MANAGEMNET OF BACTERIAL WILT OF CHILLI CAUSED BY *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al.* USING AMF AND FLUORESCENT PSEUDOMONADS

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DECLARATION

I herby declare that this thesis entitled "Management of bacterial wilt of chilli caused by *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al.* using AMF and Flourescent Pseudomonads" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society

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CERTIFICATE

Certified that the technical programme of the Msc. thesis work entitled "Management of bacterial wilt of chilli caused by *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al*. using AMF and Fluorescent Pseudomonads" of Mrs. Sonia Basheer (2002-11-16),Msc. student of the Department of Plant Pathology, College of Agriculture, Vellayani, approved by the FRC, Kerala Agricultural University has been carried out successfully and completely.

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Sonia Basheer

LIST OF ABBREVIATIONS

%	Per cent
µ	Micro
˚C	Degree Celsius
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of variance
CD	Critical difference
cm	Centimeter
CRD	Completely Randomised Design
et al	And others
Fig.	Figure
g	Gram
h	Hours
ha	Hectare
kg	Kilogram
m	Metre
mg	Milligram
ml	Millilitre
nm	Nanometer
lb	Pounds
t	Tonnes

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Introduction

1. Introduction

Chilli (*Capsicum annuum* L.) is an important spice cum vegetable crop of India. It is preferred for its pungency, spicy taste besides the appealing colour it imparts to the food. India is the largest producer and exporter of chilli which is grown over an area of 9.15 lakh ha with a production of 10.18 lakh t and accounts for an earning of over Rs. 30400 lakh foreign exchange. It is rich in ascorbic acid and known for two important quality parameters, the biting pungency due to capsicin and captivating red colour due to pigment capxanthin.

One of the reasons for the poor productivity is attributed to the incidence of several diseases. Bacterial wilt caused by *Ralstonia solanacearum* is the most lethal disease of chilli. The disease is widespread throughout the west coast from Kerala to Gujarat, Karnataka, western Maharashtra and Madhya Pradesh, eastern plains of Assam, West Bengal and Orissa and in Chottanagpur plateau area of Bihar. Commonly used antibiotics such as agrimycin and streptomycin often fail to control the disease and their economic viability is questionable (Sinha, 1986). In this context, management of the disease using bioagents become significant. Microbial antagonists such as *Bacillus* sp., *P. fluorescens* and arbuscular mycorrhizal fungi (AMF) are being successfully exploited for the management of diseases of crop plants including bacterial diseases. In the present study, the two microbial antagonists viz., *P. fluorescens* and AMF were evaluated for their potential in suppressing the bacterial wilt disease and enhance the yield of chilli. Among the PGPR group of organisms, fluorescent pseudomonads have emerged as the most promising group and are being commercially exploited for plant disease control and growth promotion world over (Kloepper and Schroth, 1978). The beneficial effect of *P. fluorescens* fall in two categories : growth promotion and plant disease suppression. Use of fluorescent pseudomonads for the management of wilt

incidence in potato (Singh and Rana, 2000), tomato (Pradeepkumar and Sood, 2001) and chilli (Yeole and Dube, 2001) is found highly successful.

AMF association improves plant growth and increases plant resistance against soil-borne pathogens. It positively affects nitrate uptake from soil (Hildebrandt *et al*., 2002), increases P uptake and accumulation (Al-Karaki, 2002b), promotes plant growth (Scagel, 2004), helps in acclimatization and growth of tissue culture plantlets (Estrada-Luna and Davies, 2003) and imparts resistance against stress conditions (Al-Karaki, 2000). However, not much work has been done to exploit AMF against bacterial pathogens.

Dual inoculation of AMF and *P. fluorescens* ensures biological control as well as better root growth and development of plant (Budi *et al*., 1999). The scope of utilizing these two ecofriendly microbes together for the management of bacterial wilt of chilli has not been investigated so far. In the present study, it is envisaged to isolate and develop efficient strains of fluorescent pseudomonads and AMF for the management of bacterial wilt of chilli. The study was undertaken with the following objectives:

- Isolation of the pathogen associated with bacterial wilt of chilli
- Evaluation of AMF for disease suppression and growth promotion
- Isolation of native *P. fluorescens*
- Evaluation of isolates of *P. fluorescens*
- Characterization of bacterial isolates antagonistic to *R. solanacearum*
- Dual inoculation of AMF and *P. fluorescens* for the management of *R. solanacearum*

Review of Literature

2. REVIEW OF LITERATURE

Chilli (*Capsicum annuum* L.) is one of the important solanaceous vegetable crops of Kerala grown for its green as well as ripe dried fruits. One of the reasons for low production in the state is the losses caused by various diseases. Among these, bacterial wilt incited by *Ralstonia solanacearum* is a major threat to the chilli crop causing much economic loss to the farmers. Once infested the crop is completely wiped off.

Ralstonia solanacearum attacks wide range of crops like tobacco, potato (Kelman, 1953), tomato (Dass and Chattopadhyay, 1955), banana (Gnanamanickam *et al*., 1979), ginger (Hayward, 1991), chilli (Yeole and Dube,2001) etc. The survival of *R. solanacearum* and its chemical control has already been studied (Remadevi, 1978; Nayar, 1982). Management of the disease using chemical methods is expensive and often ineffective in decreasing the population of pathogen (Remadevi, 1978). In this context, the relevance of management of the disease by resorting to alternative ecofriendly strategies such as the use of bioagents becomes significant.

Gadewar and Shekhawat (1988) studied field persistence of *R. solanacearum* in fallow and cultivated fields at three locations in Karnataka state. In spite of large amount of work done in this area, little has been achieved in the biocontrol of bacterial wilt due to difficulty of transporting large amount of viable cells from laboratory to field (Ciampi – Panno *et al*., 1989). *R. solanacearum* is listed as a quarantine organism in European Union, where new legislation has been introduced to control and eradicate the organism (Weller *et al*., 2000).

Autoradiography and radio assay techniques were utilized to study the entry, movement, growth and multiplication, colonization and infection of *R. solanacearum* in chilli (Markose *et al*., 2001). In India, the pathogen has been reported from 47 plant families and many of them

remained symptomless (Gadewar *et al*., 2003). James *et al*. (2003) isolated nine strains of *R. solanacearum* from bacterial wilt affected plants of brinjal, chilli and tomato from different agroclimatic zones of Kerala. Bacterial wilt pathogen, *R. solanacearum* were isolated from ginger, tomato, chromalena, chilli and potato (Kumar and Sarma, 2004).

2.1 CHARACTERIZATION OF *Ralstonia solanacearum*

R. solanacearum causing bacterial wilt of over 200 species of cultivated plants is a very heterogeneous species comprising of four biovars and three races. Kelman (1954) distinguished colony variants on TTC medium. *R. solanacearum* is gram negative bacteria with true polar flagella, 1 to 4 in number, colonies on solid media are usually small, irregularly round, slightly raised, glistening white and smooth, 3-5 mm diameter and appear within 36 to 48 hours at 28°C (Kelman, 1954; Hodgkiss, 1964; Hayward, 1994). Characterization of isolates into biovars was made through tests for utilization of C sources (Hayward, 1960). Virulence of the culture could be retained by preserving in sterile distilled water (Kelman, 1967). The bacterium does not hydrolyse starch or produce indole, liquifies gelatin slowly, reduces nitrate to nitrite, produces ammonia, clears milk without coagulation and turns it alkaline (He, 1985).

The pathogen was characterized by biochemical tests and plant inoculation. The biochemical tests include test for levan, oxidase production, test for arginine dihydrolase production, tests for utilization of carbon sources (Sivamani and Gnanamanickam, 1988). It produces acid from glucose and fructose, sucrose and sugar alcohols like glycerol and meso-inositol in 2 to 6 days (Schaad, 1992). Isolates of *R. solanacearum* produced fluidal colonies of 75 mm in TTC media, had less than 2 per cent tolerance for NaCl and utilized 97 per cent of C sources like glucose, sucrose, galactose, glycerol, mannose, ribose (Eden-Green, 1994).). Aley and Elphinstone (1995) used several culture media like TTC agar, soil

isolation medium and carbohydrate media for isolation, identification and maintenance of *R. solanacearum.*

. Some isolates of *R. solanacearum* form brown to black pigmentation of melanin in culture and tyrosine is used to detect this property (Gadewar and Shekhawat, 2003). James *et al*. (2003) obtained creamy white mucoid colonies with pink centre on TTC medium. Kumar and Sarma (2004) could isolate *R. solanacearum* in 2, 3, 5 TTC media which produced highly fluidal colonies with characteristic spiral pink centre.

2.2 ROLE OF AMF IN PLANT GROWTH PROMOTION

Mycorrhizae are symbiotic associations between specific groups of soil fungi and higher plants. Pot culture trials with chilli plants have proved that inoculation with efficient strains of AM fungi resulted in three fold increase in growth (Sreeramulu and Bagyaraj, 1986). Bolan *et al*. (1987) found that mycorrhizae are able to explore the soil thoroughly and are able to locate and use the point sources of P in the insoluble iron phosphate.

The hyphae of AMF attached to the root extended beyond the zone of phosphate depletion in P deficient soils and the roots absorb phosphate ion faster and which can diffuse from the soil to the hyphae and arbuscules inside the root cortex, where transfer to the plant occurs (Kapoor and Mukherji, 1987). Champawat (1989) proved that inoculation with AMF increased shoot dry weight, root dry weight and P uptake of chickpea.

AMF associations in plants are formed by a group of Zygomycetous fungi belonging to the order Glomales (Morton and Benny, 1990). The beneficial effects of AMF associations in plant growth and nutrition are well documented (Sivaprasad *et al*., 1990; Meenakumari and Nair, 1992; Kavitha, 2001; Kumar *et al*., 2003). *Glomus mosseae* increased the shoot

growth of beans, broad beans and chickpea by 52, 117 and 190 per cent respectively (Almomany, 1991).

The root shoot ratio was modified by endomycorrhizal inoculated, pineapple plants which showed a greater increase in shoot production when compared to root production (Guillemin *et al*., 1992). Sreenivasa (1992) found that the isolate *G. macrocarpum* was the best for improving growth, P, Zn, Cu, Mn and Fe nutrition in chilli plants. The contents of micronutrients like Zn, Cu, Fe and Mn were higher in AMF inoculated plants than uninoculated plants at all growth stages upto maturation irrespective of soil fertilizers and water regimes (Solaiman and Hirata, 1996). Mycorrhizal symbiosis significantly increased above ground dry mass, root length, P content and yield of tomato under low P condition (Bryla and Koide, 1998). Synergistic interaction between VAM and tissue culture plantlets reduced hardening period and improve *ex vitro* growth and development (Sivaprasad *et al*., 1998). The enhanced growth and gas exchange of mycorrhizal plants was particularly due to greater uptake of P and greater extraradical hyphae development (Aguilera-Gomez *et al*., 1999).

Enhancement in plant dry matter and mineral acquisition rates occurred in AMF inoculated barley plants under water stress (Al-Karaki and Clark, 1999a). Al-Karaki and Clark (1999b) found that the presence and degree of AMF root colonization increased seed lipid metabolism in Durum wheat genotypes. Leaf mass, protein concentration, nitrogen percentage was higher in leaves of maize plants colonized by *G. etunicatum* (Boucher *et al*., 1999).

AMF isolates were highly effective in overcoming acidic soil constraints. Good growth of switch grass in acidic soil depends on root-AMF symbiosis (Clark *et al*., 1999). Inoculation of *G. mosseae* enhanced growth and grain yield in kidney beans under low available P condition (Isobe and Tsuboki, 1999). Improved growth and nutrient acquisition was

observed in AMF colonized tomato plants under salt stress conditions in arid and semi arid areas (Al-Karaki, 2000). According to Singh *et al*. (2000), AMF are 'universal plant symbionts' and they help plants in phosphate uptake, nitrogen uptake and disease resistance. A simple inoculation technique by growing certain highly mycorrhizal annual plants with AMF inoculation so as to achieve better root colonization of the introduced AMF in established black pepper was reported by Sivaprasad *et al*. (2000).

AM fungi was found to enhance mineral uptake, specifically P, K, and Zn etc. (Abdul-Khaliq *et al*., 2001). Leaf water potential, assimilation rate, leaf surface area and specific leaf area were higher in mycorrhizal maize plants subjected to water stress (Amerian *et al*., 2001). Papaya plants inoculated with *G. mosseae* recorded maximum NPK content (Kennedy and Rangarajan, 2001). Mycorrhizal inoculation increased leaf area and shoot dry weight when inoculum was placed below seed at planting than dispersal in *Phaseolus vulgaris* (Oliviera and Sanders, 2001). AM inoculated *Casuarina equisetifolia* seedlings grew faster and healthier with longer root and shoot as well as higher dry matter and P uptake (Rajeswari *et al*., 2001).

According to Taichenyang *et al*. (2001), brinjal seedlings inoculated with AMF could increase dry weight of whole plant. Introduction of AMF into garlic plots ensure satisfactory yields and reduced P fertilization (Al-Karaki, 2002a). AMF increased the P availability by mobilizing the P with the help of their extra matrical hyphae, particularly in soil with less available P (Gill *et al*., 2002; Dwivedi *et al*., 2003). AMF colonization in tomato positively affected nitrate uptake from soil and nitrate allocation to plant partner (Hildebrandt *et al*., 2002) Results indicate that contribution of mycorrhizae to K, Ca and Mg nutrition of maize can be significant in a field situation and that the extent of contribution depends on availability of these nutrients

particularly of P in soils (Liu *et al*., 2002). *Glomus clarum* inoculation in sour orange increased plant P, Zn, Cu uptake (Ortas *et al*., 2002). Mycorrhizal infection enhanced fitness in tomato through improved vegetative growth and female and male functions (Poulton *et al*., 2002). Ryan and Graham (2002) found that AMF had a crucial role in maintaining soil structural stability and enhancing micronutrient concentration. *Gigaspora margarita* was efficient in producing maximum number of primary roots, dry weight of roots, P content, percentage root colonization and spore count in black pepper (Thanuja *et al*., 2002). Rapid AMF colonization enhanced physiological parameters, which helped plantlets recover rapidly during acclimatization and obtain greater growth during post-acclimatization (Estrada-Luna and Davies, 2003). Mycorrhizal plants have ability to benefit in condition that are otherwise deleterious to root growth and development particularly in terms of counteracting toxicities and disease compensation (Jalali, 2003). Percentage variation in growth rate and nutrient uptake in leguminous plants under drought and salinity condition were directly proportional to percentage of mycorrhization (Kumar and Kurup, 2003). Lata *et al*. (2003) proved that mycorrhization is a valuable tool to overcome micropropogated *Echinacea pallida* root shock.

