CONTROL OF BACTERIAL WILT

A pot culture experiment using wilt-sick soil was conducted to determine the optimum inoculation time and optimum inoculum density of AMF in checking the incidence of bacterial wilt. The three standard commercial species of *Glomus viz.*, *G. mosseae*, *G. fasciculatum* and *G. intraradices* were used to determine the optimum inoculation time and optimum inoculum density. The experiment was laid out in Completely Randomised Design (CRD) with three replication and each replication had 3 plants. The treatment details are as follows:

Factor 1: Time (t)

$t_0$ - Control

$t_1$ – At the time of sowing

$t_2$ 15 days before transplanting

$t_3$ – At the time of transplanting

Factor 2: Inoculum density (Id)

$Id_0$ Control

$Id_1$ – AMF @ 25g $kg^{-1}$ soil

$Id_2$ – AMF @ 50g $kg^{-1}$ soil

$Id_3$ - AMF @ 75g $kg^{-1}$ soil

The treatment combinations were

$t_0Id_0$ Control

$t_1Id_1$ At the time of sowing + 25g $kg^{-1}$ soil

$t_1Id_2$ At the time of sowing + 50g $kg^{-1}$ soil

$t_1Id_3$ - At the time of sowing + 75g $kg^{-1}$ soil

$t_2Id_1$ 15 days before transplanting + 25g $kg^{-1}$ soil

$t_2Id_2$ – 15 days before transplanting + 50g $kg^{-1}$ soil

$t_2Id_3$ - 15 days before transplanting + 75g $kg^{-1}$ soil

$t_3Id_1$ At the time of transplanting + 25g $kg^{-1}$ soil

$t_3Id_2$ At the time of transplanting + 50g $kg^{-1}$ soil

$t_3Id_3$ At the time of transplanting + 75g $kg^{-1}$ soil

### 3.4.1 Raising of Pusa Ruby seedlings

Surface sterilized Pusa Ruby seeds were raised in sterile soil : sand : cowdung mixture (1:1:1) filled in plastic pots (20.32 cm capacity 3 kg). The seedlings for each treatment combination were raised separately.

#### *3.4.1.1 AMF inoculation at the time of sowing*

In the case of the treatment combination $t_1Id_1$, $t_1Id_2$ and $t_1Id_3$, where the mycorrhizal inoculum need to be added at the time of sowing, the mycorrhizal inoculum was added to the respective plastic pots @ 25g $kg^{-1}$, 50g $kg^{-1}$ and 75g $kg^{-1}$ respectively and the seeds of Pusa Ruby were sown in poly bags filled with sterile soil.

#### *3.4.1.2 AMF inoculation at 15 days before transplanting*

The same procedure was followed as described in section 3.4.1.1 except that the inoculum were added 15 days before transplanting (15-day-old seedlings) to the plastic pots. The soil in the rhizosphere region was gently raked using a spatula and the inoculum was added. In the case of the treatment combinations $t_2Id_1$, $t_2Id_2$ and $t_2Id_3$, the

mycorrhizal inoculum was added at 15 days before transplanting to the respective plastic pots @ 25g $kg^{-1}$, 50g $kg^{-1}$ and 75g $kg^{-1}$.

*3.4.1.3* ***AMF inoculation at the time of transplanting***

The same procedure as described in section 3.4.1.1 was followed except that the inoculum were added to the earthern pots at the time of transplanting (30-day-old seedlings). The earthern pots were filled up with the potting mixture of wilt sick soil : sand : cowdung (2 : 1 : 1) and to this the required dosage of inoculum was added and mixed thoroughly before the seedlings were transplanted. The treatment combinations used were $t_3Id_1$, $t_3Id_2$ and $t_3Id_3$ where the mycorrhizal inoculum was added @ 25g $kg^{-1}$, 50g $kg^{-1}$ and 75g $kg^{-1}$ to the respective earthern pots.

### 3.4.2 Transplanting

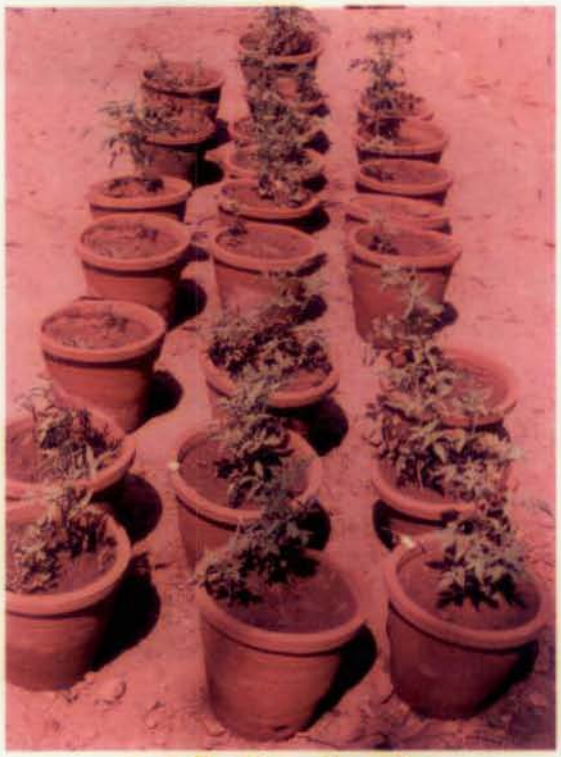
The seedlings from each treatment *viz.*, at the time of sowing, at 15 days before transplanting and at the time of transplanting with inoculum density @ 25g $kg^{-1}$, 50g $kg^{-1}$ and 75g $kg^{-1}$ were transplanted to earthern pots (capacity 5 kg) filled with potting mixture 2:1:1 (wilt sick soil : sand : cowdung) (Plate 1). The cultural practices as per package of practices recommendations of 'Crops' (KAU, 1996) were followed.

### 3.4.3 Application of nutrient solution

Plants were given Ruakara nutrient solution (Smith *et al.*, 1983) without P @ 25ml polybag$^{-1}$ at 10 days interval upto 30 days.

### 3.4.4 Observations

The AMF root colonization of the plants, the rhizosphere AMF spore count, percent wilt incidence, number of days of plant survival and biometric characters were recorded till all the plants wilted as described in the sections 3.3.5.5 and 3.3.5.6.



a. at the time of sowing

b. at 15 days before transplanting

c. at the time of transplanting

**Plate 1. A view of an experiment on determination of optimum inoculation time and inoculum density of AMF under pot culture**

## 3.5 SCREENING OF EFFECTIVE NATIVE AMF CULTURES AGAINST BACTERIAL WILT

A pot culture experiment using potting mixture of wilt-sick soil : sand : cowdung (2 : 1 : 1) was conducted to screen the best five native AMF cultures in combination obtained from screening trial (section 3.3) at 15 days before transplanting and @ 75 g $kg^{-1}$ soil against bacterial wilt along with the commercial culture. The treatments details were as follows:

| Treatment | AMF cultures used |
|---|---|
| $T_1$ | *Glomus* sp. (OT) + *Glomus* sp. (ER) |
| $T_2$ | *Glomus* sp. (OT)+ *Glomus* sp. (VM) |
| $T_3$ | *Glomus* sp. (OM) + *Glomus* sp. (VBT) |
| $T_4$ | Commercial culture (TN) |
| $T_5$ | Control |

Note : AMF was inoculated 15 days before transplanting @ 75 g $kg^{-1}$ soil

The experiment was laid out in Completely Randomized Design (CRD) with three replications. Each replication had a total of 3 plants.

### 3.5.1 Raising of Pusa Ruby seedlings

Pusa Ruby seedlings were raised in sterile soil : sand : cowdung mixture (1:1:1) filled in pots. The seedlings for each treatment were raised separately and inoculated with AMF at 15 days before transplanting @ 75 g $kg^{-1}$ soil.

### 3.5.2 Transplanting

The seedlings were transplanted to earthern pots of capacity 5kg filled with potting mixture 2:1:1 (wilt sick soil : sand : cowdung). The cultural practices as per package of practices recommendations of 'Crops' (KAU, 1996) were followed.

### 3.5.3 Observations

The AMF root colonization of the plants, the rhizosphere AMF spore count, percent wilt incidence, number of days of plant survival and biometric characters *viz.*, plant height, root length, root weight, fresh weight and dry weight were recorded.

#### *3.5.3.1 Plant height*

The height of the plant was measured from the soil level to the tip of the plant using metre scale and expressed as centimetre.

#### *3.5.3.2 Root length*

The root length was taken from the collar region to the tip of the longest root using a metre scale and expressed as centimetre.

#### *3.5.3.3 Root weight*

The root system of the plants were separated, washed and dry weight was recorded.

#### *3.5.3.4 Fresh weight of plant*

The fresh weight of the plants were recorded after uprooting the plant at the time of harvest.

#### *3.5.3.5 Dry weight of plant*

The dry weight of the plants were recorded after drying the plant sample to a constant weight at $60^0$C.

## 3.6 FIELD EXPERIMENT

A field experiment was conducted to study the efficacy of five promising AMF cultures and its combinations (obtained from pot culture studies) along with commercial culture. The study was undertaken at the Vegetable Research Farm of College of Horticulture, Vellanikkara using the varieties Pusa Ruby (susceptible) and Mukthi (moderately resistant) (Plate 2). The experiment was laid out in Randomised Complete Block Design with four replications. Each replication had 12 plants each of Pusa Ruby and Mukthi. Each plot size was 3 m x 2.7 m. The treatments were as follows:

$T_1$ -- *Glomus* sp. (OT) + *Glomus* sp. (ER)
$T_2$ -- *Glomus* sp. (OT) + *Glomus* sp. (VM)
$T_3$ -- *Glomus* sp. (OM) + *Glomus* sp. (VBT)
$T_4$ – Commercial culture (TN)
$T_5$ – Chemical control with Kocide (0.2 per cent)
$T_6$ – Absolute control

### 3.6.1 Nursery

Seedlings of Pusa Ruby and Mukthi were raised on nursery beds (0.5 $m^2$). Each mycorrhizal inoculum (@75g $kg^{-1}$ soil) was added at 15 days before transplanting as band application in the nursery (Mamtha, 1999).

### 3.6.2 Transplanting

The 30-day old mycorrhizal seedlings of Pusa Ruby and Mukthi were transplanted to the field. For the treatment $T_5$, Kocide @ 0.2 per cent was drenched on the ridges at the time of transplanting. In this treatment no mycorrhizal inoculation was done. The cultural practices as per package of practices recommendations of 'Crops' (KAU, 1996) were followed.

**Plate 2. Field view**

### 3.6.3 Observations

The AMF spore count and *R. solanacearum* population were determined at monthly intervals as described in sections 3.2 and 3.2.4. The per cent root colonization, percent wilt incidence and number of days of plant survival were recorded as described in sections 3.3.5.1, 3.3.5.3 and 3.3.5.4. The biometric characters *viz.*, plant height, root length, root weight, fresh weight and dry weight were recorded as described in sections 3.5.3.1, 3.5.3.2, 3.5.3.3, 3.5.3.4 and 3.5.3.5. The number of fruits and yield were also recorded.

#### *3.6.3.1 Fruit number*

The fruit number of each plant were recorded at the time of harvest.

#### *3.6.3.2 Yield*

The yield of fruits were recorded at the time of harvest to determine the yield per plant.

## 3.6 STATISTICAL ANALYSIS

The recorded data were statistically analysed using MSTAT package (Freed, 1986) and the different treatments were subjected to DMRT. The percent wilt incidence data were transformed using the transformation procedure available in the package.

# RESULTS

# 4. RESULTS

The studies on "Biocontrol of bacterial wilt in tomato using arbuscular mycorrhizal fungi" were studied at the College of Horticulture, during the period 2000-2002. The soil samples from high and low wilt incidence areas of Thrissur and Palakkad districts were collected and the AMF were isolated and identified. The native AMF alongwith the commercial culture was screened under sterile and unsterile pot culture conditions to test their efficacy in reducing the infection of bacterial wilt pathogen. The efficacy of the selected AMF isolates under field conditions were also carried out. The results obtained during the studies are given below.

## 4.1 ANALYSIS OF RHIZOSPHERE SOIL SAMPLES FROM HIGH AND LOW WILT INCIDENCE AREAS

### 4.1.1 Soil pH

The soil samples collected from tomato and maize rhizospheres of low wilt areas of Ozhalapathy and Eruthiampathy had pH of 6.3 - 7.1. Whereas, the soil samples of high wilt (Vellanikkara) areas were acidic in nature and the rhizosphere soil of maize from Vellanikkara recorded the lowest pH of 5.2 (Table 4.1). The soil pH from Ozhalapathy area recorded a pH > 6.0. However, the Vellanikkara soils recorded a pH ranging between 5.2 - 6.5. The rhizosphere soil from BT-1 tomato variety (highly susceptible) recorded the highest pH of 6.5 at Vellanikkara.

### 4.1.2 Population of *Ralstonia solanacearum*

The population of *R. solanacearum* was the lowest (1 x $10^4$ cfu $g^{-1}$ soil) in the case of rhizosphere soil of tomato from Ozhalapathy, followed by rhizosphere soil of Mukthi variety of tomato from Vellanikkara (2 x $10^4$ cfu $g^{-1}$ soil). The highest population of *R. solanacearum* (14 x $10^4$ cfu $g^{-1}$ soil) was recorded in the case of rhizosphere soil of tomato from Eruthiampathy (Table 4.1).

**Table 4.1 Soil pH and *Ralstonia solanacearum* population in the soil samples collected from high and low wilt incidence areas**

| Sl. No: | Location | Host | pH | *Ralstonia solanacearum* population ($10^4$ cfu $g^{-1}$ soil) |
|---|---|---|---|---|
| 1 | Ozhalapathy (low wilt) – (OT) | Tomato | 6.3 | 1 |
| 2 | Eruthiampathy (low wilt) – (ER) | Tomato | 7.1 | 18 |
| 3 | Ozhalapathy (low wilt) – (OM) | Maize | 6.8 | 11 |
| 4 | Vellanikkara (high wilt) – (VTM) | Tomato (Mukthi- moderately resistant variety) | 5.6 | 2 |
| 5 | Vellanikkara (high wilt) – (VBT) | Tomato (BT-1 - Susceptible variety) | 6.5 | 14 |
| 6 | Vellanikkara (high wilt) – (VM) | Maize | 5.2 | 14 |

Note: Each value represents an average of three replications

### 4.1.3 Soil nutrient status

The available nitrogen content was highest (360.64 kg $ha^{-1}$) for the rhizosphere soil of tomato from Ozhalapathy followed by the rhizosphere soil of tomato from Eruthiampathy (337.12 kg $ha^{-1}$). The lowest available nitrogen status (176.64 kg $ha^{-1}$) was recorded for the rhizosphere soil of maize from Ozhalapathy (Table 4.2). In the case of available P, the rhizosphere soil of Mukthi tomato variety recorded the highest (19.78 kg $ha^{-1}$) followed by rhizosphere soil of tomato from Ozhalapathy (14.25 kg $ha^{-1}$). The lowest available P content (1.99 kg $ha^{-1}$) was recorded for the rhizosphere soil of maize from Ozhalapathy followed by the rhizosphere soil of tomato from Eruthiampathy (4.66 kg $ha^{-1}$). The available K content was maximum (727.74 kg $ha^{-1}$) for the maize rhizosphere soil from Ozhalapathy. The available K content of Vellanikkara soils were generally low and the maize rhizosphere soil from Vellanikkara recorded the lowest (78.4 kg $ha^{-1}$).

The available Ca status of Ozhalapathy and Eruthiampathy soil were generally higher when compared to the Vellanikkara soil. The highest available calcium (10.2 meq. $L^{-1}$) in soil was recorded for the tomato rhizosphere soil from Eruthiampathy followed by maize rhizosphere soil from Ozhalapathy (8.4 meq. $L^{-1}$). The available calcium was lowest (4.4 meq. $L^{-1}$) for the tomato rhizosphere soil from Ozhalapathy followed by the rhizosphere soil of the tomato variety BT-1 from Vellanikkara (4.8 meq. $L^{-1}$).

The Mg content also recorded the same pattern as was seen in the case of calcium with the Ozhalapathy and Eruthiampathy soils recording a higher magnesium content when compared to Vellanikkara soils. The highest available magnesium (2.6 meq. $L^{-1}$) was recorded for the maize rhizosphere soil from Ozhalapathy followed by maize rhizosphere soil from Vellanikkara (2.2 meq. $L^{-1}$). The lowest magnesium content was recorded for the rhizosphere soil of the tomato variety Mukthi from Vellanikkara (1.2 meq. $L^{-1}$) followed by tomato rhizosphere soil from Ozhalapathy (1.6 meq. $L^{-1}$). The rhizosphere soils of tomato from Eruthiampathy and that of the tomato variety BT-1 from Vellanikkara recorded the same magnesium status (2.0 meq. $L^{-1}$).

**Table 4.2 Soil nutrient status of high and low wilt incidence areas**

| Sl. No. | Soil sample | N (kg $ha^{-1}$) | P (kg $ha^{-1}$) | K (kg $ha^{-1}$) | Ca (meq.$L^{-1}$) | Mg (meq. $L^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | Ozhalapathy (OT) | 360.64 | 14.25 | 597.12 | 4.4 | 1.6 |
| 2 | Eruthiampathy (ER) | 337.12 | 4.66 | 653.10 | 10.2 | 2.0 |
| 3 | Ozhalapathy (OM) | 176.40 | 1.99 | 727.74 | 8.4 | 2.6 |
| 4 | Vellanikkara (VTM) | 320.32 | 19.78 | 197.12 | 5.0 | 1.2 |
| 5 | Vellanikkara (VBT) | 284.48 | 7.28 | 197.12 | 4.8 | 2.0 |
| 6 | Vellanikkara (VM) | 197.12 | 5.38 | 78.40 | 5.6 | 2.2 |

### 4.1.4 AM fungal spore count

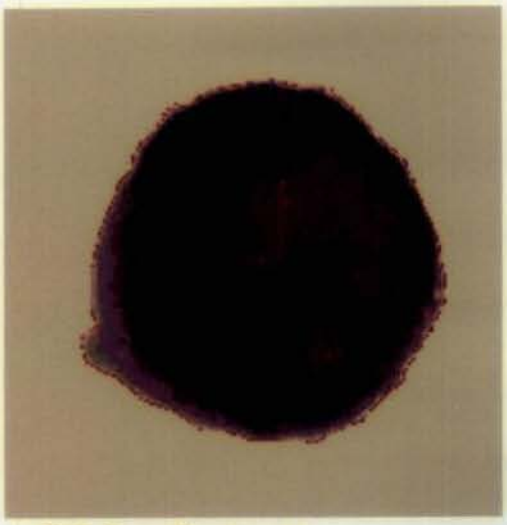
The total AM fungal spore count (48 10g $^{-1}$ soil) was highest for the maize rhizosphere soil from Ozhalapathy and the lowest (28 10g $^{-1}$ soil) was recorded for the rhizosphere soil of the tomato variety BT-1 (wilt susceptible) from Vellanikkara and the rhizosphere soil of tomato from Eruthiampathy (Table 4.3). The maize rhizosphere soil from Vellanikkara recorded a spore count of 40 10g $^{-1}$ and that from Ozhalapathy recorded a spore count of 48 10g $^{-1}$ soil. The rhizosphere soil from Mukthi tomato variety showed a spore count of 42 10g $^{-1}$ soil.

## 4.2 IDENTIFICATION OF AM FUNGI

The AM fungal spores in each rhizosphere soil sample were identified by their colour, shape, dimension, surface configuration, wall layers, number of hyphae, alignment of hyphae and form of hyphae and compared with synoptic keys (Trappe, 1982) (Table 4.4). The tomato rhizosphere soil from Ozhalapathy had AMF spores belonging to *Glomus* sp., and *Acaulospora* sp. The most predominant AMF spore was identified as *Glomus* sp. with a spore count of 30 10g $^{-1}$ soil (Plate 3a). It was globose, brownish black in colour with dimensions ranging between 50-60 µm and a smooth surface configuration at maturity. The crushed spores had a single wall layer. There was single, straight, cylindrical, small, aseptate hypha that was aligned straight with the spore axis.

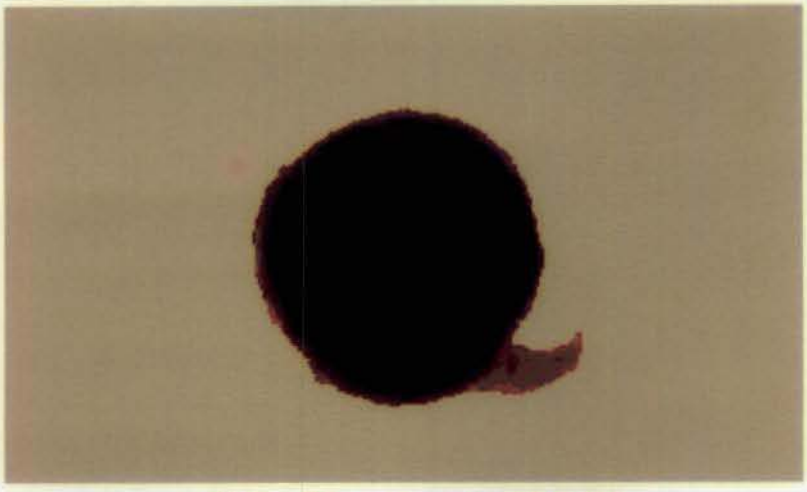
The AM fungal spores belonging to *Glomus* sp. and *Acaulospora* sp. were recorded from the tomato rhizosphere soil from Eruthiampathy. *Glomus* sp. with a spore count of 23 10g $^{-1}$ soil was the most predominant AMF spore. It was globose, brownish black in colour with dimension ranging between 40-60 µm. The surface configuration at maturity was smooth and the crushed spores had a single wall layer. The hypha was absent.

The maize rhizosphere from Ozhalapathy recorded the presence of AMF spores of *Glomus* sp. which had a spore count of 42 10g $^{-1}$soil (Plate 3b). It was ovoid, brownish

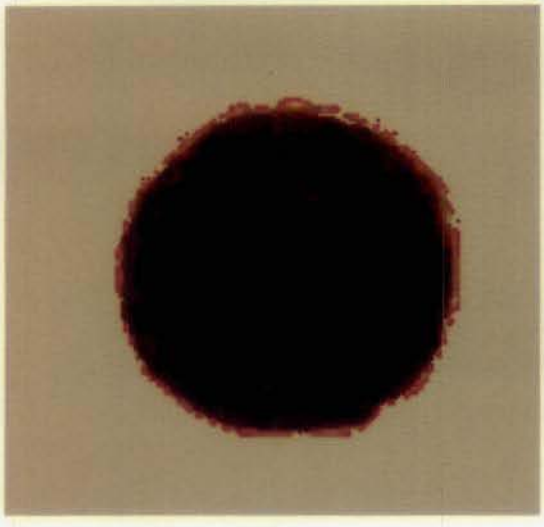


a. Ozhalapathy tomato

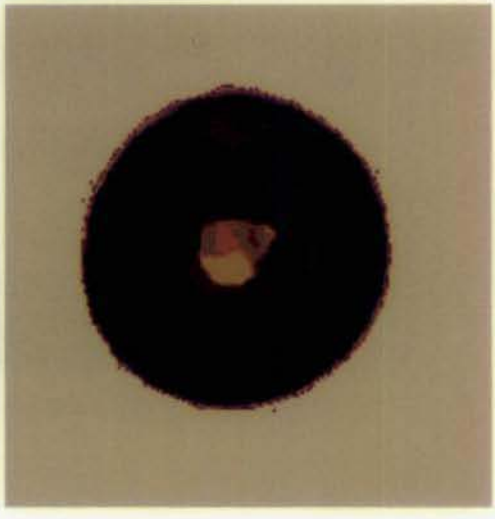
b. Ozhalapathy maize



c. Vellanikkara tomato (Mukthi)



d. Vellanikkara BT-1 tomato



e. Tamil Nadu commercial culture

Plate 3. Predominant AMF spores in different locations

**Table 4.3 Total AM fungal spore count in rhizosphere soils of high and low wilt incidence areas**

| Sl.No | Location | Host | Total spores $10g^{-1}$ of soil |
|---|---|---|---|
| 1 | Ozhalapathy (low wilt) OT | Tomato | 35 |
| 2 | Eruthiampathy (low wilt) – ER | Tomato | 28 |
| 3 | Ozhalapthy (low wilt) – OM | Maize | 48 |
| 4 | Vellanikkara(high wilt) – VTM | Tomato | 42 |
| 5 | Vellanikkara (high wilt) - VBT | Tomato | 28 |
| 6 | Vellanikkara (high wilt) - VM | Maize | 40 |

**Table 4.4 Morphological characters and identification of AM fungal spores isolated from high and low wilt incidence areas**

| Sl. No | Location | Colour | Shape | Dimension (µm) | Surface configuration at maturity | Wall layers (crushed spore) | No. of hyphae attached to the spore | Alignment of hyphae with spore axis | Form of hyphae | Spore count $10g^{-1}$ soil | AMF Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ozhalapathy Tomato (OT) | Brown | Globose | 50-70 | Smooth | Single | Single | Straight | aseptate, constricted at point of attachment | 1 | *Glomus* sp. |
| | | Black | Globose | 40-70 | Smooth | Single | Absent | - | - | 1 | *Acaulospora* sp. |
| | | Brownish black | Globose | 50-60 | Smooth | Single | Single | Straight | straight, cylindrical, small aseptate | 30 | *Glomus* sp.* |
| | | Yellowish brown | Globose | 70-80 | Smooth | Single | Absent | - | - | 1 | *Glomus* spp. |
| | | Yellow | Ovoid | 50-60 | Smooth | Single | Single | Straight | aseptate, bulbous base | 1 | *Glomus* sp. |
| | | Light brown to yellow | Globose | 40-50 | Smooth | Single | Absent | - | - | 1 | *Glomus* sp. |
| 2 | Eruthiampathy Tomato (ER) | Brownish black | Globose | 40-60 | Smooth | Single | Absent | - | - | 23 | *Glomus* sp.* |
| | | Brown | Globose | 40-60 | Smooth | Single | Absent | - | - | 1 | *Glomus* sp. |
| | | Black | Globose | 30-50 | Smooth | Single | Absent | - | - | 1 | *Acaulospora* sp. |
| | | Light brown | Globose | 40-60 | Smooth | Single | Single | Straight | straight, aseptate, small | 2 | *Glomus* sp. |
| | | Yellow | Ovoid | 30-50 | Smooth | Single | Absent | | - | 1 | *Glomus* sp. |

| Sl. No | Location | Colour | Shape | Dimension (µm) | Surface configuration at maturity | Wall layers (crushed spore) | No. of hyphae attached to the spore | Alignment of hyphae with spore axis | Form of hyphae | Spore count $10g^{-1}$ soil | AMF Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | Ozhalapathy Maize (OM) | Brown | Globose | 40-60 | Smooth | Single | Single | Straight | straight, cylindrical, small aseptate | 2 | *Glomus* sp. |
| | | Brownish black | Ovoid | 50-70 | Smooth | Single | Absent | - | - | 42 | *Glomus* sp. |
| | | Yellow | Ovoid | 40-60 | Smooth | Single | Absent | - | - | 1 | *Glomus* sp. |
| | | Light brown | Globose | 40-60 | Smooth | Single | Absent | - | - | 1 | *Glomus* sp. |
| | | Black | Irregula | 40-60 | Smooth | Single | Absent | - | - | 2 | *Glomus* sp |
| 4 | Vellanikkara Mukthi Tomato (VTM) | Black | Globose | 40-60 | Smooth | Single | Single | Straight | aseptate straight, cylindrical, small | 2 | *Scutellospora* sp |
| | | Brown | Globose | 50-70 | Smooth | Single | Single | Straight | aseptate straight, cylindrical, small | 1 | *Glomus* sp. |
| | | Brownish black | Globose | 60-70 | Smooth | Single | Single | Straight | straight, aseptate cylindrical, small | 35 | *Glomus* sp. |
| | | Yellow | Globose | 50-60 | Smooth | Single | Single | Straight | aseptate straight, cylindrical, small | 2 | *Glomus* sp |
| | | Light brown | Ovoid | 20-30 | Smooth | Single | Single | Straight | aseptate small, straight | 2 | *Glomus* sp |

| Sl. No | Location | Colour | Shape | Dimension (µm) | Surface configuration at maturity | Wall layers (crushed spore) | No. of hyphae attached to the spore | Alignment of hyphae with spore axis | Form of hyphae | Spore count $10g^{-1}$ soil | AMF Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | Vellanikkara BT-1 Tomato (VBT) | Brown | Globose | 60-70 | Smooth | Single | Single | Straight | straight, cylindrical aseptate, small | 1 | *Glomus* sp. |
| | | Brownish black | Ovoid | 50-60 | Smooth | Single | Absent | - | - | 25 | *Glomus* sp.* |
| | | Yellow | Globose | 40-60 | Smooth | Single | Absent | - | - | 2 | *Glomus* sp. |
| 6 | Vellanikkara Maize (VM) | Brownish black | Globose | 50-70 | Smooth | Single | Single | Straight | aseptate straight, cylindrical, small | 34 | *Glomus* sp.* |
| | | Brown | Globose | 40-60 | Smooth | Single | Absent | - | - | 2 | *Glomus* sp. |
| | | Light brown | Globose | 50-70 | Smooth | Single | Single | Straight | aseptate straight, cylindrical, small | 1 | *Glomus* sp. |
| | | Black | Ovoid | 30-50 | Smooth | Single | Absent | - | - | 1 | *Glomus* sp. |
| | | Yellow | Globose | 20-40 | Smooth | Single | Single | Straight | aseptate straight, cylindrical, small | 2 | *Glomus* sp. |

Note: (-) means hypha is absent

* - indicates the most predominant AMF

black in colour with dimension ranging between 50-70 µm, a smooth surface configuration and a single wall layer. The hypha was absent.

*Sclerocystis* sp. and *Glomus* sp. were the AMF spores recorded from the rhizosphere soil of the tomato variety Mukthi from Vellanikkara. *Glomus* sp. with a spore count of 35 $10g^{-1}$ soil was the most predominant AM fungal spore (Plate 3c). It was globose in shape, brownish black in colour with dimension ranging between 60-70 µm and a smooth surface configuration at maturity. The crushed spores had only a single wall layer. The hypha was single, straight, cylindrical, small and aseptate with its alignment being straight with the spore axis.

The AM fungal spores belonging to *Glomus* sp. were recorded in the rhizosphere soil of the tomato variety BT-1 from Vellanikkara (Plate 3d). They had a spore count of 25 $10g^{-1}$ soil. It was globose, brownish black in colour with dimension ranging between 50-60 µm. The surface configuration at maturity was smooth and the crushed spores had a single wall layer. The hypha was absent.

The maize rhizosphere soil from Vellanikkara recorded the presence of *Glomus* sp. with a spore count of 34 $10g^{-1}$ soil. The *Glomus* sp. spores were globose, brownish black in colour with a dimension ranging between 50-70 µm. The surface configuration at maturity was smooth and the crushed spores had a single wall layer. The hypha was single, straight, small, cylindrical and aseptate with its alignment being straight with the spore axis.

## 4.3 SCREENING OF DIFFERENT PREDOMINANT AMF CULTURES AGAINST *R. SOLANACEARUM*

The screening of different AMF cultures against *R. solanacearum* under pot culture using sterile potting mixture were carried out using the six predominant native isolates individually and in combinations along with one commercial culture.

### 4.3.1 Per cent AMF root colonization

The per cent root colonization for the treatments ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) and ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) was highest (50 per cent) followed by ($T_6$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and commercial culture (TN) which recorded 30 per cent root colonization. The control plants recorded only 10 per cent root colonization (Table 4.5).

### 4.3.2 AMF spore count

The spore count was found to be highest in the case of ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (85 spores $10g^{-1}$ soil) followed by ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (80 spores $10g^{-1}$ soil). The control plants recorded the lowest spore count of 10 spores $10g^{-1}$ soil (Table 4.5).

