POTENTIAL OF FORTIFIED SPENT MUSHROOM SUBSTRATE FOR THE MANAGEMENT OF SOIL BORNE DISEASES OF TOMATO

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Potential of fortified spent mushroom substrate for the management of soil borne diseases of tomato"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society

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INTRODUCTION

INTRODUCTION

Mushrooms are known for their delicacy, nutritional values and medicinal properties The substrate left after the harvest of one full crop of mushroom, beyond which extension of crop becomes unremunerative is called as 'spent mushroom substrate' (SMS) One of the major environmental problems in the mushroom production remains the treatment and disposal of SMS About five kg of SMS are produced for each kilogram of mushroom An obvious solution to the disposal problem is to increase the demand for SMS through the exploration and development of new application as in organic cultivation

Tomato (*Lycopersicon esculentum* Mill) is a tropical vegetable belonging to the family solanaceae India has total area (ha) 879 64 (MOFPI, 2013) The bacterial wilt and damping off are two serious soil borne diseases of this crop The damping off caused by *Pythium aphanidermatum*, is a serious problem, causing mortality of seedlings This is a serious problem for the plant producers and has caused severe economic losses in commercial nurseries

The bacterial wilt of tomato caused by *Ralstonia solanacearum* can cause destruction at any stage of the plant The ability of the pathogen to survive in the soil for a period up to 10 years even in the absence of any vegetation also contributes to the devastating nature of the disease It spreads quickly causing reduction in yield and income Control of the disease is challenging as the use of chemical products including antibiotics, fertilizers and fungicides are not effective in managing the disease

The management of diseases using chemicals is not safer to environment due to residual problem Sometimes the pathogens develop new resistant strains

Increasing concern regarding food safety and environmental pollution has generated an interest in ecofriendly practices like soil amendment and application of biocontrol agents to manage the plant diseases

Many beneficial uses for SMS are currently being evaluated In horticulture as a component of potting mixture, in agriculture or landscape trades to enrich soil, as an ingredient in the cultivation of other mushroom species, as fuel, as a medium for vermiculture, as a matrix for bioremediation The SMS contains nutrients which could be used for the growth of plants These materials are generally non toxic to plants and therefore, could be employed as a soil amendment for different crops

The SMS contains a diverse range of soil microorganisms This is proven by its disease suppressing properties and its effectiveness in bioremediation The suppressive effect extends to pathogenic microbes on plants which cast a new light on SMS as a potentially valuable organic disease control additive for potting mixtures According to Sendi *et al* (2013) SMS is considered as a source of humus formation, which provides plant nutrients, improves soil water holding capacity, soil aeration and helps to maintain soil structure In addition to primary nutrients SMS also supplies secondary, micro nutrients and improves physical property The advantages of using SMS as a soil fertilizer over chemical fertilizer is that SMS provides slow-release nutrients that will not burn crops upon application Furthermore, SMS contains a wealth of micronutrients that usually are not present in straight NPK chemical fertilizers It has been found that when SMS is added to soil it affects the levels of P, K and Mg but does not raise the level of NO_3-N (Maher, 1994)

The unsieved or coarse SMS had low water holding capacity so potting media dries rapidly and which may cause inhibition of seeds, so unsieved or coarse SMS is not suitable for potting media The SMS generally have ideal moisture content The

organic matter content varies from 40 to 60 per cent The C N ratio generally well below 30 1 Also it has small amount of quick release nitrogen The pH range of most SMS product is between 6 to 8 This range is favourable for plant growth

Under these circumstances this study has been taken up with the following objectives

1 Evaluation on biocontrol efficiency and mutual compatibility of selected antagonists

2 Biosoftening of paddy straw SMS from oyster mushroom with selected antagonists

3 Estimation of nutrient contents of SMS

4 Evaluation of fortified SMS against soil borne diseases of tomato

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Spent mushroom substrate (SMS) is a byproduct of the mushroom production industry Many researchers have reported different uses of SMS Among them the disease controlling property against plant pathogens remains more attractive Apart from the disease controlling property, SMS also improves the physical property of soil, which directly influence crop growth Zadrazil (1976) suggested that the paddy straw SMS increases soil fertility by the presence of humic acid Shukry *et al* (1999) reported that addition of straw in the soil caused an increase in the number of total bacteria, actinomycetes and fungi of the rhizosphere According to Rinker *et al* (2004) SMS contains higher percentage of three primary nutrients *viz* nitrogen, phosphorus and potassium as a fertilizer Castro *et al*, (2008) stated that *Pleurotus* waste was adequate to sustain the growth of *Salvia officinalis* by improving air porosity and mineral content of the soil

The scanning of literature on diseases of tomato showed that one of the major nursery disease is damping-off This disease is complex usually involved other pathogens, including the major fungus, *Pythium aphanidermatum* followed by *Phytophthora* sp *Rhizoctonia* sp *Fusarium* sp and *Sclerotium* sp Among them *Pythium* sp threaten tomato production because of their ability to travel through water and to survive long harsh periods as resting spores

In case of field, the most devastating diseases attacking tomato is bacterial wilt caused by *Ralstonia solanacearum* (Smith) It is considered the world's single most destructive bacterial plant disease The bacterium entered host plant roots from the soil and colonizes the xylem vessels in the vascular system Infected plants suffer yellowing, stunting and wilting, and often die rapidly (Perez *et al*, 2008)

2.1 DAMPING-OFF

Damping-off is a serious nursery disease, caused by *P. aphanidermatum* Edson (Fitzp.). Two types of damping-off disease occur in susceptible plants, pre emergence and post emergence. Accoding to Parveen and Sharma (2015) *Pythium aphanidermatum* was one of the most aggressive species in the genus and had a wide host range causes many economically important diseases. It also causes seedling rots, root rot, cottony-leak, cottony blight, stalk rot etc.

Pre-emergence damping-off results in death of the germinating seed and therefore no emergence. When the seed is able to germinate and the seedling emerges from the soil, the plant may still be susceptible to infection. Infection at this stage is referred to as post-emergence damping-off and is characterized by chlorosis in cotyledon and leaf, it is a watery rot in the taproot and hypocotyl at or near the soil line. Plants with damaged root systems may continue to grow, and possibly remain green for a few weeks, or appear stunted to varying degrees and eventually the seedling will collapses (Chin, 2003).

2.1.2 Causal agent

Pythium species mainly cause seedling damping-off, root infections and in general rotting of soft tissues in soil and aquatic habitats (Plaats- Nikerink, 1981). Mao *et al.* (1998) studied the soil borne diseases of tomato caused by *Rhizoctonia solani* and *Pythium ultimum* alone or in combination with *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *lycopersici* in the greenhouse and field. According to Tsahouridou and Thanassoulopoulos (2001) *Sclerotium rolfsii* caused severe damping-off in tomato plants in the green house.

2.1.3 Management of damping off

2.1.3.1 Biological control

Biological control of *Pythium* is a promising approach, seeing that it is comparatively benign towards the environment (Paulitz and Belanger, 2001). Farmers use the common synthetic fungicides which lead into ill effect. Many of the commonly used synthetic fungicides are unable to control *Pythium* species as it has got resistance against these synthetic fungicides. Hence there is an urgent need to replace the chemical fungicides by bio-fungicides, which are economical to the farmers and will not leave any ill effect in the soil, water and to the environment (Parveen and Sharma, 2015).

Ongenaa *et al.* (1999) evaluated four *Pseudomonas* strains for intrinsic properties conferring their ability to protect long English cucumber against *Pythium aphanidermatum* in hydroponic culture. Two of the strains, BTP1 and its siderophore negative mutant M3, increased plant yield as compared with the non-inoculated control plants. Strain BTP7 was intermediate in its biocontrol activity while strain ATCC 17400 failed to reduce disease development.