AM fungi enhanced plant water uptake in *Latuca sativa* plants which was related to amount of external mycelium produced by fungus and frequency of root colonization (Marulanda *et al*., 2003). Elongated arbuscules and globose, cylindrical vesicles were found in root samples of turmeric colonized by AMF (Reddy *et al*., 2003). AMF improved plant mineral nutrition and increased stress tolerance of host plants (Takacs and Voros, 2003). Al-Karaki *et al*. (2004) demonstrated improved growth, yield and nutrient uptake in wheat plants under drought stress in semi-arid areas. *G. fasciculatum* increased shoot length, internode number, internode length, number of leaves, stem diameter, root length and root number (Ananthakrishnan *et al*., 2004). AM fungal colonization improved nitrogen

nutrition of plants mainly when growing at low levels of nutrients (Cruz *et al*., 2004). Experiments indicated that AM association can influence grass nutrient content and species responses, plant diversity and productivity in boreal grasslands (Dhillion and Gardsgord, 2004). AMF inoculated plants had higher specific soil respiration than non mycorrhizal plants regardless of temperature treatment (Martin and Stutz, 2004). Neumann and George (2004) conducted research on *G. mosseae* inoculation in *Sorghum bicolor* in dry soil and proved that mycorrhizal colonization is beneficial to P uptake from dry soil. Mycorrhizal symbiosis in tropical leguminous trees of tribe Mimosae under nursery condition can affect growth and consequently post planting success (Patreze and Cordeiro, 2004).

AMF symbiosis enhanced osmotic regulation in roots, which could contribute to maintaining a water potential gradient favourable to water entrance from soil into roots (Porcel and Ruiz-lozano, 2004). Inoculation with *G. margarita* in cowpea increased plant growth and nutrient uptake which was correlated with colonized root length (Rohyadi *et al*., 2004). Phosphate solubilizers and AMF increased grain and straw yield and decreased CN and CP ratio (Saini *et al*., 2004). AMF colonization of soybean roots induced an increase in alkaline increase activity which could provide hexoses for fungal symbiont and for development of colonized cells (Schubert *et al*., 2004).

2.3 AMF AS BIOCONTROL AGENT

Arbuscular mycorrhizal fungal association enables better plant growth and reduces the infection caused by many soil borne fungal and nematode pathogens. The severity of diseases caused by root pathogenic fungi, bacteria and nematodes are reduced by AMF (Bagyaraj and Sreeramulu, 1982). A significant inhibition of sclerotial bodies of *Sclerotium rolfsii* was observed in chilli plants inoculated with *G. fasciculatum* and *G. macrocarpum* (Sreenivasa *et al*., 1992). Mycorrhizal fungi can

deter or significantly reduce the effect of soil-borne pathogens (Sivaprasad, 1993; Quarles, 1999; Kavitha, 2001).

Thomas *et al*. (1994) obtained biocontrol of damping off caused by *F. moniliforme* and *R. solani* in cardamom using AMF. In addition to disease suppression, AMF increased plant growth characteristics and P uptake of roots also. Several results have been obtained on the potential of AMF in promoting resistance against important plant pathogenic fungi. *G. mosseae* was found effective in controlling azhukal disease of cardamom caused by *Phytophthora nicotianae* (Sivaprasad, 1995).

The inoculation of *Glomus mosseae* decreased propagule number of *Fusarium* sp. in rhizosphere and decreased root rot in common bean by 34 to 77 per cent (Dar *et al*., 1997). AMF conferred protection to cardamom plants highly susceptible to *Phytophthora nicotianae* (Thomas *et al*., 1994) and potato plants infected with root rot (Yao *et al*., 1998). AMF are used in the management of damping off of chilli incited by *Pythium* (Kavitha, 2001) and apple seedlings infected with root rot (Narender *et al*., 2004)

AMF isolates of *Glomus* sp. are used for the control of rhizome rot of ginger, azhukal disease of cardamom, foot rot of black pepper and a mixed inoculum of AMF was found effective for root disease suppression and growth improvement without host specificity (Sivaprasad, 2002).

Bioprotection of AMF colonized plants is the outcome of complex interaction between plant, pathogen and AM fungi. AMF protects the plants against *Aphanomyces* sp., *Cylindrocladium* sp., *Fusarium* sp., *Macrophomina* sp., *Phytophthora* sp., *Pythium* sp., *Rhizoctonia* sp., *Sclerotinium* sp., *Verticillium* sp., *Thielaviopsis* sp. and various nematode (Harrier and Watson, 2004).

2.3.1 AMF as biocontrol agents against wilt diseases

The study on the influence of AMF (*Glomus mosseae*) on vascular wilt of tomato caused by *Fusarium oxysporum* fsp. *lycopersici* showed a reduction of disease in plants pre-inoculated with the AMF and the spread of the pathogen in the host was arrested (Dehne and Schoenbeck, 1975). Jalali and Thareja (1981) found that association of *G. fasciculatum* and *G. mosseae* in chickpea plants reduced wilt incidence by *F. oxysporum* f. sp. *ciceri*. Seedlings of alfalfa inoculated with AMF had a lower incidence of *Verticillium* and *Fusarium* wilt with a lower propagule number of both pathogens (Hwang *et al*., 1992). In cardamom, the biocontrol efficiency of AMF in suppressing *F. moniliforme* was investigated by Thomas *et al*. (1994).

Eventhough AMF has the potential to suppress both fungal and bacterial pathogens, not much work has been done to exploit AMF against bacterial pathogens. Sood *et al*. (1997) found that *Glomus mosseae* was highly effective in promoting germination, seedling vigour and controlling the wilt disease of tomato.

AMF are used in the management of wilt disease of guava caused by *Fusarium oxysporum* (Srivastava, 2001). Karagiannidis *et al*. (2002) conducted research on the effect of *Verticillium* wilt and mycorrhiza on root colonization, growth and nutrient uptake in tomato and egg plant seedlings. Beneficial effects of AMF supercedes the pathogenic effect of *Verticillium dahliae*.

Raji *et al*. (2003) observed 58 per cent reduction in wilt incidence in tomato due to AMF inoculation in variety Sakthi. AMF reduced deleterious effect of *Verticillium dahliae* on growth and yield of pepper (Idoia *et al*., 2004).

In pot cultures, *R. solanacearum* population in rhizosphere on root surfaces and in xylem were reduced by 26.7, 79.3 and 81.7 percentage

respectively following inoculation of tomato plants with *Glomus versiforme* (Zhu and Yao, 2004).

2.4 PGPR ACTIVITY OF FLUORESCENT PSEUDOMONADS

Fluorescent pseudomonads have emerged as the largest and potentially most promising group of PGPR for plant disease control and growth promotion (Kloepper and Schroth, 1978). PGPR activity of fluorescent pseudomonads is achieved through their general biological activities that include competition for space and nutrients, production of volatile and antimicrobial substances, iron chelating siderophores and HCN (Kloepper *et al*., 1980; Chet *et al*., 1990). Colonization of plant growth resulted in significant increase in stolon length two weeks after plant emergence in potato (Kloepper and Schroth, 1980). Ability of PGPR to induce plant growth is related to antibiosis that occurs in root zones and subsequent displacement of certain root colonizing microbes (Kloepper and Schroth, 1981). Sakthivel *et al*. (1986) reported that siderophore producing strains of fluorescent pseudomonads showed antagonism to *F. oxysporum* f. sp. *cubense*, *F. oxysporum* f. sp. *vasinfectum*, *R. solani*, *Acrocylindrium oryzae* and bacteria *X. campestris* pv. *oryzae* and *P. syringae* pv. *Phaseolicola* under *in vitro* conditios. Plugs containing cell-free siderophore were isolated from King B medium inoculated with *Pseudomonas fluorescens* (Sivamani *et al*., 1987).

One of the mechanisms of plant growth promotion by *P. fluorescens* is production of siderophores. Siderophores are low molecular weight ferric ion specific ligands designed for solubilization, transport and storage of Fe in microorganisms. Siderophores are involved in suppression of minor pathogens like deleterious rhizobacteria that colonize root surfaces and retard plant growth (Dave and Dube, 2000). Chincholkar and Chaudhari (2000) observed that the most important naturally occuring chelates for increasing iron availability to plants are microbially produced

siderophores. Chemical characterization of siderophores of rhizobacterial fluorescent pseudomonads was done (Dave and Dube, 2000).

2.5 *Pseudomonas fluorescens* AS BIOCONTROL AGENTS

Fluorescent pseudomonads are the most promising group of plant growth promoting rhizobacteria which can effectively control many soil-borne plant pathogens (Anuratha and Gnanamanickam, 1990; Yeole and Dube, 2001; Sivaprasad, 2002). Metabolites like hydrogen cyanide, phenazine-1-carboxylic acid, 2, 4-diacetyl phloroglucinol produced by *P. fluorescens* are involved in the control of many fungal pathogens (Maurhofer *et al*., 1992).

Besides suppression of plant diseases by many mechanisms, fluorescent pseudomonads induce systemic resistance in plants against attack by a wide range of pathogens (Whipps, 1997). Many strains of fluorescent pseudomonads with biocontrol activity against soil borne plant pathogen produced a polyketide metabolite 2, 4-diacetyl phloro glucinol (Bangera and Thomashow, 1999). Bora *et al*. (2000) reported that *P. fluorescens* produced siderophores which has the capacity of protecting plants from pathogen. *Pseudomonas fluorescens* produce phenazine-1- carboxylicacid and 2-acetamidophenol to control take-all disease of wheat (Sliniger, 2000).

Management of major foliar diseases of rice such as sheath blight and bacterial blight using fluorescent pseudomonads was obtained (Heera, 2001). Yeole and Dube (2001) isolated siderophore producing strains of *P. fluorescens* from rhizosphere of chilli, cotton, groundnut and soybean. Fluorescent pseudomonad isolate P-1 was found effective for the control of *Phytophthora capsici, Phytophthora maedii* and *Pythium aphanidermatum* in black pepper (Sivaprasad, 2002).

According to Amruthesh *et al*. (2003), seed treatment with PGPR suspension resulted in growth promotion of chilli seedlings. Mechanism of

plant growth promotion include phosphate solubilization, siderophore production, nitrogen fixation by rhizobacteria and their effect on growth and yield of crop plants (Bhawsar and Chopade, 2003). Deepak *et al*. (2003) reported that *P. fluorescens* is one of the PGPR that has beneficial effects including plant growth promotion, biocontrol of various diseases by antagonizing seed borne pathogens, activation of defense response of host plants.

Bacterized seeds with *P. fluorescens* lead to accumulation of higher phenolic compounds and higher activity of polyphenol oxidase, peroxidase and phenyl alanine ammonia which may play a role in defense mechanism in plants against pathogen. Increase in yield and phosphate uptake in wheat plants inoculated with *P. fluorescens* was reported (Dwivedi *et al*., 2003). Fluorescent pseudomonads produced siderophores by formation of yellow halo zones in CAS plates (Jagadesh and Kulkarni, 2003).

Increased phenolic content was observed in maize plants raised from *P. fluorescens* treated seeds inoculated with *R. solani* (Sivakumar and Sharma, 2003).

2.5.1 *Pseudomonas fluorescens* as Biocontrol Agents for Bacterial Wilts

Kempe and Sequeira (1982) found that treatment of *R. solanacearum* with *P. fluorescens* strains gave significant reduction in disease severity in bacterial wilt of potatoes. *In vitro* toxicity of *P. fluorescens* to *R. solanacearum* was reported by Sivamani *et al*. (1987). Schmidt (1988) obtained control of bacterial wilt of Italian rye grass by pre-inoculation with *P. fluorescens*. Ciampi-Panno *et al*. (1989) established that the antagonistic *P. fluorescens* BC8 isolate induced significant reduction in severity of potato bacterial wilt when applied as amendment to seed coating. Hartman and Elphinstone (1994) conducted trials in major food crops and found that *P. fluorescens* decreased wilt incidence in tomato under greenhouse condition. Varshney and Chaube (1999) studied the

biocontrol potential of some selected isolates of fluorescent pseudomonads naturally occurring in rhizosphere of tomato. Seed treatment with *P. fluorescens* along with solarization decreased the wilt incidence caused by *R. solanacearum* in ginger to 7.42 per cent and increased the yield to 29.42 t ha^{-1} compared to 19.5 t ha^{-1} in control (Anith *et al*., 2000). Studies on biocontrol of bacterial wilt of tomato using *P. fluorescens* were done by Bora *et al*. (2000). Tuber bacterization with *P. fluorescens* gave significant reduction in bacterial wilt incidence in potato (Singh and Rana, 2000).

Significant reduction in wilt incidence in tomato was obtained due to incorporation of antagonistic bacteria, *P. fluorescens* in soil (Pradeepkumar and Sood, 2001). *P. fluorescens* isolated from rhizosphere and rhizoplane of chilli showed rhizosphere competence with wilt pathogen (Yeole and Dube, 2001).

Fluorescent pseudomonads isolate P-1 was found effective for the control of bacterial wilt of ginger and solanaceous plants (Sivaprasad, 2002). Anith *et al*. (2003) obtained plant growth promotion and biological control of tomato bacterial wilt caused by *R. solanacearum*. Jagadesh and Kulkarni (2003) observed that the inhibition of *R. solanacearum* in tomato by *P. fluorescens* is due to production of antibacterial metabolites. *Pseudomonas fluorescens* J3 could colonize the root system strongly and controlled bacterial wilt in Capsicum (Jianhua *et al*., 2003). *P. fluorescens* provided systemic resistance against *X. axonopodis* and enhanced growth and disease suppression ability (Khabbaz *et al*., 2003). *P. fluorescens* inoculated to tomato by stem injection and root soaking could inhibit growth of *R. solanacearum* (Li QuiQin, 2003). Meena and Jayaraman (2003) isolated *P. fluorescens* from rhizosphere of rice, screened for antagonism against *X. oryzae pv. oryzae*. *P. fluorescens* could suppress *R. solanacearum* in brinjal by induction of systemic resistance (Ramesh and Korikamthimath, 2003). Sarmah and Bora (2003) observed the

efficiency of *P. fluorescens* in suppressing the bacterial wilt pathogen, *R. solanacearum* in tomato.

Double application of the isolates of *P. fluorescens* by seed bacterization and seedling rebacterization prominently enhanced seed germination and seedling vigour of tomato with significant inhibition of bacterial wilt caused by *R. solanacearum* (Sood and Parashar, 2003).