### 4.3.3 Dry weight of plant

The treatments did not show any significant differences with respect to dry weight of the plant. The highest dry weight (9.13 g) was recorded in ($T_2$) *Glomus* sp. (OT)+ *Glomus* sp. (ER). However, the control plants ($T_{29}$) recorded the lowest dry weight of 2.16 g (Table 4.6).

### 4.3.4 Root length

There was no significant difference between the treatments with respect to root length. The highest root length (7.67 cm) was recorded in ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) and ($T_{20}$) *Glomus* sp. (VTM) + *Glomus* sp. (VBT) followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (6.5 cm) (Table 4.6). The treatment ($T_1$) *Glomus* sp. (OT) (2.67 cm) showed the lowest root length. The control plants ($T_{29}$) had a root length of 5 cm.

**Table 4.5 Effect of different species of *Glomus* on percent root colonization and rhizosphere spore count of tomato under sterile conditions**

| Sl. No. | Treatment | Per cent root colonization | Spore count $10g^{-1}$ of soil |
|---|---|---|---|
| 1 | $T_1$ (OT) | 10 | 51 |
| 2 | $T_2$ (OT + ER) | 50 | 85 |
| 3 | $T_3$ (OT + OM) | 10 | 53 |
| 4 | $T_4$ (OT + VTM) | 20 | 58 |
| 5 | $T_5$ (OT + VBT) | 10 | 67 |
| 6 | $T_6$ (OT + VM) | 30 | 68 |
| 7 | $T_7$ (OT + TN) | 20 | 60 |
| 8 | $T_8$ (ER) | 20 | 65 |
| 9 | $T_9$ (ER + OM) | 10 | 63 |
| 10 | $T_{10}$ (ER + VTM) | 10 | 61 |
| 11 | $T_{11}$ (ER + VBT) | 20 | 53 |
| 12 | $T_{12}$ (ER + VM) | 20 | 64 |
| 13 | $T_{13}$ (ER + TN) | 20 | 58 |
| 14 | $T_{14}$ (OM) | 10 | 58 |
| 15 | $T_{15}$ (OM + VTM) | 10 | 60 |
| 16 | $T_{16}$ (OM + VBT) | 50 | 80 |
| 17 | $T_{17}$ (OM + VM) | 10 | 63 |
| 18 | $T_{18}$ (OM + TN) | 10 | 57 |
| 19 | $T_{19}$ (VTM) | 10 | 55 |
| 20 | $T_{20}$ (VTM + VBT) | 10 | 58 |
| 21 | $T_{21}$ (VTM + VM) | 20 | 57 |
| 22 | $T_{22}$ (VTM + TN) | 20 | 53 |
| 23 | $T_{23}$ (VBT) | 10 | 63 |
| 24 | $T_{24}$ (VBT + VM) | 10 | 55 |
| 25 | $T_{25}$ (VBT + TN) | 20 | 61 |
| 26 | $T_{26}$ (VM) | 10 | 59 |
| 27 | $T_{27}$ (VM + TN) | 10 | 55 |
| 28 | $T_{28}$ (TN) | 30 | 56 |
| 29 | Control | 10 | 10 |

Note : Each value represents an average of 3 replication

OT-Ozhalapathy tomato, ER - Eruthiampathy tomato, OM - Ozhalapathy maize,

VTM- Vellanikkara Mukthi (tomato), VBT- Vellanikkara BT -1 (tomato),

VM- Vellanikkara maize, TN- Commercial culture

**Table 4.6 Effect of different species of *Glomus* on dry weight and root length of tomato under sterile conditions**

| Sl. No. | Treatment | Dry weight (g) | Root length (cm) |
|---|---|---|---|
| 1 | $T_1$ (OT) | 0.66 $^{b}$ | 2.67 $^{c}$ |
| 2 | $T_2$ (OT + ER) | 9.13 $^{a}$ | 6.50 $^{ab}$ |
| 3 | $T_3$ (OT + OM) | 2.46 $^{b}$ | 4.33 $^{abc}$ |
| 4 | $T_4$ (OT + VTM) | 4.92 $^{ab}$ | 5.83 $^{abc}$ |
| 5 | $T_5$ (OT + VBT) | 5.41 $^{ab}$ | 5.00 $^{abc}$ |
| 6 | $T_6$ (OT + VM) | 1.59 $^{b}$ | 3.17 $^{bc}$ |
| 7 | $T_7$ (OT + TN) | 4.38 $^{ab}$ | 5.17 $^{abc}$ |
| 8 | $T_8$ (ER) | 3.68 $^{ab}$ | 6.17 $^{abc}$ |
| 9 | $T_9$ (ER + OM) | 3.30 $^{ab}$ | 4.33 $^{abc}$ |
| 10 | $T_{10}$ (ER + VTM) | 2.58 $^{b}$ | 5.00 $^{abc}$ |
| 11 | $T_{11}$ (ER + VBT) | 4.96 $^{ab}$ | 4.67 $^{abc}$ |
| 12 | $T_{12}$ (ER + VM) | 1.98 $^{b}$ | 4.33 $^{abc}$ |
| 13 | $T_{13}$ (ER + TN) | 6.33 $^{ab}$ | 5.17 $^{abc}$ |
| 14 | $T_{14}$ (OM) | 1.30 $^{b}$ | 4.50 $^{abc}$ |
| 15 | $T_{15}$ (OM + VTM) | 4.31 $^{ab}$ | 6.17 $^{abc}$ |
| 16 | $T_{16}$ (OM + VBT) | 6.54 $^{ab}$ | 7.67 $^{a}$ |
| 17 | $T_{17}$ (OM + VM) | 3.01 $^{ab}$ | 4.83 $^{abc}$ |
| 18 | $T_{18}$ (OM + TN) | 2.72 $^{ab}$ | 5.00 $^{abc}$ |
| 19 | $T_{19}$ (VTM) | 3.42 $^{ab}$ | 3.67 $^{bc}$ |
| 20 | $T_{20}$ (VTM + VBT) | 2.09 $^{b}$ | 7.67 $^{a}$ |
| 21 | $T_{21}$ (VTM + VM) | 6.88 $^{ab}$ | 4.50 $^{abc}$ |
| 22 | $T_{22}$ (VTM + TN) | 3.83 $^{ab}$ | 4.17 $^{abc}$ |
| 23 | $T_{23}$ (VBT) | 4.78 $^{ab}$ | 4.17 $^{abc}$ |
| 24 | $T_{24}$ (VBT + VM) | 4.61 $^{ab}$ | 6.00 $^{abc}$ |
| 25 | $T_{25}$ (VBT + TN) | 3.59 $^{ab}$ | 5.33 $^{abc}$ |
| 26 | $T_{26}$ (VM) | 1.89 $^{b}$ | 3.17 $^{bc}$ |
| 27 | $T_{27}$ (VM + TN) | 0.71 $^{b}$ | 3.33 $^{bc}$ |
| 28 | $T_{28}$ (TN) | 1.23 $^{b}$ | 4.50 $^{abc}$ |
| 29 | Control | 2.16 $^{b}$ | 5.00 $^{abc}$ |

Note : Each value represents an average of 3 replication

OT-Ozhalapathy tomato, ER - Eruthiampathy tomato, OM - Ozhalapathy maize,

VTM- Vellanikkara Mukthi (Tomato), VBT - Vellanikkara BT - 1 (tomato),

VM- Vellanikkara maize, TN- Commercial culture

### 4.3.5 Number of days of plant survival

There was no significant differences among treatments with respect to the number of days of plant survival. The treatment ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the maximum number of days of plant survival (11 days) followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (10.83 days). The minimum number of days of plant survival (4.5 days) was recorded in the case of ($T_1$) *Glomus* sp. (OT). The control plants ($T_{29}$) survived for 8 days (Table 4.7).

### 4.3.6 Percent wilt incidence

There was no significant difference between the treatments for percent wilt incidence. The treatments ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT), ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) and ($T_6$) *Glomus* sp. (OT)+ *Glomus* sp. (VM) recorded the lowest (83.33 per cent) percent wilt incidence (Table 4.7). All the other treatments, including control ($T_{29}$), recorded 100 percent wilt incidence.

### 4.3.7 Selection of most effective AMF cultures against *R. solanacearum*

The AMF cultures ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) and ($T_{20}$) *Glomus* sp. (VTM) + *Glomus* sp. (VBT) recorded the maximum root length (7.67 cm). The lowest root length was recorded for ($T_1$) *Glomus* sp. (OT) (2.67 cm). The AMF culture ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded the maximum dry weight (9.13 g) and the lowest dry weight was with ($T_1$) *Glomus* sp. (OT) (0.66 g). The maximum number of days of plant survival was recorded in ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (11.00 days). The AMF cultures ($T_1$) *Glomus* sp. (OT) recorded minimum number of days of plant survival (4.5 days). The treatments ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER), ($T_6$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and ($T_{16}$) *Glomus* sp. (OM)+ *Glomus* sp. (VBT) recorded the lowest percent wilt incidence (83.33 per cent) (Table 4.8). Based on the dry weight of plant, root length, number of days of plant survival and percent wilt incidence the AMF cultures ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER), ($T_6$) *Glomus* sp. (OT)+ *Glomus* sp. (VM) and ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) were selected for the further studies (Table 4.8).

**Table 4.7 Effect of different species of *Glomus* on number of days of plant survival and percent wilt incidence under sterile conditions**

| Sl. No. | Treatment | Number of days of plant survival | Percent wilt incidence |
|---|---|---|---|
| 1 | $T_1$ (OT) | 4.50 $^{c}$ | 100.0 $^{a}$ (1.37$^{a}$) |
| 2 | $T_2$ (OT + ER) | 10.83 $^{a}$ | 83.33 $^{a}$ (1.17$^{a}$) |
| 3 | $T_3$ (OT + OM) | 6.67 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 4 | $T_4$ (OTVTM) | 8.17 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 5 | $T_5$ (OT + VBT) | 8.00 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 6 | $T_6$ (OT + VM) | 9.33 $^{ab}$ | 83.33 $^{a}$ (1.17 $^{a}$) |
| 7 | $T_7$ (OT + TN) | 9.83 $^{ab}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 8 | $T_8$ (ER) | 8.50 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 9 | $T_9$ (ER + OM) | 6.83 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 10 | $T_{10}$ (ER + VTM) | 8.00 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 11 | $T_{11}$ (ER + VBT) | 9.33 $^{ab}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 12 | $T_{12}$ (ER + VM) | 8.83 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 13 | $T_{13}$ (ER + TN) | 10.00 $^{ab}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 14 | $T_{14}$ (OM) | 5.83 $^{bc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 15 | $T_{15}$ (OM + VTM) | 7.83 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 16 | $T_{16}$ (OM + VBT) | 11.00 $^{a}$ | 83.33 $^{a}$ (1.17 $^{a}$) |
| 17 | $T_{17}$ (OM + VM) | 7.00 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 18 | $T_{18}$ (OM + TN) | 7.83 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 19 | $T_{19}$ (VTM) | 8.17 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 20 | $T_{20}$ (VTM + VBT) | 7.00 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 21 | $T_{21}$ (VTM + VM) | 8.33 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 22 | $T_{22}$ (VTM + TN) | 9.17 $^{ab}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 23 | $T_{23}$ (VBT) | 7.17 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 24 | $T_{24}$ (VBT + VM) | 7.50 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 25 | $T_{25}$ (VBT + TN) | 8.33 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 26 | $T_{26}$ (VM) | 5.50 $^{bc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 27 | $T_{27}$ (VM + TN) | 6.17 $^{bc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 28 | $T_{28}$ (TN) | 6.17 $^{bc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |
| 29 | Control | 8.00 $^{abc}$ | 100.0 $^{a}$ (1.37 $^{a}$) |

Note : Each value represents an average of 3 replication

OT-Ozhalapathy tomato, ER – Eruthiampathy tomato, OM – Ozhalapathy maize,

VTM- Vellanikkara Mukthi (Tomato), VBT- Vellanikkara BT –1 (tomato),

VM- Vellanikkara maize, TN- Commercial culture

Values in parantheses represent transformed data

**Table 4.8 Effect of different species of *Glomus* on dry weight, root length, number of days of plant survival and percent wilt incidence under sterile conditions**

| Sl. No. | Treatment | Dry weight (g) | Root length (cm) | No. of days of plant survival | Percent wilt incidence |
|---|---|---|---|---|---|
| 1 | $T_1$ (OT) | 0.66 [b] | 2.67 | 4.50 [c] | 100.0 [a] (1.37[a]) |
| 2 | $T_2$ (OT + ER) | 9.13 [a] | 6.50 [ab] | 10.83 [a] | 83.33 [a] (1.17[a]) |
| 3 | $T_3$ (OT + OM) | 2.16 [b] | 4.33 [abc] | 6.67 [abc] | 100.0 [a] (1.37[a]) |
| 4 | $T_4$ (OT + VTM) | 4.92 [ab] | 5.83 [abc] | 8.17 [abc] | 100.0 [a] (1.37[a]) |
| 5 | $T_5$ (OT + VBT) | 5.41 [ab] | 5.00 [abc] | 8.00 [abc] | 100.0 [a] (1.37[a]) |
| 6 | $T_6$ (OT + VM) | 1.59 [b] | 3.17 [bc] | 9.33 [ab] | 83.33 [a] (1.17[a]) |
| 7 | $T_7$ (OT + TN) | 4.38 [ab] | 5.17 [abc] | 9.83 [ab] | 100.0 [a] (1.37[a]) |
| 8 | $T_8$ (ER) | 3.68 [ab] | 6.17 [abc] | 8.50 [abc] | 100.0 [a] (1.37[a]) |
| 9 | $T_9$ (ER + OM) | 3.30 [ab] | 4.33 [abc] | 6.83 [abc] | 100.0 [a] (1.37[a]) |
| 10 | $T_{10}$ (ER + VTM) | 2.58 [b] | 5.00 [abc] | 8.00 [abc] | 100.0 [a] (1.37[a]) |
| 11 | $T_{11}$ (ER + VBT) | 4.96 [ab] | 4.67 [abc] | 9.33 [ab] | 100.0 [a] (1.37[a]) |
| 12 | $T_{12}$ (ER + VM) | 1.98 [b] | 4.33 [abc] | 8.83 [abc] | 100.0 [a] (1.37[a]) |
| 13 | $T_{13}$ (ER + TN) | 6.33 [ab] | 5.17 [abc] | 10.00 [ab] | 100.0 [a] (1.37[a]) |
| 14 | $T_{14}$ (OM) | 1.30 [b] | 4.50 [abc] | 5.83 [bc] | 100.0 [a] (1.37[a]) |
| 15 | $T_{15}$ (OM + VTM) | 4.31 [ab] | 6.17 [abc] | 7.83 [abc] | 100.0 [a] (1.37[a]) |
| 16 | $T_{16}$ (OM + VBT) | 6.54 [ab] | 7.67 [a] | 11.00 [a] | 83.33 [a] (1.17[a]) |
| 17 | $T_{17}$ (OM + VM) | 3.01 [ab] | 4.83 [abc] | 7.00 [abc] | 100.0 [a] (1.37[a]) |
| 18 | $T_{18}$ (OM + TN) | 2.72 [ab] | 5.00 [abc] | 7.83 [abc] | 100.0 [a] (1.37[a]) |
| 19 | $T_{19}$ (VTM) | 3.42 [ab] | 3.67 [bc] | 8.17 [abc] | 100.0 [a] (1.37[a]) |
| 20 | $T_{20}$ (VTM + VBT) | 2.09 [b] | 7.67 [a] | 7.00 [abc] | 100.0 [a] (1.37[a]) |
| 21 | $T_{21}$ (VTM + VM) | 6.88 [ab] | 4.50 [abc] | 8.33 [abc] | 100.0 [a] (1.37[a]) |
| 22 | $T_{22}$ (VTM + TN) | 3.83 [ab] | 4.17 [abc] | 9.17 [ab] | 100.0 [a] (1.37[a]) |
| 23 | $T_{23}$ (VBT) | 4.78 [ab] | 4.17 [abc] | 7.17 [abc] | 100.0 [a] (1.37[a]) |
| 24 | $T_{24}$ (VBT + VM) | 4.61 [ab] | 6.00 [abc] | 7.50 [abc] | 100.0 [a] (1.37[a]) |
| 25 | $T_{25}$ (VBT + TN) | 3.59 [ab] | 5.33 [abc] | 8.33 [abc] | 100.0 [a] (1.37[a]) |
| 26 | $T_{26}$ (VM) | 1.89 [b] | 3.17 [bc] | 5.50 [bc] | 100.0 [a] (1.37[a]) |
| 27 | $T_{27}$ (VM + TN) | 0.71 [b] | 3.33 [bc] | 6.17 [bc] | 100.0 [a] (1.37[a]) |
| 28 | $T_{28}$ (TN) | 1.23 [b] | 4.50 [abc] | 6.17 [bc] | 100.0 [a] (1.37[a]) |
| 29 | Control | 2.16 [b] | 5.00 [abc] | 8.00 [abc] | 100.0 [a] (1.37[a]) |

Note : Each value represents an average of 3 replication

OT-Ozhalapathy tomato, ER Eruthiampathy tomato, OM Ozhalapathy maize,

VTM- Vellanikkara Mukthi (tomato), VBT Vellanikkara B T-1 (tomato),

VM- Vellanikkara maize, TN- Commercial culture

Values in parantheses represent transformed data

## 4.4 OPTIMUM INOCULATION TIME AND INOCULUM DENSITY OF AMF FOR THE CONTROL OF BACTERIAL WILT

The optimum time for AMF inoculation and the optimum inoculum density for the control of bacterial wilt in tomato under pot culture conditions were carried out using the three standard commercial species *of Glomus viz., G. mosseae, G. fasciculatum* and *G. intraradices* at the time of sowing, 15 days before transplanting and at the time of transplanting @ 25g $kg^{-1}$ soil, 50g $kg^{-1}$ soil and 75g $kg^{-1}$ soil respectively.

### 4.4.1 AMF inoculation at the time of sowing

*4.4.1.1 Dry weight of plant*

There was no significant difference between the treatments for dry weight of the plant at the time of sowing. The maximum dry weight was recorded by AMF inoculation ($T_7$) @ 25g $kg^{-1}$ (2.73 g) followed by inoculum density ($T_2$) @ 50g $kg^{-1}$ (2.31 g). However, control plants ($T_{10}$) recorded a dry weight of 0.93g (Table 4.9).

*4.4.1.2 Root length*

The treatments showed no significant differences for the root length due to AMF inoculation at the time of sowing. The maximum root length was recorded with inoculum density ($T_2$) @ 50g $kg^{-1}$ (5.44 cm) followed by AMF ($T_4$) @ 25g $kg^{-1}$ (4.78 cm). The control plants ($T_{10}$) recorded a root length of 4.67cm (Table 4.9).

*4.4.1.3 Number of days of plant survival*

The treatments showed no significant differences for the number of days of plant survival. The AMF inoculation ($T_9$) @ 75g $kg^{-1}$ recorded the maximum number of days (53.22 days) of plant survival followed by inoculum density ($T_1$) @ 25g $kg^{-1}$ (47.89 days). The control plants ($T_{10}$) survived for 16 days (Table 4.9).

**Table 4.9 Effect of different species of *Glomus* at the time of sowing on dry weight, root length, number of days of plant survival and percent wilt incidence under wilt-sick soil**

| Treatment | *Glomus* sp. | Dry weight (g) | Root length (cm) | Number of days of plant survival | Percent wilt incidence |
|---|---|---|---|---|---|
| $T_1$ | *Gm* ($Id_1$) | $0.147^{b}$ | $2.56^{ab}$ | $47.89^{a}$ | $66.66\ (0.95)^{b}$ |
| $T_2$ | *Gm* ($Id_2$) | $2.31^{ab}$ | $5.44^{a}$ | $34.89^{ab}$ | $100.0\ (1.40)^{a}$ |
| $T_3$ | *Gm* ($Id_3$) | $1.70^{ab}$ | $3.78^{ab}$ | $32.22^{ab}$ | $66.66\ (0.95)^{b}$ |
| $T_4$ | *Gf* ($Id_1$) | $0.32^{ab}$ | $4.78^{ab}$ | $6.55^{b}$ | $100.0\ (1.40)^{a}$ |
| $T_5$ | *Gf* ($Id_2$) | $0.10^{b}$ | $2.00^{b}$ | $44.22^{ab}$ | $66.66\ (0.95)^{b}$ |
| $T_6$ | *Gf* ($Id_3$) | $0.64^{ab}$ | $4.11^{ab}$ | $39.00^{ab}$ | $77.77\ (1.14)^{ab}$ |
| $T_7$ | *Gi* ($Id_1$) | $2.73^{a}$ | $5.00^{ab}$ | $23.55^{ab}$ | $88.88\ (1.25)^{ab}$ |
| $T_8$ | *Gi* ($Id_2$) | $2.08^{ab}$ | $4.67^{ab}$ | $40.34^{ab}$ | $88.88\ (1.25)^{ab}$ |
| $T_9$ | *Gi* ($Id_3$) | $1.61^{ab}$ | $4.00^{ab}$ | $53.22^{a}$ | $100.0\ (1.40)^{a}$ |
| $T_{10}$ | Control | $0.93^{ab}$ | $4.67^{ab}$ | $16.00^{ab}$ | $100.0\ (1.40)^{a}$ |

Note : Each value represents an average of 3 replications with 3 plants in each replication

Gm - *Glomus mosseae*, Gf- *Glomus fasciculatum*, Gi- *Glomus intraradices*

$Id_1$ - 25g $kg^{-1}$ soil, $Id_2$ - 50g $kg^{-1}$ soil, $Id_3$ - 75g $kg^{-1}$ soil

Values in paranthese represent transformed data

*4.4.1.4 Percent wilt incidence*

The treatments showed significant differences for percent wilt incidence. The inoculum density ($T_1$) @ 25g $kg^{-1}$, ($T_3$) @ 50g $kg^{-1}$ and ($T_5$) @ 75g $kg^{-1}$ recorded the minimum percent wilt incidence (66 per cent). However, control plants ($T_{10}$) recorded the maximum (100 per cent) percent wilt incidence (Table 4.9).

### 4.4.2 AMF inoculation at 15 days before transplanting

*4.4.2.1 Dry weight of the plant*

The treatments showed significant differences for dry weight of the plant inoculated with AMF at 15 days before transplanting. The AMF inoculation ($T_5$) @ 50g $kg^{-1}$ recorded the maximum dry weight (6.74 g). The control plants ($T_{10}$) recorded a dry weight of 3.07 g (Table 4.10).

*4.4.2.2 Root length*

The root length recorded significant differences among the treatments for AMF inoculation at 15 days before transplanting. The inoculum density ($T_3$) @ 75g $kg^{-1}$ (7.88 cm) recorded the maximum root length followed by inoculum density ($T_5$) @ 50 g $kg^{-1}$ (7.67 cm). The control plants ($T_{10}$) recorded a root length of 7 cm (Table 4.10).

*4.4.2.3 Number of days of plant survival*

The treatments recorded significant differences for the number of days of plant survival. The AMF inoculation ($T_6$) @ 75g $kg^{-1}$ recorded the maximum number of days (105.8 days) of plant survival followed by inoculum density ($T_8$) @ 50g $kg^{-1}$ (104.1 days). In the control, plants ($T_{10}$) survived only for a period of 54.3 days (Table 4.10).

*4.4.2.4 Percent wilt incidence*

The percent wilt incidence recorded significant differences between treatments at 15 days before transplanting. The AMF inoculation ($T_7$) @ 25g $kg^{-1}$, ($T_8$) @50g $kg^{-1}$ and

**Table 4.10 Effect of different species of *Glomus* 15 days before transplanting on dry weight, root length, number of days of plant survival and percent wilt incidence under wilt sick soil**

| Treatment | *Glomus* sp. | Dry weight (g) | Root length (cm) | Number of days of plant survival | Percent wilt incidence |
|---|---|---|---|---|---|
| $T_1$ | *Gm* ($Id_1$) | $3.68^{ab}$ | $6.55^{ab}$ | $64.89^{ab}$ | $77.77(1.14)^{ab}$ |
| $T_2$ | *Gm* ($Id_2$) | $4.43^{ab}$ | $7.33^{ab}$ | $67.78^{ab}$ | $77.77(1.14)^{ab}$ |
| $T_3$ | *Gm* ($Id_3$) | $4.54^{ab}$ | $7.88^{a}$ | $49.67^{b}$ | $88.88(1.25)^{ab}$ |
| $T_4$ | *Gf* ($Id_1$) | $2.82^{ab}$ | $6.89^{ab}$ | $58.22^{ab}$ | $77.77\ (1.14)^{ab}$ |
| $T_5$ | *Gf* ($Id_2$) | $6.74^{a}$ | $7.67^{ab}$ | $63.33^{ab}$ | $77.77\ (1.10)^{ab}$ |
| $T_6$ | *Gf* ($Id_3$) | $0.81^{ab}$ | $1.55^{ab}$ | $105.8^{a}$ | $22.22\ (0.46)^{b}$ |
| $T_7$ | *Gi* ($Id_1$) | $0.70^{ab}$ | $1.33^{b}$ | $95.78^{ab}$ | $22.22\ (0.46)^{b}$ |
| $T_8$ | *Gi* ($Id_2$) | $0.80^{ab}$ | $1.77^{ab}$ | $104.1^{ab}$ | $22.22\ (0.46)^{b}$ |
| $T_9$ | *Gi* ($Id_3$) | $2.94^{ab}$ | $4.77^{ab}$ | $67.11^{ab}$ | $77.77\ (1.10)^{ab}$ |
| $T_{10}$ | Control | $3.07^{ab}$ | $7.00^{ab}$ | $54.30^{ab}$ | $100.00\ (1.40)^{a}$ |

Note : Each value represents an average of 3 replications with 3 plants in each replication

Gm – *Glomus mosseae*, Gf- *Glomus fasciculatum*, Gi- *Glomus intraradices*

$Id_1$ – 25g $kg^{-1}$ soil, $Id_2$ – 50g $kg^{-1}$ soil, $Id_3$ – 75g $kg^{-1}$ soil

Values in paranthesesrepresent transformed data

($T_6$) @ 75g $kg^{-1}$ were on par and recorded the minimum bacterial wilt index (22 per cent). The control plants ($T_{10}$) alone recorded 100 percent wilt incidence (Table 4.10).

### 4.4.3 AMF inoculation at the time of transplanting

*4.4.3.1 Dry weight of the plant*

The treatments recorded significant differences for dry weight of the plant at the time of transplanting. The control plants ($T_{10}$) recorded the highest dry weight (4.49 g). The minimum dry weight (0.16 g) was recorded with AMF inoculation ($T_4$) @ 25g $kg^{-1}$ (Table 4.11).

*4.4.3.2 Root length*

The root length among the treatments recorded significant differences. The control plants ($T_{10}$) recorded the maximum (10 cm) root length. The minimum root length (1.55 cm) was recorded by AMF inoculation ($T_9$) @ 75g $kg^{-1}$ (Table 4.11).

*4.4.3.3 Number of days of plant survival*

The treatments showed significant differences for number of days of plants survival. The AMF inoculation ($T_9$) @ 75g $kg^{-1}$ recorded the maximum number of days of plants survival (98.89 days) followed by inoculum density ($T_7$) @ 25g $kg^{-1}$ (85.45 days). The control plants ($T_{10}$) recorded 56 days plant survival (Table 4.11).

*4.4.3.4 Percent wilt incidence*

The treatments showed significant differences for percent wilt incidence with AMF inoculation at the time of transplanting. The inoculum density ($T_9$) @ 75g $kg^{-1}$ recorded minimum percent wilt incidence (33 per cent). The control plants ($T_{10}$) recorded maximum percent wilt incidence (100 per cent) (Table 4.11).

**Table 4.11 Effect of different species of *Glomus* at the time of transplanting on dry weight, root length, number of days of plant survival and percent wilt incidence under wilt sick soil**

| Treatment | *Glomus* sp. | Dry weight (g) | Root length (cm) | Number of days of plant survival | Percent wilt incidence |
|---|---|---|---|---|---|
| $T_1$ | *Gm* ($Id_1$) | $0.88^{a}$ | $5.77^{abc}$ | $17.00^{c}$ | $100.0\ (1.40)^{a}$ |
| $T_2$ | *Gm* ($Id_2$) | $1.10^{a}$ | $8.33^{ab}$ | $19.56^{c}$ | $100.0\ (1.40)^{a}$ |
| $T_3$ | *Gm* ($Id_3$) | $1.19^{a}$ | $4.88^{abc}$ | $51.55^{abc}$ | $66.66\ (0.99)^{ab}$ |
| $T_4$ | *Gf* ($Id_1$) | $0.16^{a}$ | $5.11^{abc}$ | $9.11^{c}$ | $100.0\ (1.40)^{a}$ |
| $T_5$ | *Gf* ($Id_2$) | $2.01^{a}$ | $5.99^{abc}$ | $34.11^{bc}$ | $100.0\ (1.40)^{a}$ |
| $T_6$ | *Gf* ($Id_3$) | $2.49^{a}$ | $6.33^{abc}$ | $60.22^{abc}$ | $77.77\ (1.14)^{ab}$ |
| $T_7$ | *Gi* ($Id_1$) | $2.17^{a}$ | $3.55^{bc}$ | $85.45^{a}$ | $55.55\ (0.84)^{ab}$ |
| $T_8$ | *Gi* ($Id_2$) | $1.77^{a}$ | $4.33^{bc}$ | $72.00^{ab}$ | $66.66\ (0.99)^{ab}$ |
| $T_9$ | *Gi* ($Id_3$) | $1.42^{a}$ | $1.55^{c}$ | $98.89^{a}$ | $33.33\ (0.58)^{b}$ |
| $T_{10}$ | Control | $4.49^{a}$ | $10.00^{a}$ | $56.00^{abc}$ | $100.0\ (1.40)^{a}$ |

Note : Each value represents an average of 3 replications with 3 plants in each replication

Gm – *Glomus mosseae*, Gf- *Glomus fasciculatum*, Gi- *Glomus intraradices*

$Id_1$ – 25g $kg^{-1}$ soil, $Id_2$ – 50g $kg^{-1}$ soil, $Id_3$ – 75g $kg^{-1}$ soil

Values in parantheses represent transformed data

### 4.4.4 Selection of best time of AMF inoculation and inoculum density

The AMF treatments (at the time of sowing, at 15 days before transplanting and at the time of transplanting) clearly showed that the percent wilt incidence ($T_6$) @ 75g $kg^{-1}$ was the least (22 per cent) at 15 days before transplanting followed by AMF inoculation ($T_9$) @ 75g $kg^{-1}$ at the time of transplanting (33 per cent). The number of days of plant survival was maximum (105.8 days) @ 75g $kg^{-1}$ ($T_6$) followed by inoculum density ($T_8$) @ 50g $kg^{-1}$ (104.1 days) at 15 days before transplanting whereas at the time of transplanting the AMF inoculation ($T_9$) @75g $kg^{-1}$ recorded the maximum number of days of plant survival (98.89 days). The minimum percent wilt incidence (66 per cent) at the time of sowing was recorded by inoculum density ($T_1$) @ 25g $kg^{-1}$, ($T_5$) @ 50g $kg^{-1}$ and ($T_3$) @ 75g $kg^{-1}$ whereas the maximum number of days of plant survival was recorded by AMF inoculation ($T_9$) @ 75g $kg^{-1}$ (53.22 days). Based on dry weight of plant, percent wilt incidence and number of days of plant survival AMF inoculation @ 75g $kg^{-1}$ at 15 days before transplanting was found to be effective. Hence, the optimum inoculation time and inoculum density of AMF at 15 days before transplanting @ 75g $kg^{-1}$ soil were selected for further studies (Table 4.12).