Hultberg *et al.* (2000) found that bacterization of tomato seeds with *Pseudomonas fluorescens* 5.014 and its mutant 5-2/4 strains reduced the development of *P. ultimum*-induced damping-off expressed as plant weight, disease index, and fungal colonization. Tsahouridou and Thanassoulopoulos (2001) reported that *Trichoderma koningii* successfully colonized the tomato rhizospere and inhibited *S. rolfsii*.

Sabaratnam and Traquair (2002) observed suppression of *Rhizoctonia* damping-off by the seed-coated talcum powder formulation of *Streptomyces* sp. Di-944 compared to *S. griseoviridis* (Mycostop), even at lower rate of application than Mycostop, which is primarily attributed to the rhizosphere competence of

Streptomyces sp. Di-944. Kipngeno *et al.* (2015) recorded reduced damping off disease in tomato, when the seeds were treated with either *T. asperellum* TRC 900 or *B. subtilis* BS 01.

2.1.3.2 Chemical control

Diphenamid (N, N-dimethyl-2, 2- diphenylacetamide) reduced the growth of *Rhizoctonia solani* and *Pythium aphanidermatum in vitro* and decreased the incidence of pre-emergence damping off in soil artificially infested with *Rhizoctonia solani* or *Pythium aphanidermatum* (Cole and Batson, 1974).

Song *et al.* (2004) observed that Prochloraz and carbendazim were the most effective fungicides in inhibiting mycelial growth of *Fusarium oxysporum* in tomato..

2.2 BACTERIAL WILT

Bacterial wilt caused by *R. solanacearum* was reported for the first time at the end of the 19th century on potato, tobacco, tomato and groundnut in Asia, southern USA and South America. The bacterium was first described as *Bacillus solanacearum*. This disease was found to be challenging due to its destructive nature, wide host range and geographical distribution, and it is believed to be the most important bacterial disease of plants in tropical, subtropical and warm temperate zones of the world (Hayward, 1991; Kelman, 1954). In addition to the loss of plants and yield, the disease was blamed for abandonment of cultivation of tomatoes on previously productive farms due to serious infestation of the disease as the pathogen persists in a wide range of crop and weed hosts (Kelman, 1963).

The pathogen *R. solanacearum* is both soil borne and water borne, once infects the soil it easily spreads within the field as well as to adjacent fields not only affecting the crop but also rendering the farm unusable for production of any solanaceous crops (Sequeira, 1993). The disease was known to spread very quickly through furrow irrigation as well as rain (Taylor *et al.*, 2011).

Plants affected by the disease shows wilted leaves and stems usually visible at the warmest time of the day. The wilting persists until the plant dies where youngest leaves are the first to be affected and have a flaccid appearance. In most cases the stem near the root produces many adventitious root buds and roots indicating infection to the vascular bundle (EPPO, 2004). Plants may exhibit discoloration of the vascular system by showing a streaky brown to yellow cream discoloration (Agrios, 2005). The permanent wilting usually occurs due to a massive invasion of the cortex which may result to water-soaked lesions on the external surface of the stem; if an infected stem is cut crosswise tiny drops of dirty white or yellowish viscous ooze exude which indicates presence of bacterial cell from several vascular bundles (Champoiseau and Momol, 2009; EPPO, 2004). In some cases the plant may have latent infection where none of these symptoms, even under typical environmental conditions that are ideal for the pathogen are expressed (Adebayo and Ekpo, 2005; Aribaud *et al.*, 2015).

2.2.1 Causal agent

The *R. solanacearum* is an important pathogen of many crops (Tahat and Kumaruzaman, 2010). Formerly called *Pseudomonas solanacearum,* it was reported for the first time at the end of the 19th Century on potato, tobacco, tomato and groundnut in Asia, southern USA and South America (EPPO, 2004). The disease is highly infectious both in soil and in soilless culture causing wilt of plants especially

in solanaceae family. Affected crops include: tomato, eggplant and sweet pepper (Jenkins and Averre, 1983).

The bacterium *R. solanacearum* is both a soil borne and waterborne pathogen, and is known to survive and disperse in infected soil or water which can form a reservoir source of inoculum from season to season (Fajinmi and Fajinmi, 2010). High temperatures (29-35°C) play a major role in pathogen growth and disease development as temperatures below 18°C inhibit disease development (Hayward, 1991) but the pathogen can survive in a physiological latent state (Champoiseau and Momol, 2009).

2.2.3 Management of bacterial wilt

2.2.3.1 Biological control

The use of biological control agents against soil borne pathogen gained popularity in recent years. This is mainly due to environmental concerns raised by inadequate use of chemical products in disease control (Haas and Defago, 2005). Biological control methods have been widely accepted as key practice in sustainable agriculture with the biggest potential of the biological control being microorganisms, arbuscular mycorrhizal fungi (AMF) (Sharma and John, 2002; Tahat *et al.*, 2010) and some naturally occurring antagonistic rhizobacteria such as *Bacillus* sp., *Pseudomonas* sp. (Guo and Chorever, 2004). Murthy et al. (2013) found that *Trichoderma asperellum* was used as a biological control agent against bacterial wilt disease caused by *R. solanacearum*. The disease suppression was achieved by activity of induction and increased activity of defense related enzymes.

Elyousr and Asran (2009) proved the antibacterial activity of extract of Datura, Garlic and Nerium against *R. solanacearum in vitro* and *in vivo*. Garlic exhibited the strongest antibacterial activity against bacterial wilt *in vitro* and *in vivo*

followed by Datura and then Nerium. Murthy *et al* (2014) tested the biological control agent *P. fluorescens* against the bacterial wilt causing *R. solanacearum* and observed an increased enzyme activity.

2.2.3.2 Chemical control

Controlling of bacterial wilt using chemicals was a challenge because of the localization of the pathogen inside the xylem and its ability to survive in the soil. There is no known eradication bactericides available for chemical control of the bacterial wilt disease (Hartman and Elphinstone., 1994). Recent studies have shown that phosphorous acid salts (PAS) could be promising chemicals for inclusion in integrated packages to manage tomato bacterial wilt (Lin and Wang, 2011). Wu *et al.* (2012) studied that the hydrazone derivatives containing a substituted pyridine ring could inhibit the growth of *R. solanacearum.*

2.3 Effect of isolated organisms on the pathogens

Technical, economical and environmental factors were forcing to adopt new sustainable methods, such as the use of microbial antagonists, for the control of soil borne pathogens. Research mainly focussing on antagonistic fungi and bacteria, often not provides consistent or satisfying results. (Spadaro and Gullino, 2004)

2.3.1 Fungal antagonists against fungal pathogen

Elad *et al.*(1980) found that an isolate of *Trichoderma harzianum* capable of lysing mycelia of *Sclerotium rolfsii* and *Rhizoctonia solani* was isolated from a soil naturally infested with those pathogens. In culture, *T. harzianum* grew better than *S. rolfsii* and invaded its mycelium under growth conditions adverse to the pathogen. Bello *et al.* (1997) reported the production of volatile compounds by *T. hamatum* against wide range of fungal pathogens, which might be one possible mechanism of

biological control. Some heavy metal-resistant mutants of *Trichoderma* spp. selected on heavy metal-rich artificial media were effective antagonists of *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp. (Kredics *et al.*, 2001). Yuquab and Shahazad (2005) found that *T. harzianum* and *T. longibrachiatum* showed inhibition in growth of *S. rolfsii* and produced coils around its mycelium. Jayaraj *et al.* (2006) evaluated by dual culture technique a carbendazim resistant *Trichoderma harzianum* strain M1, inhibitory to the growth of the damping-off pathogen *Pythium aphanidermatum*

Rini and Sulochana(2007) studied twenty-six local isolates of *Trichoderma* spp. from Kerala for their antagonistic activity against *R. solani* and *F. oxysporum* under *in vitro* conditions. Different isolates showed varying degrees of antagonism. The two most antagonistic isolates against *R. solani* were *T. pseudokoningii* TR17 and *T. harzianum* TR20. Likewise, *T. viride* TR19 and TR22 formed the most effective isolates against *F. oxysporum.* Production of volatile and non-volatile antibiotic compounds varied among these isolates. Morsy (*et al.*, 2009) evaluated *Trichoderma viride* for its antagonistic effect against *Fusarium solani*, and succeeded in reducing the radial growth of *F. solani* by dual culture method. Mpika *et al.* (2009) evaluated 43 isolates of *Trichoderma* sp. against *P. palmivora*. Among them *T. virens* was the best which reduced the mycelial growth upto 97.90 per cent.