2.5.2 Characterization of *P. fluorescens*

Blazevic *et al*. (1973) tested the growth of *P. fluorescens* at 4, 35 and 42°C on heart infusion agar and incubated for 10 days. Other tests included oxidase test, oxidation of dextrose, test for maltose, gelatin liquifaction and nitrate reduction. Gelatin test could differentiate *P. fluorescens* from *P. putida*. Gnanamanickam and Mahadevan (1988) characterized fluorescent pseudomonads as a group which grows on Kings B medium, produces levan in nutrient agar medium containing five percent sucrose and which usually lacks accumulation of poly β-hydroxy butyrate granules. The pseudomonads associated with plants could be generally assigned to one of three species *i.e., P. fluorescens*, *P. putida* or *P. aeruginosa* using tests for characterization including levan test, oxidase test, arginine dihydrolase test, nitrate test, growth at 41°C, growth in different sugars like sucrose, arabinose, rhamnose (Schaad, 1992). Most of the plant associated strains belong to *P. fluorescens* - *P. putida* complex of organisms. Tang *et al*. (1998) used identification system based on carbon source utilization to evaluate different strains of *P. fluorescens*. According to Bossis *et al*. (2000), the identification is based on phenotypic characters as metabolic test, fatty acid composition and protein profiles.

2.6 SYNERGISTIC INTERACTION BETWEEN MYCORRHIZAL FUNGI AND PSEUDOMONADS

Klyuchinikov and Kozhevin (1991) found that AMF rapidly stimulate the development of *P. fluorescens* population on surface of extra radicular

mycelium. *P. fluorescens* and *Glomus mosseae* treatments enhanced N, P, Mn and Cu concentration in leaf fresh matter of grapevine root stock (Bavaresco and Fogher, 1992).

Dual inoculation of an antagonistic bacteria *Pseudomonas* sp. and AMF ensure biological control against root pathogens and promote root growth and development (Budi *et al*., 1999). Inoculation of AMF and rhizosphere bacteria *P. fluorescens* stimulated both fungal root colonization, accumulation of secondary compounds and blumenin in roots of barley and wheat thus acting as mycorrhiza-helper bacteria (Fester *et al*., 1999). Filion *et al*. (1999) studied direct interaction between the AMF, *G. intraradices* and *P. fluorescens* and results indicated that the growth of *P. fluorescens* was stimulated in presence of AM fungal extract.

Co-inoculation of a helper bacterium *P. fluorescens* together with an ectomycorhizal fungus can be an efficient way of optimizing controlled mycorrhization technique for production of high quality Douglas fir planting stocks (Frey – Klett *et al*., 1999).

Studies were conducted by Sastry *et al*. (2000) to assess the role of AMF – *P. fluorescens* interaction in growth promotion and nursery establishment of Eucalyptus hybrid. Interaction between ectomycorrhizal symbiosis and fluorescent pseudomonads on *Acacia holostericea* increased the shoot biomass of seedlings (Founoune *et al*., 2002). Bacterial isolates frequently stimulated development of mycorrhiza, particularly the growth and dehydrogenase activity of extraradical mycorrhizal mycelium. Co-inoculation of *P. fluorescens,* AGR1 and *G. mosseae* BEG 12 in root system of tomato promoted plant growth and increased total root length, surface area and volume in soil: sand mixture (Gamalero *et al*., 2002).

Inoculation with bacterial isolate M 30 (*P. fluorescens*) and with mycorrhizal fungus, *G. fasciculatum* significantly increased plant growth and mineral nutrition of strawberry (Gryndler *et al*., 2002). Interaction between AMF and soil bacteria affect nutrient dynamics in the environment. *G. intraradices*

when inoculated along with *P. aureginosa,* increased the level of soluble P in the medium containing nitrate as nitrogen source (Villegas and Fortin, 2002). Chemotactic responses of plant growth promoting bacteria, *P. fluorescens* to roots of arbuscular mycorrhizal tomato plants were studied (Sood, 2003).

Co-inoculation of two microorganisms synergistically increased plant growth compared to single inoculation. Gamalero *et al*. (2004) studied the impact of inoculation of two fluorescent pseudomonads and an AMF on plant growth, root architecture and P acquisition of tomato and recommended the potential use of fluorescent pseudomonad and AMF as mixed inoculants for tomato. Report of gene induction by fluorescent pseudomonads and AMF in roots of *Medicago truncatula* supports the hypothesis that some plant cell programmes may be shared during root colonization by these beneficial microorganisms (Sanchez *et al*., 2004).

Inoculation with AMF and rhizobacterial fluorescent pseudomonads alters nutrient allocation and flowering of ornamental plants. Inoculation with AMF could promote shoot emergence, leaf production, flower production of harlequin flowers while co-inoculation of AMF and rhizobacteria alters biomass partitioning and corin composition that play important role in production of this crop for corms and cormels (Scagel, 2004).

The scope of utilizing these two ecofriendly microbes for the management of bacterial wilt of chilli has not been investigated so far in Kerala. Hence in the present study it was proposed to isolate and develop efficient strains of fluorescent pseudomonads and AMF for the management of bacterial wilt of chilli.

Materials and Methods

3. MATERIALS AND METHODS

3.1 ISOLATION OF THE PATHOGEN

Chilli plants showing typical symptoms of bacterial wilt were collected from infested areas of Thiruvananthapuram district. The plants were cut at the collar region and examined for bacterial ooze. Infected stem tissues were cut into bits of approximately 0.5 cm size and surface sterilized with 0.1 per cent $HgCl_2$ for one minute. These stem bits were washed in a series of changes of sterile water and plated on CPG-TTC agar medium and incubated for 48 h at room temperature (27 ± 3°C). Single colonies from the plates were subcultured on to CPG-TTC (Appendix 1) slants. Pure culture of the bacterium was maintained in sterile water at 4°C.

Isolation of the pathogen was done from the bacterial ooze also. Surface sterilized bits were transferred to a drop of sterile distilled water taken on a sterile glass slide. The bits were then teased apart using sterilized blade and forceps and kept for one minute so that the bacterial ooze got mixed with the sterile water. The bacterial suspension thus obtained was streaked on TTC medium and isolated colonies of the bacterium obtained after 48 h of incubation were transferred to TTC medium in slants.

3.1.1 Pathogenicity Test

The pathogenicity test was conducted with sterile distilled water suspensions of bacteria prepared from 48 h old cultures grown on TTC medium. Vigorously growing young chilli seedlings of Jwalamukhi variety were inoculated by seedling dip. The pathogen was reisolated from the infected plants as described under 3.1. The most virulent isolate was selected and maintained for further studies.

3.2 CHARACTERIZATION OF THE PATHOGEN

The morphological, cultural, physiological and biochemical characteristics of the isolate were studied.

3.2.1 Gram Reaction

Gram staining was done using 24 h old culture of the pathogen.

3.2.1 Colony Characteristics

Dilute sterile distilled water suspension of pathogen was streaked over TTC medium and incubated for 48 h for the development of isolated colonies (Aley and Elphinstone, 1995). Colony characteristics with respect to time of appearance, shape, texture, consistency, colour and transparency were recorded.

3.2.3 Water Soluble Pigment Production

Water soluble fluorescent pigment production was tested in King's medium B (King *et al*., 1954) (Appendix 1). The test culture was spot inoculated into medium in petriplates. The petridishes were incubated for 48 h and examined for fluorescence under UV light.

3.2.4 Production of Levan

The culture was tested for the production of levan on peptone-beef extract medium (Appendix 1) with five per cent sucrose (Hildebrandt *et al*., 1992). Dilute suspension of test culture was streaked over sterilized media in petriplates and incubated for 48 h.

3.2.5 Growth at Different Temperatures

A loopfull of the 24 h old culture of the pathogen was streaked on to nutrient agar slants (Appendix 1) and incubated for 48 h at 4°C, 20°C, 30°C, 37°C and 40°C and optimum growth was recorded (Hildebrandt *et al*., 1992).

3.2.6 Growth at Different Time Intervals

A loopfull of the 24 h old culture was transferred to peptone – water broth (Appendix 1) in test tubes and incubated at room temperature (27 ± 3°C). The growth (OD value) was measured every 24 h after inoculation upto ten days using UV-VIS spectrophotometer 118 at 405 nm.

3.2.7 Starch Hydrolysis

Nutrient agar containing 0.2 per cent starch (Appendix 1) was used for this test. The test culture was spot inoculated at the centre of the petridish containing the medium. After 2, 3 and 7 days, the agar surface was flooded with Lugol's iodine solution and allowed to react for a few minutes. A colourless or reddish brown zone around the bacterial growth in contrast to blue background of the medium is interpreted as a positive test for starch hydrolysis (Hildebrandt *et al*., 1992).

3.2.8 Gelatin Utilization

Nutrient agar containing 0.4 per cent gelatin (Appendix 1) was used for the test. The test culture was spot inoculated on to the medium and incubated for 48 h. The agar surface was flooded with a 0.2 per cent mercuric chloride in 20 per cent hydrochloric acid and allowed to react for a few minutes to precipitate the gelatin in the medium. A clear zone surrounding the bacterial growth is interpreted as a positive test for gelatin utilization (Hildebrandt *et al*., 1992)

3.2.9 Production of Ammonia

The test culture was grown in peptone water in test tubes. The accumulation of ammonia was detected by using Nessler's reagent which gives a brown to yellow precipitate with ammonia (Eden-Green, 1994).

3.2.10 Catalase Test

Smear of the test culture was made from 24 to 48 h old slant growth and covered with a few drops of 20 volume hydrogen peroxide

(Hildebrandt *et al*., 1992). Catalase activity could be detected for gas production by examining the slides under microscope. Catalase converts hydrogen peroxide to water by removal of oxygen.

3.2.11 Sodium Chloride Tolerance

Different concentrations of NaCl *viz*., 0.5, 1.00, 2.00, 3.00 and 5.00 per cent were prepared in distilled water to which one ml of bacterial suspension from 24 h old culture of pathogen was added (Eden-Green, 1994). One loopfull of the mixture was streaked on to TTC medium and observations were recorded every 24 h upto 72 h.

3.2.12 Sensitivity to Streptomycin

Filter paper method was used to study the sensitivity of the isolate to different concentrations of streptomycin *viz*., 500, 1000, 2000 and 3000 ppm. Sterilized filter paper discs soaked in the respective concentration of antibiotic suspension was placed at the centre of petridishes containing nutrient agar seeded with the pathogen maintaining four replications each. The extent of inhibition zone formed was measured after 48 h.

3.3 MULTIPLICATION OF AMF INOCULUM

Different isolates of AM fungus were grown in sterilized soil : sand (1 : 1) mixture for three months using sorghum (*Sorghum vulgare* Pers.) as host plant in pots (30x30). Mycorrhizal spores, root fragments and hyphae served as mycorrhizal inoculum. 50 g of inoculum of the respective AMF culture was used for inoculation per pot.

3.3.1 Evaluation of AMF for Disease Suppression and Growth Improvement in Chilli

A pot culture experiment was conducted in CRD using three replications with Jwalamukhi variety. Ten numbers of native AMF isolates *viz*., M_1, M_2, M_3, M_4, M_5, M_6, M_7, M_8, M_9 and M_{10} developed and maintained in the Department of Plant Pathology, College of Agriculture, Vellayani were used to evaluate the effect of AMF on suppression of

bacterial wilt and growth improvement in chilli. Seedlings were raised in nursery pre-inoculated with the respective AMF cultures and transplanted into pots containing 2:1:1 mixture of soil: sand: cowdung. The experiment was conducted with the following treatments.

T_1	–	M_1R_1	T_{13}	–	M_7R_1
T_2	–	M_1R_0	T_{14}	–	M_7R_0
T_3	–	M_2R_1	T_{15}	–	M_8R_1
T_4	–	M_2R_0	T_{16}	–	M_8R_0
T_5	–	M_3R_1	T_{17}	–	M_9R_1
T_6	–	M_3R_0	T_{18}	–	M_9R_0
T_7	–	M_4R_1	T_{19}	–	$M_{10}R_1$
T_8	–	M_4R_0	T_{20}	–	$M_{10}R_0$
T_9	–	M_5R_1	T_{21}	–	M_0R_1
T_{10}	–	M_5R_0	T_{22}	–	M_0R_0
T_{11}	–	M_6R_1			
T_{12}	–	M_6R_0			

M_1, M_2, M_3, M_4, M_5, M_6, M_7, M_8, M_9, M_{10} – Native cultures of AMF

M_0 – Without AMF

R_1 – With inoculation of *R. solanacearum*

R_0 – Without inoculation of *R. solanacearum*

3.3.2 Inoculation with the pathogen

Artificial inoculation with the pathogen was done at the time of transplanting to incite the disease. Inoculation was done by seedling dip using 24 h old culture of the pathogen. Four days after inoculation, wilting

symptoms were noted and percentage disease incidence was recorded. Based on the ability of disease suppression, two best native AMF isolates were selected for further studies.

3.3.3 Biometric characteristics

Observations on biometric characters such as fresh and dry weight of plants, length of root and shoot, number of secondary branches, girth of stem and fresh weight of fruits were recorded at the time of harvest.

3.4 ISOLATION OF NATIVE *Pseudomonas fluorescens*

P. fluorescens were isolated from phyllosphere and rhizosphere of healthy chilli plants grown in different areas of Thiruvananthapuram district following serial dilution plate technique (Waksman, 1992).

3.4.1 Isolation of *P. fluorescens* from Rhizosphere

Chilli plants were uprooted gently and soil adhering to the root surface was collected. One gram soil was taken from this and shaken in 250 ml Erlen Meyer Flask containing 100 ml of sterile distilled water at 150 rpm for 30 minutes. From this dilutions were prepared and one ml of the aliquots from 10^{-5} and 10^{-6} were transferred to sterilized petridishes and plated with melted and cooled King's medium B. The plates were rotated clockwise and anticlockwise and incubated at 27 ± 3°C for 48 hours. Isolated colonies of bacteria were transferred to KMB slants and maintained at 4°C.

3.4.2 Isolation of *P. fluorescens* from Phyllosphere

Endophytic pseudomonads were isolated from healthy leaf samples collected from disease free areas. One gram leaf sample was surface sterilized with 0.1 per cent mercuric chloride for one minute, rinsed in sterile distilled water and ground with sterile pestle and mortar. Stock solution was prepared by mixing the extract with 100 ml of sterile distilled water. From the stock solution, serial dilutions were prepared and one ml

aliquots from 10^{-3} and 10^{-4} were transferred to sterilized petridishes and plated with KMB, rotated gently and incubated for 48 h. Isolates obtained from the serial dilution plate technique were maintained in KMB slants.