## 4.5 SCREENING OF SELECTED AMF CULTURES AT THE OPTIMUM INOCULATION TIME (15 DAYS BEFORE TRANSPLANTING) AND INOCULUM DENSITY (@ 75G $KG^{-1}$ SOIL) FOR THE CONTROL OF BACTERIAL WILT

The effective native AMF cultures obtained from screening trial for the control of bacterial wilt were carried out under unsterile pot culture conditions to assess the suitability of optimum inoculation time and inoculum density.

### 4.5.1 Plant height

The treatments showed no significant differences with respect to plant height. The treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the maximum plant height (46.66 cm) followed by ($T_4$) commercial culture (TN) (41.33 cm). The control plants ($T_5$) recorded the lowest plant height (35.66 cm) (Table 4.13).

**Table 4.12 Comparison of different species of *Glomus* at the time of sowing, 15 days before transplanting and at the time of transplanting**

| Treatment | *Glomus* sp. | At time of sowing ($t_1$) | | | | 15 days before transplanting ($t_2$) | | | | At the time of transplanting ($t_3$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dry weight (g) | Root length (cm) | Number of days plants survived | Percent wilt incidence | Dry weight (g) | Root length (cm) | Number of days plants survived | Percent wilt incidence | Dry weight (g) | Root length (cm) | Number of days plants survived | Percent wilt incidence |
| $T_1$ | *Gm* ($Id_1$) | $0.147^{b}$ | $2.56^{ab}$ | $47.89^{a}$ | $66.66\ (0.95)^{b}$ | $3.68^{ab}$ | $6.55^{ab}$ | $64.89^{ab}$ | $77.77\ (1.14)^{ab}$ | $0.88^{a}$ | $5.77^{ab}$ | $17.00^{c}$ | $100.0\ (1.40)^{a}$ |
| $T_2$ | *Gm* ($Id_2$) | $2.31^{ab}$ | $5.44^{a}$ | $34.89^{ab}$ | $100.0\ (1.40)^{a}$ | $4.43^{ab}$ | $7.33^{ab}$ | $67.78^{ab}$ | $77.77\ (1.14)^{ab}$ | $1.10^{a}$ | $8.33^{ab}$ | $19.56^{c}$ | $100.0\ (1.40)^{a}$ |
| $T_3$ | *Gm* ($Id_3$) | $1.70^{ab}$ | $3.78^{ab}$ | $32.22^{ab}$ | $66.66\ (0.95)^{b}$ | $4.54^{ab}$ | $7.88^{a}$ | $49.67^{b}$ | $88.88\ (1.25)^{ab}$ | $1.19^{a}$ | $4.88^{ab}$ | $51.55^{ab}$ | $66.66\ (0.99)^{ab}$ |
| $T_4$ | *Gf* ($Id_1$) | $0.32^{ab}$ | $4.78^{ab}$ | $6.55^{b}$ | $100.0\ (1.40)^{a}$ | $2.82^{ab}$ | $6.89^{ab}$ | $58.22^{ab}$ | $77.77\ (1.14)^{ab}$ | $0.16^{a}$ | $5.11^{ab}$ | $9.11^{c}$ | $100.0\ (1.40)^{a}$ |
| $T_5$ | *Gf* ($Id_2$) | $0.10^{b}$ | $2.00^{b}$ | $44.22^{ab}$ | $66.66\ (0.95)^{b}$ | $6.74^{a}$ | $7.67^{ab}$ | $63.33^{ab}$ | $77.77\ (1.10)^{ab}$ | $2.01^{a}$ | $5.99^{ab}$ | $34.11^{bc}$ | $100.0\ (1.40)^{a}$ |
| $T_6$ | *Gf* ($Id_3$) | $0.64^{ab}$ | $4.11^{ab}$ | $39.00^{ab}$ | $77.77\ (1.14)^{ab}$ | $0.81^{ab}$ | $1.55^{ab}$ | $105.8^{a}$ | $22.22\ (0.46)^{b}$ | $2.49^{a}$ | $6.33^{ab}$ | $60.22^{ab}$ | $77.77\ (1.14)^{ab}$ |
| $T_7$ | *Gi* ($Id_1$) | $2.73^{a}$ | $5.00^{ab}$ | $23.55^{ab}$ | $88.88\ (1.25)^{ab}$ | $0.07^{b}$ | $1.33^{b}$ | $95.78^{ab}$ | $22.22\ (0.46)^{b}$ | $2.17^{a}$ | $3.55^{bc}$ | $85.45^{a}$ | $55.55\ (0.84)^{ab}$ |
| $T_8$ | *Gi* ($Id_2$) | $2.08^{ab}$ | $4.67^{ab}$ | $40.34^{ab}$ | $88.88\ (1.25)^{ab}$ | $0.80^{ab}$ | $1.77^{ab}$ | $104.1^{ab}$ | $22.22\ (0.46)^{b}$ | $1.77^{a}$ | $4.33^{bc}$ | $72.00^{ab}$ | $66.66\ (0.99)^{ab}$ |
| $T_9$ | *Gi* ($Id_3$) | $1.61^{ab}$ | $4.00^{ab}$ | $53.22^{a}$ | $100.0\ (1.40)^{a}$ | $2.94^{ab}$ | $4.77^{ab}$ | $67.11^{ab}$ | $77.77\ (1.10)^{ab}$ | $1.42^{a}$ | $1.55^{c}$ | $98.89^{a}$ | $33.33\ (0.58)^{b}$ |
| $T_{10}$ | Control | $0.93^{ab}$ | $4.67^{ab}$ | $16.00^{ab}$ | $100.0\ (1.40)^{a}$ | $3.07^{ab}$ | $7.00^{ab}$ | $54.30^{ab}$ | $100.0\ (1.40)^{a}$ | $4.49^{a}$ | $10.00^{a}$ | $56.00^{abc}$ | $100.0\ (1.40)^{a}$ |

Note : Each value represents an average of 3 replications with 3 plants in each replication

Gm – *Glomus mosseae*, Gf - *Glomus fasciculatum*, Gi – *Glomus intraradices*

$Id_1$ – 25g $kg^{-1}$ soil, $Id_2$ - 50g $kg^{-1}$ soil, $Id_3$ – 75gkg soil

Values in paranthesesrepresent transformed data

**Table 4.13 Effect of AMF inoculation 15 days before transplanting @ 75 g $kg^{-1}$ soil on the plant height, root length, root weight, fresh weight and dry weight under wilt sick soil**

| Treatments | Plant height (cm) | Root length (cm) | Root weight (g) | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|---|---|
| $T_1$ | 38.33 [a] | 5.44 [a] | 0.25 [a] | 5.59 [ab] | 1.40 [ab] |
| $T_2$ | 40.55 [a] | 6.00 [a] | 0.21 [a] | 4.00 [ab] | 1.00 [ab] |
| $T_3$ | 46.66 [a] | 5.32 [a] | 0.23 [a] | 5.45 [ab] | 1.36 [ab] |
| $T_4$ | 41.33 [a] | 6.00 [a] | 0.42 [a] | 6.93 [a] | 1.73 [a] |
| $T_5$ | 35.66 [a] | 4.33 [a] | 0.22 [a] | 1.72 [b] | 0.43 [b] |

Note : Each value represents an average of 3 replications with 3 plants per replication

$T_1$ *Glomus* sp. (OT) + *Glomus* sp. (ER)

$T_2$ – *Glomus* sp. (OT) + *Glomus* sp. (VM)

$T_3$ – *Glomus* sp. (OM) + *Glomus* sp. (VBT)

$T_4$ – Commercial culture (TN)

$T_5$ Control

### 4.5.2 Root length

There was no significant difference among the treatments for root length. The treatments ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and ($T_4$) commercial culture (TN) recorded the maximum root length (6.00 cm) followed by ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (5.44 cm) and ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (5.32cm). The control plants ($T_5$) recorded the lowest root length (4.33 cm) (Table 4.13).

### 4.5.3 Root weight

The treatments did not differ significantly with respect to the root weight. The treatment ($T_4$) commercial culture (TN) recorded the maximum root weight (0.42 g) followed by ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (0.25 g). The control plants ($T_5$) recorded a root weight of 0.22 g. (Table 4.13).

### 4.5.4 Fresh weight

The treatments did not differ significantly with respect to the fresh weight of the plant. The treatment ($T_4$) commercial culture (TN) recorded the maximum fresh weight (6.93 g). The native cultures ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER), *Glomus* sp. (OT) + *Glomus* sp. (VM) and *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded fresh weights which were on par. Among the native cultures, ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded the maximum fresh weight (5.59 g). The control plants ($T_5$) recorded the minimum fresh weight (1.72 g) (Table 4.13).

### 4.5.5 Dry weight

The treatments did not differ significantly with respect to the dry weight of the plant. The treatment ($T_4$) commercial culture (TN) recorded the maximum dry weight (1.73 g). The control plants ($T_5$) recorded the minimum dry weight (0.43 g). The dry weight of the plants inoculated with the native cultures ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER), ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and ($T_3$) *Glomus* sp. (OM) + *Glomus*

sp. (VBT) were on par and among them ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded the highest dry weight (1.39 g) (Table 4.13).

### 4.5.6 Number of days of plant survival

The treatments differed significantly with respect to the number of days of plant survival. The treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the maximum number of days of plant survival (82.11 days), followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (80.11 days) which were on par. The control plants ($T_5$) recorded the least number of days the plants survived (51.0 days) (Table 4.14).

### 4.5.7 Percent wilt incidence

The treatments did not differ significantly with respect to percent wilt incidence. The minimum percent wilt incidence (55.55 per cent) was recorded by ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT). The control plants ($T_5$) recorded 100 percent wilt incidence. The treatments ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER), ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and ($T_4$) commercial culture (TN) were on par and among them ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the minimum bacterial wilt incidence (66.66 per cent) (Table 4.14).

### 4.5.8 Per cent root colonization

The per cent root colonization was maximum (40 per cent) for the treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) followed by ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) and ($T_3$) *Glomus* spp. (OM) + *Glomus* spp. (VBT) which recorded 30 per cent root colonization. The treatment ($T_4$) commercial culture (TN) recorded only 20 per cent root colonization. The per cent root colonization of control plants ($T_5$) was zero (Table 4.15).

### 4.5.9 AM fungal spore count

The AM fungal spores were maximum for the treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (175 spores $10g^{-1}$soil) followed by ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) and ($T_4$) commercial culture (TN) which recorded spore count of 155 spores

**Table 4.14 Effect of AMF inoculation 15 days before transplanting @ 75 g $kg^{-1}$ soil on the number of days of plant survival and percent wilt incidence under wilt sick soil**

| Treatment | Number of days of plant survival | Percent wilt incidence |
|---|---|---|
| $T_1$ | 65.22 $^{ab}$ | 88.88 $^{ab}$ ($1.25^{ab}$) |
| $T_2$ | 80.11 $^{a}$ | 66.66 $^{ab}$ ($0.95^{ab}$) |
| $T_3$ | 82.11 $^{a}$ | 55.55 $^{b}$ ($0.84^{b}$) |
| $T_4$ | 62.78 $^{ab}$ | 88.88 $^{ab}$ ($1.25^{ab}$) |
| $T_5$ | 51.00 $^{b}$ | 100.0 $^{a}$ ($1.40^{a}$) |

Note : Each value represents an average of 3 replications with 3 plants per replication

$T_1$ – *Glomus* sp. (OT) + *Glomus* sp. (ER)

$T_2$ – *Glomus* sp. (OT) + *Glomus* sp. (VM)

$T_3$ – *Glomus* sp. (OM) + *Glomus* sp. (VBT)

$T_4$ – Commercial culture (TN)

$T_5$ – Control

Value in paranthesis represent transformed data

**Table 4.15 Effect of AMF inoculation 15 days before transplanting @ 75 g $kg^{-1}$ soil on spore count and percent root colonization**

| Treatment | Spore count ($10\ g^{-1}$ soil) | Percent root colonization |
|---|---|---|
| $T_1$ | 155 | 30 |
| $T_2$ | 175 | 40 |
| $T_3$ | 139 | 30 |
| $T_4$ | 155 | 20 |
| $T_5$ | 80 | 0 |

Note: Each value represents an average of 3 replication

$T_1$ - *Glomus* sp. (OT) + *Glomus* sp. (ER)

$T_2$ - *Glomus* sp. (OT) + *Glomus* sp. (VM)

$T_3$ - *Glomus* sp. (OM) + *Glomus* sp. (VBT)

$T_4$ – Commercial culture (TN)

$T_5$ – Control

$10g^{-1}$soil. The spore count from control ($T_7$) was the least (80 spores $10g^{-1}$soil) (Table 4.15).

### 4.5.10 Selection of the AMF cultures for field studies

The treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the lowest percent wilt incidence (55.55 per cent) followed by ($T_2$) *Glomus* sp. (OT)+ *Glomus* sp. (VM) (66.66 per cent). The number of days of plant survival was maximum for the treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (82.11 days) followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (80.11 days). The plant height recorded by ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (46.66 cm) was the maximum followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (40.55 cm). The treatments ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER), ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) for fresh weight, dry weight, root length and root weight did not differ significantly and were on par. Based on the number of days of plant survival, percent wilt incidence and plant height the native cultures ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) were selected as the best two among the three native cultures (Table 4.16).

## 4.6 FIELD EXPERIMENT

A field experiment to determine the efficacy of the selected AMF cultures obtained from the pot culture experiment under both sterile and un-sterile conditions were carried out at the Vegetable Research Farm of College of Horticulture, Vellanikkara with the tomato variety Pusa Ruby (highly susceptible). The tomato variety Mukthi (moderately resistant) was used for comparison.

### 4.6.1 Plant height

The treatments differed significantly for plant height in the case of Pusa Ruby. The treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum (23.75 cm) plant height. The control plants ($T_6$) recorded the lowest (8.75 cm) plant height.

**Table 4.16 Evaluation of effective AMF cultures at the optimum inoculation time and inoculum density**

| Treatment | Plant height (cm) | Root length (cm) | Root weight (g) | Fresh weight (g) | Dry weight (g) | Number of days of plant survival | Percent wilt incidence |
|---|---|---|---|---|---|---|---|
| $T_1$ | 38.33 [a] | 5.44 [a] | ,0.25 [a] | 5.59 [ab] | 1.39 [ab] | 65.22 [ab] | 88.88 [ab] (1.25[ab]) |
| $T_2$ | 40.55 [a] | 5.99 [a] | 0.21 [a] | 4.00 [ab] | 1.00 [ab] | 80.11 [a] | 66.66 [ab] (0.95[ab]) |
| $T_3$ | 46.66 [a] | 5.32 [a] | 0.23 [a] | 5.45 [ab] | 1.36 [ab] | 82.11 [a] | 55.55 [b] (0.84[b]) |
| $T_4$ | 41.33 [a] | 5.99 [a] | 0.42 [a] | 6.93 [a] | 1.73 [a] | 62.78 [ab] | 88.88 [ab] (1.25[ab]) |
| $T_5$ | 35.66 [a] | 4.33 [a] | 0.22 [a] | 1.72 [b] | 0.43 [b] | 51.00 [b] | 100.0 [a] (1.40[a]) |

Note : Each value represents an average of 3 replications with 3 plants per replication

$T_1$ – *Glomus* sp. (OT) + *Glomus* sp. (ER)

$T_2$ – *Glomus* sp. (OT) + *Glomus* sp. (VM)

$T_3$ – *Glomus* sp. (OM) + *Glomus* sp. (VBT)

$T_4$ – Commercial culture (TN)

$T_5$ – Control

Value in paranthesesrepresent transformed data

There was no significant difference between treatments with respect to plant height in the case of Mukthi. The treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the maximum (44.22 cm) plant height followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (42.27 cm) and ($T_4$) commercial culture (TN) (41.51 cm) which were on par. The control plants ($T_6$) recorded the minimum (31.03 cm) plant height (Table 4.17).

### 4.6.2 Root length

The treatments differed significantly for root length in the case of Pusa Ruby. The treatment ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded the maximum (13.0 cm) root length followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (8.75 cm). The control plants ($T_6$) with 5 cm root length was the lowest.

The root length of Mukthi variety differed significantly between treatments. Among the native AMF, ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded maximum root length (15.13 cm). The chemical treatment ($T_5$) with Kocide (0.2 per cent) recorded the maximum (22.45 cm) root length. The control plants ($T_6$) recorded the lowest (6.25 cm) root length (Table 4.17).

### 4.6.3 Root weight

The treatments differed significantly with respect to plant root weight in the case of Pusa Ruby. The treatment ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded the maximum root weight (3.51 g) which was followed by the treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (1.92 g). The control plants ($T_6$) recorded the lowest (1.05 g) root weight.

There was significant difference among treatments for the plant root weight in the case of Mukthi. The chemical treatment ($T_5$) with Kocide (0.2 per cent) recorded the maximum (10.32 g) root weight followed by ($T_4$) commercial culture (TN) (7.17 g). The

**Table 4.17 Effect of AMF inoculation on the plant height, root length, root weight, fresh weight, dry weight, fruit number and fruit weight for Pusa Ruby and Mukthi tomato varieties under field conditions**

| Treatment | Pusa Ruby | | | | | | | Mukthi | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | Root length (cm) | Root weight (g) | Fresh weight (g) | Dry weight (g) | Fruit number | Fruit weight (g) | Plant height (cm) | Root length (cm) | Root weight (g) | Fresh weight (g) | Dry weight (g) | Fruit number | Fruit weight (g) |
| $T_1$ | 18.25 $^{b}$ | 13.00 $^{a}$ | 3.51 $^{a}$ | 15.56 $^{ab}$ | 3.11 $^{ab}$ | - | - | 36.19 $^{b}$ | 9.63 $^{cd}$ | 4.80 $^{d}$ | 32.19 $^{ab}$ | 9.95 $^{ab}$ | 38.75 $^{ab}$ | 49.74 $^{ab}$ |
| $T_2$ | 23.75 $^{a}$ | 8.75 $^{b}$ | 1.92 $^{b}$ | 17.09 $^{a}$ | 3.42 $^{a}$ | - | - | 42.27 $^{a}$ | 6.75 $^{d}$ | 1.80 | 33.22 $^{a}$ | 5.63 $^{c}$ | 48.00 $^{a}$ | 28.16 $^{c}$ |
| $T_3$ | 16.25 $^{c}$ | 8.00 $^{bc}$ | 1.20 $^{c}$ | 17.09 $^{a}$ | 3.42 $^{a}$ | - | - | 44.22 $^{a}$ | 15.13 $^{b}$ | 5.60 $^{bc}$ | 29.22 $^{bc}$ | 7.63 $^{bc}$ | 34.75 $^{ab}$ | 38.15 $^{bc}$ |
| $T_4$ | 15.25 $^{c}$ | 7.25 $^{bc}$ | 1.41 $^{bc}$ | 13.91 $^{ab}$ | 2.78 $^{ab}$ | - | - | 41.57 $^{a}$ | 12.18 $^{bc}$ | 7.17 $^{b}$ | 22.91 $^{c}$ | 6.53 $^{bc}$ | 34.25 $^{ab}$ | 32.63 $^{bc}$ |
| $T_5$ | 17.25 $^{c}$ | 6.00 $^{cd}$ | 1.10 $^{c}$ | 15.45 $^{ab}$ | 3.09 $^{ab}$ | - | - | 39.42 $^{ab}$ | 22.45 $^{a}$ | 10.32 $^{a}$ | 24.20 $^{bc}$ | 11.67 $^{a}$ | 33.75 $^{ab}$ | 58.33 $^{a}$ |
| $T_6$ | 8.750 $^{c}$ | 5.00 $^{d}$ | 1.05 $^{c}$ | 10.50 $^{b}$ | 2.10 $^{b}$ | - | - | 31.03 $^{b}$ | 6.25 $^{d}$ | 3.60 $^{cd}$ | 11.24 $^{d}$ | 7.12 $^{bc}$ | 5.75 $^{b}$ | 35.58 $^{bc}$ |

Note : Each value represents an average of 4 replications with 12 plants per replication

$T_1$ – *Glomus* sp. (OT) + *Glomus* sp. (ER)

$T_2$ – *Glomus* sp. (OT) + *Glomus* sp. (VM)

$T_3$ – *Glomus* sp. (OM) + *Glomus* sp. (VBT)

$T_4$ – Commercial culture (TN)

$T_5$ – Chemical control (Kocide 0.2%)

$T_6$ – Control

treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the lowest root weight (1.80 g). The control plants ($T_6$) recorded a root weight of 3.59 g (Table 4.17).

### 4.6.4 Fresh weight

The treatments did not differ significantly for fresh weight in the case of Pusa Ruby. The treatments ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) and ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum fresh weight (17.09 g) and were on par. The control plants ($T_6$) recorded the lowest fresh weight (10.5 g).

There was no significant difference between the treatments with respect to fresh weight in the case of Mukthi. The treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum fresh weight (33.22 g) followed by ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (32.19 g). The control plants ($T_6$) recorded the lowest fresh weight (11.24 g).

The fresh weight of Mukthi variety was higher for all the treatments when compared to Pusa Ruby variety (Table 4.17).

### 4.6.5 Dry weight

The dry weight of Pusa Ruby did not differ significantly between treatments. The treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) and ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum (3.42 g) dry weight and they were on par. The control plants ($T_6$) recorded the lowest dry weight (2.10 g).

The treatments differed significantly with respect to plant dry weight in the case of Mukthi. Among the treatments with native AMF, ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded maximum dry weight (9.95 g). The treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the lowest plant dry weight (5.63 g). However, the chemical treatment ($T_5$) with Kocide (0.2 per cent) recorded the maximum dry weight (11.67 g) in the case of Mukthi. The control plants ($T_6$) recorded plant dry weight of 7.12 g (Table 4.17).

### 4.6.6 Fruit number

The plants of the variety, Pusa Ruby wilted 32 days after transplanting and thus was not able to bear any fruits. So, the data for fruit number and fruit weight could not be collected.

The treatments did not differ significantly for fruit number in the case of Mukthi. The treatment ($T_2$) *Glomus* sp. (OT)+ *Glomus* sp. (VM) recorded the maximum (48.0) fruit number. The control plant ($T_6$) (5.75) recorded the lowest fruit number. The treatments ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT), ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER), ($T_4$) commercial culture (TN) and the chemical treatment ($T_5$) with Kocide (0.2 per cent) were on par and among them the treatment ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded the maximum (38.75) (Table 4.17).

### 4.6.7 Fruit weight

The Pusa Ruby plants did not survive and wilted within 32 days of transplanting and hence fruit weight could not be collected.

The fruit weight of Mukthi differed significantly between treatments. The chemical treatment (T5) with Kocide (0.2 per cent) recorded the maximum (58.33 g) fruit weight followed by treatment ($T_1$) *Glomus* sp. (OT)+ *Glomus* sp. (ER) (49.74 g). The lowest fruit weight (28.16 g) was recorded by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM). The control plants ($T_6$) recorded fruit weight (35.58 g) which was on par with the treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (38.15 g) and ($T_4$) commercial culture (TN) (32.63 g) (Table 4.17).

### 4.6.8 Number of days of plant survival

The plants did not differ significantly with respect to the number of days of survival in the case of Pusa Ruby. The chemical treatment ($T_5$) with Kocide (0.2 per cent) recorded the maximum (32.67 days) number of days in the case of Pusa Ruby. The

control plants ($T_6$) with 15.25 days recorded the least number of days. Among the native AMF, ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum number of days (31.65).

The treatments differed significantly with respect to the number of days of plant survival in the case of Mukthi. The treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum (50.33 days). The least number of days (45.04 days) was recorded by the control plants ($T_6$). All the treatments were on par (Table 4.18) (Plate 4a & 4b).

### 4.6.9 Percent wilt incidence

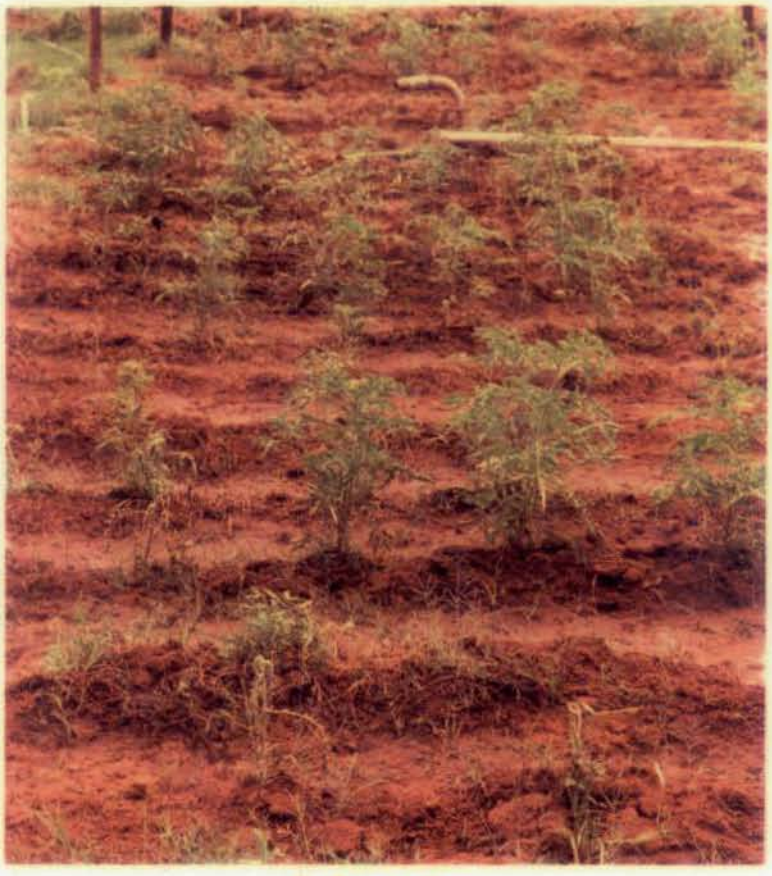
The treatments did not differ significantly with respect to percent wilt incidence in the case of Pusa Ruby. The Pusa Ruby plants recorded 100 percent wilt incidence for all the treatments, including the treatments inoculated with native AMF.

The treatments did not show any significant difference with respect to percent wilt incidence in the case of Mukthi. The treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT), ($T_1$) *Glomus* sp. (OT)+ *Glomus* sp. (ER) and control ($T_6$) recorded 100 per cent wilt incidence in the case of Mukthi. The treatment ($T_4$) commercial culture (TN) recorded the lowest (93.72 per cent) percent wilt incidence followed by ($T_5$) Kocide (0.2 per cent) (95.80 per cent) and (T2) *Glomus* sp. (OT) + *Glomus* sp. (VM) (97.90 per cent). All the treatments were on par. (Table 4.18).

### 4.6.10 Per cent root colonization

In the case of Pusa Ruby variety, the per cent root colonization was maximum (40 per cent) for ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) followed by ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT), ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) and ($T_4$) commercial culture (TN) which all recorded 30 per cent root colonization. Control plants ($T_6$) recorded zero per cent root colonization.

**a. Close view**



**b. Long view**

**Plate 4. Wilted plants in the wilt sick field**

**Table 4.18 Effect of AMF inoculation on the number of days of plant survival and percent wilt incidence for Pusa Ruby and Mukthi tomato varieties under field conditions**

| Treatments | Pusa Ruby | | Mukthi | |
|---|---|---|---|---|
| | Number of days of plants survival | Percent wilt incidence | Number of days of plant survival | Percent wilt incidence |
| $T_1$ | 28.16 [a] | 100.0 [a] (1.40 [a]) | 48.60 [a] | 100.0 [a] (1.40 [a]) |
| $T_2$ | 31.65 [a] | 100.0 [a] (1.40 [a]) | 50.33 [a] | 97.90 [a] (1.37 [a]) |
| $T_3$ | 27.33 [a] | 100.0 [a] (1.40 [a]) | 46.11 [a] | 100.0 [a] (1.40 [a]) |
| $T_4$ | 26.24 [ab] | 100.0 [a] (1.40 [a]) | 47.08 [a] | 93.72 [a] (1.31 [a]) |
| $T_5$ | 32.67 [a] | 100.0 [a] (1.40 [a]) | 48.51 [a] | 95.80 [a] (1.34 [a]) |
| $T_6$ | 15.25 [b] | 100.0 [a] (1.40 [a]) | 45.04 [a] | 100.0 [a] (1.40 [a]) |

Note : Each value represents an average of 4 replications with 12 plants per replication

$T_1$ – *Glomus* sp. (OT) + *Glomus* sp. (ER) $T_4$ – Commercial culture (TN)

$T_2$ – *Glomus* sp. (OT) + *Glomus* sp. (VM) $T_5$ – Chemical control (Kocide 0.2 per cent)

$T_3$ – *Glomus* sp. (OM) + *Glomus* sp. (VBT) $T_6$ – Control

Value in paranthesis represent transformed data

In Mukthi variety, the per cent root colonization was maximum (60 per cent) for ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) followed by ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) and ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) which recorded 50 per cent root colonization. The treatment ($T_4$) commercial culture (TN) recorded 40 per cent root colonization. The control plants ($T_6$) recorded 10 per cent root colonization (Table 4.19).

### 4.6.11 AMF spore count

The initial AMF count in the field at the time of transplanting was 132.75 spores $10g^{-1}$soil. The AMF spore count at 30 DAT for Pusa Ruby was maximum for ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (144.25 spores $10g^{-1}$soil) followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (141.5 spores $10g^{-1}$soil) and ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (140 spores $10g^{-1}$ soil). The minimum spore count (107.5 spores 10 $g^{-1}$soil) was recorded for ($T_6$) control (Table 4.19).

At 60 DAT, the maximum spore count was for ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (176.5 spores $10g^{-1}$soil) followed by ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (150 spores $10g^{-1}$soil) and ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (144.75 spores $10g^{-1}$soil). The control plants ($T_6$) recorded the lowest spore count (109.5 spores $10g^{-1}$soil). The spore count for all the treatments including control showed a higher value at 60 DAT when compared to the spore count at 30 DAT.

In the case of Mukthi variety, the initial AMF count in the field at the time of transplanting was 132.75 spores $10g^{-1}$soil. The AMF spore count at 30 DAT was maximum for ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (162.5 spores $10g^{-1}$soil) followed by (T3) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (152.5 spores $10g^{-1}$ soil) and ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (149.0 spores $10g^{-1}$soil) and. The minimum spore count (115.5 spores 10 $g^{-1}$soil) was recorded for ($T_6$) control.

At 60 DAT, the maximum spore count was for (T2) *Glomus* sp. (OT)+ *Glomus* sp. (VM) (210.0 spores $10g^{-1}$soil) followed by ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) (192.75 spores $10g^{-1}$soil) and ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (172.5

**Table 4.19 Effect of different AMF cultures on percent root colonization, spore count and *R. solanacearum* population for Pusa Ruby and Mukthi tomato varieties under field conditions**

| Treatments | Pusa Ruby | | | | | Mukthi | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Percent root colonization | Total AMF spore count (10 g $^{-1}$ soil) | | *Ralstonia* population ( x $10^4$ cfu g$^{-1}$ soil) | | Percent root colonization | Total AMF spore count (10 g $^{-1}$ soil) | | *Ralstonia* population ( x $10^4$ cfu g$^{-1}$ soil) | |
| | | 30 DAT | 60 DAT | 30 DAT | 60 DAT | | 30 DAT | 60 DAT | 30 DAT | 60 DAT |
| $T_1$ | 30 | 144.25 | 150.0 | 68.5 | 82.75 | 50 | 149.0 | 172.5 | 60 | 76 |
| $T_2$ | 40 | 141.5 | 176.75 | 74.75 | 88.5 | 60 | 162.5 | 210.0 | 70 | 82.5 |
| $T_3$ | 30 | 140.0 | 144.75 | 75 | 97.5 | 50 | 152.5 | 192.75 | 68.5 | 85.75 |
| $T_4$ | 30 | 123.5 | 127.5 | 75 | 84 | 40 | 135.5 | 160.5 | 70.5 | 81 |
| $T_5$ | 10 | 125.0 | 129.75 | 78 | 96.5 | 10 | 125.0 | 129.75 | 72 | 89.5 |
| $T_6$ | 0 | 107.5 | 109.5 | 99 | 125 | 10 | 115.5 | 128.5 | 97 | 120 |

Note :Each value represents an average of 4 replications

$T_1$ – *Glomus* sp. (OT) + *Glomus* pp. (ER)

$T_2$ – *Glomus* sp. (OT) + *Glomus* sp. (VM)

$T_3$ – *Glomus* sp. (OM) + *Glomus* sp. (VBT)

$T_4$ – Commercial culture (TN)

$T_5$ – Chemical control (Kocide 0.2 per cent)

$T_6$ – Control

spores $10g^{-1}$soil). The control plants ($T_6$) recorded the lowest spore count (128.5 spores $10g^{-1}$soil). The spore count for all the treatments including control showed a higher value at 60DAT when compared to the spore count at 30 DAT. (Table 4.19).