Haque and Nandkar (2012) evaluated seven isolates of *Trichoderma* spp. obtained from rhizosphere soils of tomato plants for antagonism against *Fusarium oxysporum* f.sp. *lycopersici*, which causes tomato damping-off by means of dual culture method, and among these isolates, *T. virens* showed the strongest inhibition of the growth of *Fusarium oxysporum* f.sp. *lycopersici*.

Singh *et al.*(2014) evaluated the effect of four isolates of *Trichoderma* species *viz. Trichoderma harzianum (Th Azad), Trichoderma viride (01PP), Trichoderma*

asperellum (Tasp/CSAU) and *Trichoderma longibrachiatum* (21PP) on *Pythium aphanidermatum* (both grown on PDA). *T. harzianum* recorded maximum growth inhibition (60.38%) against *P. aphanidermatum* and produced more amounts of volatile and non-volatile metabolites. The culture filtrate of *Trichoderma harzianum* recorded complete inhibition on the mycelia growth of pathogen.

2.3.2 Bacterial anatagonists against fungal pathogens

Chen *et al.* (1998) investigated the effect of induced systemic resistance (ISR) by *Pseudomonas* rhizobacteria on the pre- and post-infection development of *Pythium aphanidermatum* on cucumber roots. Hultberg *et al.* (2000) studied *in vitro* and *in vivo* interactions between different strains (5.014 and its mutant 5-2/4) of *P. fluorescens* and *P. ultimum* in tomato seedlings. Strain 5.014 inhibited *P. ultimum* to a larger extent than the mutant 5-2/4 on King's medium B, a medium that promotes siderophore production. On yeast–malt medium, mutant strain 5-2/4 inhibited the growth of *P. ultimum* to a larger extent than 5.014 and produced a high amount of the antibiotic 2, 4-diacetylphloroglucinol.

Garbeva *et al* (2004) found that *Pseudomonas* strains have antagonistic properties against the soil-borne pathogen *Rhizoctonia solani* AG3. Jayaraj *et al.* (2005) reported formulations of a strain of *Bacillus subtilis* AUBS-1, have an inhibitory action towards the growth of damping-off pathogen, *Pythium aphanidermatum*. According to Haung *et al.* (2012), in the dual culture with *B. subtilis* (SQR – N43) and *R. solani*, at 48 hr after inoculation hyphal deformation, enlargement of cytoplasmic vacuoles and cytoplasm leakage were observed.

Rini and Sulochana (2007) evaluated 56 isolates of *P. fluorescens* from Kerala against *F. oxysporum* and *R. solani*. They found that the isolates P20 and P28

were most effective against *F. oxysporum*, whereas isolates P28 and P51 showed the greatest inhibition against *R. solani*. According to Baysal *et al.* (2008), among the two strains of *B. subtilis* against *F. oxysporum.* f. sp. *redicis- lycopersici,* the strain EU07 has showed more suppression on growth of the pathogen compared to the strain QST 713.

Ahmadzadeh and Tehrani (2009) carried out fungal inhibition by a plate assay. And found that *Pseudomonas fluorescence* inhibited *Rhizoctonia solani* by producing DAPG. Morsy *et al.* (2009) found that *Trichoderma* and *Bacillus* genera were most feasible biocontrol microorganisms to suppress *Fusarium solani* on tomato by pot culture experiment as well as in field. According to Seema and Devaki (2012), *in vitro* evaluation of *B. subtilis* gave 50 per cent inhibition against *R. solani* and on 5^{th} day the colour of the media turned to brown at the antagonized portion. This may be due to the presence of antibiotic like inturin A and surfactin produced by *B. subtilis*.

2.3.3 Fungal antagonists against bacterial pathogen

Seventy-nine plant growth-promoting fungi (PGPFs) were isolated from rhizosphere soil of healthy vegetables, sunflower, legumes, and cereals. Among them, nine revealed saprophytic ability, root colonization, phosphate solubilization, IAA production, and plant growth promotion. An agar disc diffusion method was performed to determine the ability of each PGPF isolate to inhibit growth of *R. solanacearum* YA12-1(Jogaiah *et al*, 2013). According to Murthy *et al.* (2013) two isolates of *Trichoderma asperellum* (T4 and T8) exhibited high antagonistic activity against a virulent strain of *R. solanacearum*.

2.3.4 Bacterial antagonists against bacterial pathogen

Among seventy-six bacterial isolates were screened for their ability to inhibit growth of *R. solanacearum* PT1J, five showed high potential for minimizing growth of the pathogen; namely *Bacillus* sp. Ba4, N9C8 and N11IV, and enterobacteria N9A and Ba3 (Thongwai and Kumaruzaman, 2007). Aliye *et al.* (2008) screened one hundred and twenty rhizosphere bacterial isolates against virulent strain of *Ralstonia solanacearum* (PPRC-Rs). Among the bacterial isolates, *Bacillus subtilis* PFMRI, *Paenibacillus macerans* BS-DFS, *Paenibacillus macerans* PF9 and *P. fluorescens* PF20 were found to inhibit the growth of the pathogen significantly. Wei *et al.* (2011) isolated the antagonistic *Bacillus amyloliquefaciens* strains QL-5 and QL-18 from rhizosphere soil of tomato and used separately or together to fortify organic fertilisers to control bacterial wilt of tomato.

Murthy *et al.* (2014) investigated the bio control agent *Pseudomonas fluorescens* against the bacterial wilt causing *R. solanacearum*. The seedling treatment of *P. fluorescens* isolates induced a significant increase in the activities of peroxidase (POX), Polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and β-1, 3-glucanase.

2.4 SPENT MUSHROOM SUBSTRATE (SMS)

2.4.1 Spent mushroom substrate for disease management

Spent Mushroom Substrate (SMS) often referred to as, Mushroom Compost is the growing medium that results from the mushroom growing process. Mushroom Compost is made from agricultural materials, such as hay, straw, straw horse

bedding, poultry litter, cottonseed meal, cocoa shells and gypsum. Spent mushroom substrate is rich with many potential antagonists. Scanning of literatures showed that many researchers have exploited the potential effect of SMS in disease control.

Kleyn and Wetzler (1981) found the most common microflora associated with SMS are bacterial isolate like *Bacillus licheniformis,* actinomycete isolates *viz., Streptomyces diastaticus* and *Thermoactinomyces vulgaris*. Other actinomycete isolates included *Streptomyces albus*, *Streptomyces griseus*, *Thermoactinomyces thalpophilis*, *Thermomonospora chromogena*, and *Thermomonospora fusca*, and the fungal isolates like *Aspergillus fumigatus* and *Humicola grisea* var. *thermoidea*, Other fungal isolates included *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillu sterreus*, *Aspergillus versicolor* group, *Chrysosporium luteum*, *Mucor spp.*, *Nigrospora* spp., *Oidiodendron* spp., *Paecilomyces* spp., *Penicilliumchrysogenum*, *Penicillum expansum*,*Trichoderma viride*, and *Trichurus* spp.