In all, thirty number of isolates were obtained, 18 isolates from the rhizosphere and 12 isolates from the phyllosphere. These were maintained in KMB at 4°C. These thirty isolates along with 15 number of isolates developed and maintained in the Department of Plant Pathology, College of Agriculture, Vellayani were screened under *in vitro* conditions.

3.5 EVALUATION OF ISOLATES OF *P. fluorescens*

Thirty number of isolates obtained from serial dilution technique were subjected to *in vitro* screening along with 15 number of isolates developed in the Department of Plant Pathology, College of Agriculture, Vellayani following dual culture technique (Burgers *et al*., 1999) for selecting the most efficient isolate. One ml of sterile distilled water was poured into 24 h old culture of bacterial pathogen for suspending the bacterial cells. This bacterial suspension was added to 15 ml melted and cooled KMB, swirled and poured into sterile petridishes. Sterilized filter paper discs of 1 cm diameter, dipped in a suspension of 24 h old culture of the antagonist was placed at the centre of the seeded KMB plates. Three replications were maintained for each isolate. Petriplates seeded with the sterile water served as control. The inoculated plates were incubated at room temperature (27 ± 3°C). The inhibition zone was measured after 48 h. The extent of inhibition zone formed was measured for assessing the efficiency of fluorescent pseudomonads.

3.6 CHARACTERIZATION OF BACTERIAL ISOLATES ANTAGONISTIC TO *Ralstonia solanacearum*

Efficient native isolates obtained from dual culture technique were subjected to morphological, cultural and biochemical characterization

following standard procedures. Based on their properties, each isolate was identified as separate species of saprophytic fluorescent pseudomonads.

3.6.1 Levan Formation

Dilute suspension of the bacterial isolates was streaked over the peptone beef extract medium containing five per cent sucrose (Appendix 1)

The plates were incubated for 48 h at room temperature (27 ± 3°C). Presence of large, white dome and mucoid colonies on the medium indicated production of levan from sucrose.

3.6.2 Gelatin Liquifaction

Nutrient gelatin medium of the following composition was dispensed into tubes to a depth of 4 cm and autoclaved at 1.5 kg cm^{-2} pressure for 20 minutes.

Forty eight hour old cultures of the isolates were stab inoculated to these gelatin columns and incubated. These tubes were checked for gelatin liquifaction at regular intervals for one month.

3.6.3 Growth at Different Temperatures

King's medium B broth of the following composition was used for studying the growth of the bacteria at different temperatures.

Peptone	–	20.0 g
$K_2 HPO_4$	–	1.5 g
$MgSO_4.7H_2O$	–	1.5 g
Glycerol	–	10.0 ml
Distilled water	–	1000 ml
pH	–	7.2

Five ml of King's medium B broth was dispensed into test tubes and autoclaved at 1.5 kg cm^{-2} pressure for 20 minutes. One loopfull of the

test isolates were transferred into the broth and incubated at 4°C and 41°C along with control. The absorbance of inoculated broth was measured at regular intervals for four days.

3.6.4 Growth at Different Time Intervals

A loop full of 24 h old culture was transferred to peptone-water broth in tubes and incubated at room temperature (27 ± 3°C). The growth (OD value) was measured every 24 h using UV-VIS spectrophotometer 118 at 405 nm for 10 days.

3.6.5 Utilization of Sugars

The basal medium for this test was Hayward's medium (Hayward, 1964) of the following composition.

Peptone	–	1.0 g
$NH_4 H_2PO_4$	–	1.0 g
KCl	–	0.2 g
$MgSO_4 . 7H_2O$	–	0.2 g
Bromothymol blue	–	0.03 g
Agar-agar	–	3.0 g
Distilled water	–	1000 ml
pH	–	7.2

The sugars used for the test were arabinose, galactose and sugar alcohol sorbitol, inositol.

Ninety ml of basal medium was dispensed in 250 ml conical flask and autoclaved at 1.5 kg cm^{-2} pressure for 20 minutes. Ten per cent of sugar and sugar alcohols were prepared in sterile distilled water and sterilized by filtration. Ten ml of each of the filtered solution was transferred to melted and cooled basal medium. The medium containing

sugar was dispensed into tubes to a depth of 4 cm. The medium was then stab inoculated with the isolates. These tubes were incubated and observations recorded for a period of one month. Change in colour of the medium from blue to yellow indicated positive utilization of carbon compounds with the production of sugars.

3.6.6 Utilization of Alcohol

Ten per cent ethanol was added to the medium to get ten per cent concentration. This was inoculated with the bacteria as described under 3.6.5

3.6.7 Utilization of n-butyl Amine

n-butyl amine was added to Hayward's medium so as to get concentration of one per cent. This was inoculated with test bacteria.

3.6.8 Utilization of Phenyl Acetate

Phenyl acetate was added to Hayward's medium so as to get a concentration of one per cent

3.6.9 Utilization of testosterone, Propylene glycol, Nicotinate

Hayward's media was used in this test also with the addition of testosterone, proplylene glycol and nicotinate as described in 3.6.8 in separate experiments containing one per cent each of the chemical.

3.7 EFFECT OF DUAL INOCULATION OF AMF AND *P. fluorescens* ON DISEASE SUPPRESSION IN CHILLI

Two efficient isolates each of AMF and *P. fluorescens*, viz., M_5 and M_7, Pf-14 and P-1 respectively were used for the experiment. The seeds of Jwalamukhi variety were bacterized with the *P. fluorescens* culture and sown in pots pre-inoculated with the selected AMF cultures. A pot culture experiment was conducted to study the effect of inoculation of AMF and *P. fluorescens* on disease suppression and growth improvement in chilli.

The experiment was laid out in CRD with the following treatments with three replications each.

T_1	–	$M_1P_1R_1$	T_{11}	–	$M_0P_2R_1$
T_2	–	$M_1P_1R_0$	T_{12}	–	$M_0P_2R_0$
T_3	–	$M_1P_2R_1$	T_{13}	–	$M_0P_0R_1$
T_4	–	$M_1P_2R_0$	T_{14}	–	$M_0P_0R_0$
T_5	–	$M_2P_1R_1$	T_{15}	–	$M_1P_0R_1$
T_6	–	$M_2P_1R_0$	T_{16}	–	$M_1P_0R_0$
T_7	–	$M_2P_2R_1$	T_{17}	–	$M_2P_0R_1$
T_8	–	$M_2P_2R_0$	T_{18}	–	$M_2P_0R_0$
T_9	–	$M_0P_1R_1$	T_{19}		$M_0P_0R_1$ + Agrimycin-100
T_{10}	–	$M_0P_1R_0$			

M_1, M_2 – Best isolates of AMF

M_0 – Without AMF

P_1, P_2 – Best isolates of *P. fluorescens*

P_0 – Without *P. fluorescens*

R_1 – With *R. solanacearum*

R_0 – Without *R. solanacearum*

3.7.1 Inoculation of AMF and *P. fluorescens*

Two best AMF cultures *viz*., M_5 and M_7 were inoculated to pots @ 50 g inoculum per pot. Seeds bacterized with the *P. fluorescens* cultures, Pf-14 and P-1 were sown into pots pre-inoculated with AMF.

3.7.2 Inoculation of the pathogen

Bacterial pathogen was inoculated at the time of transplanting of the seedlings by root dip method. After four days of inoculation, the plants

were observed for wilting symptoms and percentage disease incidence was recorded at weekly intervals.

3.7.3 Application of *P. fluorescens*

Soil drenching was done using the two best cultures of *P. fluorescens viz*., Pf-14 and P-1. Two per cent talc based formulation of the antagonist was prepared and applied to the rhizosphere region. Foliar application was done using two per cent suspension of talc based formulation of the respective bacterial isolates.

3.7.4 Application of Antibiotic

Agrimycin-100 was applied as soil drench at a concentration of 200 ppm which served as check along with the untreated check.

3.8 EFFECT OF DUAL INOCULATION OF AMF AND *P. fluorescens* ON BIOMETRIC CHARACTERISTICS IN CHILLI

Observations on important biometric characters such as fresh and dry weight of plants, length of shoot and root, fresh weight of fruits, stem girth and number of secondary branches were recorded at the time of harvest.

3.9 STATISTICAL ANALYSIS

The data generated from the experiment were subjected to Analysis of Variance (ANOVA) technique after appropriate transformation wherever needed.

Results

4. RESULTS

4.1 ISOLATION OF THE PATHOGEN

Bacterial wilt pathogen *Ralstonia solanacearum* was isolated from chilli plants grown in highly infested areas of Thiruvananthapuram district. The infected plants showed bacterial ooze from the vascular tissues with brownish discolouration at the collar region. On isolation, colonies in TTC medium appeared brownish white, slimy with entire margin and have no surface markings and resting structures. The bacterium was gram negative, straight rod having polar flagella.

4.1.1 Pathogenicity Test

Different isolates of *R. solanacearum* were tested for virulence and the most virulent isolate was selected. The pathogen was inoculated to chilli plants of Jwalamukhi variety and symptom development was monitored. Sudden drooping of the leaves and rotting of the stem was observed in infected seedlings (Plate 1). Greyish-white bacterial ooze came out when the stem was cut across and squeezed. On reisolation, the pathogen produced growth characters similar to those described in 4.1. The pure culture of the pathogen was maintained in sterile distilled water in screw capped bottles at 4°C.

4.2 CHARACTERIZATION OF THE PATHOGEN

The most virulent isolate was subjected to morphological, cultural, physiological and biochemical characterization (Table 1).

4.2.1 Gram Reaction

Morphological studies revealed that the bacteria are gram negative, rods, motile with polar flagellation.

1. *R. solanacearum* inoculated chilli plant.
2. Control

Plate 1 Effect of inoculation of *Ralstonia solanacearum* in chilli seedlings

Table 1 Characterization of *R. solanacearum*

Characterization test	Isolate
Growth in TTC medium	+
Water soluble pigment production	-
Production of levan	+
Growth at 4°C	+
20°C	+
30°C	+
37°C	+
40°C	-
Starch hydrolysis	-
Gelatin utilization	-
Production of ammonia	+
Catalase test	+
Sodium chloride tolerance 0.5 %	+
1.0 %	+
2.0 %	+
3.0 %	+
5.0 %	-

4.2.2 Colony Characteristics

Colony characteristics were studied in TTC media. The presence of discrete fluidal colonies with definite pink centres characteristic of the virulent strains of the bacterium was noted.

4.2.3 Water Soluble Pigment Production

The bacterial isolate did not produce any fluorescence under UV light which indicated that there is no water soluble pigment production in King's medium B.

4.2.4 Production of Levan

Large, white, domed and mucoid colonies were produced in peptone – beef extract medium which indicated production of levan.

4.2.5 Growth at Different Temperatures

Growth at different temperatures was scored in nutrient agar media. The culture produced good growth at 30° and 37°C but not at 40°C. Moderate growth was observed at 20°C and 4°C.

4.2.6 Growth at Different Time Intervals

Survival of the pathogen in peptone – water was measured from time of inoculation upto a period of 10 days in UV-VIS spectrophotometer 118. To standardize the wavelength for measuring growth a concentration curve was drawn using different wavelengths. The wavelength which gave maximum absorbance at a particular concentration of the sample was selected.

The inoculation of *R. solanacearum* in peptone-water broth resulted in significant increase in growth. Maximum OD value was recorded on the fifth day of inoculation. A steady increase in growth was observed from the zero day to the fifth day after inoculation. The bacterial growth remained in a stationary state for the days till the eighth day. A decline in OD value was observed after eight days (Table 2). A growth curve was

Table 2 Growth of *R. solanacearum* in peptone-water broth at (27 ± 2°C)

Days after inoculation	OD value (405 nm)
0	0.25
1	0.64
2	0.84
3	1.14
4	1.95
5	2.00
6	2.01
7	2.01
8	1.65
9	1.02
10	0.75
CD	0.14

drawn taking the OD along the Y axis and number of days along the X axis (Fig.1).

4.2.7 Starch Hydrolysis

The bacterial isolate was tested for starch hydrolysis and the results indicated that the isolate was unable to hydrolyse starch.

4.2.8 Gelatin Utilization

The bacterial isolate did not utilize gelatin from the media.

4.2.9 Production of Ammonia

The production of ammonia was tested using Nessler's reagent. 1111111111111111111111111111111A positive reaction was detected by the formation of an yellow precipitate.

4.2.10 Catalase Test

The bacterial isolate was found to be catalase positive.

4.2.11 Sodium Chloride Tolerance

The isolate was found to tolerate sodium chloride at 0.5 and 1.0 per cent concentration for 72 h. At 2.0 and 3.0 per cent concentrations of sodium chloride, the isolate was found to survive for 24 h. Sodium chloride at 5.0 per cent concentration was lethal to the bacterium and the culture was not capable of growing at this concentration.

4.2.12 Sensitivity to Streptomycin

Sensitivity to streptomycin at different concentrations was measured based on the extent of inhibition zone formation after 48 h of incubation. Maximum inhibition was obtained with 3000 ppm concentration of streptomycin (Table 3).

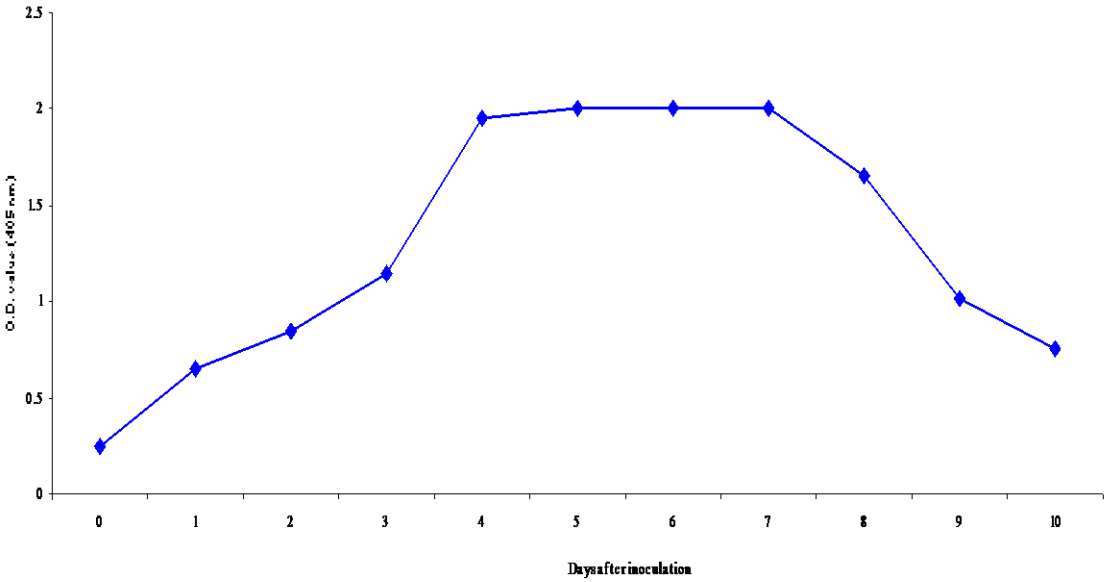


Fig. 1 Survival of *R. solanacearum* in peptone-water broth at room temperature (27 ± 3°C)

Table 3 Sensitivity of *R. solanacearum* to different concentrations of streptomycin

Concentration of streptomycin (ppm)	Inhibition zone(mm)
500	5
1000	11
2000	15
3000	20
CD	0.044

4.3 MULTIPLICATION OF AMF INOCULUM

AMF inoculum was multiplied in sterilized soil : sand (1 : 1) mixture using sorghum as host plant. 50 g of inoculum consisting of mycorrhizal spores, root fragments and hyphae was applied to each pot for mass multiplication of each of the AMF cultures for application in the screening trial.