### 4.6.12 Enumeration of *Ralstonia solanacearum*

The initial *Ralstonia* population in the field at the time of transplanting was 53 $x10^4$ cfu $ml^{-1}$. The pathogen population in the field did not show much variation for Pusa Ruby and Mukthi varieties of tomato.

The *R. solanacearum* population in Pusa Ruby at 30 DAT was maximum (99 x $10^4$ cfu $ml^{-1}$) for ($T_6$) control and the lowest was for (T1) *Glomus* sp. (OT) + *Glomus* sp. (ER) (68.5 x $10^4$ cfu $ml^{-1}$). The pathogen population registered an increase for all the treatments including control at 60 DAT when compared to their population at 30 DAT. The *R. solanacearum* population was maximum for ($T_6$) control (125 x $10^4$ cfu $ml^{-1}$) at 60 DAT and the lowest was recorded for (T1) *Glomus* sp. (OT) + *Glomus* sp. (ER) (82.75 x $10^4$ cfu $ml^{-1}$).

Similarly, in the case of Mukthi, the *R. solanacearum* population at 30 DAT was maximum (97 x $10^4$ cfu $ml^{-1}$) for ($T_6$) control and the lowest for ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (60 x $10^4$ cfu $ml^{-1}$). The pathogen population recorded an increase at 60 DAT when compared to their population at 30 DAT for all the treatments. The maximum population of *R. solanacearum* at 60 DAT was recorded for ($T_6$) control (120 x $10^4$ cfu $ml^{-1}$) and the lowest for ($T_1$) *Glomus* spp. (OT) + *Glomus* spp. (ER) (76 x $10^4$ cfu $ml^{-1}$) (Table 4.19).

The pre-inoculated seedlings of tomato varieties Pusa Ruby and Mukthi were found to delay the incidence of bacterial wilt disease. In the case of Pusa Ruby the treatment ($T_5$) *Glomus* sp. (OT) + *Glomus* sp. (VM) delayed the bacterial wilt incidence upto 45.65 days after AMF inoculation. The same treatment recorded maximum percent root colonization (40 per cent) and spore count (176.75 spores 10 $g^{-1}$ soil). However, the minimum *Ralstonia* population (82.75 x $10^4$ cfu $ml^{-1}$) was recorded by the treatment ($T_1$)

*Glomus* sp. (OT) + *Glomus* sp. (ER) at 60 DAT. The percent wilt incidence was 100 percent for the tomato variety Pusa Ruby.

Similarly, in the case of Mukthi variety the treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded maximum number of days of plant survival (65.33 days after AMF inoculation). The treatment also recorded maximum percent root colonization (60 per cent) and spore count (201.0 spores 10 $g^{-1}$ soil). The fruit number was maximum (48.0) for ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM), whereas the fruit weight recorded the maximum (49.74 g) for the treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT). The *Ralstonia* population was minimum (76 x $10^4$ cfu $ml^{-1}$) for ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) at 60 DAT.

# DISCUSSION

# 5. DISCUSSION

The AM fungi are ubiquitous in nature and are found in symbiotic association with the root system of higher plants. The role of AMF in promoting plant growth through nutrient absorption especially phosphorus has been extensively studied. But its role as a biocontrol agent in controlling soil borne diseases has not been studied in detail. In the present study "Biocontrol of bacterial wilt in tomato using arbuscular mycorrhizal fungi", the importance of native AMF in reducing bacterial wilt in tomato under pot culture and field conditions has been highlighted. Moreover, the importance of optimum time of AMF inoculation and optimum inoculum density in reducing the wilt disease has been discussed in detail in this chapter.

The bacterial wilt pathogen is known to survive in soil in a viable and virulent form in the absence of its host for more than 2 years over a wide range of pH. There are no reports of pH having a direct influence on the pathogen population in the soil. But there are reports of increase in nutrient content in the soil induced by pH, reducing the population of the pathogen in the soil and thereby reducing the wilt incidence.

The pH of Vellanikkara soils was in the acidic range with pH ranging between 5.2 – 6.5. However, the Ozhalapathy and Eruthiampathy soils had pH towards the neutral range of 6.3 – 7.1. The low wilt incidence in Ozhalapathy and Eruthiampathy soils may be due to high pH induced increase in calcium content in the soil. The calcium content for Ozhalapathy and Eruthiampathy soils (8.4 meq. $L^{-1}$ and 10.2 meq. $L^{-1}$ respectively) was higher when compared to the Vellanikkara soil (4.8 meq. $L^{-1}$). This is in confirmation with the findings of Yamazaki (2001) who reported that increased calcium concentration in the nutrient solution reduced the bacterial wilt severity in the seedlings and reduced the pathogen population in stem. This result indicated that the resistance of tomato to bacterial wilt is markedly affected by calcium nutrition of the host.

The AMF are symbiotic associations of fungi with the roots of higher plants. They help the plants in absorbing nutrients from the soil. Therefore, the soil nutrient content plays a major role in determining the AMF population in the soil, with the P content exerting the major influence on the AMF. The results of the nutrient status of the six soil samples with respect to N, P, K, Ca and Mg in relation to their influence on AMF is discussed in this section.

The low P content (1.99 kg $ha^{-1}$) in Ozhalapathy and Eruthiampathy (4.66 kg $ha^{-1}$) soils indicated that such soil types are suitable for the growth and development of AMF which in turn imparts resistance to the plant. In a similar study, Jalali and Thareja (1985) reported that in phosphate rich soils, mycorrhizal density was poor, while root samples from soils of low phosphate status had extensive mycorrhizal colonization. The P content in Vellanikkara soils was higher when compared to Ozhalapathy and Eruthiampathy areas. The high P content in these soils might have affected the efficiency of AMF in controlling the bacterial wilt.

In the present study, it was also observed that nitrogen content in Vellanikkara soils were generally lower when compared to that of Ozhalapathy and Eruthiampathy soils. However, the lowest nitrogen content (176.4 kg $ha^{-1}$) was recorded by the Ozhalapathy maize rhizosphere soil which also recorded the lowest P content (1.99kg $ha^{-1}$). It was observed by Hayman (1970) that application of nitrogen fertilizer not only reduced root colonization but suppressed spore germination as well. The low soil fertility of Ozhalapathy maize rhizosphere which was indicated by their low N and P content might have stimulated the AMF spore production. The K content of Vellanikkara soil was lower when compared to Ozhalapathy and Eruthiampathy areas. The K status of the soils did not stimulate the AMF activity which is in agreement with reports of Daniel and Trappe (1980) and Siqueria *et al.*, (1982).

The soil factors such as soil pH, soil nutrient status and soil fertility influenced the AMF spore population in the soil. Since the AMF are symbiotic fungi, a soil pH range

favouring plant growth is most suited for AMF multiplication and growth. The results on soil pH, soil nutrient status and soil fertility of the six soil samples used in the present study is discussed.

The AMF spore count was highest (48 $10g^{-1}$ of soil) in the case of maize rhizosphere soil of Ozhalapathy (OM) due to the low N and P content coupled with a near neutral pH of 6.8. The soils of low fertility has a pronounced positive effect on the AMF spore germination and colonization (Hayman, 1970). With respect to the effect of pH on spore germination, it was found that a pH range favouring plant growth also influenced AMF spore germination (Green *et al.*, 1976; Young *et al.*, 1985). A pH of 6.8 can be considered as near neutral and this together with the low fertility status of the maize rhizosphere soil of Ozhalapathy (OM) might have resulted in the increased AMF spore production. In the case of the rhizosphere soil of the tomato variety BT-1 from Vellanikkara (VBT), the AMF spores were the lowest indicating that a lower soil pH (6.53) and higher N and P content than the maize rhizosphere soil from Ozhalapathy resulted in reduced AMF spore production. This indicated the need for screening AMF cultures suitable for a particular edaphic factor in a given situation.

The AM fungi are ubiquitous in nature and are seen in symbiotic association with the roots of higher plants. The host, soil and environmental factors together influence the AMF diversity in the soil. The most predominant AMF genus belongs to *Glomus*. The AMF spores identified from the soil samples from Palakkad and Thrissur districts confirmed the findings of earlier workers.

The AMF spore isolated from high and low wilt incidence areas indicated the presence of *Glomus* sp., *Acaulospora* sp. and *Sclerocystis* sp. in all the rhizosphere soils. In a similar study, Harikumar and Potty (1999) reported the presence of AMF isolates belonging to *Acaulospora* sp., *Glomus* sp., *Sclerocystis* sp. and *Gigaspora* sp. from soils of north Kerala which is in concurrence with the present results obtained from high wilt and

low wilt incidence areas of Thrissur and Palakkad districts. As there is AMF diversity in soils with host preference, it is very essential to identify AMF and screen for a suitable host.

However, *Glomus* sp. was the most predominant in all the locations indicating the adaptability of *Glomus* sp. to a wide range of soil and environmental factors. These results are in concurrence with earlier studies of Harikumar and Potty (1999), Beena (1999) and Raji (2002), where the predominance of *Glomus* spp. in the soils of Kerala has been reported. The predominant AMF from each location were selected for further studies.

The screening of different AMF cultures against *Ralstonia solanacearum* under pot culture using sterile potting mixture were carried out using the six predominant native isolates and one commercial culture individually and in combinations. This was done to determine the most suitable AMF culture (individual or in combination) capable of reducing the wilt incidence and improve the plant dry weight and root length. There are reports of AMF cultures in combination performing better than individual cultures. The present study was carried out to determine the most efficient AMF culture which could reduce the wilt incidence and increase the plant dry weight and root length.

The per cent AMF root colonization was highest (50 per cent) in the case of the treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) when compared to ($T_{29}$) control plants. The AMF spore count was also found to be highest (85spores $10g^{-1}$ soil) in the case of *Glomus* sp. (OT) + *Glomus* sp. (ER) when compared to control plants (10 spores $10g^{-1}$ soil). These results indicated that mixed cultures of AMF performed better when compared to individual AMF. Miller *et al.* (1989) also observed that a mixture of two AMF species, viz. *Glomus mosseae* and *Glomus macrocarpum* promoted plant growth better than individual fungus which confirms with the present findings. Moreover, the spore number is an indication of the per cent root colonization as reported by Daft and Nicolson (1972). The commercial culture (TN) recorded only 30 per cent root colonization, which suggested that indigenous fungi adapted to particular edaphic conditions possess some survival advantage over introduced species in soils of similar conditions such as their ability to colonize better,

spread through the plant or sporulate, greater inoculum potential and competitive ability (Lambert *et al.*, 1980). This study indicated that the native AMF isolated from the low wilt (Palakkad) and high wilt (Thrissur) incidence areas were better than the commercial culture as they could adapt much faster than the introduced AMF. Moreover, the study also indicated the importance of mixed cultures over individual cultures as the former could bring about better percent root colonization and also increased spore population in the rhizosphere region.

The AMF association with the root system of higher plants helps in the absorption of more nutrients from the soil. This in turn lead to an increase in growth of the AMF inoculated plants which was evident from their increased dry weight. Moreover, since the AMF are associated with the root system, it effectively increased the root length.

The dry weight of plant recorded the maximum (9.13 g) in the case of the treatment ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) when compared to ($T_{29}$) control (2.16 g) (Fig. 2). The treatment ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the maximum root length (7.67 cm) when compared to ($T_{29}$) control (5 cm) (Fig. 3). The treatment ($T_{28}$) commercial culture (TN) recorded a dry weight of 1.23 g and a root length of 4.5 cm. These results indicated that mixed cultures of AMF performed better when compared to individual cultures. This is in confirmation with the findings of Miller *et al.* (1989), where it was observed that a mixture of two species was better that individual fungus alone. This also indicated a reciprocal stimulation between fungi, or in a given environment, they have different abilities in nutrient uptake and transfer to the plant, which eventually added up promoting plant growth.

The results also confirmed that fungi are often more efficient at increasing plant growth in soils to which they have become adapted. In a similar study, Lambert *et al.*, (1980) also reported the adaptation of indigenous VAM fungi to particular edaphic conditions over introduced species in soils of similar conditions. This might be due to their better colonization, sporulation, greater inoculum potential and competitive ability over the

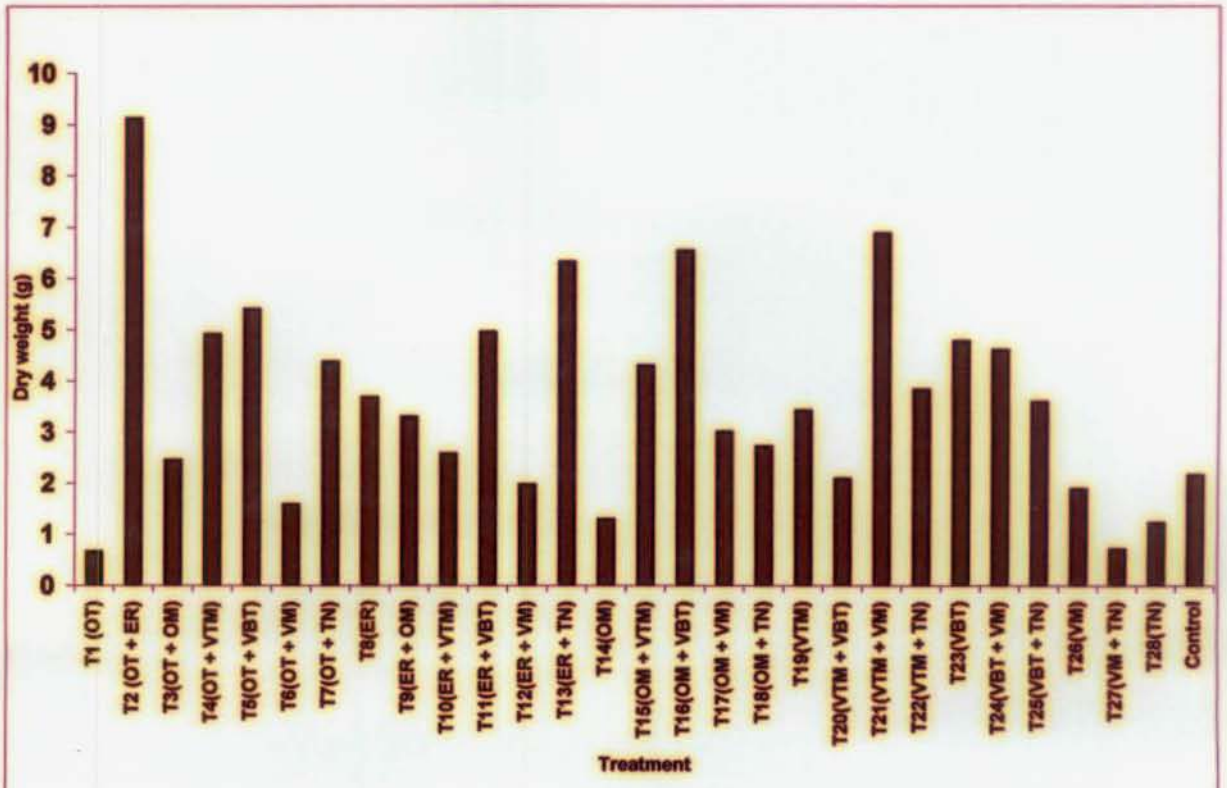



**Fig. 2 Effect of different species of *Glomus* on dry weight under sterile condition**

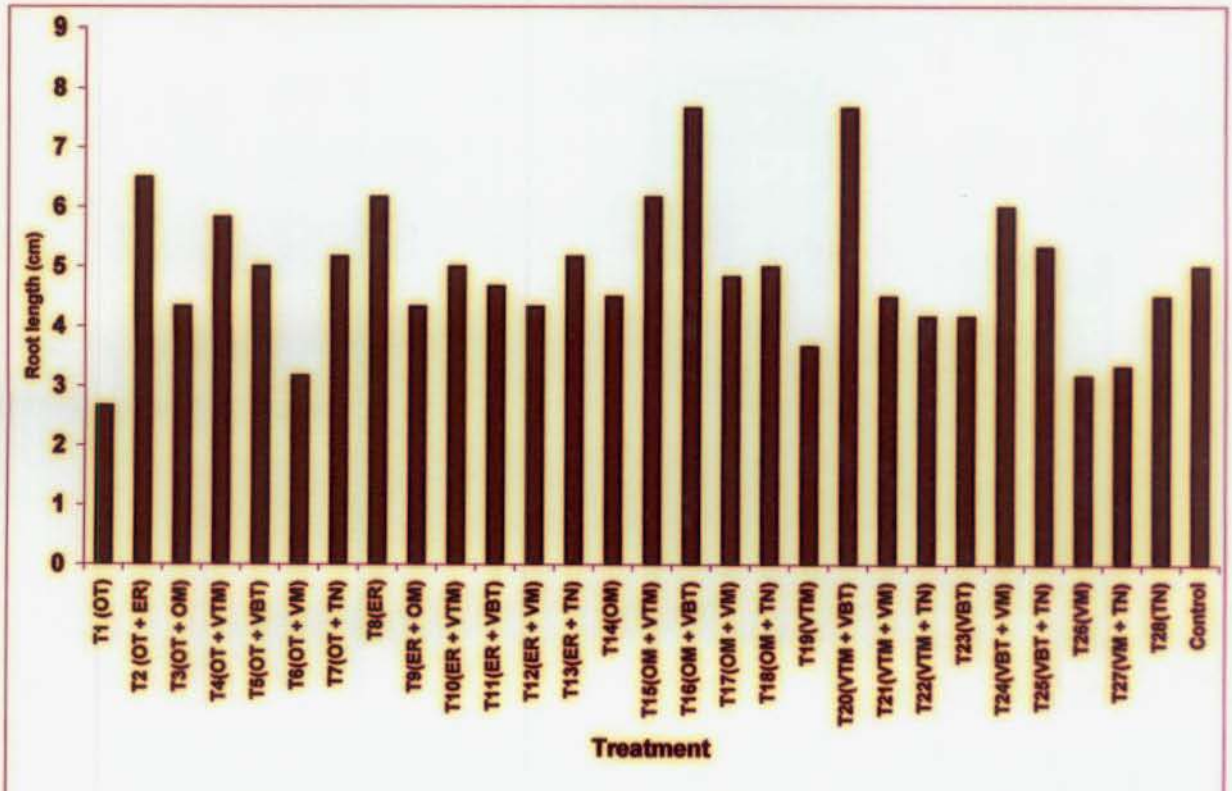



**Fig. 3 Effect of different species of *Glomus* on root length under sterile condition**

non indigenous AM fungi. This study indicated that the native AMF isolates promoted plant growth to a much greater extent when compared to the commercial culture. This once again confirmed the better adaptability of the native AMF isolates to a particular edaphic situation when compared to the commercial cultures.

The treatment ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the maximum number of days of plant survival (11 days) followed by ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (10.83 days) and ($T_6$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (9.33 days) when compared to ($T_{29}$) control (8 days) (Fig. 4). The treatment ($T_{28}$) commercial culture (TN) inoculated plants survived for only 6.17 days and the lowest was recorded by ($T_1$) *Glomus* sp. (OT) (4.50 days). The percent wilt incidence for the treatments ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT), ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) and ($T_6$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the lowest (83.33 per cent). All the other treatments recorded 100 per cent wilt incidence (Fig. 5). These results indicate that the dual inoculation of different AMF species resulted in increased biomass and also survival of the plants (Thomas *et al.*, 1994; Sivaprasad *et al.*, 1995).

These results also indicated the importance of indigenous AMF in reducing the per cent wilt incidence and increasing the number of days of plant survival. This is in confirmation with the findings of Nandakumar *et al.*, (2003) who reported that the native AMF reduced the percent wilt incidence and increased the number of days of plant survival when compared to the commercial cultures. This is because indigenous AMF are better adapted to a particular edaphic situation and posses some survival advantage over introduced spores such as their colonization ability, competitive ability etc. In a similar study Lambert *et al.*, (1980) demonstrated the efficiency of indigenous AMF in root colonization and enhancing plant growth compared to non-indigenous AMF.

Based on the number of days of plant survival and the percent wilt incidence the AMF cultures ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT), ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) and ($T_6$) *Glomus* sp. (OT) + *Glomus* sp. (VM) were found to be effective against *R.*

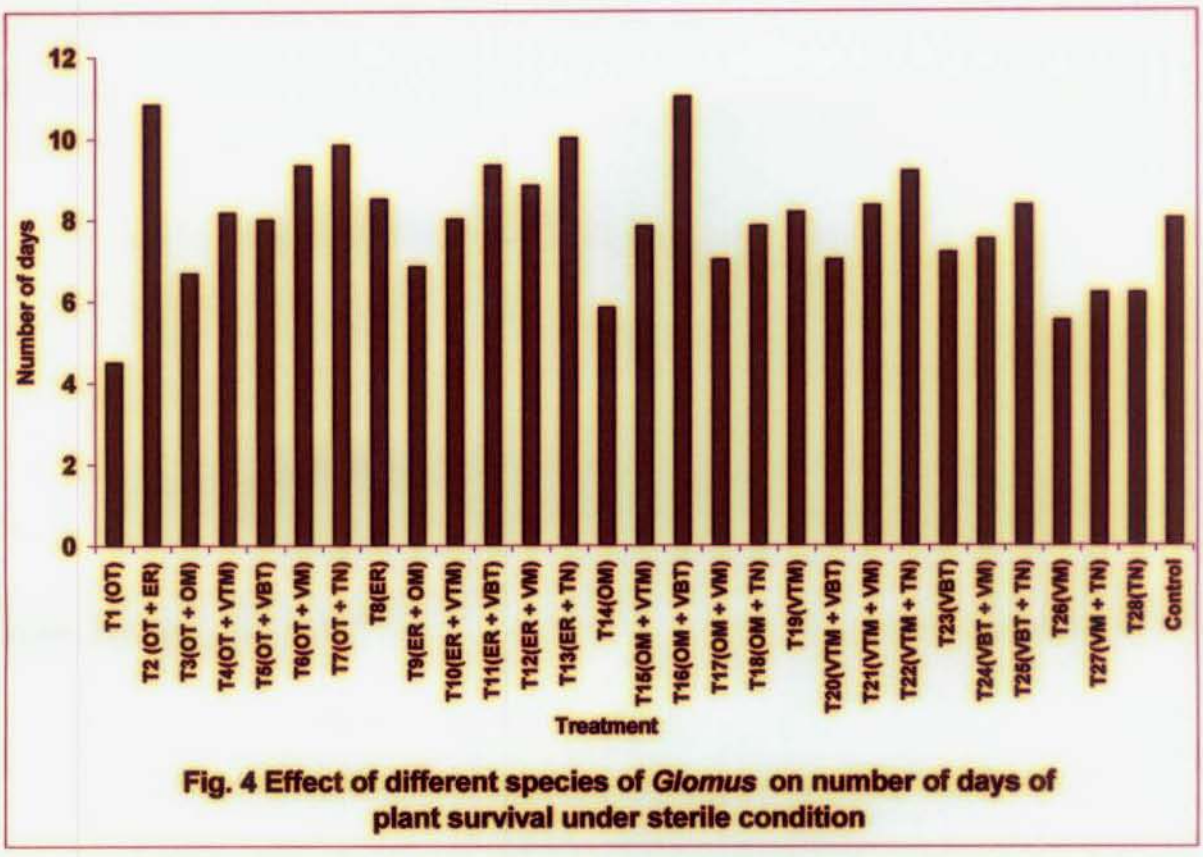



**Fig. 4 Effect of different species of *Glomus* on number of days of plant survival under sterile condition**

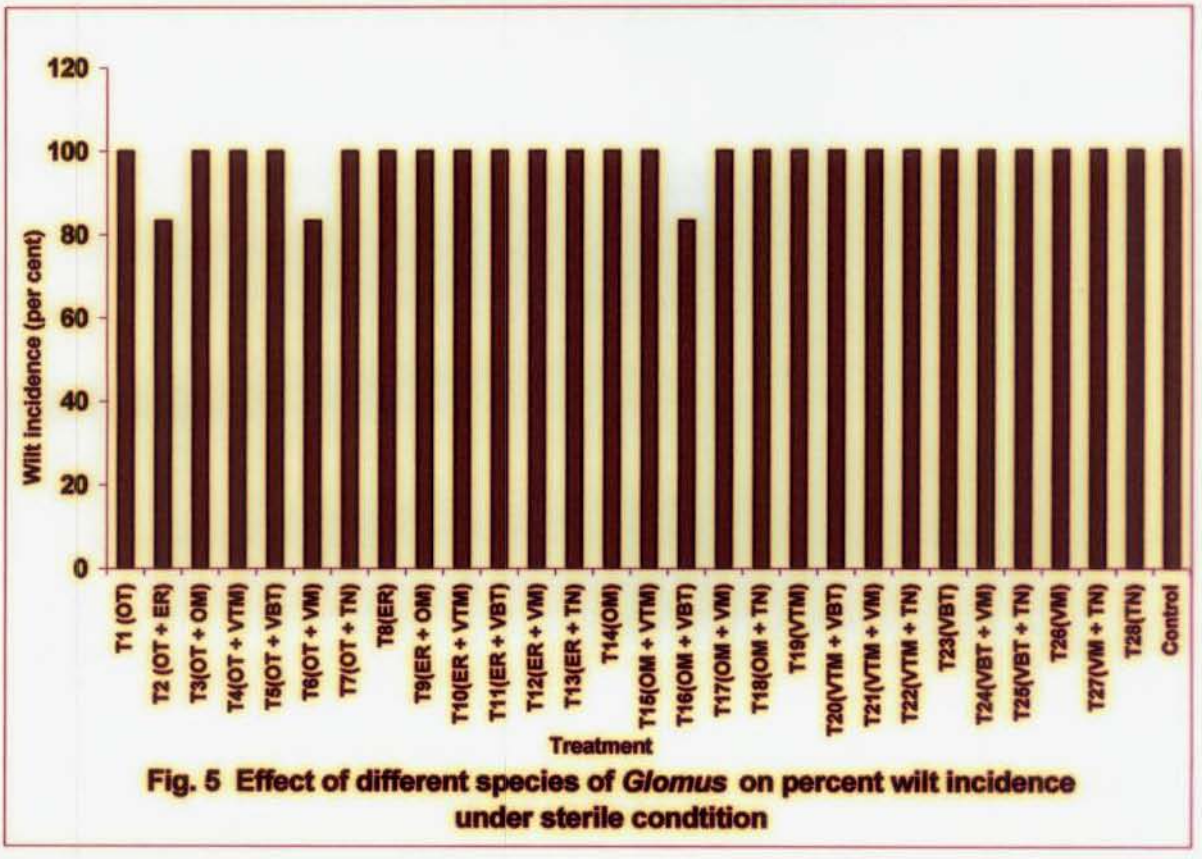



**Fig. 5 Effect of different species of *Glomus* on percent wilt incidence under sterile condtition**

*solanacearum*. Among these native cultures, the treatment ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) delayed the wilting upto 41 days (after AMF inoculation). These results indicated that dual inoculation is better than individual inoculation. This indicated that there was reciprocal stimulation between fungi and in a given environment, they have different abilities in nutrient uptake and transfer to the plant and that these abilities eventually combine to give a better result (Miller *et al.*, 1989). In the case of dual inoculation, the inoculum density of AMF spores was more which in turn increased the root colonization thereby reducing the bacterial wilt incidence. This is in confirmation with the findings of Daft and Nicolson (1968); Ferguson (1981). But, all the dual inoculation treatments were not effective which may be due to the fact that the inoculum potential per unit of inoculum, of each species varies (Daniels *et al.*, 1981). Thus, the dual inoculation of treatments ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT), ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (ER) and ($T_6$) *Glomus* sp. (OT) + *Glomus* sp. (VM) were selected as they gave less percent wilt incidence and maximum number of days of plant survival when compared to other treatments. Among these treatments, the treatment ($T_{16}$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) was the best as it gave the least percent wilt incidence (83.33 per cent), maximum number of days of plant survival (11.0 days after bacterial inoculation) and maximum root length (7.67 cm).

The determination of the optimum inoculation time and inoculum density are equally important for the screening of the best AMF. The optimum inoculation time and inoculum density varied with the host and soil factors. The inoculation time and inoculum density are particularly important in the case of short duration crops like tomato where we can go for pre-inoculation of the tomato seedlings in the nursery before transplanting to the field so that they have the potential to survive for a longer time and thereby give more yield.