Cronin *et al.* (1996) noticed clarified water extracts of slurries of spent mushroom substrate (SMS) inhibited conidial germination of the apple scab pathogen *Venturia inaequalis* up to 98% by *in vitro* method and the extracts produced from sterile SMS were virtually ineffective compared with those from non-sterile SMS.

According to Ntougias *et al.* (2004), the bacterial diversity in SMC is greatly affected by the origin of the initial material, its thermal pasteurization treatment and the potential unintended colonization of the mushroom substrate during the cultivation process. SMC contain Gram-positive bacteria, associated with the genera *Bacillus*, *Paenibacillus*, *Exiguobacterium*, *Staphylococcus*, *Desemzia*, *Carnobacterium*, *Brevibacterium*, *Arthrobacter* and *Microbacterium* of the bacterial divisions *Firmicutes* and *Actinobacteria.*

According to Davis *et al.* (2005) Mushroom compost (spent mushroom substrate, SMS) exhibits suppressive characteristics against various fungi, as well as

against plant diseases caused by fungi. In addition, mushroom compost has physical and chemical characteristics that make it ideal for blending with landscape mulch to enhance growth of horticultural plants.

Ntougias *et al.* (2008) conducted pot experiment to assess suppressiveness against soil-borne and foliar pathogens of tomato with spent mushroom substrate and peat at 1:3 w/w ratios. The effect of SMS on disease suppression (damping off caused by *P. aphanidermatum*) and plant growth promotion in tomato was also reported by Sanam and Gokulapalan (2010). Qiao *et al.* (2011) reported that SMS hydrolysates could be useful for the cultivation of microorganisms and the production of high value compounds such as nisin and lactic acid.

Egein *et al.* (2011) carried out a pot culture experiment with two tomato varieties tropimech (TT3) and tomate U82B(TT2), 4-6 weeks old seedlings were transplanted into potted soil amended with spent mushroom substrate at the ratio 1:3, 1:4, 1:5 and 1:6 in plastic pots. Stem diameter, plant height, number of leaves, disease incidence and disease severity were observed against *Fusarium oxysporum.*

Parada *et al.* (2011) reported that plants treated with water extract from SMS or autoclaved water extract against anthracnose in cucumber reduced the disease. According to them water-soluble and heat-stable compounds in the SMS enhance the state of systemic acquired resistance and protect cucumbers from anthracnose.

The phylogenetic study proved that the SMS from different mushrooms harbour diverse microbial population. Out of different SMS, the one from *P. sajor-caju* harbor fungi (*Schizophyllum commune* and *Pezizomycotina* sp.) and bacteria (*Bacillus licheniformis*, *P. fluorescens* and *B. pumilus*) with appreciable level of ligninolytic enzymes activities and decolorization potential against textile effluent (Singh *et al.*, 2012).

Zhu *et al.* (2012) noticed the antibacterial activity of polysaccharide from SMS against *E. coli*. Phan and Sabaratnam (2012) concluded that SMS was no longer regarded as a waste but as a renewable resource from the mushroom industry. Not only SMS can be employed in a number of green technology endeavours, three enzymes recovered are potentially useful for the bioremediation of pollutants and other industrial biotechnology purposes. One of them is production of lignocellulosic enzymes such as laccase, xylanase, lignin peroxidase, cellulase and hemicellulase.

Remya (2012) conducted a pot culture experiment and reported that among the various SMS used, paddy straw SMS of *P. sajor- caju* and isolated antagonists from SMS, were found to be most effective for the management of soil borne diseases of ginger. Roshna (2013) reported that the isolates of *T. hamatum* and *B.subtilis* from pleurotus SMS provided luxuriant growth and was found effective against phytophthora diseases of pepper cuttings.

Shitole *et al.* (2013) reported the combination of *Trichoderma viride* @ 5 g/kg SMS + *Pseudomonas fluorescens* @ 5 g/kg SMS where SMS and soil proportion was75:25 found highly effective with minimum pre emergence (6.82%) and postemergence (9.09%) damping off as against control (36.36 and 50% pre and post emergence damping off) in tomato.

2.4.2 SMS as soil amendment

The advantages of using SMS as a soil fertilizer over chemical fertilizer is that SMS provides slow-release nutrients that will not burn crops upon application. Furthermore, SMS contains a wealth of micronutrients that usually are not present in standard NPK chemical fertilizers. It has been found that when SMS is added to soil it affects the levels of P, K and Mg but does not raise the level of NO_3-N (Maher, 1994). Romaine and Holocomb (2000) found that tomato seeds did not germinate well in perlite mixture with unsieved or coarse SMS. Because unsieved and coarse

SMS had low water holding capacity and hence the media dried rapidly and inhibited seed germination.

Sangwan *et al.* (2002) conducted an experiment to test whether spent mushroom compost (SMC) could be used as organic manure for wheat cultivation. Substantial masses of solutes were released during SMS weathering. High dissolved N, Direct Organic Matter (DOM), Ca^{2+}, K^{+}, Mg^{2+}, $SO4^{2-}$and Cl^{-} fluxes from weathering of SMS illustrated a remarkable loss of plant nutrients and a potential source of contamination for soils and water sources. The related environmental implications should be considered, particularly in large SMS weathering operations (Guo and Chorover, 2004).

Polat *et al.* (2004) reported statistically significant differences among different levels of spent mushroom compost applications, and two and four tons/ha gave the best result in terms of total and marketable yield in lettuce. According to Guo and Chorover (2004) weathering of piled material in the field is a popular method to treat spent mushroom substrate of Agaricus (SMS) before reuse. During the weathering process, rainfall and snowmelt pass through SMS piles and a large amount of solutes is released in the leachate.

Spent mushroom compost of *Agaricus* contains a lot of salt and unstable organic material, so it should be aged for about two years before applying. This allows for leaching of organic solutes and decomposition of organic matter. Spent mushroom compost contains about 12% nitrogen, 0.2% phosphorus and 1.3% potassium. After being aged for 18 months, phosphorus and nitrogen do not change, but potassium can decrease (Uzun, 2004). Chong (2005) noticed that the spent material is rich in certain nutrients and has physical properties, such as aeration, porosity and water retention capacity. Spent mushroom compost can be used as a soil

or potting mix supplement to grow crops including fruit, vegetables, corn (*Zea mays*), and potted foliage and greenhouse flowers.

Suess and Curtis (2006) observed Value-Added Strategies for Spent Mushroom Substrate as it contains a diverse range of soil microorganisms. This was proven by its disease suppressing properties and its effectiveness in bioremediation. The biological properties of SMS enhance its marketability as a soil conditioner. Addition of microorganisms to soil would enhance and accelerate regular soil processes such as nutrient mobilization and aggregate formation. SMS might have variable chemical and physical properties due to variability of ingredients and processing; however, it is generally regarded as a neutral soil amendment. SMS will neither add a great amount of the macronutrients like nitrogen, phosphorous and potassium (NPK) to the soil nor will it tie up nutrients. The P^H of SMS is around 7 which is suitable for most crops as all essential nutrients are available to plants at this P^H. SMS has the potential to play an important role as a biological disease suppressant. SMS is rich in microorganisms, such as disease fighting bacteria and fungus. It naturally suppresses pathogens in the soil that cause plant damage and decline in yields.