4.3.1 Evaluation of AMF for Disease Suppression and Growth Improvement in Chilli

Chilli seedlings pre-inoculated in the nursery with the native AMF cultures from M_1 to M_{10} were used to evaluate the suppression of wilt incidence and growth improvement.

4.3.2 Inoculation of the Pathogen and Incidence of Bacterial Wilt

Bacterial wilt pathogen, *R. solanacearum* was inoculated at transplanting stage by seedling dip. The data on percentage disease incidence is presented in Table 4

Bacterial pathogen was inoculated @ 2.5 ml per plant containing 8 x 10^7 cfu/ml. Chilli seedlings pre-inoculated with M_5 culture recorded no disease incidence (0.00) when compared to other AMF cultures (Plate 2). The next efficient AMF culture identified was M_7 which recorded a percentage disease incidence of 15.92 as against 91.75 percent recorded in uninoculated control. The AMF culture, M_6 recorded 70.58 per cent disease incidence which was statistically on par with M_8 (67.75). Culture M_3 recorded 47.83 per cent disease incidence which was on par with M_4, M_2, and M_{10} with 47.83 , 45.17, and 43.67 per cent respectively. The AMF culture M_9 (22.58) and M_1 (27.30) were statistically on par.

Maximum percentage reduction over control was recorded in chilli seedlings pre-inoculated with M_5 culture (100.00) followed by M_7 (90.91).

Table 4 Effect of AMF inoculation on suppression of *R.solanacearum* in chilli

AMF isolate	Percentage disease incidence	Percentage reduction over control
M_0R_1	91.67 (91.75)	-
M_1R_1	16.67 (27.30)	81.82
M_2R_1	33.33 (45.17)	63.64
M_3R_1	25.00 (47.83)	72.73
M_4R_1	25.00 (47.83)	72.73
M_5R_1	**0.00 (0.00)**	**100.00**
M_6R_1	58.33 (70.58)	36.37
M_7R_1	8.33 (15.92)	90.91
M_8R_1	50.00 (67.75)	45.46
M_9R_1	16.67 (22.58)	81.82
$M_{10}R_1$	33.33 (43.67)	63.64
CD (5%)	5.11	

Figures in brackets are values after angular transformation

1. Control
2. M_5R_1

Plate 2 Effect of native AMF (M_5R_1) on suppression of wilt in chilli

1. Control
2. M_7R_0

Plate 3 Effect of native AMF (M_7R_0) on growth characteristics and yield in chilli

These efficient native cultures of AMF *viz*., M_5 and M_7 were selected for further studies.

4.3.3 Effect of AMF on Biometric Characters of Chilli

The effect of AMF on growth characteristics of chilli plants are presented in Table 5.

Significant increase in plant height was noticed in plants pre-inoculated with AMF when compared to uninoculated control. The treatment M_3R_0 recorded the highest shoot length of 34.00 cm which was statistically on par with treatments M_8R_1 (32.30 cm), M_1R_0 (31.30 cm), M_5R_0 (30.00 cm), M_7R_0 (30.00 cm), M_6R_0 (28.67 cm), M_4R_1 (28.33 cm), M_9R_1 (28.33 cm), M_4R_0 (28.00 cm), $M_{10}R_1$ (28.00 cm), M_5R_1 (27.33 cm), M_7R_1 (23.33 cm), and These treatments were significantly superior to the control which recorded shoot length of 15.30 cm.

The root length of AMF inoculated plants was significantly higher compared to control. The maximum root length was recorded in treatment M_6R_1 (27.67 cm) which was statistically on par with treatments M_6R_0 (26.33 cm), $M_{10}R_0$ (21.00 cm), M_7R_0 (20.33 cm), M_1R_0 (19.80 cm), M_8R_1 (19.33 cm), M_7R_1 (18.67 cm), M_9R_1 (18.30 cm), M_5R_1 (17.67 cm), M_5R_0 (17.00 cm). These treatments were significantly superior to the uninoculated control M_0R_0 (8.30 cm).

At the time of harvest the number of secondary branches were significantly higher in AMF inoculated plants. Maximum number of secondary branches were recorded in M_5R_0 (30.80) which was on par with M_2R_0 (30.00) followed by $M_{10}R_0$ (18.30). These were significantly higher than the number of secondary branches in control M_0R_0 which recorded 13.30.

Maximum stem girth was recorded in $M_{10}R_0$ (6.00 cm) followed by M_5R_1 (3.67 cm) which was statistically on par with M_4R_1 (3.50 cm), M_7R_1 (3.17 cm), M_4R_0 (3.16 cm), M_3R_0 (3.00 cm), M_6R_1 (2.67 cm), M_6R_0 (2.66 cm),

Table 5 Effect of AMF inoculation on growth and yield in chilli

Treatments	Shoot length (cm)	Root length (cm)	Number of secondary branches(no plant^{-1})	Stem girth (cm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Yield (g plant^{-1})
M_0R_0	15.30	8.30	13.30	1.17	40.00	3.67	16.67
M_1R_1	6.21	15.0	2.30	2.30	46.67	8.67	30.00
M_1R_0	31.30	19.80	14.20	2.17	60.00	15.33	16.67
M_2R_1	19.67	15.00	19.00	2.31	53.33	12.67	20.00
M_2R_0	15.67	6.67	30.00	2.00	50.00	10.33	5.00
M_3R_1	20.00	15.00	15.00	1.33	60.00	16.00	15.00
M_3R_0	**34.00**	15.27	14.60	3.00	**66.67**	**18.33**	33.33
M_4R_1	28.33	13.30	14.00	3.50	26.67	5.33	8.30
M_4R_0	28.00	12.67	15.30	3.16	26.67	5.00	21.67
M_5R_1	27.33	17.67	13.60	3.67	46.67	8.00	73.33
M_5R_0	30.00	17.00	**30.80**	2.33	30.00	3.33	31.67
M_6R_1	22.00	**27.67**	14.00	2.67	16.67	5.00	26.67
M_6R_0	28.67	26.33	13.60	2.66	23.30	4.00	13.33
M_7R_1	23.33	18.67	14.60	3.17	21.67	2.67	61.67
M_7R_0	30.00	20.33	16.00	2.83	56.67	16.67	**83.33**
M_8R_1	32.30	19.33	14.60	2.33	36.67	15.00	33.30
M_8R_0	22.33	15.32	13.60	2.50	33.33	6.67	31.67
M_9R_1	28.33	18.30	16.30	2.00	53.33	11.67	60.00
M_9R_0	22.67	9.90	13.60	1.83	30.00	6.33	50.00
$M_{10}R_1$	28.00	13.50	15.60	1.33	53.33	9.33	56.67
$M_{10}R_0$	18.67	21.00	18.30	**6.00**	50.00	8.67	26.67
M_0R_1	10.00	3.30	2.00	1.33	5.00	1.00	1.00
CD(5%)	10.98	11.78	6.45	1.80	17.40	8.00	35.50

M_8R_0 (2.50 cm), M_5R_0 (2.33 cm), M_7R_0 (2.83 cm), M_8R_1 (2.33 cm), M_2R_1 (2.31 cm), M_1R_1 (2.30 cm), M_1R_0 (2.17 cm), M_2R_0 (2.00 cm), M_9R_1 (2.00 cm). These treatments were significantly superior to control M_0R_0 (1.17 cm).

The fresh weight of chilli plants inoculated with AMF were higher compared to the untreated control. The treatment M_3R_0 (66.67g) recorded maximum fresh weight which was on par with M_1R_0 (60.00 g), M_3R_1 (60.00 g), M_7R_0 (56.67 g), M_2R_1 (53.33 g), M_9R_1 (53.33 g), $M_{10}R_1$ (53.33 g), M_2R_0(50.00 g) and $M_{10}R_0$ (50.00 g). These treatments were significantly higher than the fresh weight of control plant M_0R_0 (40.00 g).

The dry weight of inoculated plants were significantly higher when compared to uninoculated control. The maximum dry weight of 18.33 g was recorded in the treatment M_3R_0 which was statistically on par with M_3R_1 (16.00 g), M_1R_0 (15.33g), M_8R_1(15.00 g), M_2R_1 (12.67 g) and M_9R_1 (11.67 g) compared to control M_0R_0 which recorded 3.67 g.

The yield of chilli plants inoculated with AMF were significantly higher compared to control (Plate 3). Maximum yield was recorded in M_7R_0 (83.33 g) which as statistically on par with M_5R_1 (73.33 g), M_7R_1 (61.67 g), M_9R_1 (60.00 g), $M_{10}R_1$ (56.67 g), M_9R_0 (50.00 g) compared to control M_0R_0 (16.67 g).

4.4 ISOLATION OF NATIVE *Pseudomonas fluorescens*

In all, thirty isolates of fluorescent *Pseudomonas* spp. were obtained from chilli growing areas of Thiruvananthapuram district. Out of these isolates 18 were obtained from rhizosphere and 12 isolates from the phyllosphere of chilli plants.

4.4.1 Isolation of *P. fluorescens* from Rhizosphere

Eighteen number of isolates of fluorescent pseudomonads were obtained from rhizosphere at a dilution of 10^{-5} and 10^{-6} in KMB after an incubation period of 48 h. The colonies were circular, flat or convex shaped, elevated with entire margin and produced fluorescent pigment.

Single colonies were transferred to KMB slants and maintained for further studies.

4.4.2 Isolation of *P. fluorescens* from Phylloplane

Twelve number of isolates were obtained from phylloplane at a dilution of 10^{-3} and 10^{-4} in KMB. Typical colonies of fluorescent pseudomonads as described under 4.4.1 appeared after 48 h. Single colonies were transferred to KMB slants and maintained for further studies.

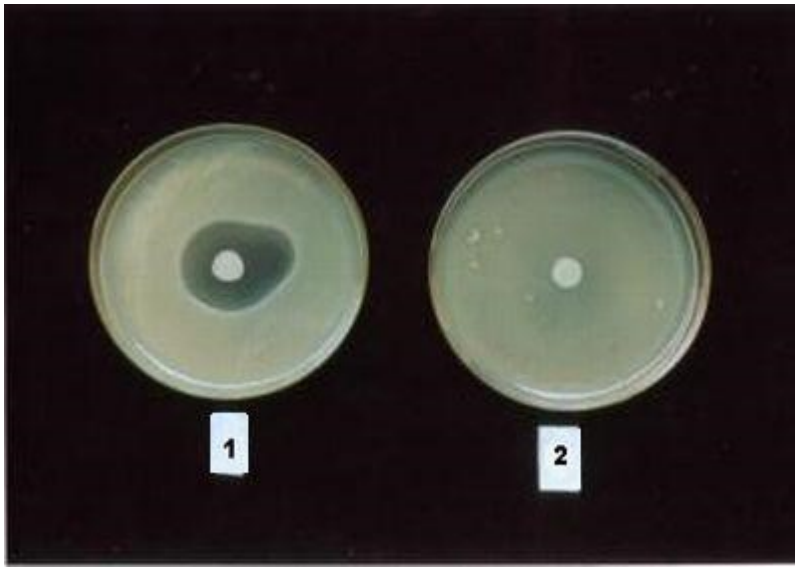
4.5 SCREENING OF ISOLATES OF *P. fluorescens*

Along with the 30 isolates collected, 15 number of isolates developed and maintained in the Department of Plant Pathology, College of Agriculture, Vellayani were also used for *in vitro* screening. These isolates were P-1, P-2, $B_{5,}$ B_{22}, B_{25}, B_{28}, $B_{31,}$ $B_{34,}$ B_{39}, B_{52}, $B_{53,}$ $B_{57,}$ $B_{63,}$ $B_{65,}$ and $B_{70.}$

Maximum inhibition zone was produced by isolate Pf-14 (20.0mm) which was statistically on par with P-1 (19.00 mm) (Plate 4 and Plate 5). The isolates Pf-11, B-31, Pf-2, P-2 recorded an inhibition zone of 14.0, 11.6, 11.6 and 11.3 mm respectively. The isolates Pf-15, Pf-19 and Pf-20 did not produce any inhibition zone. The isolates which produced maximum inhibition *viz*., Pf-14 and P-1 were identified as efficient isolates and maintained for further studies (Table 6).

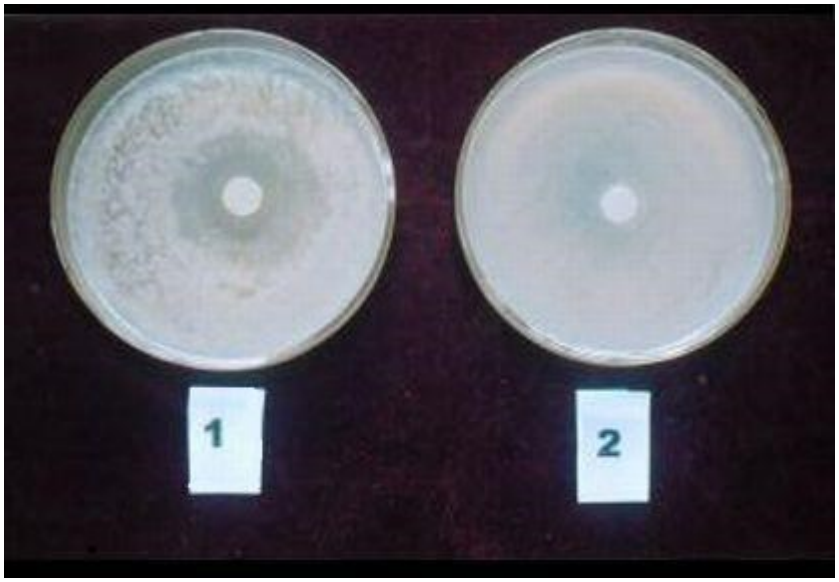
4.6 CHARACTERIZATION OF BACTERIAL ISOLATES ANTAGONISTIC TO *R. solanacearum*

The best isolates obtained from dual culture technique namely Pf-14 and P-1 were subjected to characterization following standard procedures prescribed by Schaad (1992) (Table 7).