The AMF inoculation at the time of sowing showed that the dry weight and root length of the plant was increased when compared to control. The AMF treatment ($T_2$) with inoculum density @ 50 g $kg^{-1}$ recorded a dry weight (2.31 g), when compared to ($T_{10}$) control (0.93 g) (Fig. 6). The maximum root length (5.44 cm) was also recorded for the treatment ($T_2$) with AMF inoculation @ 50 g $kg^{-1}$ (5.44 cm) when compared to ($T_{10}$) control

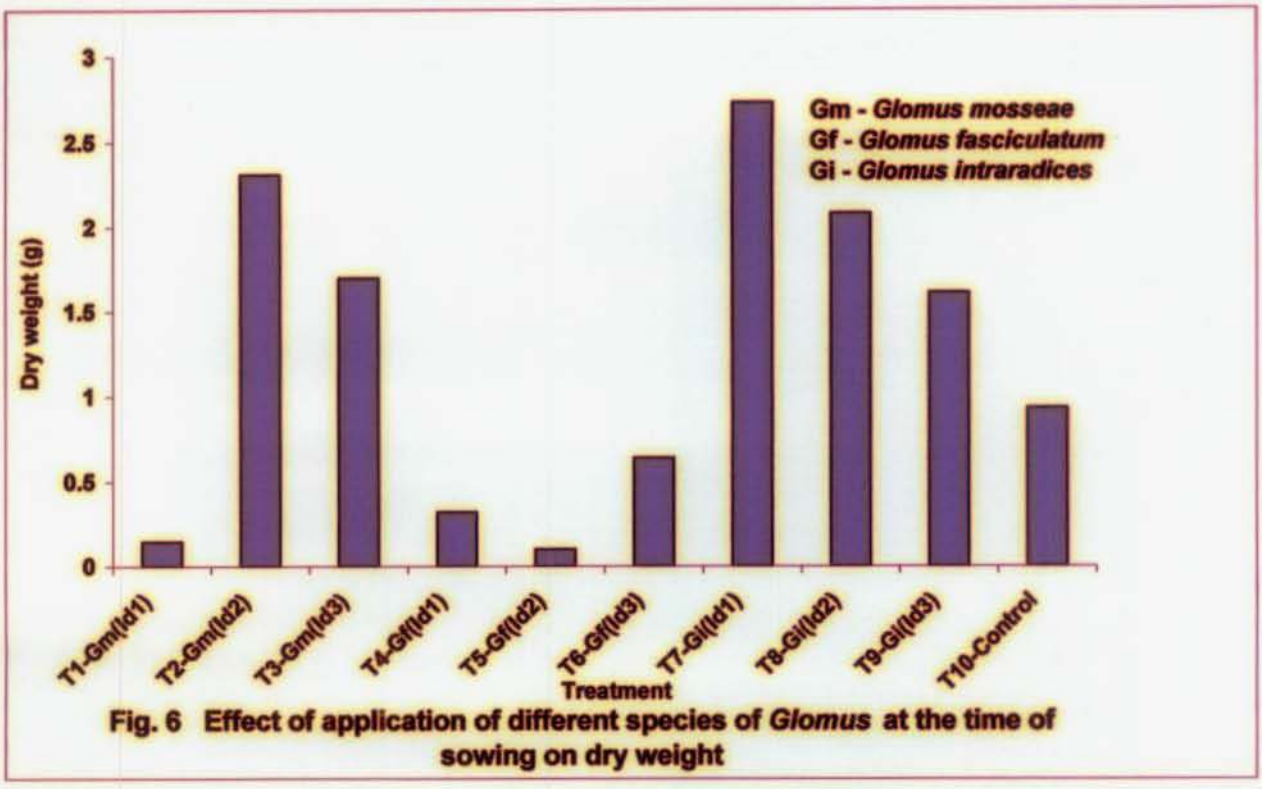



**Fig. 6 Effect of application of different species of *Glomus* at the time of sowing on dry weight**

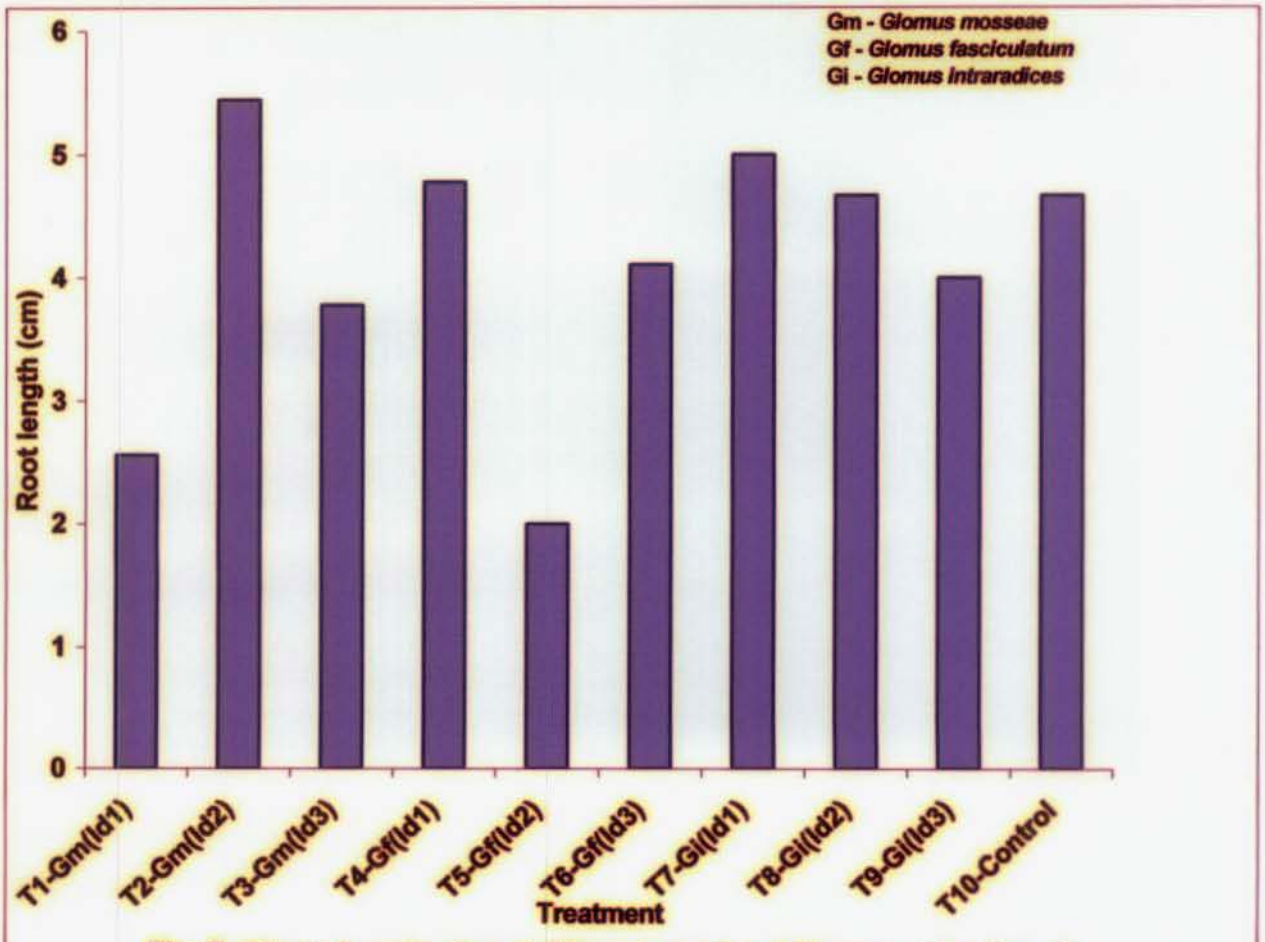



**Fig. 7 Effect of *application of different species of Glomus* at the time of sowing on root length**

(4.67 cm) (Fig. 7). These results indicated that a higher AMF inoculum density is required for increasing the percentage root colonization which in turn increased the root length. Moreover, being non-indigenous AMF they required a higher inoculum density to establish on the roots as they have to compete for space with the indigenous AMF spores in the soil. This work is in confirmation with the results of Daft and Nicolson (1968) and Ferguson (1981) who examined the influence of inoculum dosage on rate of colonization and observed that increased inoculum dosage resulted in increased colonization and thereby increased the root length. The increase in root colonization helped in better absorption of nutrients as the absorptive capacity of the root system was enhanced due to the AMF colonization. This in turn contributed to the improvement in plant growth which in turn is highlighted by the increase in dry weight of the plant. This was especially important with respect to annual crops like tomato with short growing seasons where high inoculum dosages may be required if maximum plant growth stimulation has to be achieved (Wallace, 1973; Hayman, 1982; Harley and Smith, 1983; Reid, 1984).

The treatment ($T_9$) with inoculum density @ 75g $kg^{-1}$ also recorded the maximum number of days of plant survival (53.22 days after transplanting to wilt sick soil), when compared to ($T_{10}$) control (16 days) (Fig. 8). The treatment ($T_3$) with inoculum density @ 75 g $kg^{-1}$ recorded minimum percent wilt incidence (66 per cent) when compared to 100 per cent wilt incidence in the case of ($T_{10}$) control (Fig. 9). These results clearly indicated that the higher inoculum density enhanced root growth through an increase in phosphate uptake by AM fungi. The increased root growth in turn expanded the absorptive capacity of the root system for nutrients and water and this explains the increased tolerance of mycorrhizal and P-fertilized plants as they could compensate for loss of root mass or function caused by the soil borne pathogens. Cordier *et al.*, (1996) also observed that mycorrhiza induced root growth could compensate for the root loss caused by soil borne pathogens. This explains the lower percent wilt incidence and maximum number of days of plant survival for the treatment with inoculum density @ 75 g $kg^{-1}$ soil. Moreover, the findings of Sood *et al.*, (1997) is in confirmation with the results obtained in the present study. He reported that *Glomus mosseae* was highly effective in controlling the bacterial wilt disease in tomato c.v.

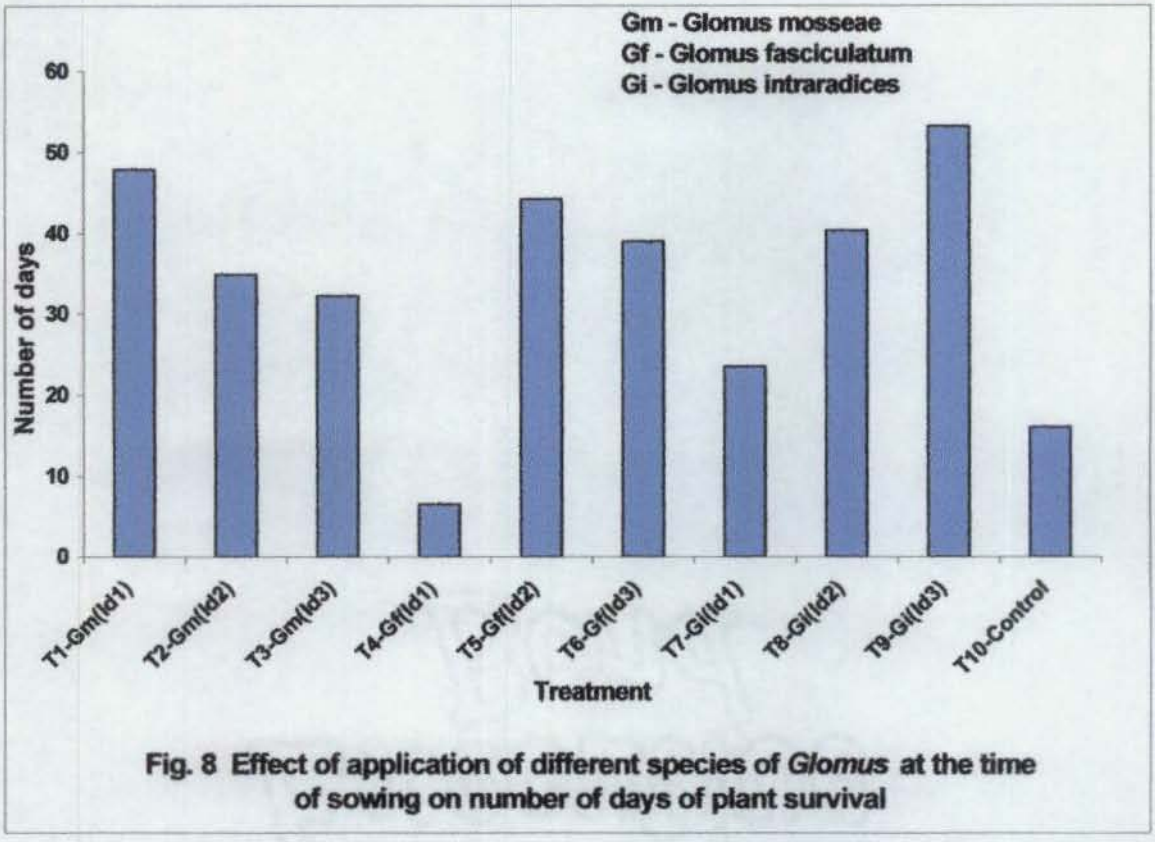



**Fig. 8 Effect of application of different species of *Glomus* at the time of sowing on number of days of plant survival**

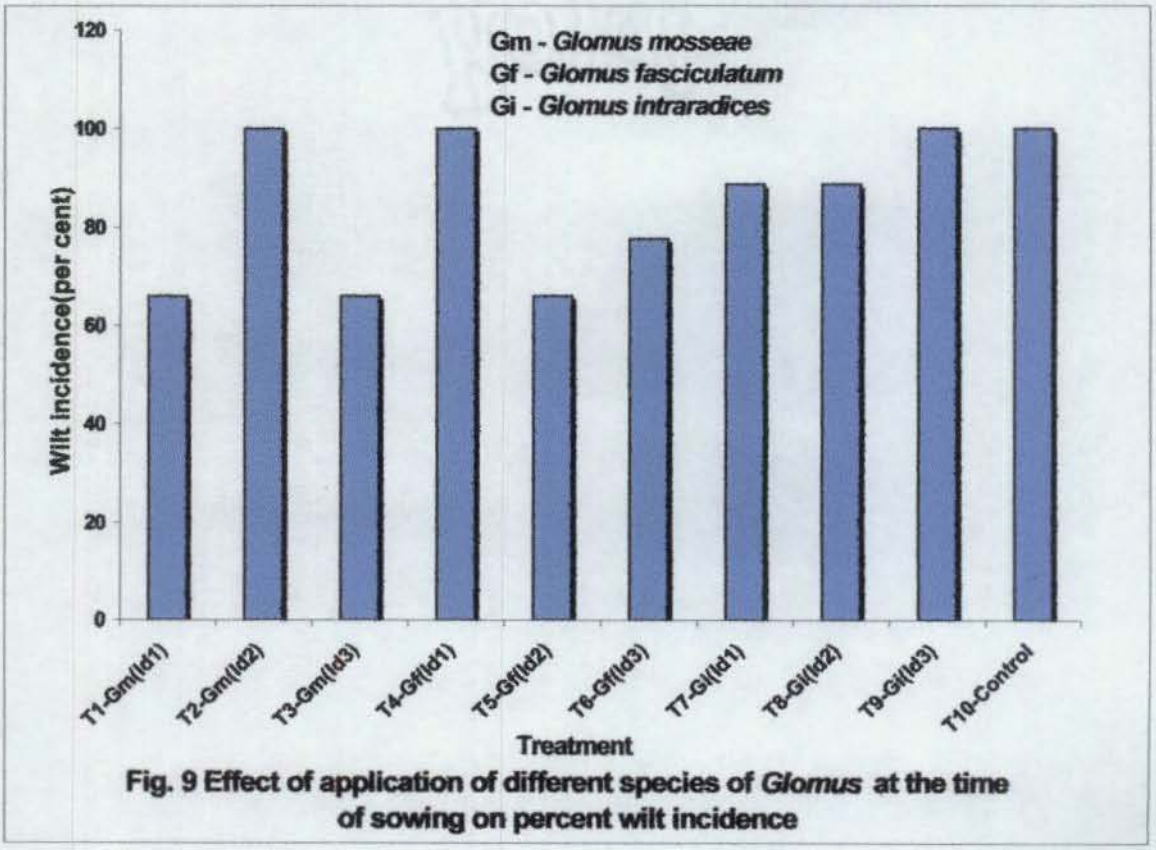



**Fig. 9 Effect of application of different species of *Glomus* at the time of sowing on percent wilt incidence**

Roma till the termination of potted experiment, 48 days after challenge inoculation with *R. solanacearum*. Kumar and Sood (2002) observed that the wilt incidence in the *G. mosseae* treated susceptible tomato cultivar Solan Gola was 44.4 per cent when compared to 83.3 per cent in control which further confirmed the effectiveness of AMF in reducing the wilt incidence. Nandakumar *et al.*, (2003) reported that the native AMF prolonged the disease incidence by as much as 40 days after inoculation with the wilt pathogen, when compared to 18 days in control. Moreover, they also observed that the percent disease incidence was reduced in native AMF treated plants, when compared to those treated with the commercial culture.

The treatment ($T_5$) with inoculum density @ 50g $kg^{-1}$ soil recorded maximum dry weight (6.74 g) when compared to ($T_{10}$) control plants (3.07 g) (Fig. 10). The treatment with inoculum density @ 75g $kg^{-1}$ ($T_3$) and 50 g $kg^{-1}$ ($T_5$) recorded maximum root length (7.88 cm and 7.67 cm respectively) when compared to ($T_{10}$) control (1.33 cm) (Fig. 11). This might be due to the higher AMF inoculum density. As the AMF inoculum density was more the ability of the AMF spores to form symbiotic associations with the root system was increased resulting in higher per cent root colonization. This in turn increased the absorptive capacity of thc root system resulting in the absorption of more nutrients and water, thereby enhancing the plant growth. This is in concurrence with the earlier studies (Wallace, 1973; Hayman, 1982; Harley and Smith, 1983; Reid, 1984). The results of the present study therefore explained for the increase in plant dry weight and root length with increase in inoculum density.

The number of days of plant survival was also found to be maximum (105.8 days) for the treatment ($T_6$) with inoculum density @ 75 g $kg^{-1}$ (Fig. 12). The treatment ($T_6$) with inoculum density @ 75g $kg^{-1}$ recorded the lowest percent wilt incidence (22 %) when compared to 100 per cent in ($T_{10}$) control plants (Fig. 13). The root colonization percentage was increased due to the increase in inoculum density. This in turn was found to increase the absorptive capacity of the roots which helped the plants to compensate for the loss of root mass or function caused by the attack of soil borne pathogens. This finding is in

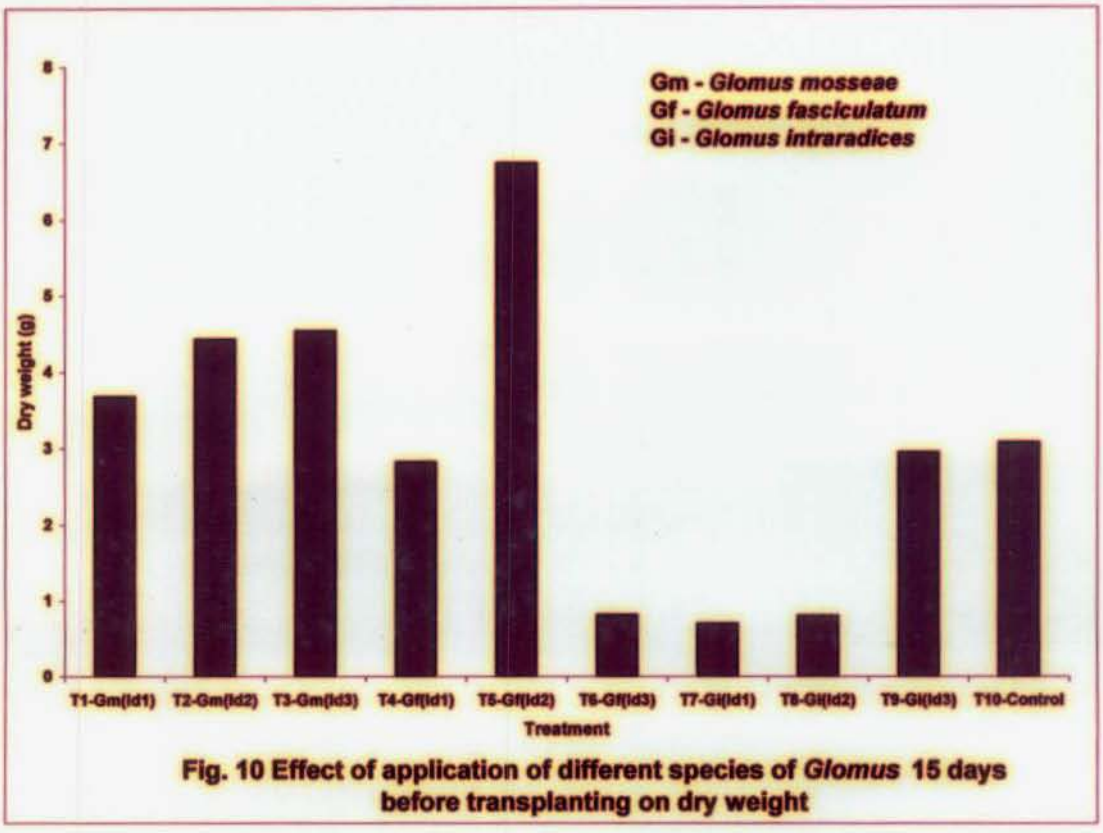



**Fig. 10 Effect of application of different species of *Glomus* 15 days before transplanting on dry weight**

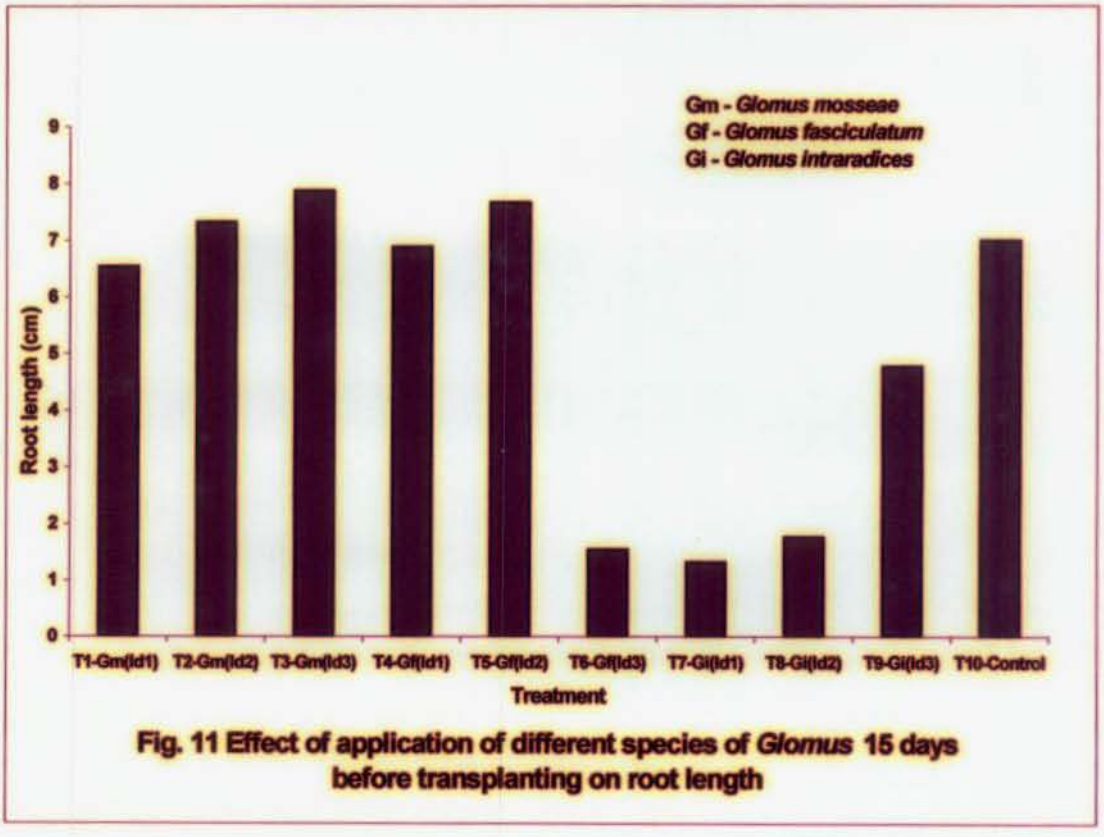



**Fig. 11 Effect of application of different species of *Glomus* 15 days before transplanting on root length**

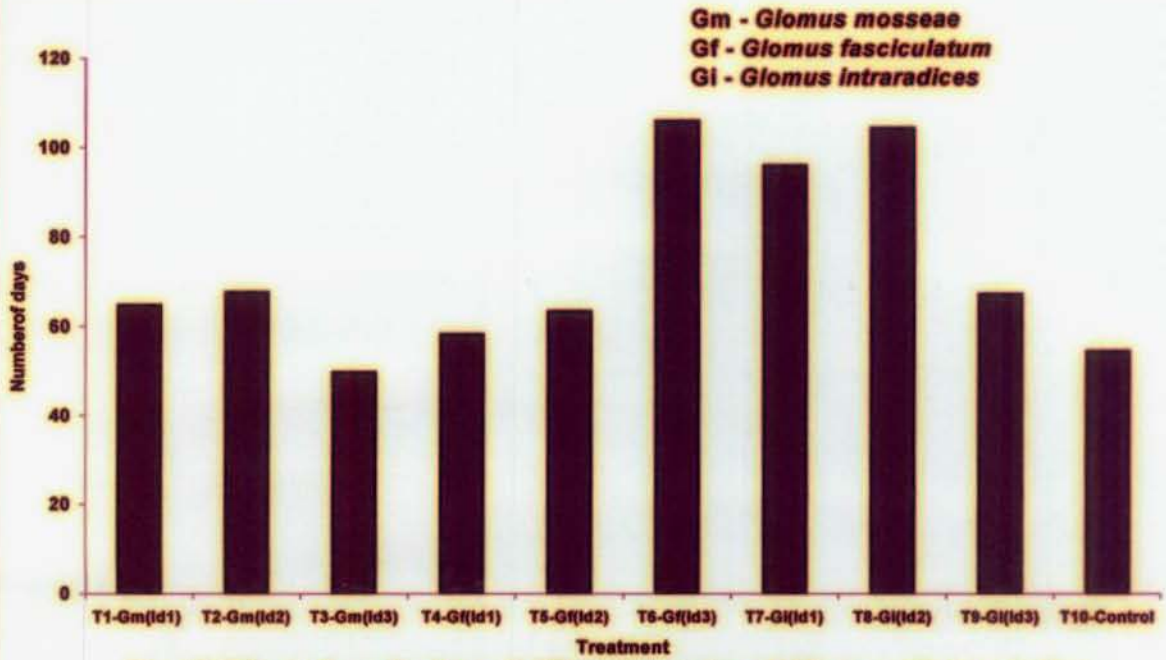



**Fig. 12 Effect of application of different species of *Glomus* 15 days before transplanting on number of days of plant survival**

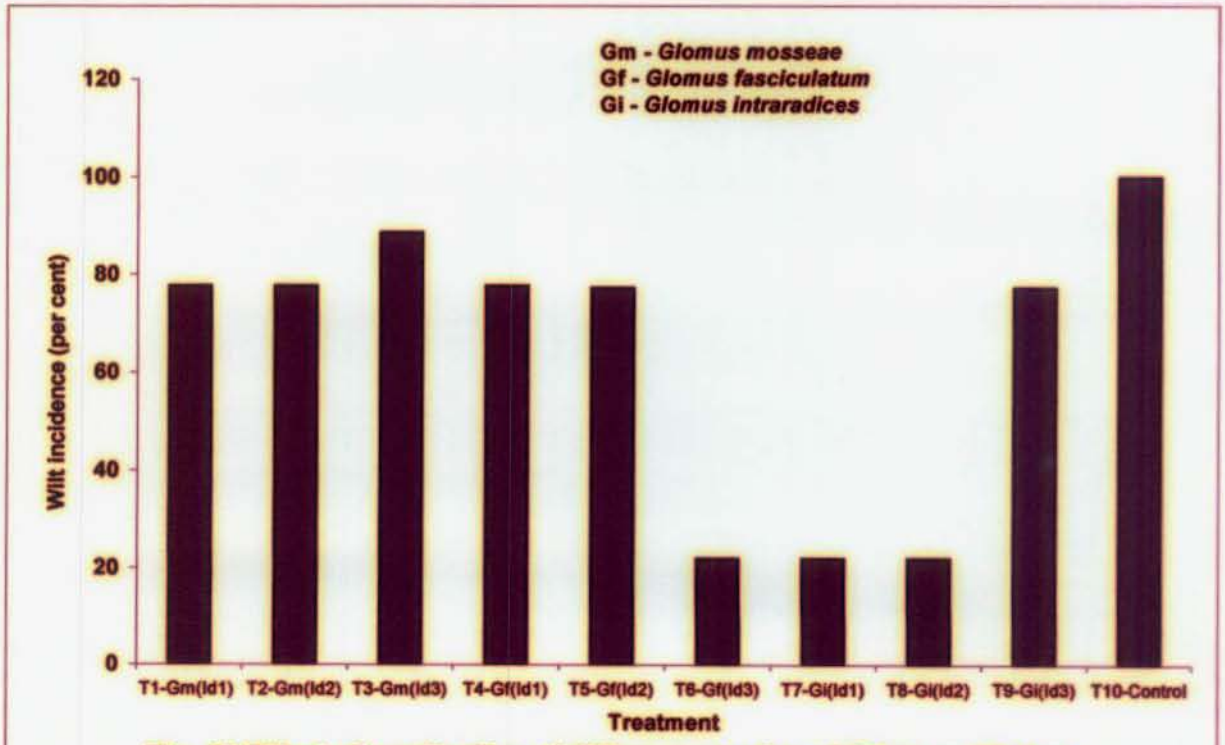



**Fig. 13 Effect of application of different species of *Glomus* 15 days before transplanting on percent wilt incidence**

confirmation with the results reported by Cordier *et al.*, (1996), where the role of mycorrhizal induced root growth compensating for the root loss caused by soil borne pathogens is highlighted. Sood *et al.*, (1997) reported that *Glomus mosseae* was highly effective in controlling the bacterial wilt disease in tomato c.v. Roma till the termination of potted experiment 48 days after challenge inoculation with *R. solanacearum*. In a related experiment, Kumar and Sood (2002) reported that the per cent wilt incidence of the susceptible tomato cultivar Solan Gola was reduced to 44.4 per cent when compared to 83.3 per cent in control when they were inoculated with *G. mosseae*. Nandakumar *et al.*, (2003) reported that the native AMF prolonged the disease incidence by as much as 40 days after inoculation with the wilt pathogen when compared to 18 days in control. Moreover, they also observed that the percent disease incidence was reduced in native AMF treated plants when compared to those treated with the commercial culture. This is in confirmation with the results of the present study where the disease was delayed upto 105.8 days. The maximum survival of plants and lower wilt incidence observed in the present findings is thus explained due to the increase in inoculum density of mycorrhiza.

The control plants ($T_{10}$) recorded maximum dry weight (4.49 g) (Fig. 14) and root length (10 cm) (Fig. 15) when compared to AMF treatments with different inoculum densities. The reason for the increased dry weight and root length of control plants may be due to the fact that the seedlings were raised without AMF inoculation and only at the time of transplanting to the wilt sick soil, AMF inoculation was carried out. The AMF that was inoculated might have faced competition from the indigenous AMF present in the wilt sick soil resulting in poor root colonization and establishment of inoculated AMF. This might have accounted for the decrease in plant dry weight and root length of the AMF treated plants when compared to control. This is in confirmation with the findings of Lambert *et al.* (1980) where it was reported that indigenous VAM fungi were more adapted to the particular edaphic conditions as they possessed some survival advantage over the non-indigenous ones of introduced species.