Trygve *et al.* (2007) used *Agaricus* spent mushroom substrate (SMS) in turf industry in the northeastern United States for soil improvement. When tilled into soil at high rates, some turf grass managers claim that SMS inhibits turf seed germination. Incorporation of high rates of SMS represents a potential problem for turf grass establishment.

Castro *et al.* (2008) reported the use of a substrate composed of two parts soil with high organic matter content and one part oyster mushroom spent substrate (obtained after two crops of *Pleurotus* cultivation on a sunflower seed hulls based

substrate) resulted in improvement of the growth and nutritional status of common sage plants *Salvia officinalis* cultivated in pots. This improvement can be due to the PSS contribution to higher air porosity and content of certain essential mineral nutrients to the substrate. It can also be suggested that washing out excess of salts present in the PSS, which demands time and labor, is not necessary for the cultivation of plants tolerant/resistant to drought or salinity.

Medina *et al.* (2009) used spent mushroom substrate (SMS) of *Agaricus bisporus* (SMS-AB) and *Pleurotus ostreatus* (SMS-PO) in different ratios for growing tomato, courgette and pepper. For the growth of tomato all the substrates were found suitable, while for pepper and courgette all SMS-AB based substrates and the media containing low dose of SMS-PO and SMS-50 were found to be adequate for the growth. The media with SMS-PO presented lower pH and electrical conductivity values, but their low total water holding capacity could have limited the retention of nutrients from fertirrigating system of nurseries.

Polat *et al.* (2009) noticed that all of the spent mushroom compost treatments resulted in higher yield than control treatment. The highest total fruit yield was obtained at 40 ton ha-1 and it was followed by 80 and 20 ton ha^{-1} SMC applications ($p<0.05$). When the effects of SMC application on fruit width were investigated, the highest values were found at 80 kg ha^{-1} SMC application ($p<0.05$). Based on the study it was concluded that as an organic material source and amendment of greenhouse soil application of at least 6 months kept SMC was very effective and beneficiary for cucumber production, quality and recycling the spent mushroom compost.

Dar *et al.* (2009) observed supplying plant nutrients through the integrated use of Agaricus –SMC (WB- SMC) of a narrow C:N ratio and 90 kg fertilizer N ha^{-1} gave

an advantage over the use of Oyster-SMC (OY- SMC) and/or fertilizer N. The OY-SMC, with a wider C:N ratio, reduced N recovery and agronomic N efficiency but its incorporation with fertilizer N can increase yield, recovery efficiency and agronomic efficiency compared with application of OY-SMC alone. The use of WB-SMC in rice cropping enhanced C sequestration but the effect due to OY-SMC with a wider C:N ratio compared to WB-SMC with a narrow C:N ratio was higher because of low mineralization and high immobilization of its C.

Bindhu (2010) reported that as compared to soil the organic carbon content of spent mushroom substrates was high. She also reported that the spent mushroom substrate registered a pH range of 6.9 to 7.2 with average calcium content and more accumulation of nitrogen and calcium in fruits, phosphorus in roots and potassium in stem and leaves of tomato.

Eudoxie and Alexander (2011) used fine SMS as a media for growing tomato seedlings. The greater plant height and seedling vigour was attained with five week after sowing. The SMSF treatment naturally supplied all the essential nutrients required for proper growth and development. Available levels of the macronutrients were much greater in the SMS than that applied as a standard fertilizer supplement for nursery cultivation.

Medina *et al.* (2012) concluded that the soil application of spent mushroom substrates improved soil fertility, since soil organic C and N, available P and phosphatase activity were increased significantly by the organic fertilisation with these substrates, this soil fertility improvement being higher with T1 treatment(50 % V/V of agaricus and pleurotus) . Also, the addition of the spent mushroom substrate did not alter the soil salinity or the pH value. However, low N mineralisation of these materials, N immobilisation, was contributing to low N losses. These results

suggested that the organic N mineralization of spent mushroom substrate was predominantly determined by nutrient demand of the crop According to Wei *et al.* (2011) the appropriate timing for spent mushroom substrate additions to the soil should be approximately one month prior to the planting, since after this period N immobilization was not observed in the alkaline soil of this experiment.

Fidanza *et al.* (2012) reported that fresh mushroom compost had an average pH of 6.6, with an average carbon:nitrogen ratio of 13:1. Organic matter content averaged 25.86% (wet weight), 146.73 lb/yard3 (wet volume) or 60.97% (dry weight). For the primary macronutrients, average total nitrogen content averaged 1.12% (wet weight), 6.40 lb/yard3 (wet volume) or 2.65% (dry weight), phosphorus measured 0.29% (wet weight), 1.67 lb/yard3 (wet volume) or 0.69% (dry weight), and potassium was 1:04% (wet weight), 5.89 lb/yard3 (wet volume) or 2.44% (dry weight). Average soluble salt content was 13.30 mmho/cm (wet weight basis).

According to Sendi *et al.* (2013) spent mushroom waste (SMW), otherwise known as spent mushroom substrate (SMS) contains nutrients which could be used for the growth of plants. SMS is generally nontoxic to plants and therefore, could be employed as soil amendment for different crops. The SMW is claimed to be a source of humus formation, and humus provides plant micronutrients, improves soil water holding capacity, soil aeration, and helps maintain soil structure.

Ashrafi *et al.* (2014) observed mushroom, the fruiting body of macro fungi, as an important food item with its high nutritive and medicinal values. Mushroom cultivation has consistently been increasing and producing huge amount of mushroom waste that is spent mushroom substrate every year. About five kilograms of fresh compost are needed to produce one kilogram of mushrooms.

2.5 BIOSOFTENING

Kuhad *et al.* (1997) reported that one of nature's most important biological processes is the degradation of lignocellulosic materials to carbon dioxide, water and humic substances. This implies possibilities to use biotechnology in the pulp and paper industry and consequently, the use of microorganisms and their enzymes to replace or supplement chemical methods is gaining interest. The enzyme and enzyme mechanisms are used by fungi and bacteria to modify and degrade these components.

Rajan *et al.* (2005) carried out biosoftening in arecanut husk fibers using *Phanerochaete chrysosporium*. The fibre is composed of cellulose and varying proportions of hemicellulose, lignin, pectin and protopectin. Microbes that selectively remove lignin without loss of appreciable amounts of cellulose and fiber strength properties are extremely attractive for the biosoftening of arecanut fiber.

2.5.1 Effect of biosoftening

The biosoftening may cause increase or decrease in quality of the products, the change may occur in physico chemical properties, nutrient contents or microbial parameters. According to Weber *et al.* (2000), the bacterial community on rice straw under anoxic condition was relatively stable after 15 days of incubation. Therefore assume that the colonization process is mainly driven by multiplication of initial colonizers and the cells lose activity with age. Romaine and Holcomb (2000) reported that the SMS product has a C:N ratio well below 30:1. C:N ratio is an important indicator of N_2 availability. If C: N ratio is above 35:1, soil microorganisms can immobilize nitrogen

According to Kumar *et al.* (2007) fungal inoculation in paddy straw compost lowered the pH and pH range of 6.3 to 7.5 was acceptable value for the final product.

They also reported addition of inoculum like *Aspergillus nidulans, Scytalidium thermophilum* and *Humicola* sp. reduced the electrical conductivity of paddy straw. Ahlawat (2007) reported that weathering of button mushroom spent (8 to 16 months) leads to decrease in potasium and pH content and increase in zinc, iron, manganeese, magnesium, calcium and copper contents. According to Kumar *et al.* (2007), initial low C:N ratio caused fast degradation of cellulose and hemicelluloses, while high initial C:N ratio resulted in low degradation of both cellulose and hemicelluloses.