1. Inhibition of Pf-14
2. Control

Plate 4 Effect of fluorescent *Pseudomonas* sp. (Pf-14) in inhibiting growth of *R. solanacearum*.



1. Inhibition of P-1
2. Control

Plate 5 Effect of fluorescent *Pseudomonas* sp. (P-1) in inhibiting growth of *R. solanacearum*.

Table 6 *In vitro* screening native isolates of *P. fluorescens* against *R. solanacearum* in KMB after 48 h

Isolates	Inhibition zone (mm)
Pf-1	7.30
PF-2	11.60
Pf-3	2.30
Pf-4	1.00
Pf-5	4.00
Pf-6	8.60
Pf-7	1.60
Pf-8	8.60
Pf-9	6.00
Pf-10	10.00
Pf-11	14.00
Pf-12	2.00
Pf-13	1.00
Pf-14	**20.00***
Pf-15	0.00
Pf-16	5.00
Pf-17	7.10
Pf-18	8.00
Pf-19	0.00
Pf-20	0.00
Pf-21	1.30
Pf-22	0.70
Pf-23	2.50
Pf-24	9.00
Pf-25	0.30

Table 6 Continued

Isolates	Inhibition zone (mm)
Pf-26	1.00
Pf-27	0.30
Pf-28	0.60
Pf-29	4.00
Pf-30	8.00
P-1	19.00
P-2	11.30
B_{65}	6.60
B_{52}	6.60
B_{39}	0.60
B_{34}	6.30
B_{53}	6.60
B_{70}	8.10
B_{5}	6.50
B_{28}	5.00
B_{57}	5.00
B_{31}	11.60
B_{63}	5.00
B_{22}	6.60
B_{25}	0.60
CD	3.84

*Maximum inhibition zone

Table 7 Characterization of efficient native isolates of *P. fluorescens*

Biochemical tests	Pf-14	P-1
Levan formation	_	+
Gelatin liquifaction	+	+
Growth at 4°C	_	+
Growth at 41°C	+	_
Utilization of arabinose	_	+
Galactose	_	+
Sorbitol	_	+
Inositol	_	+
Ethanol	+	+
n-butyl amine	_	_
Propylene glycol	+	+
Phenyl acetate	_	_
Testosterone	_	_
Nicotinate	_	_

4.6.1 Levan Formation

Production of large white dome and mucoid colonies on the medium by P-1 indicated formation of levan from sucrose. The isolate Pf-14 did not give a positive reaction.

4.6.2 Gelatin Liquifaction

The culture Pf-14 and P-1 liquified gelatin columns within ten days.

4.6.3 Growth at Different Temperatures

Survival of Pf-14 and P-1 were tested in King's B broth at 4°C and 41°C. P-1 recorded positive growth at 4°C but not at 41°C while Pf-14 produced growth at 41°C but not at 4°C.

4.6.4 Growth at Different Time Intervals

The growth of *P. fluorescens* in peptone water was measured in UV-VIS spectrophotometer 118 for 10 days at 405 nm (Table 9). The OD value was maximum on the fourth day (2.12). Growth was found stabilized at fourth, fifth and sixth day after inoculation. A decline in growth was observed after the sixth day. The growth curve of Pf-14 and P-1 are represented in Fig. 2 and Fig. 3 respectively.

4.6.5 Utilization of sugars

The sugars galactose, arabinose and sugar alcohol sorbitol, inositol were utilized only by P-1. The two cultures viz., P-1 and Pf-14 did not utilize n-butyl amine, phenyl acetate, testosterone, nicotinate. Ethanol and propylene glycol were utilized by both the isolates (Table 7)

The characteristics of the isolates Pf-14 and P-1 derived from the physiological and biochemical tests were compared with the characters of saprophytic fluorescent pseudomonas described by Schaad (1992). Isolates Pf-14 and P-1 have been identified as *P. aeruginosa* and *P. fluorescens* biovar 2 respectively (Table 8).

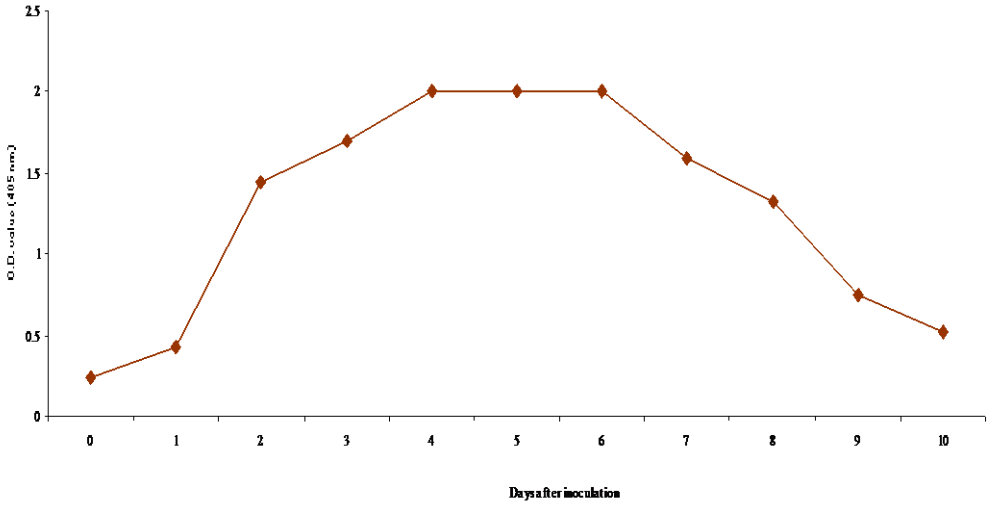


Fig. 2 Growth curve of Pf-14

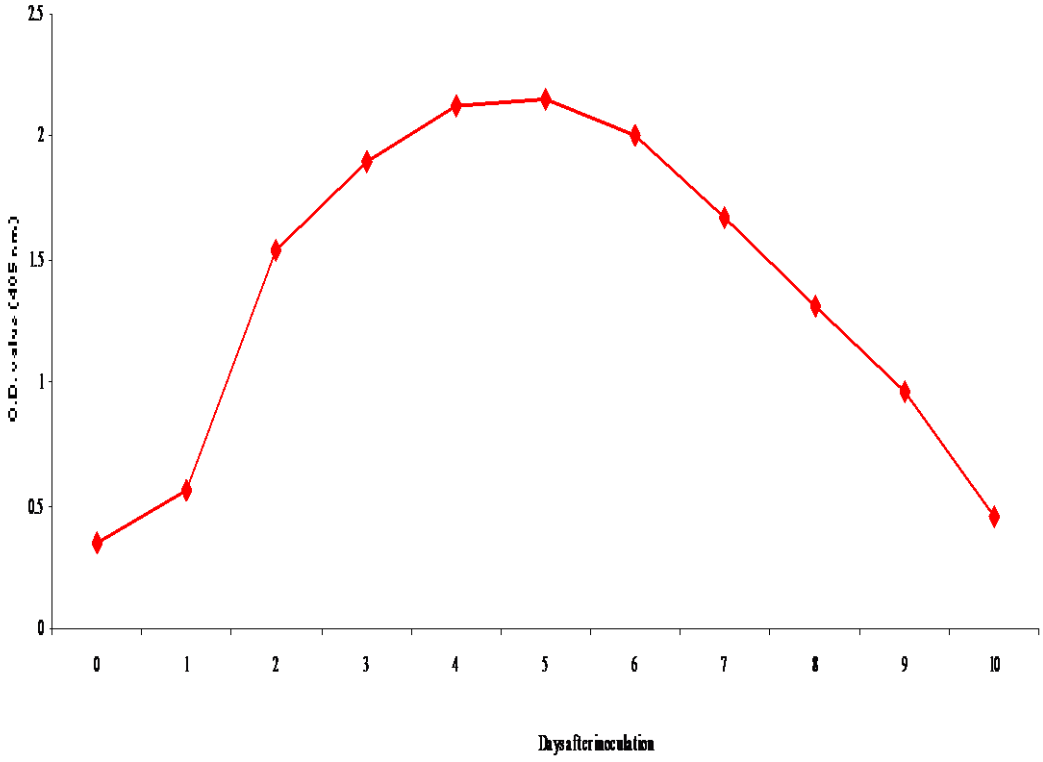


Fig. 3 Growth curve of P-1

Table 8 Characters of plant associated saprophytic fluorescent *Pseudomonas* sp. (Schaad, 1992)

	P. aeruginosa	*P. fluorescens* biovar					*P. chlororaphis*	*P. aureofaciens*	*P. putida* biovar	
		I.	I.	I.	V.	V.			A	B
Levan formation	-	+	+	-	+	-	+	+	-	-
Gelatin liquefaction	+	+	+	+	+	+	+	+	-	-
Growth at 4˚C	-	+	+	+	+	v	+	+	v	+
Growth at 41˚C	+	-	-	-	-	-	-	-	-	-
Utilization of Arabinose	-	+	+	v	+	-	-	+	v	+
Galactose	-	+	+	v	+	v	v	+	-	+
Sorbitol	-	+	+	v	+	v	-	-	-	v
Inositol	-	v	+	v	+	v	+	+	-	-
Propylene glycol	+	-	+	v	-	v	-	-	v	+
Ethanol	+	-	+	v	-	v	v	-	v	v
n-Butyl amine	-	-	-	-	-	v	-	v	+	+
Phenyl acetate	-	-	-	-	-	v	v	+	+	+
Testosterone	-	-	-	-	-	-	-	-	-	+
Nicotinate	-	-	-	-	-	v	-	-	v	+

Table 9 Growth of efficient native isolates of *P. fluorescens* at different time intervals

Days after inoculation	OD value	
	Pf-14	P-1
0	0.24	0.35
1	0.43	0.56
2	1.44	1.54
3	1.69	1.90
4	2.01	2.12
5	2.00	2.12
6	2.00	2.00
7	1.59	1.67
8	1.32	1.31
9	0.75	0.96
10	0.52	0.46
CD	0.16	0.25

4.7 EFFECT OF DUAL INOCULATION OF AMF AND *P. fluorescens* FOR THE MANAGEMENT OF *R. solanacearum* IN CHILLI

Chilli seeds of Jwalamukhi variety were bacterized with two best *P. fluorescens* cultures *viz*., Pf-14 and P-1. The inoculated seeds were sown in pots pre-inoculated with selected AMF cultures *viz*., M_5 and M_7 to evaluate the suppression of wilt incidence and growth improvement in chilli.

The bacterial pathogen was inoculated at the time of transplanting by seedling dip method. The data on percentage disease incidence is presented in Table 10.

Bacterial pathogen was inoculated @ 2.5 ml per plant containing 8×10^7 cfu/ml. Chilli seedlings inoculated with M_5 and P-1 recorded the least percentage disease incidence of 14.31 ($M_5P_2R_1$) as against the uninoculated control which recorded 90.25 (Plate 6). This was statistically on par with treatments $M_7P_2R_1$ (29.80), $M_7P_1R_1$ (25.23) and $M_5P_0R_1$ (24.47).

Two per cent suspension of the talc based formulation of the bacterial isolates *viz*., Pf-14 and P-1 were applied as soil drench and foliar application respectively, 15 days after transplanting.

Agrimycin-100 (200 ppm) was applied to the treatment $M_0P_0R_1$ which recorded a percentage disease incidence of 39.80 which was statistically on par with $M_5P_1R_1$ (39.30), $M_7P_0R_1$ (37.75) and $M_0P_2R_1$ (36.75).

4.8 EFFECT OF DUAL INOCULATION OF AMF AND *P. fluorescens* ON BIOMETRIC CHARACTERISTICS OF CHILLI

The data on the effect of combined inoculation of AMF and *P. fluorescens* on growth characters of chilli are presented in Table 11.

Dual inoculation of AMF and *P. fluorescens* resulted in significant increase in shoot length of chilli plants. It was maximum in treatment

Table 10 Effect of dual inoculation of AMF and *P. fluorescens* on suppression of *R. solanacearum* in chilli

Treatments	Percentage disease incidence	Percentage reduction over control
$M_0P_0R_1$	92.65 (90.25	_
$M_0P_0R_1$+ Agrimycin	40.41 (39.80)	55.90
$M_0P_1R_1$	55.16 (48.12)	40.46
$M_0P_2R_1$	35.26 (36.75)	61.94
$M_5P_0R_1$	25.03 (24.47)	72.98
$M_5P_1R_1$	40.31 (39.30)	56.49
$M_5P_2R_1$	**15.26 (14.31)**	**83.53**
$M_7P_0R_1$	37.15 (37.75)	59.90
$M_7P_1R_1$	26.03 (25.23)	71.99
$M_7P_2R_1$	31.47 (29.80)	66.03
CD	9.36	_

Table 11 Effect of dual inoculation of AMF and *P. fluorescens* on growth and yield in chilli

Treatments	Shoot length(cm)	Root length (cm)	Number of secondary branches (number plant^{-1})	Stem girth (cm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Yield (g plant^{-1})
$M_0P_0R_0$	17.45	7.00	12.02	1.12	24.22	6.26	13.00
$M_0P_0R_1$	24.40	2.33	1.43	2.00	3.00	1.22	1.50
$M_0P_0R_1$+ Agrimycin	20.00	2.55	4.60	2.16	6.26	2.29	2.26
$M_0P_1R_1$	26.65	21.65	18.55	3.12	25.66	6.66	7.00
$M_0P_1R_0$	29.07	**23.25**	20.62	3.56	30.29	9.55	16.00
$M_0P_2R_1$	25.66	12.22	13.16	1.34	38.69	9.99	15.00
$M_0P_2R_0$	28.17	14.66	23.67	2.12	39.21	10.00	21.67
$M_5P_0R_1$	26.33	10.69	15.26	2.50	46.20	11.66	70.00
$M_5P_0R_0$	28.00	11.25	17.99	2.66	50.33	13.99	66.00
$M_5P_1R_1$	33.67	15.99	22.17	4.12	58.17	12.21	35.66
$M_5P_1R_0$	32.62	18.26	**31.26**	3.99	49.99	14.66	26.22
$M_5P_2R_1$	30.56	17.11	29.33	3.00	42.00	10.09	17.66
$M_5P_2R_0$	**35.50**	18.13	25.63	**4.32**	60.25	15.00	35.42
$M_7P_0R_1$	20.22	9.21	11.23	1.56	58.99	15.12	66.21
$M_7P_0R_0$	29.33	11.99	19.99	1.99	59.33	15.99	71.22
$M_7P_1R_1$	30.67	12.75	22.31	2.66	44.66	13.75	80.03
$M_7P_1R_0$	34.52	14.15	24.99	2.75	55.29	14.88	**86.66**
$M_7P_2R_1$	22.00	20.96	18.60	2.12	60.99	16.33	49.66
$M_7P_2R_0$	24.88	20.99	20.00	2.31	**65.66**	**17.23**	56.22
CD(5%)	11.27	10.67	6.45	1.99	18.21	6.00	37.50

$M_5P_2R_0$ (35.50 cm). This was statistically on par with $M_7P_1R_0$ (34.52 cm), $M_5P_1R_1$ (33.67 cm), $M_7P_1R_1$ (30.67 cm), $M_5P_2R_1$ (30.56 cm), $M_7P_0R_0$ (29.33 cm), $M_0P_1R_0$ (29.07 cm), $M_0P_2R_0$ (28.17 cm), $M_5P_0R_0$ (28.00 cm), $M_0P_1R_1$ (26.65 cm), $M_5P_0R_1$ (26.33 cm) and $M_0P_2R_1$ (25.66 cm).