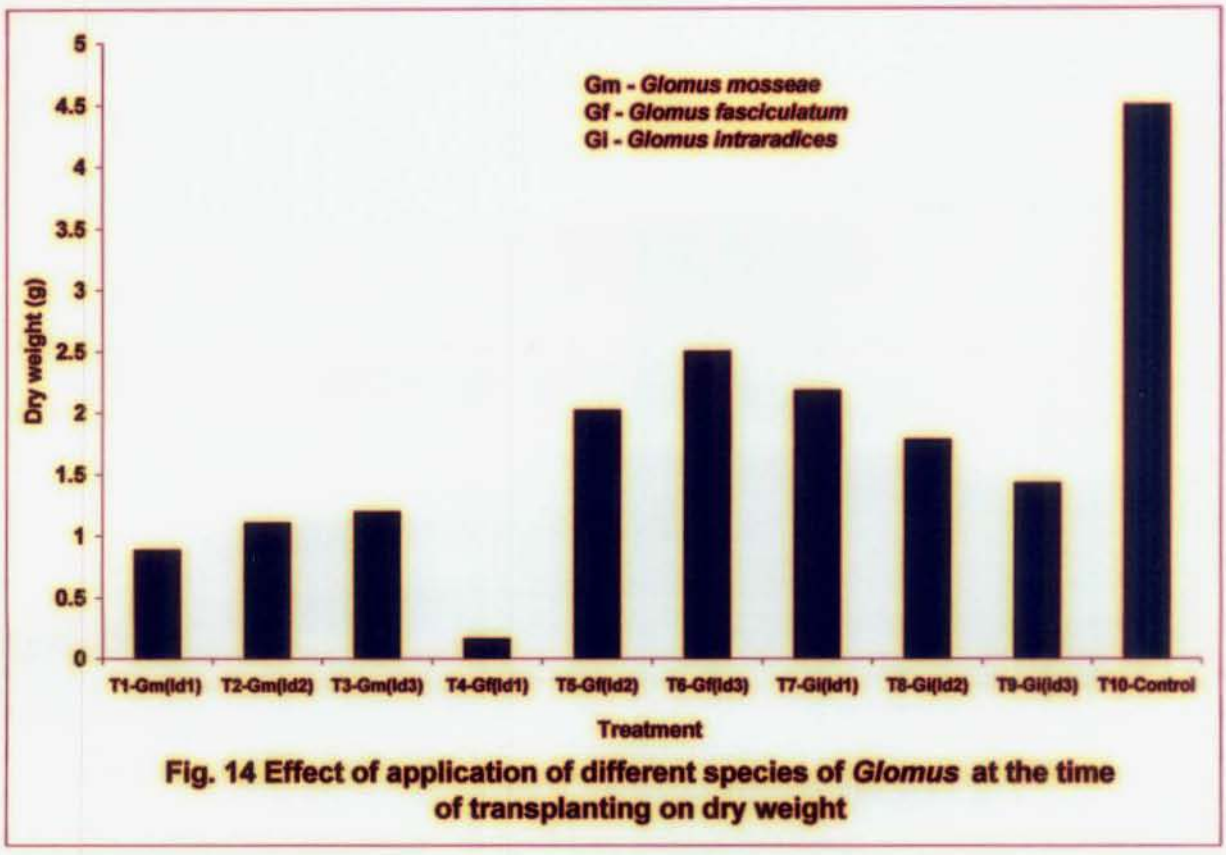



**Fig. 14 Effect of application of different species of *Glomus* at the time of transplanting on dry weight**

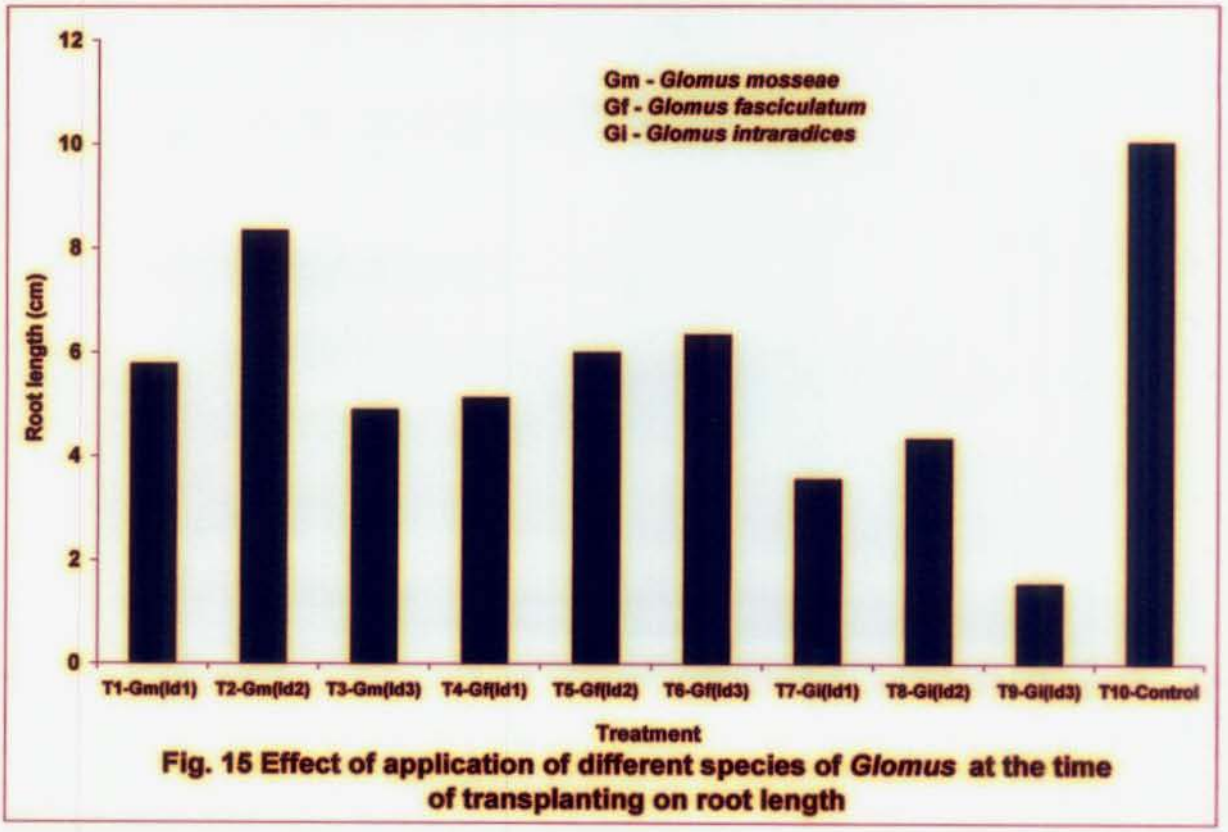



**Fig. 15 Effect of application of different species of *Glomus* at the time of transplanting on root length**

The treatments ($T_9$) with inoculum density @ 75 g $kg^{-1}$ recorded maximum number of days of plant survival (98.89 days) when compared to ($T_{10}$) control (56 days) (Fig. 16) and the same treatment ($T_9$) also recorded the least percent wilt incidence (33 per cent) when compared to ($T_{10}$) control (100 per cent) (Fig. 17). The higher inoculum density of the inoculated AMF resulted in more root colonization and thereby was able to reduce the incidence of bacterial wilt. The AMF inoculation @ 25 g $kg^{-1}$, 50 g $kg^{-1}$ and 75 g $kg^{-1}$ was done at the time of transplanting and it might have taken some time for the inoculated AMF spores to establish on the roots which resulted in less number of days the plants survived. The control plants on the other hand was colonized immediately by the indigenous AM fungi due to its better adaptability to the particular soil conditions and this accounted for the survival of control plants upto 56 days. The percent wilt incidence for the treatments @ 75 g $kg^{-1}$ was comparatively less due to the better AMF root colonization (Cordier *et al.*, 1996). Moreover, the findings of Sood *et al.*, (1997) is in confirmation with the results obtained in the present study. He reported that *Glomus mosseae* was highly effective in controlling the bacterial wilt disease in tomato c.v. Roma till the termination of potted experiment 48 days after challenge inoculation with *R. solanacearum*. Kumar and Sood (2002) observed a reduction in the percent wilt incidence of the susceptible tomato cultivar Solan Gola from 83.3 per cent in control to 44.4 per cent in *G. mosseae* treated plants. Nandakumar *et al.*, (2003) also reported delay in disease incidence for native AMF treated plants upto 40 days after inoculation with wilt pathogen *R. solanacearum* compared to 18 days in control. The higher inoculum density has led to more root colonization which in turn reduced the bacterial wilt incidence and increased the number of days the plants survived.

The optimum inoculation time and inoculum density were tried in different combinations. Based on the dry weight of the plant, root length, percent wilt incidence and maximum number of days of plant survival, the inoculation time and inoculum density were selected.

The dry weight (6.74 g) and root length (7.88 cm) of the plant was found to be higher at 15 days before transplanting when compared to the AMF inoculation at the time of

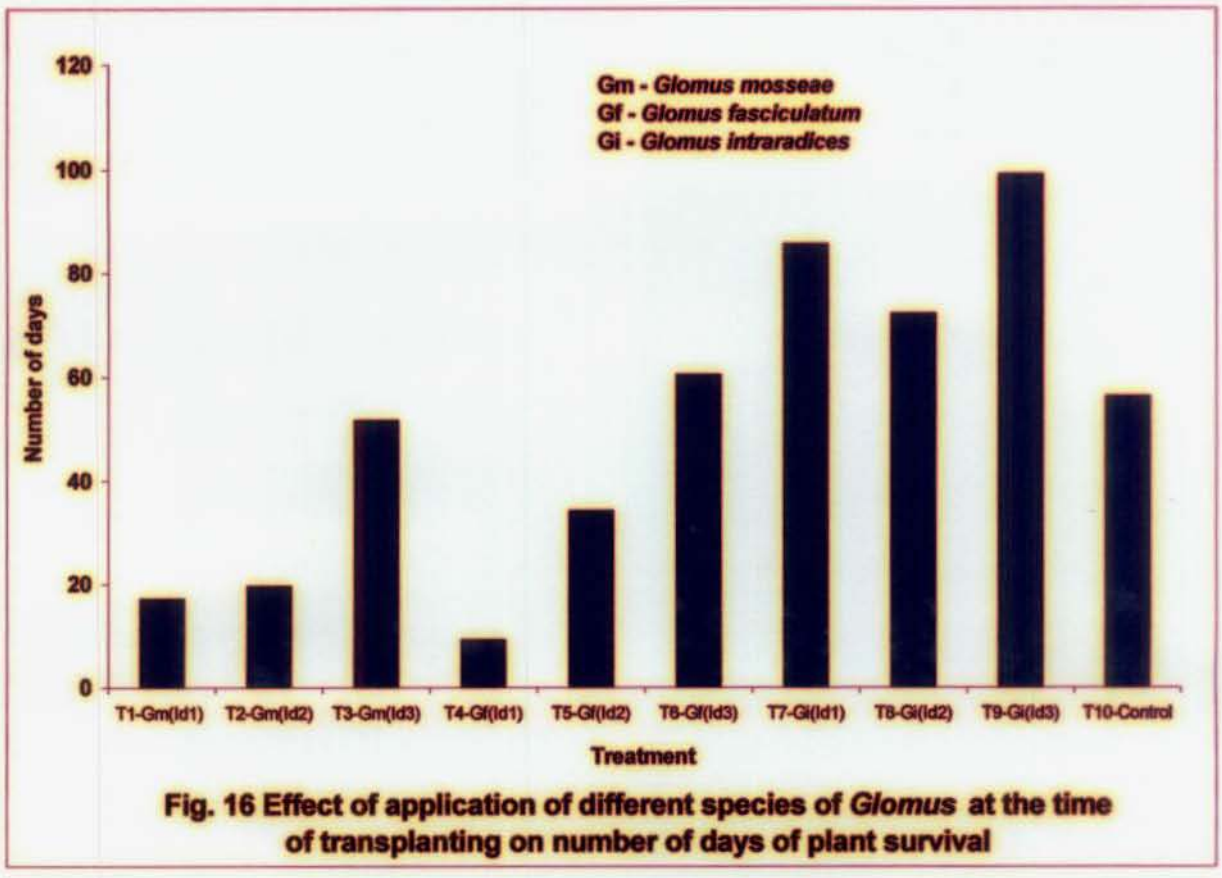



**Fig. 16 Effect of application of different species of *Glomus* at the time of transplanting on number of days of plant survival**

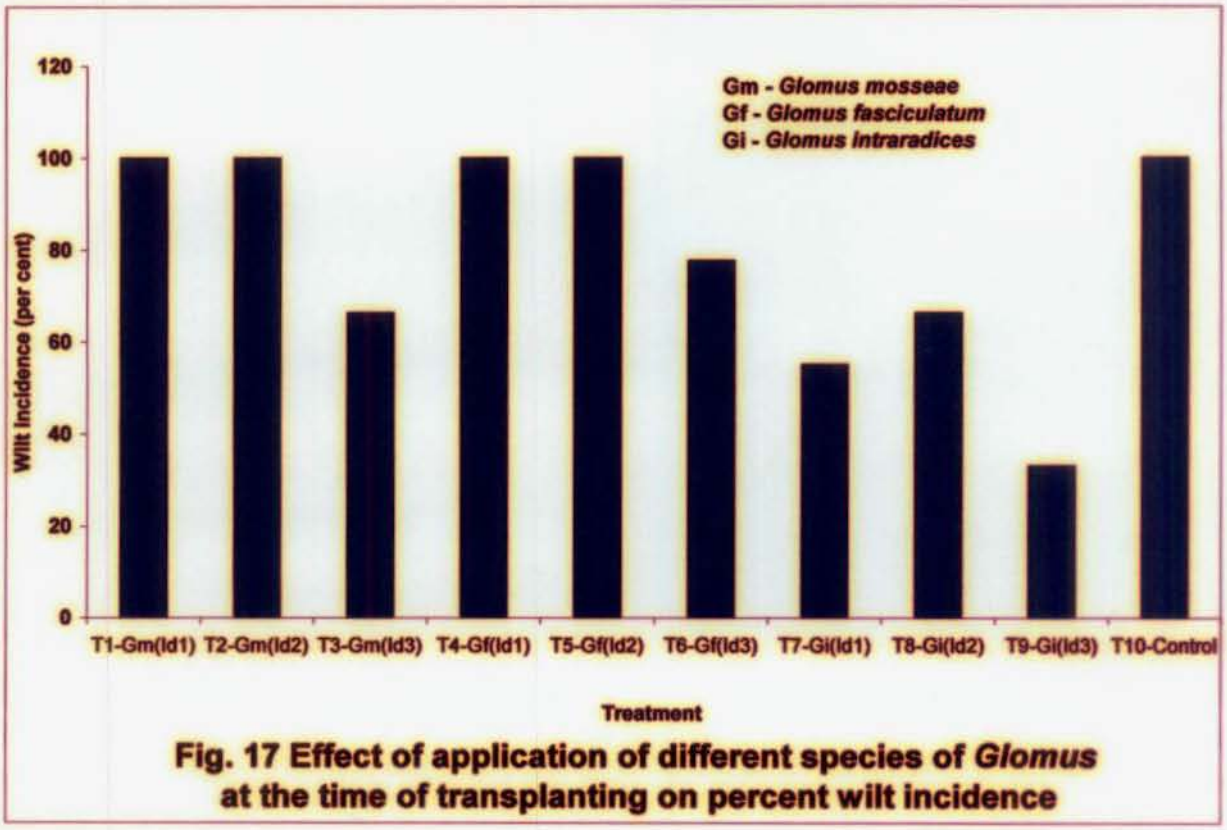



**Fig. 17 Effect of application of different species of *Glomus* at the time of transplanting on percent wilt incidence**

sowing and at the time of transplanting (Fig 18 and 19). The AMF treatments (at the time of sowing, at 15 days before transplanting, at the time of transplanting) showed that the number of days the plants survived was maximum (105.8 days) (Fig. 20) and percent wilt incidence was the lowest (22 per cent) (Fig. 21) when AMF was inoculated at 15 days before transplanting. These results clearly showed that pre-inoculation of AMF (at 15 days before transplanting) gave better plant growth and also reduced wilt incidence and increased the number of days the plants survived. The AMF inoculation at 15 days before transplanting might have allowed the AMF time to colonize roots much efficiently than at the time of sowing and at the time of transplanting thereby reducing the bacterial wilt incidence. Moreover, this also prevented the indigenous ineffective AMF from colonizing the roots. The results showed that the lower percent of wilt incidence, maximum number of days of plant survival, maximum plant dry weight and root length was noticed in the treatments where AMF was inoculated at 15 days before transplanting. The findings were in concurrence with the earlier works (Halos and Zorilla, 1979; Davis and Menge, 1979; Thomson *et al.*, 1983; Hussey and Roncadori, 1982; Grandison and Cooper, 1986; Smith *et al.*, 1986; Iyer and Sundararaju, 1993; Sivaprasad, 1995) who observed that inoculation method to pre-colonize the plants with the VAM fungus 2-4 week before pathogen inoculation was the best and it is a realistic system for transplanted crops that can be inoculated with VAM fungi before they are planted into field soils. In the present study, the pre-inoculation with AMF was carried out at the time of sowing (4 week before transplanting to wilt sick soil) and at 15 days before transplanting (2 week before transplanting to wilt sick soil).

Similarly, the percent wilt incidence was the lowest (22 per cent) and the maximum number of days of survival (105.8 days) were recorded when the AMF inoculum was applied @ 75 g $kg^{-1}$ soil. These results indicated that higher inoculum density formed a mechanical barrier in preventing the entry of pathogens as a result, the disease incidence was less. It was reported that increased inoculum dosage resulted in increased AMF colonization rate and in annual crops with short growing seasons, high inoculum dosages may be required if maximum plant growth stimulation has to be achieved which in turn gave immunity to

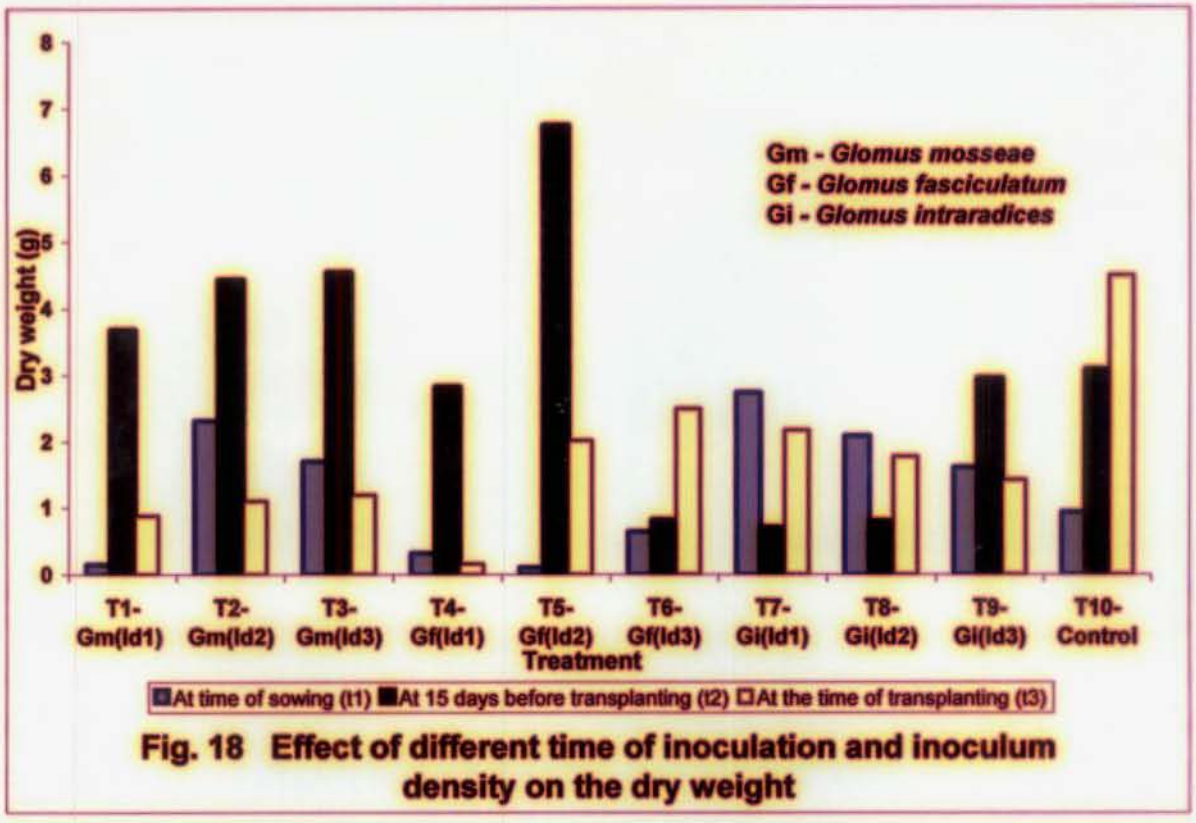



**Fig. 18 Effect of different time of inoculation and inoculum density on the dry weight**

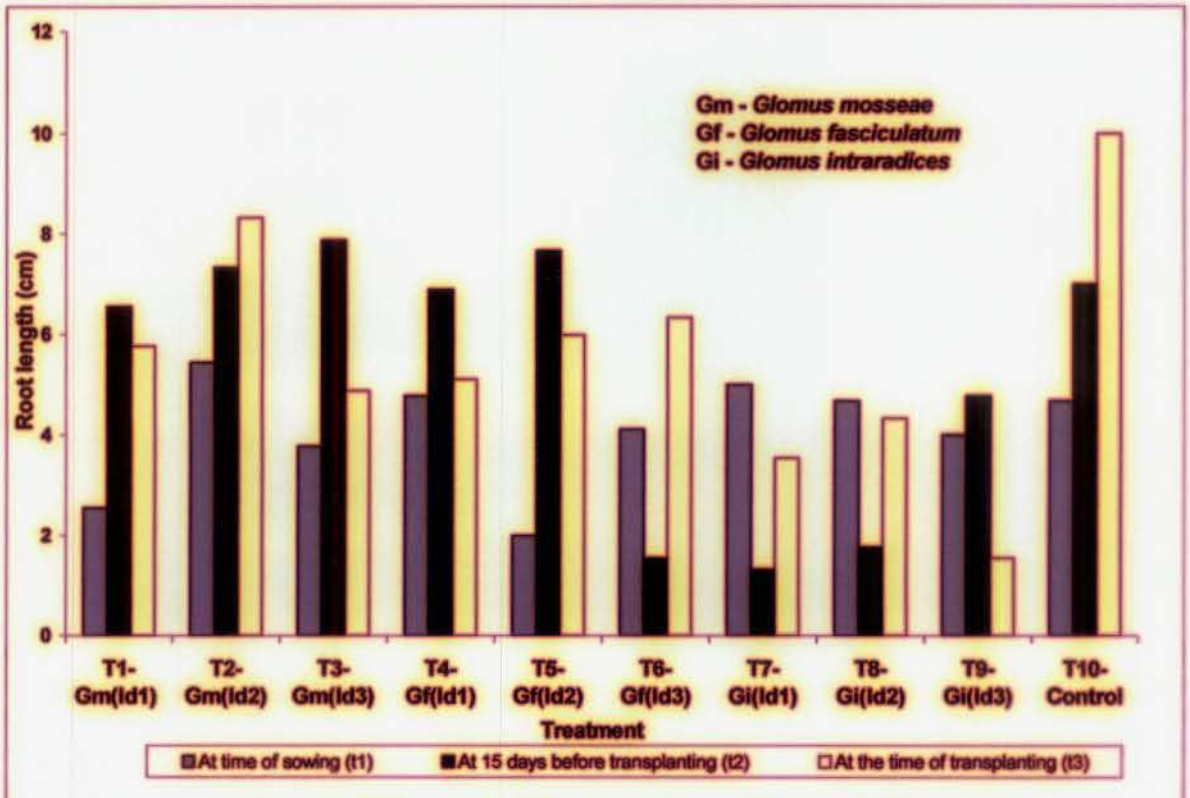



**Fig. 19 Effect of different time of inoculation and inoculum density on root length**

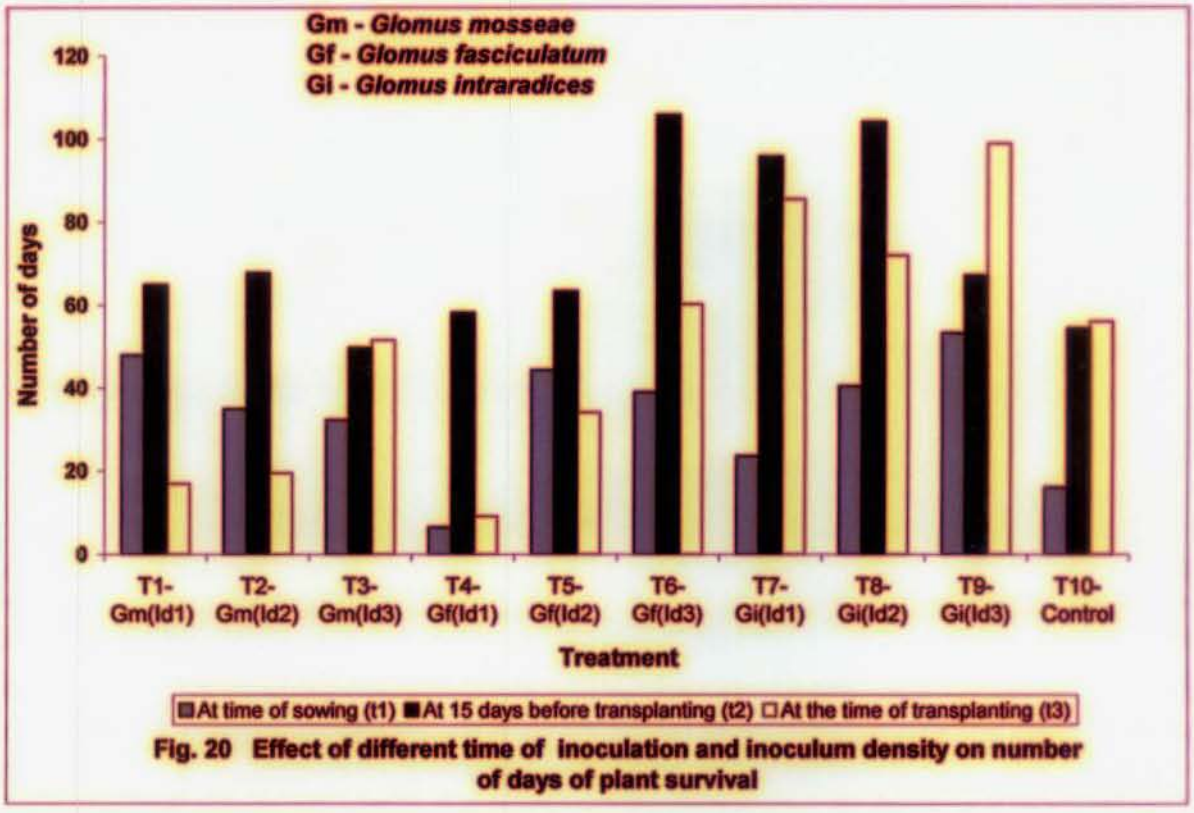



**Fig. 20 Effect of different time of inoculation and inoculum density on number of days of plant survival**

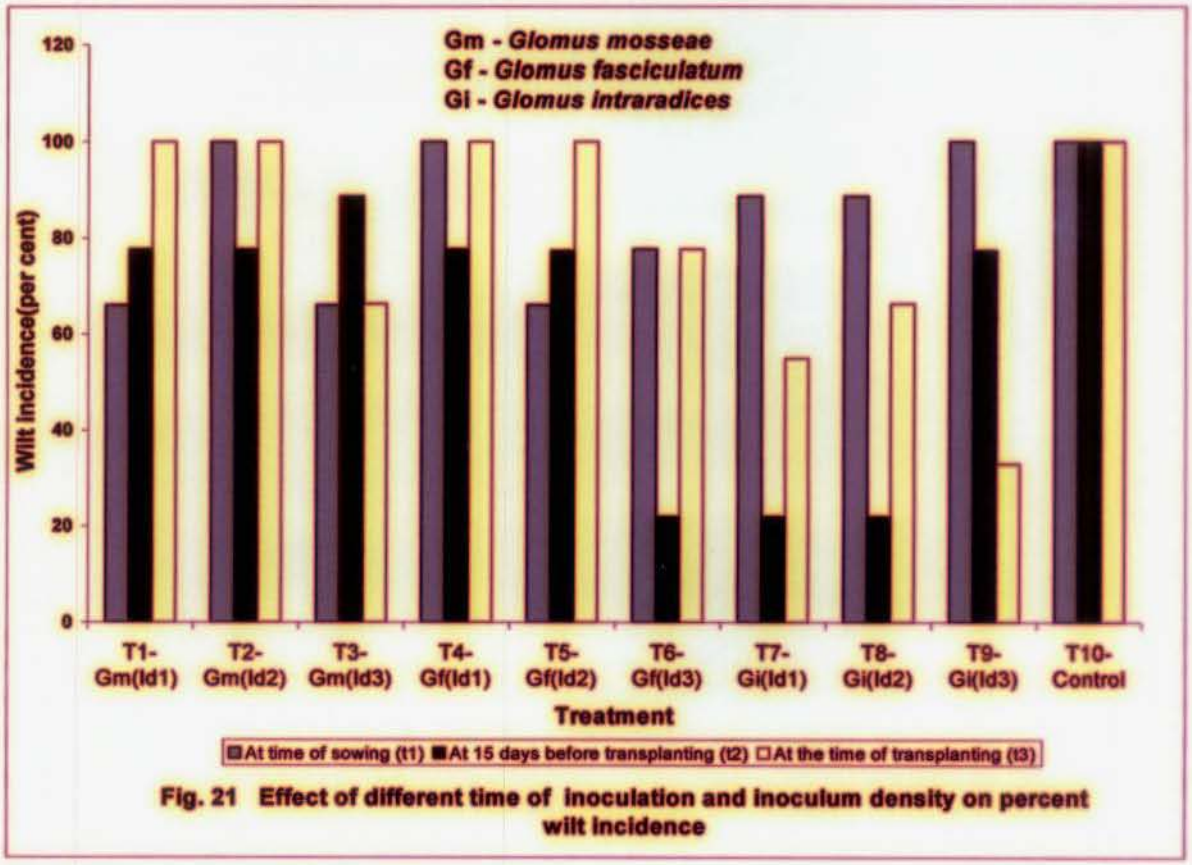



**Fig. 21 Effect of different time of inoculation and inoculum density on percent wilt incidence**

plants against the pathogen. In earlier studies, Daft and Nicolson, 1968; Johnson, 1977; Carling *et al.*, 1979 and Ferguson, 1981 have reported the influence of inoculum density on rate of colonization and suggested that higher inoculum density increased root colonization which in turn imparts resistance to the plant against the pathogen. The results of the present study very well confirmed the role played by high inoculum density and pre-inoculation of plants with AMF atleast 2 weeks before pathogen inoculation in delaying the wilt incidence and increasing the number of days of plant survival.

Based on the dry weight, root length, number of days plants survived and percent wilt incidence, the selection of the optimum inoculum time was carried out. It was found that the AMF inoculation at 15 days before transplanting recorded much higher dry weight, root length, maximum number of days the plants survived and minimum percent wilt incidence when compared to AMF inoculation at the time of sowing indicating that the AMF root colonization was delayed due to the absence of roots. Therefore, AMF could not infect hosts. It was also observed that the percent wilt incidence for the AMF inoculation at the time of transplanting was more when compared to 15 days before transplanting with the number of days of plant survival was less for AMF inoculation at the time of transplanting than at 15 days before transplanting. This indicated that AMF inoculation at the time of transplanting resulted in competition with the indigenous AMF resulting in lesser root colonization of the introduced effective AMF as it took more time to adapt itself to the particular edaphic condition. Hence, it can be seen that even though plants survived and percent wilt incidence was reduced, the AMF inoculation at the time of sowing and at the time of transplanting are not suitable as indicated in the present study.

After finding out best time of inoculation and inoculum density, further studies were conducted with the effective AMF obtained from screening trial so as to assess the effectiveness of AMF at standardized inoculum density and best time of inoculation.

Among the native isolates, the treatment ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded the maximum fresh weight (5.58 g), dry weight (1.39 g) and root weight (90.25 g)

(Fig. 22). This might be due to the competitive ability of the indigenous AMF isolates. This result is in concurrence with the findings of many research workers (Daft and Nicolson, 1972; Lambert *et al.*, 1980; Miller *et al.*, 1989). They observed increased plant growth from inoculation of indigenous VAM species over the non-indigenous VAM species (commercial culture).

The treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded maximum number of survival days (82.11 days) (Fig. 23) and the least percent wilt incidence (55.33 per cent) (Fig 24), when compared to control which recorded less number of days (51 days) and 100 per cent wilt incidence. These results indicated that mixed cultures of native effective AMF isolated from different locations were better able to control the wilt incidence suggesting reciprocal stimulation between fungi. This is in confirmation with the findings of Miller *et al.*, (1989). They also observed a similar result where mixture of two species was better than individual fungus alone due to their stimulatory effect on each other.

Moreover, the native isolates were better adapted to the particular edaphic conditions and possessed some survival advantage over introduced species (Lambert *et al.*, 1980). This indicated that the native isolates of AMF were more beneficial in reducing the wilt incidence, and increasing the number of days the plants survived. This is in confirmation with the findings of Nandakumar *et al.*, (2003) where it was reported that the native AMF prolonged the disease incidence by as much as 40 days after inoculation with the wilt pathogen when compared to 18 days in control. Moreover, they also observed that the percent disease incidence was reduced in native AMF treated plants when compared to those treated with the commercial cultures. In addition, the native isolates were also seen to promote the plant growth. It was also observed that the mixed native cultures gave better result when compared to the application of individual native cultures.

The treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum spore count (175 spores $10g^{-1}$ soil) and maximum per cent root colonization (40 per cent) when compared to ($T_5$) control which recorded a spore count of 80 spores $10g^{-1}$ soil and zero

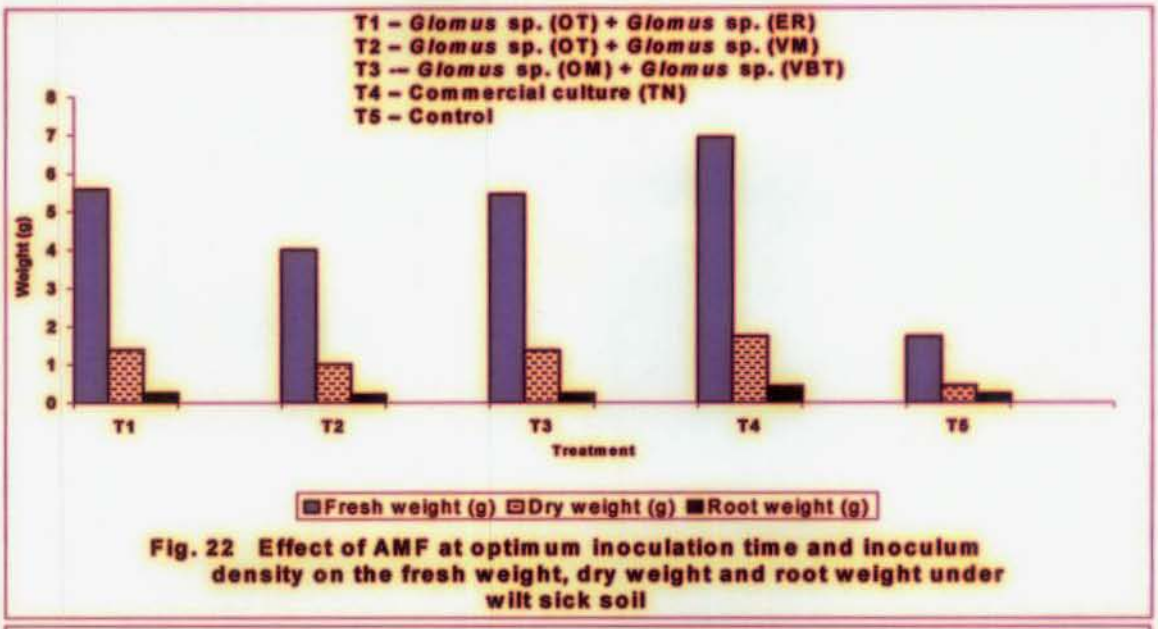



**Fig. 22 Effect of AMF at optimum inoculation time and inoculum density on the fresh weight, dry weight and root weight under wilt sick soil**

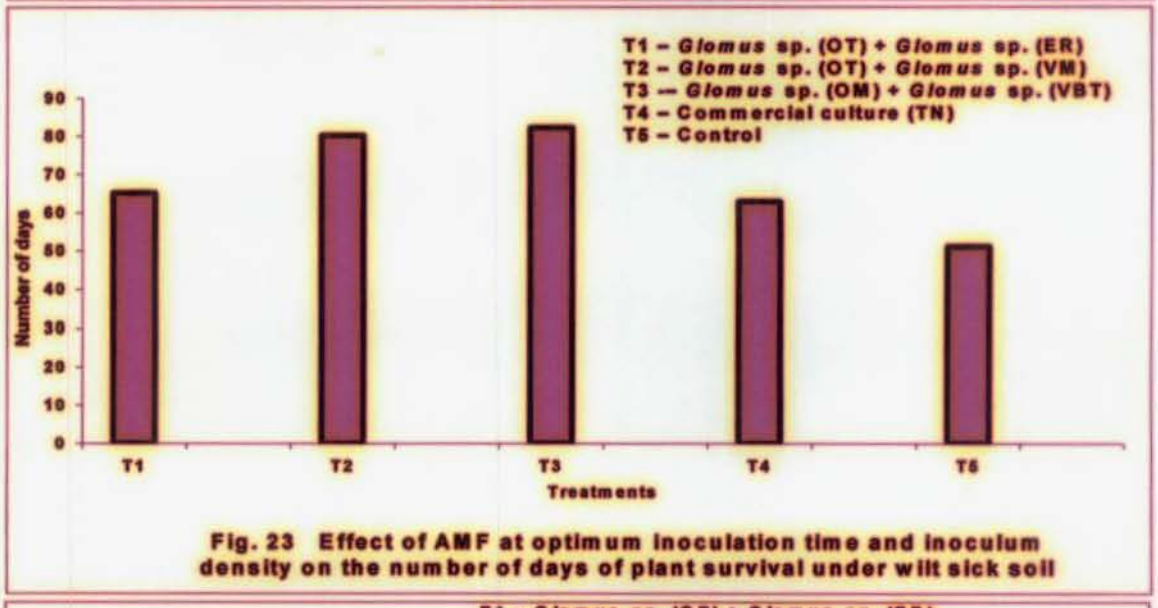



**Fig. 23 Effect of AMF at optimum inoculation time and inoculum density on the number of days of plant survival under wilt sick soil**

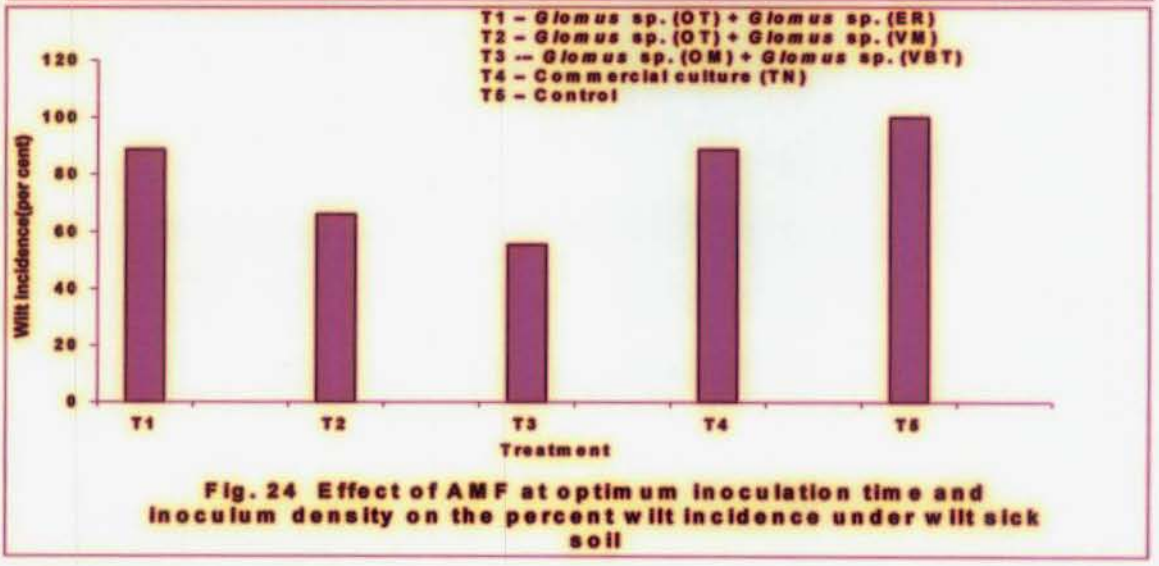



**Fig. 24 Effect of AMF at optimum inoculation time and inoculum density on the percent wilt incidence under wilt sick soil**

per cent root colonization. The treatment ($T_4$) commercial culture (TN) recorded only 20 per cent root colonization and a spore count of 155 spores $10g^{-1}$ soil. The results indicated that mixed inocula were better than either fungus alone. Moreover, spore numbers might be an effective measure of root colonization as was found in the results. The commercial culture recorded low per cent root colonization and low spore count than the native isolate. This also indicated that the non-indigenous AMF (commercial culture) failed to develop in comparison with indigenous AMF. The findings of research workers Daft and Nicolson, 1972; Lambert *et al.*, 1980; Miller *et al.*, 1989 are in confirmation with the results of the present study.

However, the corresponding per cent root colonization (30 per cent) and spore count (139 spores $10g^{-1}$ soil) of the treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) were less compared to that of ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) which recorded per cent root colonization of 40 per cent and spore count of 175 spores $10g^{-1}$ soil even though the wilt incidence was more than *Glomus* sp. (OM) + *Glomus* sp. (VBT). These results suggested that the preferential association between certain plant and fungal species can be evaluated with respect to combinations which provided the greatest root colonization or maximum sporulation but these three factors were not necessarily correlated (Mosse, 1975). The result explained why the treatment ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded a low wilt incidence and maximum days of plant survival even though the sporulation and per cent root colonization were less.

As the native AMF cultures recorded a higher spore count and per cent root colonization over the commercial culture, it was evident that the native cultures were better adapted to a particular edaphic situation when compared to the introduced cultures. Based on the number of days of plant survival, per cent wilt incidence, plant height, per cent root colonization and spore count, the native culture combinations of *Glomus* sp. (OM) + *Glomus* sp. (VBT) and *Glomus* sp. (OT) + *Glomus* sp. (VM) were selected as the best two among the three native culture combinations.

The effect of AMF on the fresh weight, dry weight, root length, root weight and plant height were studied under field conditions using the tomato variety Pusa Ruby (highly susceptible) and the tomato variety Mukthi (moderately resistant) was used for comparison.

The AMF inoculated Pusa Ruby plants recorded improved plant growth with respect to the biometric parameters *viz.*, plant height, root length, root weight, fresh weight and dry weight over the control plants. The chemically treated plants also recorded increased plant growth over control. However, the treatment ($T_4$) with commercial culture (TN) recorded much less growth promotion when compared to the native cultures (Fig. 25 and 26). The results are in confirmation with the findings of Miller *et al.*, (1989) who observed that dual inoculation of two species gave a much better stimulation for plant growth when compared to individual application of a particular species. Moreover, the reduced growth of Pusa Ruby on inoculation with the commercial culture is in concurrence with the results of Lambert *et al.*, (1980) and Raji (2002) where indigenous fungi were more efficient at increasing plant growth in soils to which they have become adapted.

The AMF inoculated Mukthi plants also recorded increased plant growth over control with respect to the biometric characters *viz.*, plant height, root length, root weight, fresh weight and dry weight. As in the case of Pusa Ruby, the native AMF inoculated Mukthi plants recorded improved plant growth over the commercial culture (TN) with respect to plant height, root length, fresh weight and dry weight (Fig. 27 and 28). Chemical control ($T_5$) also recorded increased plant height, root length, root weight, fresh weight and dry weight over control. These results indicated that the native isolates are better adapted to local conditions, when compared to the introduced AMF cultures (Lambert *et al.*, 1980; Raji, 2002).

The effect of AMF on the percent wilt incidence and number of days of plant survival were studied under field conditions using the tomato varieties Pusa Ruby (highly susceptible) and Mukthi (moderately resistant) was used for comparison.

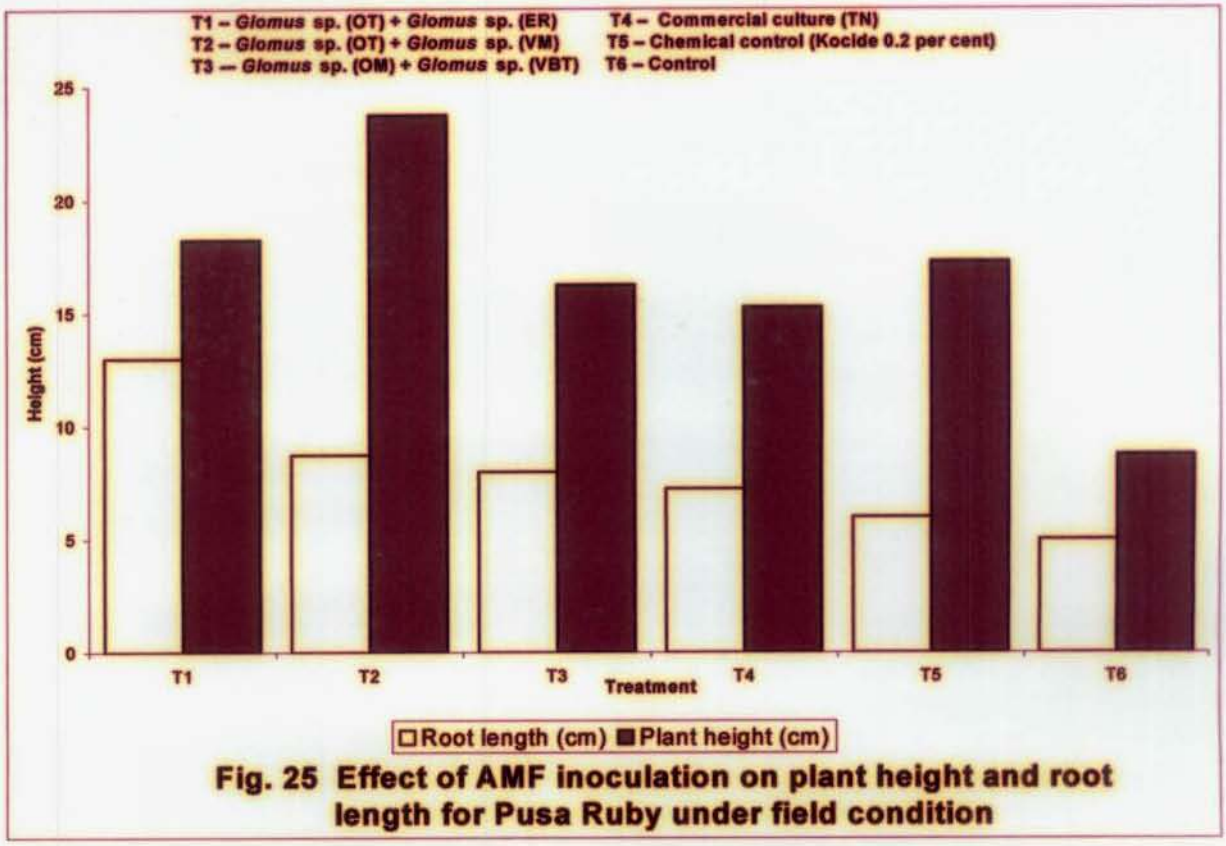



**Fig. 25 Effect of AMF inoculation on plant height and root length for Pusa Ruby under field condition**

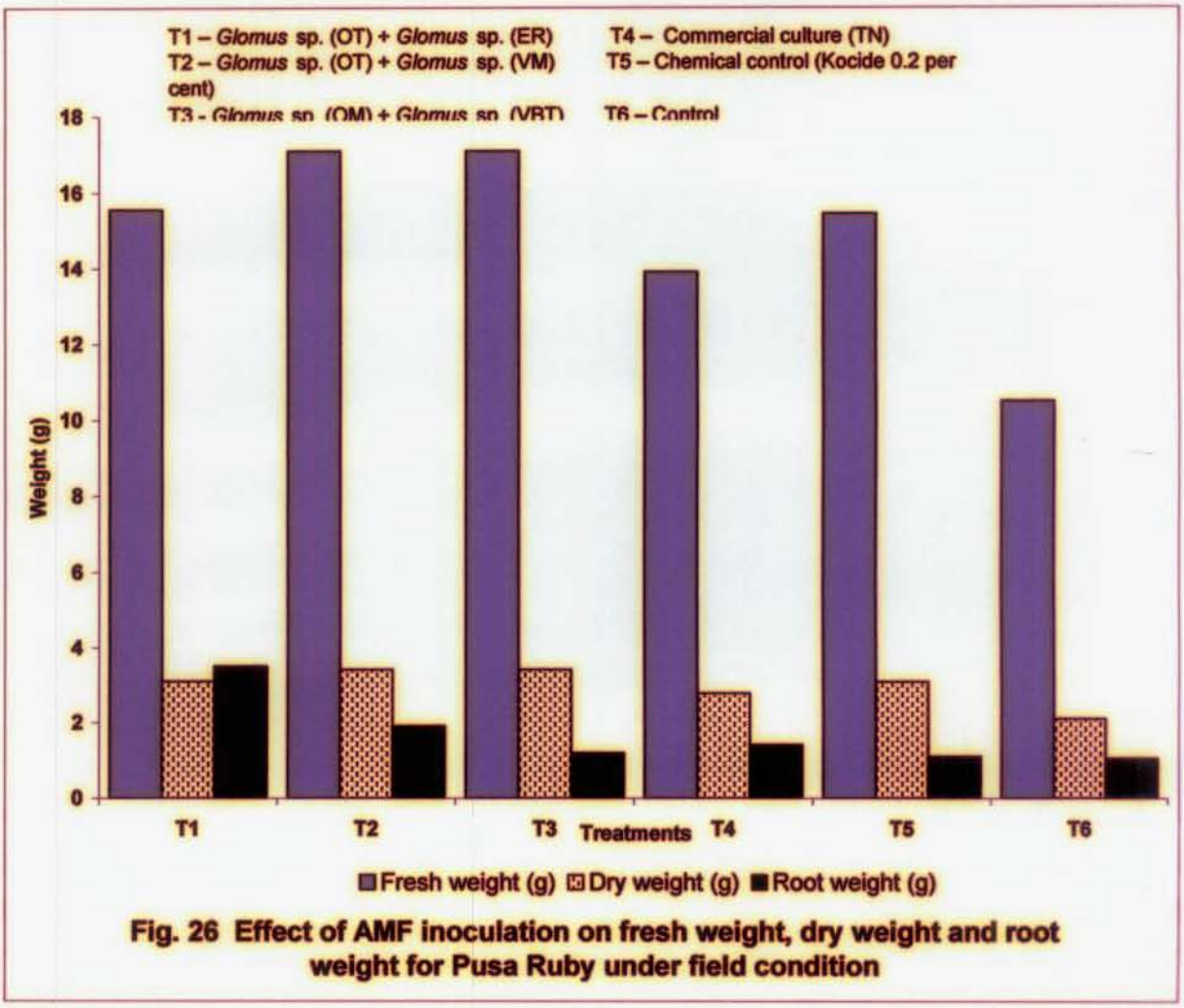



**Fig. 26 Effect of AMF inoculation on fresh weight, dry weight and root weight for Pusa Ruby under field condition**

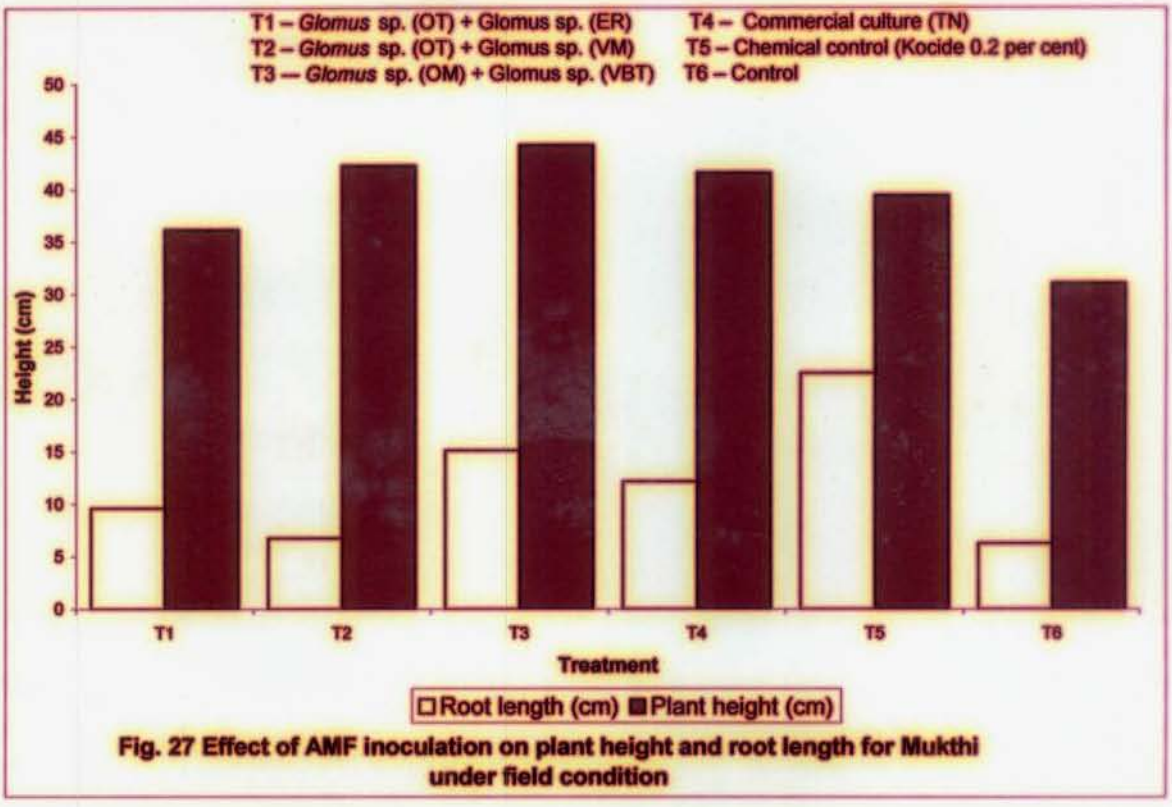



**Fig. 27 Effect of AMF inoculation on plant height and root length for Mukthi under field condition**

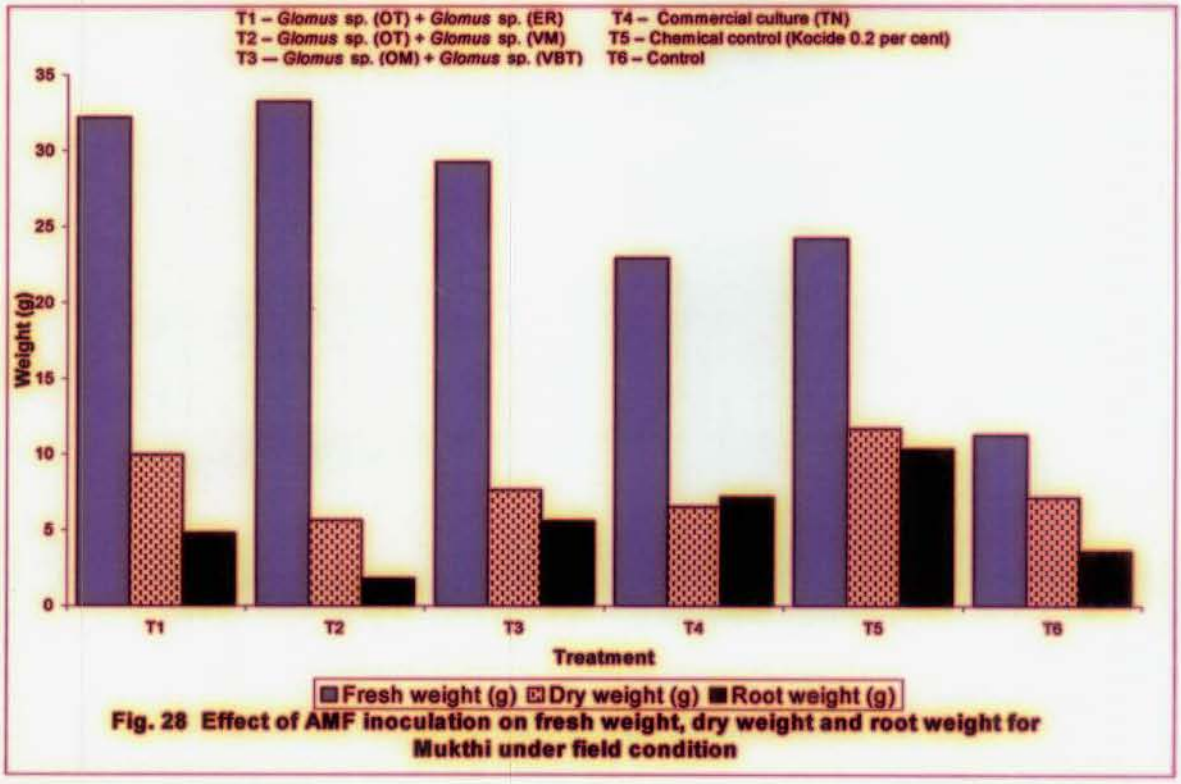



**Fig. 28 Effect of AMF inoculation on fresh weight, dry weight and root weight for Mukthi under field condition**

The Pusa Ruby plants recorded 100 per cent wilt incidence at 31.65 days after transplanting in wilt sick soil (46.65 days after AMF inoculation) (Fig. 30) for all the treatments and the maximum number of days of plant survival was recorded for the treatment ($T_5$) with kocide (0.2 per cent) 32.67 days after transplanting to wilt sick soil. It was also observed that the treatment ($T_4$) commercial culture (TN) inoculated plants survived only for 26.24 days after transplanting (41.24 days after AMF inoculation), which was less than that recorded by the native cultures (Fig. 29). However, the treatments with the native and commercial culture of AMF recorded more number of days of plant survival when compared to control (15.25 days after transplanting to wilt sick soil). This is in confirmation with the findings of Sood *et al.*, (1997) who reported that *Glomus mosseae* was highly effective in controlling the bacterial wilt disease in tomato c.v. Roma till the termination of potted experiment 48 days after challenge inoculation with *R. solanacearum*.

A similar study conducted by Nandakumar *et al.*, (2003) also reported that the native AMF was able to delay the disease incidence by 40 days after inoculation with the wilt pathogen *R. solanacearum* when compared to control where wilting occurred within 18 days which further confirms the present finding. The results also indicated that Pusa Ruby being a susceptible variety, was more prone to wilt incidence and this might be the reason for the 100 per cent wilt incidence recorded by the variety (Paul, 1998; Akbar, 2002; Raji, 2002). The reason for biological control not much effective against wilt incidence may be due to the fact that biological control with mycorrhizae, like most instances of biological control, can never confer complete immunity against root diseases, but impart a degree of resistance or tolerance against the soil borne pathogens (Wilhelm, 1973). The native isolates prolonged the disease incidence as they were more adapted to particular edaphic conditions and possess some survival advantage over introduced species in soils of similar conditions.

Although chemical control with kocide (0.2 per cent) at the time of transplanting increased the number of days of plant survival (32.67 days), the wilt incidence recorded was 100 per cent. This result is in contrast with the findings of Akbar (2002), who reported that chemical control with kocide @ 0.15 per cent as drenching at time of planting and 30 DAP

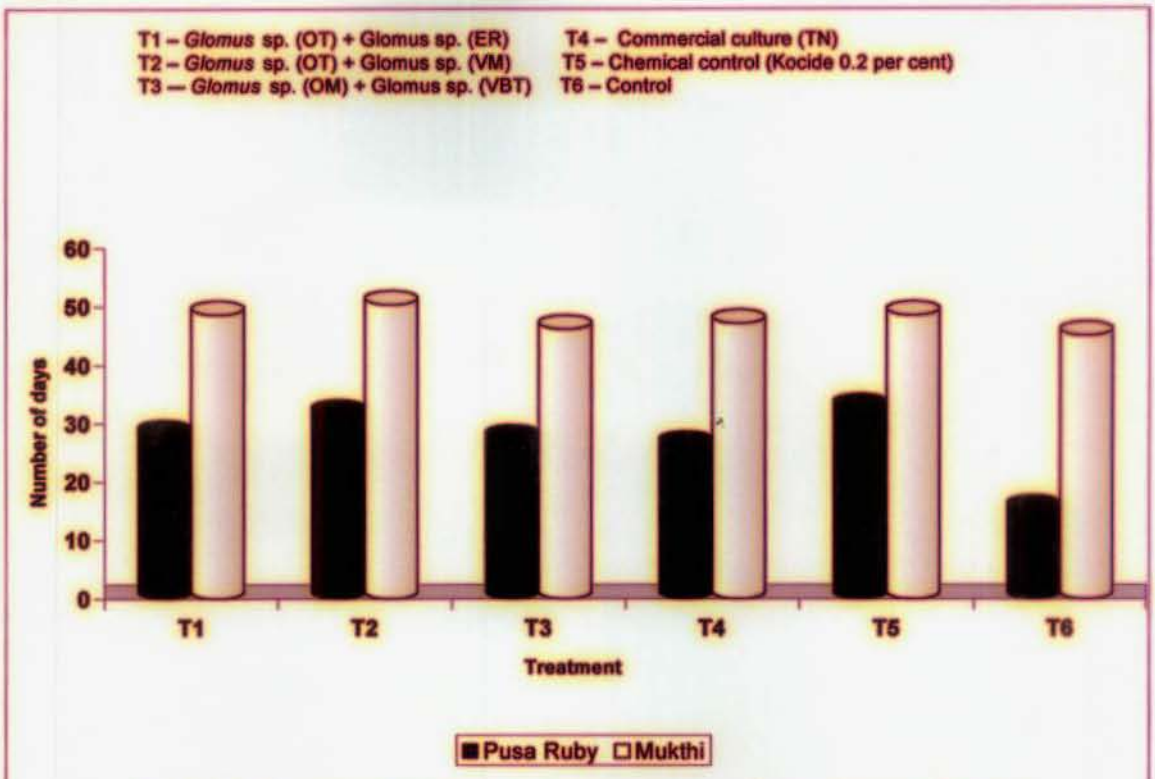



**Fig. 29 Effect of AMF inoculation on number of days of plant survival for Pusa Ruby and Mukthi under field condition**

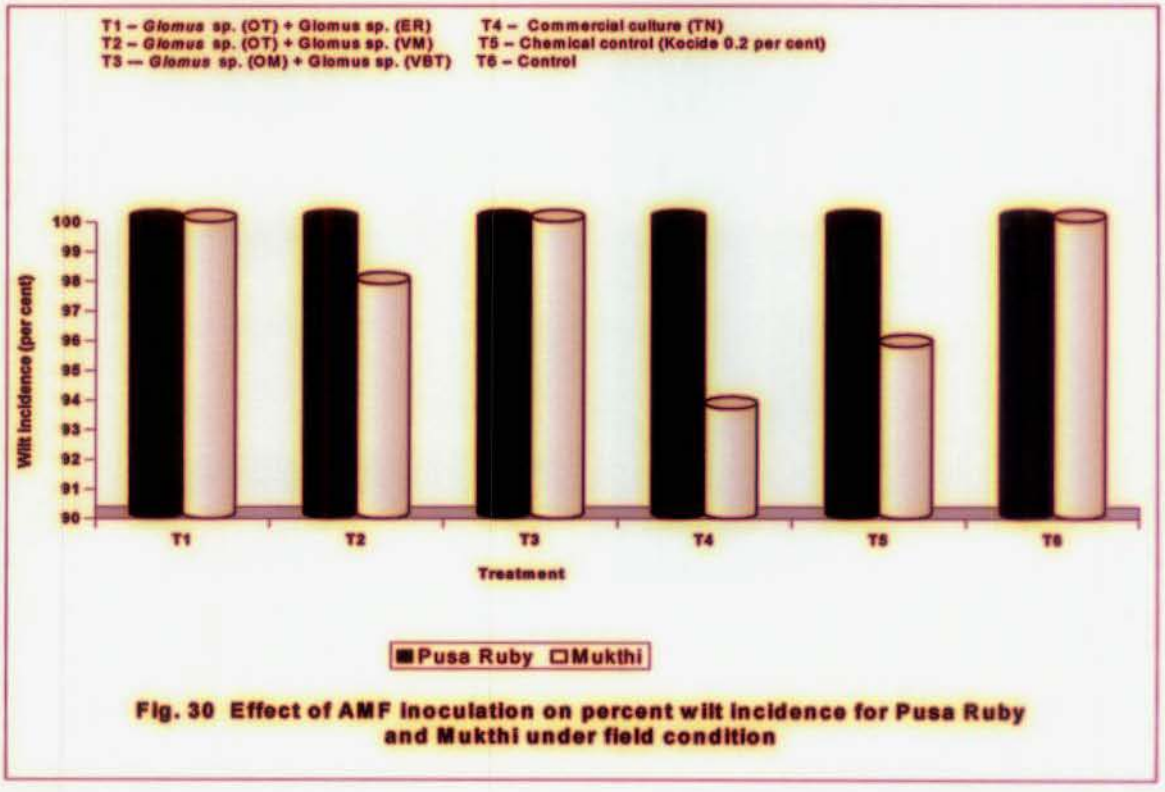



**Fig. 30 Effect of AMF inoculation on percent wilt incidence for Pusa Ruby and Mukthi under field condition**

gave the least wilt incidence for the resistant variety Sakthi under field conditions. The wilting per cent of Sakthi variety of tomato under field conditions was only 42.54 per cent at 75 DAP. The difference in result obtained in the present study with chemical control might be due to the fact that in the present study, Pusa Ruby variety of tomato was used. This variety is highly susceptible to bacterial wilt and this may have resulted in the higher wilt incidence when compared to the variety Sakthi which is resistant to the wilt pathogen resulting in lower wilt incidence. Thus, susceptibility of the Pusa Ruby variety to wilt pathogen might be due to variation in host genotype which in turn played an important role in the AMF symbiotic association and in turn influenced the incidence of bacterial wilt (Graham and Sylvertsen, 1985; Graham *et al.*, 1991; Jayaraman and Kumar., 1995; Markose, 1996; Nakaho *et al.*, 2000).