Goyal and Sindu (2011) found maximum decrease in C:N ratio of paddy straw compostable material obtained with fungal consortium. The decreased C:N ratio at the end of composting process is an indication of maturity of compost. According to Bhanu *et al.* (2014) the optimum moisture level of 60-65 per cent has to be maintained by turning at weekly interval for SMS of button mushroom to be a supporting medium for *T. harzianum* (10^4 to 8.72×10^6). Viji and Neelanarayan (2015) reported an increase in potasium and decrease of phosphorus as well as nitrogen content after composting of paddy straw.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study on 'Potential of fortified spent mushroom substrate for the management of soil borne diseases of tomato' was conducted in the department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the period 2013-2015.

The details of the materials used and the methods followed during the course of investigation are presented below.

3.1 ISOLATION OF PATHOGEN ASSOCIATED WITH THE DISEASE

The fungal pathogens associated with the damping-off of tomato *Pythium aphanidermatum, Phytophthora palmivora, Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii* were isolated from infected seedlings. The pathogens were isolated by taking a small portion of collar region of the seedlings along with some healthy area. These were cut into small bits and surface sterilized with one percent sodium hypochlorite, washed in three changes of sterile distilled water and placed in Petri dishes containing solidified Potato Dextrose Agar medium (PDA). All the dishes were incubated under room temperature for 3-5 days and observed for the growth of pathogen.

In case of bacterial pathogen *Ralstonia solanacearum*, the tomato plants which show wilting symptoms were collected from Agricultural Research Station Mannuthy. The collected specimens were thoroughly washed under tap water. Field diagnostic test for bacterial wilt was carried out by cutting a tomato stem of about eight centimeter in length picked from the root base of a wilted plant and the stem portion dipped in a clear glass beaker filled with clear water. The milky exudates coming as ooze from the cut end of the stem proved the disease causing agent as *R. solanacearum* (Goszczynska *et al.*, 2000). Samples of stem portion from the wilted

plants were taken about 15 cm above the ground level from the field and were cut into small pieces and surface sterilized with 1% sodium hypochlorite solution and triple rinsed in sterile water. The pieces were macerated in sterile distilled water and allowed to stand for two minutes. Using an inoculation loop, the extract was streaked on Triphenyl Tetrazolium Chloride (TZC) medium. The inoculated Petri dishes were incubated upside down for a period of 30 - 48 hours in room temperature and observed for the growth of bacterium.

3.1.1. Pathogenicity test

To prove the Koch's postulates all the pathogens associated with damping off disease were inoculated separately on healthy tomato seedlings and were incubated and observed for the symptom expression. The pathogens were re-isolated from the infected area and the characters of the pure cultures of these pathogens were compared with the original cultures. The pure cultures of all the isolates were maintained for further studies.

In case of bacterial wilt, the freshly wilted plants were collected from the field, collected the ooze and used for inoculation on healthy tomato seedlings. The pathogen was re-isolated from the infected plants which showed the wilting symptom and the characters of the pure cultures of these pathogen was compared with the original culture. The pure culture was maintained for further studies.

3.1.2 Purification of pathogens

The fungal pathogens *viz.*, *P. aphanidermatum, P. palmivora*, *F. oxysporum*, *R. solani* and *S. rolfsii*, grown on the PDA medium were purified by hyphal tip method. The pure cultures were maintained on PDA slants for further investigations. Bacterial culture of *R. solanacearum* isolated were purified by streak plate method and preserved in sterilized distilled water and stored under room temperature and at 4°C in screw cap bottle for experimental use.

3.2 PRODUCTION OF SPENT MUSHROOM SUBSTRATE

The Spent Mushroom Substrate (SMS), a kind of lignocellulosic material containing cellulose, hemicellulose, lignin, remnant of edible fungi and carbohydrate, is the byproduct of mushroom production industry. The mushroom species *Pleurotus florida* was selected for the study and was grown on paddy straw.

3.2.1 Preparation of spawn

Spawn was prepared as per the method suggested by Sinden (1934). Sorghum grains were used as the substrate for spawn production. The sterilized grains were inoculated aseptically with actively growing pure culture of *P. florida* and incubated at 25-26 ^{0} C for complete colonization of grains. The spawn thus obtained as mother spawn was used for further spawn production to raise mushroom beds.

3.2.2 Preparation of substrate

Paddy straw was used as substrate for the cultivation of *P. florida*. Good quality paddy straw was chopped into bits of four to five cm length and used for mushroom bed preparation.

3.2.3 Sterilization of substrate

Chemical sterilization was followed for sterilizing the substrate. The substrate was transferred to gunny bags and steeped in a solution containing 7.5g carbendazim and 50ml formaldehyde in 100 liters of water for 16 to 18 h. After the sterilization excess water drained off and then spread on a clean floor for drying to attain an optimum level of moisture content required for bed preparation.

3.2.4 Preparation of beds

The standard compact poly bag method described by Bhaskaran *et al.* (1978) was used for bed preparation using paddy straw.. The perforated bags were filled upto five cm height with sterilized substrate and pressed with hand for making it even. Then 30 g of spawn was sprinkled over the filled substrate along the peripheral region. A second layer of sterilized substrate was filled and spawned as above. This process was repeated for four times. Finally the mushroom beds were tied tightly with twine. For filling one bed 150g spawn was used. The inoculated bags were incubated in a dark room for spawn run. The room temperature and relative humidity were maintained at 26-28^0 C and 80-90 per cent respectively. After spawn run, the beds were transferred to cropping room, where the temperature and RH were maintained at 25-26^0 C and 80 to 90 per cent respectively.

3.2.5 Observations

Observations on time taken for spawn run and the microbial population of substrate at different growth stages were recorded.

3.3 ENUMERATION OF MICROORGANISMS FROM SMS

Enumeration of microorganisms from substrate at different stages of cropping as well as from SMS was carried out by serial dilution and plate count method. For the quantitative estimation of bacteria, fungi and actinomycetes, Nutrient Agar medium (NA), Martin Rose Bengal Agar medium (MRBA) and Kenknights medium (KAN) were used respectively (Johnson and Curl, 1972).

3.3.1 Enumeration of microorganisms from substrates at different stage of cropping

Quantitative assessment of microbial population from the substrate at different stages of mushroom cultivation *i.*e., at the time of spawn running (15th day),

during harvest (30^{th} day) and after harvest (50^{th} day) were carried out by serial dilution and plate count method.

Ten grams of sample was suspended in conical flask containing 90ml sterile water which serves as 10^{-1} dilution. The flasks were shaken for 15 minutes and dilutions were made to obtain the final dilution of 10^{-6}. Dilutions of 10^{-3}, 10^{-5} and 10^{-6} were used for the isolation of actinomycetes, fungi and bacteria respectively. One ml from the required dilution was poured into the Petri plates, melted cooled media was added and gently rotated in clockwise and anticlock wise direction. The inoculated Petri dishes were incubated at room temperature for a week and daily observations were recorded.

Enumeration of microbial population from each sample was carried out by multiplying the actual count with the dilution factor.

3.4. *In vitro* EFFECT OF SELECTED ANTAGONISTS AGAINST PATHOGENS

The antagonists *viz. Trichoderma hamatum, T. viride* and bacterial antagonist *Bacillus subtilis*, which were selected from the previous studies on SMS in the Department of Plant Pathology, College of Horticulture, Vellanikkara were evaluated against *P. aphanidermatum, P. palmivora, F. oxysporum, R. solani, S. rolfsii* and *R. solanaceaum* under laboratory conditions using dual culture method (Skidmore and Dickinson, 1976) and was compared with reference cultures of *T. viride* and *Pseudomonas fluorescens*.