Significant increase in root length was noticed in plants co-inoculated with AMF and *P. fluorescens* when compared with the uninoculated control. The treatment $M_0P_1R_0$ recorded maximum root length of 23.25 cm which was statistically on par with treatments $M_0P_1R_1$ (21.65 cm), $M_7P_2R_0$(20.99 cm), $M_7P_2R_1$(20.96 cm), $M_5P_1R_0$(18.26 cm), $M_5P_2R_0$ (18.13 cm), $M_5P_2R_1$ (17.11 cm), $M_5P_1R_1$(15.99 cm), $M_0P_2R_0$ (14.66 cm), $M_7P_1R_0$ (14.15 cm) and $M_7P_1R_1$ (12.75 cm).

The number of secondary branches were significantly higher in plants inoculated with AMF and *P. fluorescens*. Maximum number of secondary branches was noticed in treatment $M_5P_1R_0$ (31.26) which was statistically on par with $M_5P_2R_1$ (29.33), $M_5P_2R_0$ (25.63), $M_7P_1R_0$ (24.99).

The maximum stem girth was recorded in treatment $M_5P_2R_0$ (4.32 cm). This was statistically on par with $M_5P_1R_1$ (4.12 cm), $M_5P_1R_0$ (3.99 cm), $M_0P_1R_0$ (3.56 cm), $M_0P_1R_1$ (3.12 cm), $M_5P_2R_1$ (3.00 cm), $M_5P_0R_0$ (2.66 cm), $M_7P_1R_1$ (2.66 cm), $M_7P_1R_0$ (2.75 cm), $M_5P_0R_1$ (2.50 cm), and $M_7P_2R_0$ (2.31 cm)

At the time of harvest, the fresh weight of plants inoculated with AMF and *P. fluorescens* was higher when compared to uninoculated control. The treatment $M_7P_2R_0$ recorded maximum fresh weight of 65.66 g. This was statistically on par with $M_7P_2R_1$ (60.99 g), $M_5P_2R_0$ (60.25 g), $M_7P_0R_0$ (59.33 g), $M_7P_0R_1$ (58.99 g), $M_5P_1R_1$ (58.17 g), $M_7P_1R_0$ (55.29 g), $M_5P_0R_0$ (50.33 g) and $M_5P_1R_0$ (49.99 g).

Combined inoculation of AMF and *P. fluorescens* significantly increased the dry weight of chilli plants. The maximum dry weight was for treatment $M_7P_2R_0$ (17.23 g). This was statistically on par with

$M_7P_2R_1$ (16.33 g), $M_7P_0R_0$ (15.99 g), $M_7P_0R_1$ (15.12 g), $M_5P_2R_0$ (15.00 g), $M_7P_1R_0$ (14.88 g), $M_5P_1R_0$ (14.66 g), $M_5P_0R_0$ (13.99 g), $M_7P_1R_1$ (13.75 g), $M_5P_1R_1$ (12.21 g) and $M_5P_0R_1$ (11.66 g).

The yield of chilli plants co-inoculated with AMF and *P. fluorescens* were significantly higher compared to control (Plate 7). Maximum yield was obtained in treatment $M_7P_1R_0$ (86.66 g) which was statistically on par with treatments $M_7P_1R_1$ (80.03 g), $M_7P_0R_0$ (71.22 g), $M_5P_0R_1$ (70.00 g), $M_7P_0R_1$ (66.21 g), $M_5P_0R_0$ (66.00 g), $M_7P_2R_0$ (56.22 g), $M_7P_2R_1$ (49.66 g), and compared to control $M_0P_0R_0$ (13.00 g).

1. Control
2. $M_5P_2R_1$

Plate 6 Effect of AMF and *Pseudomonas fluorescens* ($M_5P_2R_1$) on suppression of wilt in chilli

1. $M_7P_1R_0$
2. Control

Plate 7 Effect of AMF and *Pseudomonas fluorescens* ($M_7P_1R_0$) on growth characteristics

Discussion

5. Discussion

Ralstonia solanacearum is a serious pathogen causing wilt in economically important crops in lowland, humid tropics and cooler tropical highlands. The pathogen has been reported to cause wilt disease in several solanaceous plants including tomato, chilli and brinjal (Smith, 1896).

Chilli is one of the important solanaceous vegetable crops of Kerala and bacterial wilt caused by *R. solanacearum* is the most serious constraint in the production of the crop. Management of the disease using chemical methods is expensive and often ineffective. In this context, the relevance of management of the disease by resorting to alternative ecofriendly strategies such as use of bioagents becomes significant.

The bacterial wilt pathogen for the study was isolated from infested areas of Thiruvananthapuram district and pathogenicity was proved on chilli plants. Based on the cultural, morphological and biochemical characteristics the pathogen was identified as *R. solanacearum* (Hayward, 1964). Growth in specific media like CPG-TTC agar medium, production of levan, ammonia and the positive reaction to catalase test indicated that the isolate was *R. solanacearum*.

It is well established that AMF help plants in the uptake of nutrients particularly N, P and confers disease resistance (Sullia, 1991) and thereby promotes plant growth and development (Meenakumari and Nair, 1992; Sreenivasa, 1992; Sreenivasa *et al*, 1992). AMF inoculated plants exhibit resistance towards soil borne pathogens through a number of mechanisms such as exclusion of pathogen, activating defense mechanisms of plants and by improving nutrition of plants (Sharma *et al*., 1992; Isobe and Tsuboki, 1999). In the present investigation, ten native AMF isolates were screened to identify efficient AMF cultures capable of

suppressing bacterial wilt of chilli. Eventhough the ability of AMF to suppress the disease varied with different cultures, 100 per cent disease reduction was obtained in plants pre-inoculated with AMF culture, M_5 followed by M_7 which could give 90.91 per cent reduction in disease (Fig. 4). Among the cultures screened, M_5 and M_7 were highly effective in suppressing bacterial wilt of chilli (Table 4). The ability of AMF in suppressing bacterial wilt has already been reported in tomato (Sood *et al*., 1997; Raji *et al*., 2003). Effective AMF association leads to changes in mycorhizosphere population (Graham, 1988), enhanced uptake of phosphorus (Abdul-Khaliq *et al*., 2001), competition with the pathogen for nutrients and space (Al-Karaki, 2002a) and induction of host defence mechanism (Schubert *et al*., 2004). These desirable changes brought about by AMF colonization might have restricted the infection, multiplication and spread of the pathogen.

Growth, biomass production and yield of chilli plants were enhanced uniformily with AMF inoculation. However, variations were observed in the response of chilli plants to different isolates of AMF. The AMF isolates M_3 and M_6 recorded maximum shoot length and root length respectively. Biomass production was highest for cultures M_3 and M_7 (Fig. 5). The yield was maximum in M_7 inoculated plants. Mycorrhizal symbiosis is known to increase the plant biomass and yield due to enhanced changes in physiological and biochemical nature of the plant. This has been observed in a spectrum of crop plants. AMF inoculation resulted in faster growth of seedlings of chilli (Sreenivasa, 1992), casuarinas (Rajeswari *et al*., 2001), maize (Soderberg *et al*., 2002), tomato (Hildebrandt *et al*., 2002), garlic (Al-Karaki, 2002a), avocado (da Silveira *et al*., 2002) and cashew (Ananthakrishnan *et al*., 2004) compared to uninoculated seedlings. These endophyte colonized roots enhanced plant growth by increasing their nutrient uptake, which results in increased yield (Gill *et al*., 2002).

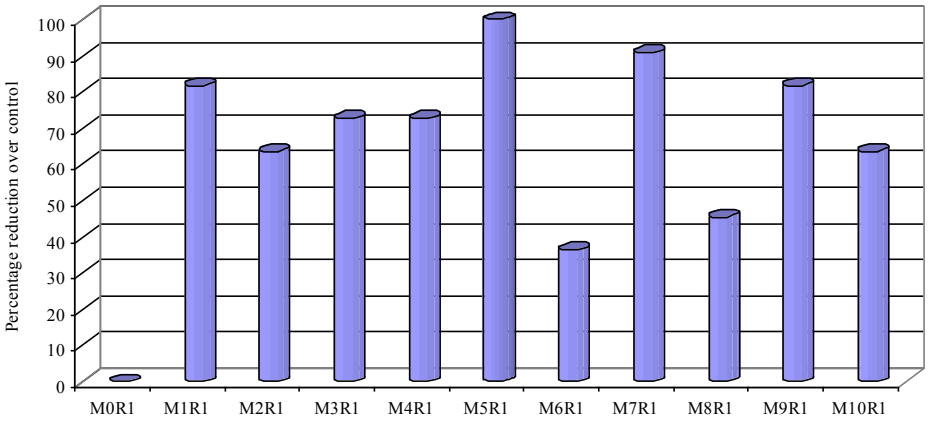


Fig. 4 Effect of AMF inoculation on suppression of *R. solanacearum* infection in chilli

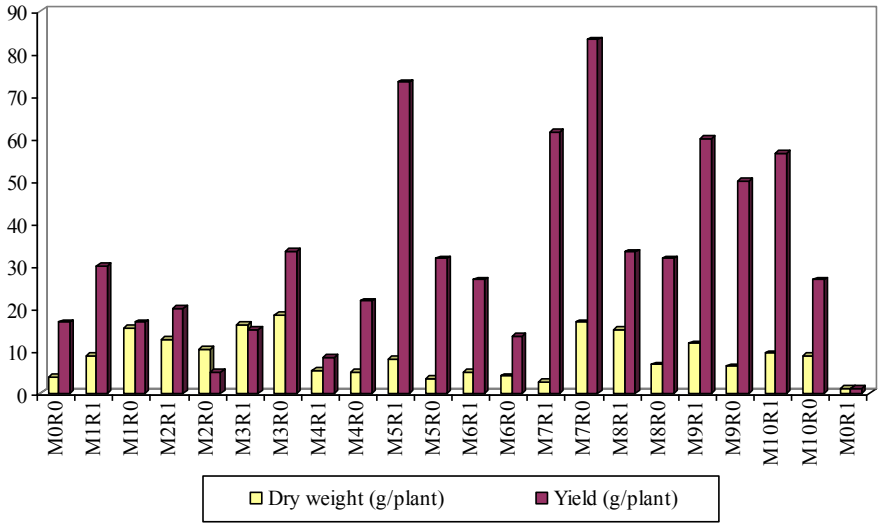


Fig. 5. Effect of AMF inoculation on biomass production and yield in chilli

The mycorrhizal plants are able to explore the soil more thoroughly and are able to locate and utilise the point source of P in the insoluble iron phosphate. Dwivedi *et al*. (2003) reported that AMF increases the phosphorus availability by mobilizing the phosphorus with the help of their extramatrical hyphae, particularly in soils with less available P. Inoculation with AMF increased root length, shoot length as well as increased level of nutrients and yield in lentil (Gill *et al*., 2002), dry weight of shoot and root in chickpea (Champawat, 1989), casuarinas (Rajeswari *et al*., 2001), brinjal (Taichenyang *et al*., 2001) and wheat (Al-Karaki *et al*., 2004).

In the present investigation, the native AMF isolate M_5 recorded 100 per cent suppression of bacterial wilt in chilli followed by M_7 with 90.91 per cent suppression. Although the cultures, M_5 was highly effective in suppressing the disease. But the crop yield was relatively less than that of M_7 which recorded maximum yield along with very effective disease suppression. It is highly desirable to obtain a culture having both biocontrol potential and growth stimulation. In this point of view isolate M_7 is most effective followed by M_5. Similar results of increased plant growth together with suppression of *Phytophthora capsici* (Sivaprasad *et al*., 2000) and *Verticillium dahliae* (Idoia *et al*., 2004) has already been reported in black pepper. In the present study, the AMF cultures M_5 and M_7 were adjudged as promising isolates capable of effectively suppressing of bacterial wilt as well as increasing growth and yield of chilli.

Management of bacterial wilt of chilli using resistant cultivars, adoption of cultural practices like crop rotation etc. provides only limited success. Cultures of *P. fluorescens* were isolated from chilli growing areas of Thiruvananthapuram district and in all 30 number of isolates were obtained. These isolates along with 15 native isolates obtained from the Department of Plant Pathology, College of Agriculture, Vellayani were evaluated *in vitro* for their antagonistic property against the wilt pathogen.

Biological control with bacterial antagonists has emerged as one of the effective alternate method for the management of soil-borne bacterial and fungal plant pathogens. Fluorescent pseudomonads are organisms with great potential in biological control and plant growth promotion (Kloepper and Schroth, 1978). *P. fluorescens* could very effectively inhibit the ginger wilt pathogen, *R. solanacearum* under *in vitro* condition (Anith *et al.,* 2000). Significant reduction in wilt incidence was also obtained in tomato plants inoculated with *P. fluorescens* (Pradeepkumar and Sood, 2001; Anith *et al*., 2004). The native isolates of fluorescent pseudomonads are found highly potential bioagents capable of effectively suppressing bacterial wilt in chilli and tomato (Meenakumari *et al*., 2003). Among the 45 isolates obtained, two isolates *viz*., Pf-14 and P-1 produced maximum inhibition against *R. solanacearum* in KMB medium.