The least bacterial wilt incidence (93.72 per cent) recorded by Mukthi variety was for the treatment ($T_4$) with the commercial culture (TN) (Fig. 30). This result indicated the efficiency of the commercial culture (TN) over the native isolates of AMF. This might be due to the competitive ability of the commercial culture over the indigenous AMF isolates. This result is in concurrence with the findings of many research workers (Powell, 1976; Powell, 1977a, 1977b, 1977c) and Powell and Daniel (1978). They observed increased plant growth from inoculation with non-indigenous VAM species even into non-sterile soils containing indigenous species. The only native isolate combination to record less than 100 per cent wilt incidence was the treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) (97.90 per cent) which also recorded the maximum number of days of plant survival (50.33 days after transplanting i.e., 65.33 days after AMF inoculation) (Fig. 29). This is in confirmation with the findings of Sood *et al.*, (1997) who observed that *Glomus mosseae* was highly effective in controlling the bacterial wilt disease in tomato c.v. Roma till the termination of potted experiment 48 days after challenge inoculation with *R. solanacearum*.

A similar finding by Nandakumar *et al.*, (2003) where the native AMF was able to prolong the disease incidence by 40 days after inoculation with *R. solanacearum* further confirm the results obtained in the present study. The chemical control ($T_5$) with kocide (0.2

per cent) also recorded less than 100 per cent wilt incidence. The native isolate combinations ($T_3$) *Glomus* sp. (OM) + *Glomus* sp. (VBT) and ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded 100 per cent wilt incidence in Mukthi. The wilt incidence being less for the commercial culture (TN) might be due to the fact that initial growth of the non-indigenous fungi (commercial culture) was faster than the native isolates. The findings of many previous workers (Powell, 1976, 1977a, 1977b, 1977c; Powell and Daniel, 1978) are in concurrence with the results of the present study. Moreover, plants may vary in their dependence on the symbiotic association with AMF due to their physiological or anatomical differences (St. John, 1980; Markose, 1996; Nakaho, 2000). This in turn affects the host response to bacterial wilt incidence. The adaptability of the indigenous isolates to a particular edaphic condition due to the possession of some survival advantage over introduced species in soils might have resulted in maximum days of plant survival recorded by the native isolates. The native isolates possess better colonizing ability, faster spread through plants and sporulate and greater inoculum potential and competitive ability (Lambert *et al.*, 1980).

The per cent wilt incidence recorded by chemical control was less than that recorded by the native isolates. This might be due to the effect of the host itself more than that of AMF. Because Mukthi is a moderately resistant variety of tomato against bacterial wilt and the host genotype itself provided some sort of resistance to the plants. The survival of the plants inoculated with AMF upto 50 days after transplanting (65 days after AMF inoculation) in unsterilized field soils also indicated the long term response of VAM inoculation. This is in confirmation with the earlier findings of Johnson and Crews, (1979); Sivaprasad, (1995). They observed that mycorrhiza inoculated plants transplanted to the field recorded much better survival and growth of the inoculated plants at 4 months after transplant. Moreover, the high bacterial wilt incidence recorded by mycorrhiza inoculated plants may be due to the fact that biological control agents do not impart complete immunity against soil borne pathogens, but only a certain degree of tolerance, which is true in the case of mycorrhiza also (Wilhelm, 1973).

The per cent root colonization of Pusa Ruby variety of tomato at 60 DAT was the highest for the treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and this corresponded with the high spore count at 60 DAT for the same treatment. The AMF infection in the chemically treated plants ($T_5$) was only 10 per cent which was only better than ($T_6$) control. The spore count showed an increase at 60 DAT from that recorded at the time of transplanting and 30 DAT. The control plants recorded a lower spore count which did not change appreciably at 60 DAT. These results indicated that spore count was related with per cent root colonization as suggested by Daft and Nicoloson (1972). Moreover, the availability of a host (Pusa Ruby) may have resulted in an increase in spore population at 60 DAT from the initial count. The root colonization and spore production of the VAM isolates especially the native ones increased during the growth period of the crop. This might be due to the availability of host roots for colonization as VAM are symbiotic fungi found in association with roots of crop plants. These results are in concurrence with the findings of Hayman (1970) who observed that root colonization and sporulation increased through out the growing season, peaking just prior to harvest.

This results have been further confirmed by the findings of Raji (2002) for the tomato varieties BWR-1 and Sakthi where an increase in spore count and root colonization during the growing season was observed which is in concurrence with the results of the present study. The chemical control using kocide (0.2 per cent) registered a lower spore count and per cent root colonization. This might be due to the harmful effect of the chemical on the indigenous and introduced AMF species. This was evident from the reduction in spore count in the chemical treated plots from the initial spore count before chemical treatment. The decrease in spore count naturally led to a decrease in per cent root colonization. This result is in confirmation with the findings of Akbar (2002) where it was observed that the per cent root colonization recorded was the lowest for the kocide (@ 0.15 per cent) treated plants.

The *R. solanacearum* population in the initial stage at the time of transplanting was 53 x $10^4$ cfu $ml^{-1}$. The *Ralstonia* population increased in all the treatments and the least

*Ralstonia* population was recorded by the treatment ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (82.75 x $10^4$ cfu $ml^{-1}$) at 60 DAT when compared to ($T_6$) control (125 x $10^4$ cfu $ml^{-1}$). The pathogen *R. solanacearum* is endemic to the Vellanikkara soils and are known to survive for long periods in soil in the absence of the host. The initial *Ralstonia* population itself was high and the presence of the Pusa Ruby variety of tomato resulted in the built up of the pathogen population which was evident from the present study. The *Ralstonia* population in the Vellanikkara soil was found to increase in soil with the growing season as reported by Raji (2002) which confirmed the results of the present study.

In the case of Mukthi variety, the percentage root colonization at 60 DAT was the highest (60 per cent) for the treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) and this also corresponded with the high spore count at 60 DAT. The AMF infection in the kocide (0.2 per cent) treated plants ($T_5$) was only 10 per cent and the control plants ($T_6$) also recorded 10 per cent root colonization. The spore count showed an increase at 60 DAT from that recorded at the time of transplanting and 30 DAT. The control plants recorded a lower spore count (128.5). These results are in confirmation with the findings of Daft and Nicolson (1972). The availability of host may have resulted in increase of spore population at 60 DAT from the initial count. The root exudates from the host plants might have stimulated the spore production of AMF which is in confirmation with the findings of Becard and Piche (1989a). They observed that the presence of a growing root significantly stimulated the growth of AM fungi even when there was no root fungal contact and active fungal growth ceased upon root removal. The root colonization of the AMF isolates especially the native ones increased during the growth period of the crop. This might be due to the availability of host roots for colonization as VAM are symbiotic fungi found in association with roots of crop plants.

These results are in concurrence with the findings of Hayman (1970) who observed that root colonization and sporulation increased through the growing season, peaking just prior to harvest which is in concurrence with the results of the present study. The chemical control with kocide (0.2 per cent) registered a lower spore count and per cent root

colonization. This might be due to the harmful effect of the chemical on AMF species. This is evident from the reduction in spore count of the chemically treated plots from the initial spore count before chemical treatment. The decrease in spore count naturally led to a decrease in per cent root colonization. This result is in confirmation of the findings of Akbar (2002) where he observed that the per cent root colonization recorded was the lowest for the kocide treated plots.

The *Ralstonia* population of Mukthi did not show much variation when compared to that observed in the Pusa Ruby rhizosphere. The least *Ralstonia* population was recorded for the treatment ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) (76 x $10^4$ cfu $ml^{-1}$) at 60 DAT when compared to ($T_6$) control (120 x $10^4$ cfu $ml^{-1}$). The Vellanikkara soils are conducive for the survival of the *Ralstonia* population and the presence of the host (Mukthi) might have resulted in the build up of the pathogen population at 60 DAT. The *Ralstonia* population in the Mukthi rhizosphere did not show much variation when compared to that found in the Pusa Ruby rhizosphere. The results observed in the present study is in concurrence with the findings of Raji (2002), where it was noticed that the *Ralstonia* population in soil increased with the growing season.

The rhizosphere spore count and per cent root colonization for Pusa Ruby was comparatively less when compared with that of Mukthi rhizosphere soil. This may be due to the fact that the Pusa Ruby being a susceptible variety, is less colonized by AMF when compared to the moderately resistant Mukthi variety which recorded a much higher colonization and spore count. This is in confirmation with the findings of Harikumar and Potty (1999) and Raji (2002) where they reported root colonization of more than 85 percent in sweet potato and tomato grown in Vellanikkara soils. Raji (2002) also reported high spore count in the AM inoculated rhizosphere of tomato variety Sakthi and BWR-1, which are resistant and moderately resistant varieties. This further confirms the higher root colonization of Mukthi variety when compared to Pusa Ruby. But, even then the root colonization and spore count in Mukthi variety were very less, when compared to the results of Raji (2002). This might be due to the climatic factors prevailing during the crop growth

period. The increase in spore count was not much at 60 DAT from what was present at 30 DAT. This might be due to the heavy rains during this period (Appendix 5).

The initial spore count was less as there was heavy rain during the time of transplanting. The spore population increased gradually for the native AMF inoculated plots at 30 DAT as the indigenous AMF are better able to adapt to natural edaphic conditions (Lambert *et al.*, 1980) and there was reduction in the rainfall. The soil temperature might also have influenced spore production rather than air temperature (Smith and Bowen, 1979; Graham *et al.*, 1982). The soil temperature from date of transplanting upto 30 DAT was between 30-35°C and this favoured spore production (Schenck and Schroder, 1974). This is in confirmation with the results obtained for spore production at 30 DAT in the present study.

However, after this period (30 DAT), the soil temperature gradually increased and reached beyond 40°C. During the period between 30 DAT and 60 DAT the soil temperature was generally more than 35°C. This might have had an adverse effect on AMF sporulation and root colonization as the optimum temperature for AMF sporulation and root colonization is 30-35°C. As a result, the per cent root colonization and sporulation decreased. This confirmed the slight increase in spore count and AMF root colonization at 60 DAT.

Moreover, heavy rains during the 6$^{th}$ week after transplanting (42 DAT) reduced the spore count drastically and this confirms with the results obtained by Trimurtulu and Johri (1998) where they reported higher spore count during the post rainy (November) and Winter (February) months when compared to summer (May) and rainy (August) months when the spore count was less. This further explains only a slight increase in spore count observed in the present study during the month of October (60 DAT).

Another factor which might have influenced the AMF spore count and root colonization is the sunshine hours (photoperiod). The sunshine hours during the period 30 DAT was 8.7 hr day$^{-1}$ and that during 60 DAT was 2.1 hr day$^{-1}$. This difference in photoperiod might have influenced the AMF spore count and root colonization as was

reported by Hayman, 1974; McCool, 1981; where they observed that a photoperiod of 12hr or more may be more important than light intensity in providing high levels of root colonization.

The *Ralstonia* population in the initial stage at the time of transplanting was 53 x $10^4$ cfu $ml^{-1}$. There was a gradual increase in the *Ralstonia* population for all the treatments in both the tomato varieties as the crop growth progressed. The *Ralstonia* population was the lowest for the treatment ($T_1$) *Glomus* sp. (OT) + *Glomus* sp. (ER) although the per cent AMF root colonization and spore count was highest for ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM). The maximum *Ralstonia* population was recorded at 60 DAT when the minimum and maximum temperature was 23.1-29.36 $^0$C. This confirms with the results obtained by Sunaina and Gupta (1998) where they observed that maximum soil population of *Ralstonia* occurred when the mean minimum and maximum air temperature ranges between 20.5-28 $^0$C. The period at 60 DAT also happened to receive heavy rainfall (268.3 mm $week^{-1}$) and this also is in concurrence with the observations of Sunaina and Gupta (1998).

The soil temperature during this period (60 DAT) at 5 cm, 10 cm and 15 cm depths varied between 26-31 $^0$C and the maximum air temperature was 29.3 $^0$C. The period between 30 DAT and 60 DAT received around 310 mm of rainfall. This data suggested that the increase in *Ralstonia* population at 60 DAT was due to the favourable environmental conditions which is in confirmation with the findings of Bora *et al.* (1996). They observed that a soil temperature range of 25-30 $^0$C accompanied by maximum air temperature 26-30 $^0$C and monthly rainfall ranging between 200-300 mm favoured bacterial growth and multiplication resulting in severe wilt incidence.

The present study clearly showed that dual inoculation of AMF selected from different locations gave better control of plant diseases and also improved plant growth when compared with inoculation of individual AMF. Another important aspect found in this study was the effect of pre-inoculation of AMF and the determination of the optimum inoculum density. It was found out that pre-inoculation of tomato seedlings 15 days before transplanting gave best control of the bacterial wilt pathogen and optimum inoculum density

was found to be 75 g $kg^{-1}$ soil. The lower inoculum densities @ 25 g $kg^{-1}$ and 50 g $kg^{-1}$ soil failed to give effective control. Moreover, in the field experiment it was found that the selected native AM fungi were able to prolong the occurrence of the disease upto 47 days after AMF inoculation in the case of Pusa Ruby (highly susceptible) and 65 days after AMF inoculation in the case of Mukthi (moderately resistant). This is in confirmation with the findings of Sood *et al.*, (1997). They observed that *Glomus mosseae* was highly effective in controlling the bacterial wilt disease in tomato c.v. Roma till the termination of potted experiment 48 days after challenge inoculation with *R. solancearum*. This brings to the fore the importance of an integrated approach in the control of bacterial wilt pathogen. The use of tolerant/resistant varieties, clean cultural practices avoiding root injury, correct planting time, removal of wilted plants from the field and the use of biocontrol agents including effective native AMF cultures could provide better control against the bacterial wilt pathogen. The most effective native AMF found to reduce wilt incidence (97.90 per cent) and prolong the occurrence of disease (65 days after AMF inoculation) was ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) in the case of Mukthi variety of tomato, whereas in the case of Pusa Ruby, the same treatment ($T_2$) *Glomus* sp. (OT) + *Glomus* sp. (VM) prolonged the disease occurrence upto 46.65 days after AMF inoculation. However, the culture *Glomus* sp. (OT) + *Glomus* sp. (VM) could not reduce the wilt incidence significantly in Pusa Ruby which recorded 100 per cent wilt incidence.

The present study clearly indicated that the AMF is a potential biocontrol agent under pot culture but under field conditions AMF could only delay the incidence of bacterial wilt which could be due to the influence of soil, host and environmental factors on the performance of the inoculated AMF. Moreover, biocontrol agents can never confer complete immunity against the root diseases but imparts resistance /tolerance against soil- borne pathogen. But there are reports of AMF and bacterial antagonists significantly reducing the wilt incidence in susceptible tomato cultivars when applied simultaneously. However, extensive studies are needed under field conditions to test the long-term effect of AMF application individually and in combination with other antagonists in biocontrol of bacterial wilt.

# SUMMARY

# 6. SUMMARY

The present study on "Biocontrol of bacterial wilt in tomato using arbuscular mycorrhizal fungi" was carried out at the Dept. of Plant Pathology, College of Horticulture, Vellanikkara during the period 2000-2002. The experiment consisted of isolating and identifying AMF fungi from high and low wilt incidence areas of Thrissur and Palakkad districts. The predominant AMF from these locations were screened under sterile conditions and the optimum inoculum density and inoculation time determined under unsterile pot culture conditions. This was tested in the field to find out the efficacy of the selected AMF at the standardized inoculation time and inoculum density under field conditions. The summary of the results obtained are given below:

The soil samples were collected from high and low wilt incidence areas of Thrissur and Palakkad district from tomato and maize rhizospheres. The soil samples were analyzed for their nutrient status as well as pH. The pH of Vellanikkara soils (Thrissur) was acidic, whereas that of Ozhalapathy and Eruthiampathy (Palakkad) were towards neutral to alkaline range. The enumeration of *Ralstonia solanacearum* population was undertaken to find out the population of the pathogen in the high wilt and low wilt soils. The maximum *Ralstonia* population was found in tomato rhizosphere soil of Eruthimapathy and the minimum in tomato rhizosphere soil of Ozhalapathy. The soil nutrient status *viz.*, N, P, K, Ca and Mg content were determined for the six soil samples in addition to their pH. The nitrogen, potassium, calcium and magnesium content of Ozhalapathy and Eruthiampathy soils were higher when compared to Vellanikkara soils. However, the P content of Ozhalapathy and Eruthiampathy soils were considerably less than that in Vellanikkara soils.

The AMF present in the six soil samples were isolated and identified. The predominant AMF in all the soils were *Glomus* sp. The other AMF genera like *Acaulospora* and *Sclerocystis* were also identified. The enumeration of the total AMF spores in the soils from high wilt and low wilt areas was carried out and the maize rhizosphere soil of Ozhalapathy recorded the maximum spore count.

The screening of the predominant AMF spores individually and in combination against *R. solanacearum* using sterile potting mixture was carried out along with a commercial culture of AMF from TNAU for comparison. The per cent root colonization and AMF spore count was maximum for *Glomus* sp. (OM) + *Glomus* sp. (VBT) and *Glomus* sp. (OT) + *Glomus* sp. (ER) with the control plants recording the least. The maximum dry weight was recorded in *Glomus* sp. (OT) + *Glomus* sp. (ER) whereas *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded maximum root length and maximum number of days of plant survival. The treatments *Glomus* sp. (OT) + *Glomus* sp. (ER), *Glomus* sp. (OT) + *Glomus* sp. (VM) and *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the least percent wilt incidence whereas all other treatment combinations recorded 100 per cent wilt incidence. Based on the dry weight, root length, maximum number of days of plant survival and percent wilt incidence, the AMF cultures *Glomus* sp. (OT) + *Glomus* sp. (ER), *Glomus* sp. (OT) + *Glomus* sp. (VM) and *Glomus* sp. (OM) + *Glomus* sp. (VBT) were selected.

The optimum inoculation time (at the time of sowing, at 15 days before transplanting and at the time of transplanting) in combination with inoculum density (@ 25g $kg^{-1}$soil, 50g $kg^{-1}$soil and 75g $kg^{-1}$soil) were carried out using AMF cultures under unsterile pot culture conditions. The treatment combination at 15 days before transplanting @ 75g $kg^{-1}$soil recorded maximum dry weight and root length, maximum number of days of plant survival and minimum percent wilt incidence. Based on these observations the treatment combination at 15 days before transplanting @ 75g $kg^{-1}$soil was selected for further pot culture studies and field experiment.

The effective native AMF isolates obtained from screening experiment were tested at the standardized inoculum density and inoculation time under unsterile pot culture conditions to determine the most effective AMF. The plant height, root length, root weight, fresh weight and dry weight recorded the maximum for the commercial culture. Among the native AMF, *Glomus* sp. (OT) + *Glomus* sp. (ER) recorded the maximum for the above mentioned parameters and was found the best. The native AMF isolates survived much

longer and gave lower wilt incidence. The native isolate combination of *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the maximum days plants survived and least percent wilt incidence. The native AMF combination *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum spore count per cent root colonization.

The field experiment was undertaken to test the efficacy of the screened effective AMF cultures. Both Pusa Ruby (highly susceptible) and Mukthi (moderately resistant) tomato varieties were used for the study. The fresh weight, dry weight, root length and plant height recorded the maximum for *Glomus* sp. (OT) + *Glomus* sp (VM) in the case of Pusa Ruby, whereas in Mukthi, the commercial culture (TN) recorded maximum dry weight, root length and root weight. The variety Mukthi alone produced fruits as Pusa Ruby did not survive upto flowering with the native AMF culture combination *Glomus* sp. (OT) + *Glomus* sp. (VM) recording the maximum. However, the fruit weight was maximum in the treatment with kocide (0.2 per cent).

The number of days of plant survival was maximum for the treatment with kocide (0.2 per cent) in the case of Pusa Ruby. Among the native isolate combination *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum number of days of plant survival. The percent wilt incidence was 100 per cent for all treatments. The treatment *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded the maximum number of days of plant survival followed by *Glomus* sp. (OT) + *Glomus* sp. (ER) in the case of Mukthi. The wilt incidence was the least for commercial culture (TN) followed by chemical control with kocide (0.2 per cent). The only native AMF isolate to record less than 100 per cent wilt incidence was *Glomus* sp. (OT) + *Glomus* sp. (VM).

The AMF spore count was maximum in *Glomus* sp. (OT) + *Glomus* sp. (ER) and *Glomus* sp. (OT) + *Glomus* sp. (VM) at 30 DAT and 60 DAT respectively in the case of Pusa Ruby. However, in the case of Mukthi the AMF spore count at 30 DAT and 60 DAT was maximum in *Glomus* sp. (OT) + *Glomus* sp. (VM). The per cent root colonization in both Pusa Ruby and Mukthi was maximum in *Glomus* sp. (OT) + *Glomus* sp. (VM). The

*Ralstonia* population in the rhizosphere did not show much variation with respect to the tomato varieties Pusa Ruby and Mukthi. The pathogen population in the rhizosphere of both varieties recorded the maximum for control with the treatment *Glomus* sp. (OT) + *Glomus* sp. (ER) recording the minimum at 30 DAT and 60 DAT.

The present study indicated that the native AMF culture combination *Glomus* sp. (OT) + *Glomus* sp. (VM) could prolong the disease incidence upto 32 DAT (45 days after AMF inoculation) in susceptible varieties and upto 50 DAT (65 days after AMF inoculation) in moderately resistant one.

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## REFERENCES


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# APPENDIX

## Appendix 1

1. Triphenyl Tetrazolium Chloride (TZC) media composition

| | |
|---|---|
| Peptone | : 10.0 g |
| Casein hydrolysate | : 1.0 g |
| Glucose | : 5.0 g |
| Agar | : 20.0 g |
| Distilled water | : 1000 ml |
| PH | : 7.0 |

1% TZC was added to a final concentration of 5 ml/l after autoclaving

## Appendix 2

1. Ruakara nutrient solution composition

| Solution A | | Solution B | |
|---|---|---|---|
| $MgNO_3$ | : 22.25 g | NaCl | : 1.5 g |
| $CaNO_3$ | : 75.5 g | $K_2SO_4$ | : 29.8 g |
| $NH_4NO_3$ | : 38.4 g | $Na_2SO_4$ | : 2.5 g |
| $KNO_3$ | : 10.25 g | Water | : 4.5 l |
| Water | : 4.5 l | | |

Minor nutrients

a) Boron : 6.3 g (500 ml)
b) Manganese Chloride : 20.2 g (dissolve in 20.26 ml of 0.1 N HCl and make upto 500 ml with water)
c) Zinc Chloride : 5.8 g (dissolve in 117.3 ml of 0.1 N HCl and make upto 500 ml with water)
d) Cupric Chloride : 1.2 g (dissolve in 12.07 ml of 0.1 N HCl and make upto 500 ml with water)
e) Ammonium molybdate : 0.2 g in 500 ml water
f) Cobalt Chloride : 0.4 g in 500 ml water

Place 5 ml of each minor nutrients in a bottle and dilute with 2.5 l water

Ferric citrate : 13.5 g in 119 ml of 1 N HCl and dilute to 2.5 l water

300 ml of Solution A + 300 ml of Solution B + 150 ml of minor nutrient solution + 22.5 ml of ferric citrate and make upto 4.5 l with water.

## Appendix 3

1. Formalin : Acetic acid : Alcohol (FAA) composition

| | |
|---|---|
| Formalin (40%) | : 5 ml |
| Glacial acetic acid | : 5 ml |
| Ethanol (95%) | : 90 ml |

## Appendix 4

1. Trypan blue composition

| | |
|---|---|
| Trypan blue | : 50 mg |
| Lactophenol | : 100 ml |

2. Lactophenol composition

| | |
|---|---|
| Lactic acid | : 10 ml |
| Phenol | : 10 ml |
| Glycerol | : 20 ml |
| Water | : 20 ml |

Appendix 5

Weather parameters during the field experiment (August – November, 2002)

| Date | Ambient temperature(°C) | | Soil temperature (°C) | | | | | | RH (%) | | Sunshine hours (h/day) | Rainfall (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5cm | | 10cm | | 15cm | | | | | |
| | Max. | Min. | Max. | Min. | Max. | Min. | Max. | Min. | Max. | Min. | | |
| August 1st week | 28.6 | 22.2 | 31.1 | 25.0 | 29.8 | 25.2 | 29.1 | 25.6 | 95 | 79 | 0.9 | 94 |
| August 2nd week | 27.9 | 22.8 | 30.3 | 24.7 | 28.8 | 24.8 | 28.2 | 25.2 | 94 | 83 | 2.6 | 337 |
| August 3rd week | 30.1 | 23.4 | 33.4 | 25.9 | 32.0 | 25.8 | 31.1 | 26.0 | 93 | 72 | 5.4 | 13.8 |
| August 4th week | 30.9 | 24.1 | 39.5 | 27.0 | 36.4 | 27.3 | 34.9 | 27.9 | 93 | 65 | 7.3 | 3.8 |
| Sept 1st week | 29.8 | 23.2 | 33.4 | 26.7 | 33.4 | 27.0 | 32.1 | 27.5 | 94 | 71 | 5.5 | 3.4 |
| Sept 2nd week | 30.7 | 22.9 | 39.0 | 26.3 | 36.1 | 26.6 | 34.4 | 27.1 | 92 | 63 | 8.7 | - |
| Sept 3rd week | 31.3 | 22.8 | 41.5 | 27.6 | 38.5 | 28.1 | 36.9 | 28.7 | 91 | 59 | 8.3 | - |
| Sept 4th week | 32.5 | 22.7 | 44.2 | 28.7 | 39.1 | 29.1 | 38.3 | 29.9 | 90 | 55 | 8.2 | - |
| Oct 1st week | 32.2 | 23.3 | 37.3 | 27.4 | 35.7 | 27.7 | 34.6 | 28.4 | 89 | 67 | 5.7 | 51 |
| Oct 2nd week | 29.3 | 23.1 | 30.9 | 26.0 | 30.2 | 26.0 | 29.7 | 26.3 | 93 | 89 | 2.1 | 268.3 |
| Oct 3rd week | 30.1 | 23.0 | 33.4 | 26 | 31.8 | 25.9 | 30.9 | 26.2 | 92 | 74 | 4.3 | 25.1 |
| Oct 4th week | 31.5 | 23.5 | 27.3 | 25.5 | 33.7 | 27.3 | 32.6 | 27.6 | 92 | 66 | 6.0 | 9.9 |
| Nov 1st week | 31.6 | 23.3 | 34.7 | 26.3 | 32.3 | 26.4 | 31.3 | 26.9 | 84 | 61 | 5.7 | 33.4 |
| Nov 2nd week | 31.8 | 23.5 | 35.3 | 26.6 | 33.6 | 26.8 | 32.6 | 27.3 | 90 | 66 | 4.7 | 8.7 |
| Nov 3rd week | 31.2 | 23.2 | 33.9 | 26.1 | 31.9 | 26.3 | 31.0 | 26.7 | 83 | 59 | 4.7 | 9.4 |
| Nov 4th week | 31.3 | 23.3 | 36.8 | 25.7 | 34.8 | 26.3 | 33.1 | 26.9 | 78 | 63 | 6.8 | 4 |

Note : (-) indicates no rainfall

# BIOCONTROL OF BACTERIAL WILT IN TOMATO USING ARBUSCULAR MYCORRHIZAL FUNGI

By

NANDAKUMAR. A.

## ABSTRACT OF THE THESIS

**Submitted in partial fulfilment of the requirement for the degree of**

# Master of Science in Agriculture

**Faculty of Agriculture**

**Kerala Agricultural University**

**Department of Plant Pathology**

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**2003**

# ABSTRACT

The studies on "Biocontrol of bacterial wilt in tomato using arbuscular mycorrhizal fungi" were carried out at the Dept. of Plant Pathology, College of Horticulture, Vellanikkara during the period 2000 – 2002. The main objectives of the study were to identify suitable native AMF from high and low wilt incidence areas of Thrissur and Palakkad districts, screen them against *Ralstonia solanacearum* in pot culture under sterile and wilt sick conditions, to determine the optimum inoculation time and inoculum density of AMF and to test the efficiency of AMF in the wilt sick field.

The soil samples collected from Thrissur and Palakkad district were analyzed for their nutrient status, pH, *Ralstonia* population and total AMF spore count. The Vellanikkara soils were acidic with low N, K, Ca and Mg and high P content when compared to Ozhalapathy and Eruthiampathy soils. The *Ralstonia* population were generally higher in Vellanikkara soils. On the other hand, Ozhalapathy soils had higher AMF spore count. The *Glomus* sp. was found to be the most predominant AMF in the soils of high wilt (Vellanikkara, Thrissur district) and low wilt incidence (Ozhalapathy and Eruthiampathy, Palakkad district).

The screening of the predominant native AMF cultures against *R. solanacearum* were carried out individually and in combinations under sterile conditions along with the TNAU commercial culture for comparison. The treatments with the native AMF combinations *viz.*, *Glomus* sp. (OT) – *Glomus* sp. (ER), *Glomus* sp. (OT) + *Glomus* sp. (VM) and *Glomus* sp. (OM) + *Glomus* sp. (VBT) recorded the least percent wilt incidence with the native AMF combination *Glomus* sp. (OM) + *Glomus* sp. (VBT) recording the maximum number of days of plant survival (11 days). The dry weight and root length were also higher for these three native AMF combinations.

The determination of the optimum inoculation time (at the time of sowing, at 15 days before transplanting and at the time of transplanting) in combination with the inoculum density (@ 25 g kg$^{-1}$ soil, 50 g kg$^{-1}$ soil and 75 g kg$^{-1}$ soil) were carried out using different

species of AMF in pot culture using wilt sick soil. The inoculation time at 15 days before transplanting 75 g $kg^{-1}$ soil was found to be optimum. This was evaluated further, under pot culture and field experiment studies using the native AMF combinations selected from the screening experiment.

The selected native AMF combinations from the screening experiment were tried at 15 days before transplanting @ 75 g $kg^{-1}$ soil in pot culture using wilt sick soil in comparison with the TNAU commercial culture to select the best two combinations for the field experiment. The native AMF combinations recorded the least percent wilt incidence and maximum number of days of plant survival when compared to the TNAU commercial culture. The AMF combinations *Glomus* sp. (OM) + *Glomus* sp. (VBT) and *Glomus* sp. (OT) + *Glomus* sp. (VM) were found to be the best.

A field experiment to test the efficacy of the selected native AMF culture combinations were carried out using the tomato varieties Pusa Ruby (susceptible) and Mukthi (moderately resistant). The native AMF combination *Glomus* sp. (OT) + *Glomus* sp. (VM) recorded maximum number of days of plant survival (32 and 50 days respectively) in both the tomato varieties. However, it could not prevent wilt incidence in the case of Pusa Ruby variety, which recorded 100 percent wilt, and Mukthi variety 97..9 % wilt incidence. The same native AMF combinations *Glomus* sp. (OT) + *Glomus* sp. (VM) also recorded maximum fruit number in Mukthi whereas Pusa Ruby did not produce fruits as they did not survive even upto flowering.

The present study clearly indicated that the native AMF combination *Glomus* sp. (OT) + *Glomus* sp. (VM) was the best in pot culture studies using wilt sick soil and could delay the disease incidence in the susceptible Pusa Ruby variety of tomato upto 82 days after transplanting. However, under field conditions it could delay the disease incidence in the susceptible variety Pusa Ruby only upto 32 days and in the moderately resistant variety Mukthi upto 50 days. So more extensive studies are needed to develop a suitable native AMF to control the bacterial wilt in tomato which is effective under field conditions.