3.4.1 *In vitro* evaluation of fungal antagonists against fungal pathogens

The fungal antagonists selected from previous studies *viz., T. hamatum, T. viride* and the reference culture *T. viride* were tested against the fungal pathogens *viz., P.apahanidermatum, P. palmivora, F. oxysporum, R. solani* and *S. rolfsii* under

in vitro condition. A mycelial disc of 8 mm diameter was cut from actively growing culture of the fungal pathogens and placed in the centre of one half of the Petri dish with PDA medium. The fungal antagonist was similarly transferred and placed at the centre of other half of the same Petri dish. Three replications were maintained for each antagonist. The pathogen and antagonists grown as monoculture served as control. The growth measurements were taken at regular intervals after 24 h of inoculation. The per cent inhibition of growth of pathogen over control was calculated by the formula suggested by Vincent (1927).

$$\text{Per cent inhibition of growth (PI)} = \frac{C - T}{C} \times 100$$

C= Growth of fungus in control (mm)

T= Growth of fungus in treatment (mm)

The nature of antagonistic reaction against pathogen was assessed by the following method suggested by Purkayastha and Battacharya (1982).

Type of reaction:-

Homogenous	: Free intermingling of hyphae
Over growth	: Pathogen over grown by antagonists
Cessation of growth	: Cessation of growth at line of contact
Aversion	: Development of clear zone of inhibition

3.4.2 *In vitro* evaluation of bacterial antagonists against fungal pathogens

The antagonistic efficiency of bacterial isolates against fungal pathogens were tested under *in vitro* condition by streaking the bacterial isolates on both side of the pathogen. Three replications were kept for each antagonist and the monoculture of each pathogen was maintained as control. The per cent inhibition of growth of pathogen over control was calculated as mentioned in 3.4.1.

3.4.2.1 Streaking on both sides

A mycelial disc of pathogen of 8 mm size was inoculated at the centre of Petri dish with PDA medium. The bacterial isolate was inoculated as a line of streak on either side of pathogen on the same day. The Petri dishes were incubated at room temperature and observations on growth of pathogen were taken at regular intervals.

3.4.3 *In vitro* evaluation fungal antagonists against *R. solanacearum*

The effect of fungal antagonists against *R. solanacearum* was studied by placing the disc of 8 mm size taken from the actively growing culture of fungal antagonists and were placed at the centre of the Petri dishes seeded with *R. solanacearum* .The antagonists and pathogen were inoculated on the same day. Three replications were maintained for each antagonist and monoculture of each organism was maintained as control.

The Petri dishes were incubated at room temperature (28 ± 2^0 C) and observations on zone of inhibition and growth of fungal antagonists were recorded upto when the growth in the control plates fully covered the 90mm diameter. The per cent inhibition of growth of pathogen over control was calculated as mentioned in 3.4.1.

3.4.4 *In vitro* evaluation of bacterial antagonists against *R. solanacearum*

The following method of inoculation was adopted for testing the effect of bacterial antagonists against *R. solanacearum.* Both the antagonist and the pathogen were inoculated on the same day. Three replications maintained for each antagonist and control.

3.4.3.1 Point inoculation of the antagonistic organism

A loopful of antagonistic oraganism was spotted at the centre of the plate seeded with the test pathogen, *R. solanacearum.* Plates were observed upto 48h and diameter of inhibition zone was recorded.

3.5 *In vitro* EVALUATION ON MUTUAL COMPATIBILITY BETWEEN THE SELECTED FUNGAL AND BACTERIAL ANTAGONISTS

The mutual compatibility between selected fungal and bacterial antagonists were tested by *in vitro* evaluation on PDA medium by dual culture method. This compatibility was tested for preparation of consortium, which was given as a treatment in the pot culture experiment.

A mycelial disc of 8 mm size of the fungal antagonist was inoculated at the centre of the Petri dish with PDA medium. On the next day bacterial isolate was inoculated as a line streak on either side of the disc, leaving 2.25 cm from the periphery of the Petri dish. The plates were then incubated at room temperature and observed daily for any type of inhibition. The absence of inhibition indicated compatibility.

3.6 BIOSOFTENING OF PADDY STRAW SMS

For biosoftening the SMS was treated with the best fungal antagonist, best bacterial antagonist and a microbial consortium, which were selected from the *in vitro* evaluation. The liquid culture, 150 ml of the respective organisms were prepared in a standard flask.

For fungus, PDA broth was prepared and sterilized and 8 mm mycelial disc of five day old actively growing culture was used as inoculum source. This was incubated for 7 days to obtain mycelial mat. After 7 days of incubation the mycelial mat was collected and macerated in the grinder along with the PDA broth to get a concentration of 10^6 cfu/ ml. This was applied to the SMS @ 300 ml / kg and mixed thoroughly (Wei *et al.*, 2011). For each treatments 5 kg of SMS was taken and four replications were maintained.

In the case of bacteria King's B broth was prepared, sterilized and inoculated with bacterial culture and incubated at room temperature. Frequent shaking was given daily by rotary shaker to about half an hour. After 48 hrs colour changes and turbidity were observed in KB broth with the concentration of 10^8 cfu/ ml. This 48 hour old bacterial broth was used @ 300 ml per kg of SMS and mixed thoroughly. For each treatments 5 kg of SMS was taken and four replications were maintained.

For microbial consortia, 150 ml of both bacterial and fungal broth were taken and made upto 300 ml and applied to SMS. For each treatment 5 kg of SMS was taken and four replications were maintained. The raw SMS served as control.

Observations were taken at monthly intervals on microbial population, physico- chemical parameters and nutrient content of SMS.

3.6.1 Enumeration of microbial population:

The procedures were same as that given in 3.3. Dilutions of 10^{-7} and 10^{-8} were used for fungus and bacteria respectively.

3.6.2 Physico - chemical parameters:

pH : The pH was analyzed at monthly intervals by using pH meter

EC : Electrical conductivity was tested using EC meter.

Moisture content : The per cent moisture content was worked out by Hot air oven method.

$$\text{Moisture percent by weight} = 100 \frac{(B-C)}{B-A}$$

A = Weight of the Petri Dish

B = Weight of the Petri dish plus material before drying

C = Weight of the Petri Dish plus material after drying

Visual observations like colour and texture of the treated SMS were also recorded.

3.7 MINERAL NUTRIENT ANALYSIS

The different treatments in biosoftening of SMS were analysed for their nutrient contents. Samples were collected, dried and powdered in grinder and used for analysis.

Table 1. Methods of Nutrient analysis

Sl. No.	Element	Method
1	Nitrogen	Modified Kjeldhal's digestion method (Jackson, 1973)
2	Phosphorus	Diacid digestion of sample followed by filtration. Vanabdomolybdate phosphoric yellow colour in nitric acid system (Piper, 1966)
3	Potassium	Diacid digestion of sample followed by filtration. Flame photometry determination (Jackson, 1973)
4	Calcium and magnesium	Diacid digestion of sample followed by filtration. The filtrate was collected, analysed for Ca and Mg using Perkin-Elmer AAS (Piper, 1966)
5	Sulphur	Diacid digestion of sample followed by filtration. The filtrate was collected, analysed for S using Perkin-Elmer AAS (Piper, 1966)
6.	Iron, manganese,zinc and copper	Diacid digestion of sample followed by filtration. The filtrate was collected, analysed for Fe, Mn,Zn and Cu using Perkin-Elmer AAS (Piper, 1966)

3.8 EVALUATION OF FORTIFIED SMS AGAINST SOIL BORNE DISEASES OF TOMATO

A pot culture experiment was conducted to evaluate the antagonistic efficiency and quality of SMS against soil borne diseases of tomato. The pot culture study was conducted as two experiments (Plate 1).