. *In vitro* antagonism by fluorescent pseudomonas may be attributed to the production of siderophores (Sakthivel *et al*., 1986) or production of inhibitory metabolites like phenazine-1-carboxylicacid, hydrogen cyanide, 2,4-diacetyl phloroglucinol (Maurhofer *et al*., 1992). Exhaustive studies have been conducted on the *in vitro* toxicity of *P. fluorescens* to *R. solanacearum* (Bora *et al*., 2000) and the inhibition may be due to the production of antibiotics, antibacterial metabolites, competition for nutrients etc. (Dave and Dube, 2000). In the present study, inhibitory property of the isolates Pf-14 and P-1 with significant inhibition zone formation reflects the inherent potential of the organism to produce inhibitory metabolites against *R. solanacearum*.

The two efficient isolates identified after *in vitro* screening were subjected to characterization following standard procedures (Schaad, 1992). Based on the reaction towards various tests, the isolates Pf-14 and P-1 were identified as *P. aeruginosa* and *P. fluorescens* biovar 2 respectively.

The selected AMF cultures *viz*., M_5 and M_7 and fluorescent pseudomonads isolates *viz*., Pf-14 and P-1 were further tested for their combined effect on disease suppression and growth improvement in chilli. Results of the study indicated that dual inoculation of AMF (M_5) and *P. fluorescens* (P-1) is beneficial and it could reduce the incidence of bacterial wilt by 83.53 per cent ($M_5P_2R_1$) over control (Fig. 6). Such positive interaction between AMF and fluorescent pseudomonas and suppression of root pathogens has already been reported (Budi *et al*., 1999; Sanchez *et al*., 2004; Scagel, 2004). The potential of the combined effect of these two beneficial microbes for the management of diseases of crop plants has not been investigated so far in Kerala. However, some work on interaction effect of AMF and bacterial antagonists in tomato plants has been reported from other countries (Gamalero *et al*., 2004).

Dual inoculation of AMF and *P. fluorescens* could enhance growth, biomass production and yield of chilli plants (Fig. 7). The maximum shoot length (35.50 cm) and stem girth (4.32 cm) were recorded for treatment $M_5P_2R_0$. The treatments $M_0P_1R_0$ and $M_5P_1R_0$ recorded maximum root length (23.25 cm) and number of secondary branches (31.26) respectively. The fresh weight (65.66 g plant^{-1}) and dry weight (17.23 g plant^{-1}) of chilli plants were maximum in $M_7P_2R_0$, whereas the yield was maximum in treatment $M_7P_1R_0$ (86.66 g plant^{-1}). Results indicated that combined inoculation of *P. fluorescens* and AMF significantly increase plant growth and yield (Gamalero *et al*., 2004; Scagel, 2004; Sanchez, 2004).

It is known that the desirable effect of AMF such as enhanced nutrient uptake and the physiological and biochemical changes in the host favours plant growth and development (Sivaprasad *et al*., 1990; Meenakumari and Nair, 1992; Kavitha, 2001). *P. fluorescens* in addition to imparting tolerance to diseases, it improves growth and development of host plant due to hormonal effect (Nautiyal, 2000; Bhawsar and Chopade, 2003). The remarkable increase in disease suppression as well as growth and yield of

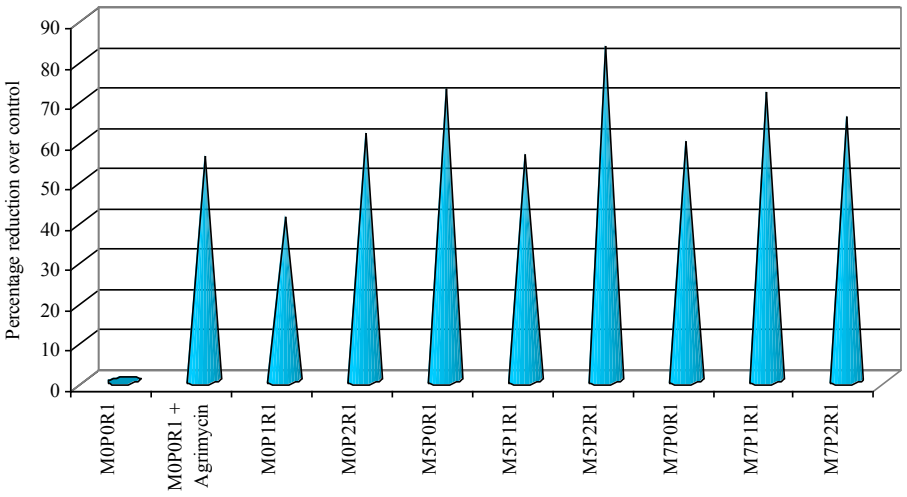


Fig. 6. Effect of dual inoculation of AMF and *P. fluorescens* on suppression of *R. solanacearum* in chilli

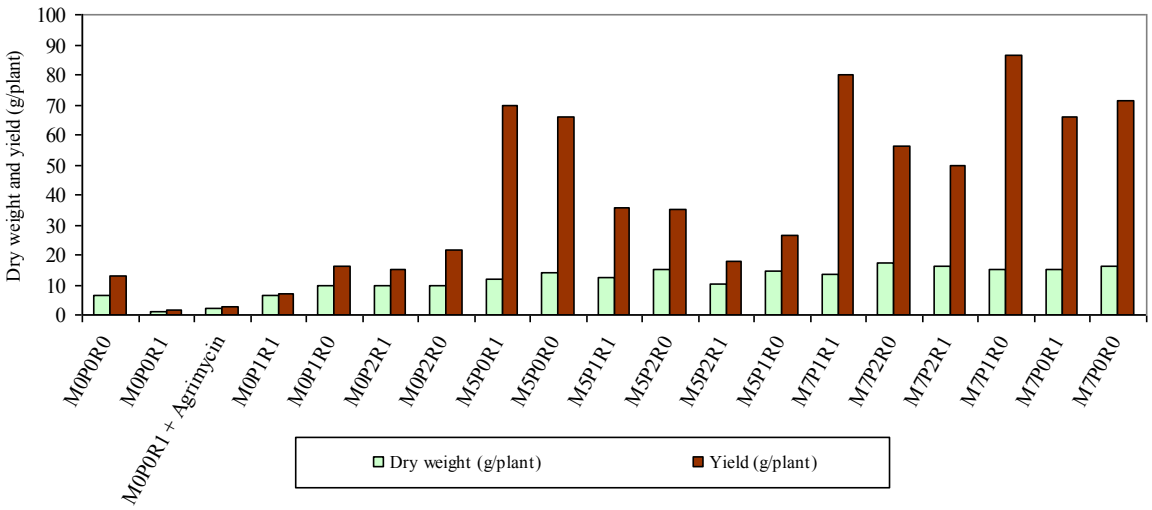


Fig. 7. Effect of dual inoculation of AMF and *P. fluorescens* on biomass production and yield in chilli

chilli observed in the present investigation could be attributed to the additive effect of the desirable traits of these microbes. Such positive interaction of AMF with *P.fluorescens* and its effect on crop growth and yield have been recorded in a spectrum of crops including grapevine (Bavaresco and Fogher, 1992) , strawberry (Gryndler *et al*., 2002), tomato (Gamalero *et al*., 2002), eucalyptus (Sastry *et al*., 2000), and acacia (Founoune *et al*., 2002). Interaction between AMF, *G. intraradices* and *P. aeruginosa* resulted in increase in the level of soluble phosphorus (Villegas and Fortin, 2002).

The present investigation emphasise the potential of native AMF and P. fluorescens in suppressing bacterial wilt of chilli and improving crop growth and yield. The native isolates of AMF and fluorescent pseudomonads selected from the study namely M_5, M_7 and Pf-14, P-1 respectively are highly promising. However it requires further testing under field conditions before recommendation to farmers.

Summary

6. SUMMARY

Chilli (*Capsicum annuum* L.) is one of the important solanaceous vegetable crops of Kerala. Bacterial wilt caused by *R. solanacearum* is a serious disease wherever the crop is grown in India. Management of the disease using antibiotics is highly expensive and often ineffective (Remadevi, 1978). Biological control methods for the management of this disease reduces the dependence on high risk chemicals and is ecologically sound and environmentally safe. In this context, the present study was proposed to isolate and develop efficient native strains of fluorescent pseudomonads and AMF for the management of bacterial wilt of chilli.

Phytopathogenic *R. solanacearum* was isolated from the diseased chilli plants grown in highly infested areas of Thiruvananthapuram district and pathogenicity was proved. The isolates were subjected to various cultural, morphological, physiological and biochemical studies and the pathogen was identified as *R. solanacearum* (E.F. Smith) Yabuuchi *et al*.

Ten native cultures of AMF developed and maintained in the Department of Plant Pathology, College of Agriculture, Vellayani were screened for disease suppression and growth improvement in chilli. Inoculation with AMF cultures M_5 and M_7 could give maximum suppression of wilt disease and this was found superior to other AMF cultures. Significant increase in biometric characters such as root length, shoot length, number of secondary branches, stem girth, fresh weight, dry weight and yield were obtained in chilli plants pre-inoculated with native AMF. From the results of this experiment, two best AMF cultures *viz*., M_5 and M_7 were selected for further studies.

Thirty isolates of fluorescent pseudomonads isolated from the phyllosphere and rhizosphere of healthy chilli growing areas were

screened for their efficiency in inhibiting *R. solanacearum*. Fifteen isolates of fluorescent pseudomonads developed and maintained in the Department of Plant Pathology, College of Agriculture, Vellayani were also used for the *in vitro* screening experiment. Native isolates of fluorescent pseudomonads *viz*., Pf-14 and P-1 were found superior in inhibiting the pathogen. The inhibition zone formed by these two isolates viz., Pf-14 (20.0 mm) and P-1 (19.0 mm) were higher compared to other isolates. The isolates tested viz., Pf-14 and P-1 were subjected to various biochemical tests and identified as *P. aeruginosa* and *P. fluorescens* biovar 2 respectively.

Dual inoculation of selected native AMF cultures (M_5 and M_7) and *P. fluorescens* isolates (Pf-14 and P-1) on bacterial wilt suppression and growth improvement in chilli was studied. Pre-inoculation of chilli seedlings with M_5 along with application of *P. fluorescens* isolate P_1 ($M_5P_2R_1$) recorded the least disease incidence. Dual inoculation of AMF and *P. fluorescens* resulted in increase in root and shoot length, number of secondary branches, stem girth, fresh and dry weight and yield of chilli plants. The treatment $M_7P_1R_0$ recorded maximum yield whereas biomass production was highest in treatment $M_7P_2R_0$. Dual inoculation of M_5 and P-1 was found to be the best in suppressing bacterial wilt, whereas for growth improvement in chilli, combination of M_7 and Pf-14 was found to be the best.

The present investigation emphasizes the importance of combined inoculation of AMF and *P. fluorescens* for the management of bacterial wilt of chilli. Efficient isolates of AMF and fluorescent pseudomonads obtained could be further tested under field conditions and made available to the farmers of Kerala.

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*Original not seen

Appendix

APPENDIX-I

COMPOSITION OF DIFFERENT MEDIA

(a) Casaminoacid peptone glucose-2,3,5-triphenyl tetrazolium chloride medium or CPG-TTC medium (Kelman, 1954)

Casaminoacid	:	1.0g
Peptone	:	10.0g
Glucose	:	5.0g
Agar	:	20.0g
Distilled water	:	1000ml
pH		6.5

200ml portions of the medium was sterilized in flasks by autoclaving at 15lb pressure for 20 minutes. Before plating, 1ml of sterile 1 percent solution of 2,3,5- triphenyl tetrazoliumchloride stored in the dark after autoclaving at 15lb pressure for 8 minutes was added to each flask to give a final concentration of 0.005 percent tetrazolium chloride.

(b) King's medium B (KMB)

Peptone	:	20.0g
Dipotassium hydrogen orthophosphate	:	1.5g
Magnesiumsulphate	:	1.5g
Glycerol	:	10ml
Distilled water	:	1000ml
pH	:	7.2

APPENDIX-I Continued

(c) Peptone – beef extract medium containing 5 percent sucrose

Peptone	:	10.0g
Beef extract	:	5.0g
Sucrose	:	50.0g
Agar	:	20.0g
Distilled water	:	1000ml
pH	:	7.0

(d) Nutrient agar medium

Beef extract	:	3.0g
Peptone	:	5.0g
Agar	:	15.0g
Distilled water	:	1000ml
pH	:	6.8

(e) Peptone water broth

Peptone	:	10.0g
Sodiumchloride	:	5.0g
Casaminoacid	:	10.0g
Distilled water	:	1000ml
pH	:	7.0

(f) Nutrient agar containing 0.2 percent starch

Peptone	:	10.0g
Beef extract	:	5.0g
Starch	:	2.0g
Agar	:	20.0g
Distilled water	:	1000ml

pH	:	7.0

(g) Nutrient gelatin medium

Peptone	:	10.0g
Beef extract	:	5.0g
Gelatin	:	4.0g
Agar	:	20.0g
Distilled water	:	1000ml
pH	:	7.0

MANAGEMNET OF BACTERIAL WILT OF CHILLI CAUSED BY *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al.* USING AMF AND FLUORESCENT PSEUDOMONADS

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Abstract of the
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ABSTRACT

Management of bacterial wilt of chilli caused by *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al*. using AMF and *Pseudomonas fluorescens* was studied. The pathogen was isolated and pathogenicity proved. Based on the cultural, morphological, physiological and biochemical characteristics, the isolate was identified as *R. solanacearum*. Out of the ten native AMF cultures screened, cultures M_5 and M_7 were found effective for disease suppression and growth improvement in chilli.

Pseudomonas spp. were isolated from phyllosphere and rhizosphere of chilli plants collected from different locations of Thiruvananthapuram district. Out of the 45 isolates subjected to *in vitro* screening by dual culture technique, two best isolates *viz*., Pf-14 and P-1 were selected. These isolates, Pf-14 and P-1 were subjected to biochemical characterization and were tentatively identified as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biovar 2 respectively.

Interaction of efficient native isolates of AMF and *P. fluorescens* isolates on disease suppression and growth improvement in chilli was studied. Talc based formulation of the two best native isolates, Pf-14 and P-1 were applied as soil drench and foliar spray. Dual inoculation of chilli seedlings with M_5 and P-1 recorded the least disease incidence. The growth, biomass production and yield was highest for the treatment $M_7P_1R_0$ which is a combination of AMF culture M_7 and Pseudomonas culture Pf-14. The application of antibiotic agrimycin was found ineffective in suppressing bacterial wilt pathogen.

The present study forms the first report of the synergistic interaction of AMF and *P. fluorescens* for the management of bacterial wilt and growth improvement in chilli. The technology of combined inoculation of AMF and *P. fluorescens* could be recommended for adoption by the vegetable farmers of Kerala after confirming the results under field conditions.