1. Management of damping off of tomato

2. Management of bacterial wilt of tomato

The SMS was incorporated along with potting mixture on v/v basis at the time of sowing/planting. SMS fortified with selected antagonists and microbial consortium was used in the pot culture experiment. The challenge inoculation with *P.aphanidermatum* was carried out at 21 DAS, while in case of *R. solanacearum* the challenge inoculation was given at 30 DAT.

The treatment details of the experiment are given below:

Design - CRD

Replication - 3

Number of treatments – 12

Variety – Pusa Ruby

Experiment no. 1: - Management of damping off of tomato

T1- Potting mixture amended with 50% fortified SMS using the best fungal antagonist.

T2- Potting mixture amended with 50% fortified SMS using the best bacterial antagonist

T3-Potting mixture amended with 50% fortified SMS using microbial consortium

T4- Potting mixture + soil drenching of best fungal antagonist

T5- Potting mixture + soil drenching of best bacterial antagonist

T6 - Potting mixture + soil drenching of microbial consortium

T7 - Potting mixture + soil drenching with reference culture of *T.viride.*

T8 - Potting mixture + soil drenching with reference culture of *P. fluorescens* .

T9 - Potting mixture + fungicide drenching using copper hydroxide (2g/lit).

T10- Potting mixture +50% SMS

T11- Potting mixture alone (control)

T12- Absolute control

Experiment no 2:- Management of bacterial wilt of tomato

The treatments T1 to T12 are same as the above experiment 1. Additionally one treatment was included which consists of potting mixture with application of chemical fertilizers as recommended in package of practices.

3.9 Preparation of potting mixture and planting

The potting mixture consisting of sand: soil: cow dung in the ratio of 1:1:1 was prepared and used along with the paddy straw SMS of *P. florida* for filling polybags of size 20 × 20cm as mentioned in the treatments. For the management of damping off disease 25 mg of seeds were sown in each bag. The fortification of SMS was done by addition of 300 ml broth of particular organism per kg of substrate and

for soil drenching 200 ml of broth with selected antagonistis as well as consortium were used (Wei *et al.*, 2011).

In case of management of bacterial wilt the bags were planted with two plants of 30 days old seedlings, the treatments were applied at the time of planting. The treatment in which chemical fertilizer applied was based on Package of Practices Recommendations, Crop 2011 (KAU, 2011).

3.9.1 Observations recorded

Observations on the germination of seeds, plant biometric characters, per cent disease incidence and yield at different intervals were recorded.

3.9.1.1 Germination of seeds

The number of seeds germinated in each treatment was recorded at 21 days after sowing and calculated the per cent germination.

3.9.1.2 Per cent disease incidence

Challenge inoculation of pathogen was done at 21 days after sowing in the case of damping off while in the case of bacterial wilt the inoculum was given at 30 days after transplanting. The wilt incidence was recorded at weekly intervals as well as at harvest. The per cent disease incidence was calculated using formula

$$PDI = \frac{\text{Number of infected plants} \times 100}{\text{Total number of plants}}$$

3.9.1.3 Biometric observations

The following observations were recorded.

a. Root length

b. Shoot length

c. Fresh weight of root and shoot

d. Plant vigour index (PVI) = (Mean root length+ Mean shoot length) ×

per cent germination.

e. Yield - The fresh fruit yield per bag from each treatment was recorded at the final stage of harvest and was expressed in g/ plant.

3.10 STATISTICAL ANALYSIS

Analysis of variance was performed on the data collected using the statistical package MSTAT (Freed, 1986). Multiple comparison among the treatment means were done using DMRT.

Plate 1: Over view of pot culture experiment

Experiment 1: Management of damping off of tomato

Experiment 2: Management of bacterial wilt of tomato

RESULTS

4. RESULTS

The present investigations were carried out to find out the potential of fortified spent mushroom substrate against soil borne diseases *viz.*, damping off and bacterial wilt of tomato. *In vitro* evaluation was carried out with antagonists *viz.*, *Trichoderma viride* (SMS), *T. hamatum* (SMS) and *Bacillus subtilis* (SMS) along with reference cultures of Kerala Agricultural University *viz.*, *T. viride* and *Pseudomonas fluorescens* against soil borne pathogens of tomato *viz.*, *Pythium aphanidermatum* and *Ralstonia solanacearum.* Mutual compatibility among the antagonists was also tested. The study also included fortification of SMS with these antagonists, its effect on biosoftening of SMS, nutritional status and pot culture evaluation. The results of the study are described below.

4.1 ISOLATION OF SOIL BORNE PATHOGENS ASSOCIATED WITH THE DISEASE

The pathogens causing damping off disease were isolated from the diseased specimens. The major pathogen causing damping off is *Pythium aphanidermatum* and other pathogens which were associated with the disease are *Phytophthora palmivora*, *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii.* The affected seedlings were characterized by cotyledon and leaf chlorosis along with watery rot in the tap root and hypocotyls at or near the soil line.

The pathogens were isolated by using potato dextrose agar medium and incubated under room temperature for 2-3 days. The isolated pathogens were further purified by hyphal tip method, subcultured and maintained on PDA slants for further studies.

In case of bacterial wilt, the diseased plants are characterized by wilted leaves and stems and vascular discolouration. They were collected from the field of Agricultural Research Station, Mannuthy, and were subjected to ooze test. Isolation of *R. solanacearum* was done in TZC agar medium. The pathogen was identified based on shape, size and colour of the colonies as circular, fluidal, slimy white with light pink pin point centre. The culture was purified by streak plate method. Single colony was selected, sub cultured and maintained in NA slants and also in sterile distilled water for further studies. Pathogenicity was also tested on tomato plants with different cultures of the pathogens and were re-isolated and maintained as pure culture.

4.2 PRODUCTION OF SPENT MUSHROOM SUBSTRATE

The isolation of oyster mushroom *viz., Pleurotus florida* was done as per the standard tissue culture technique. The actively growing cultures were used for mushroom spawn production by inoculating on sorghum grains as described under 3.2.1. After 15 days of incubation the inoculated sorghum grains became white hard mass, completely impregnated with mycelium and used for mushroom production using paddy straw as substrate. The substrate left after the harvest of mushroom was used as SMS.

4.3 ENUMERATION OF MICROORGANISMS FROM SUBSTRATE

The microbial population from the substrate was enumerated by serial dilution and plate count method at different stages of mushroom production *viz.* after spawn running (15 days), during harvest (30 days) and after harvest (50 days). The data are presented in Table 2.

Table 2: Enumeration of bacterial and fungal population from the substrate at different stages of cropping

Microorganisms	Population at different stages of cropping		
	Stage 1	Stage 2	Stage 3
Fungal population ($\times 10^5$ cfu/g of substrate)	25.33	48.67	55.33
Bacterial population ($\times 10^6$ cfu/g of substrate)	55.67	29.33	17.33

Stage 1- at the time of spawn running

Stage 2- at harvest

Stage 3- after harvest

The fungal and bacterial populations were found to be varied in each stage of mushroom growth. The actinomycetes colonies were absent in all the stages of mushroom growth.

In the case of fungus, the population was less after spawn running (25.33 × 10^5 cfu/ g of substrate) and started increasing and maximum population was observed after harvest (55.33× 10^5 cfu/ g of substrate). In all stages, the fungus observed was *Trichoderma* sp.

In case of bacteria, the population was higher in stage 1 (55.67×10^6 cfu/ g of substrate) compared to other stages. During harvest, the population was 29.33×10^6 cfu/ g of substrate and after harvest it was 17.33×10^6 cfu/ g of substrate. In all the stages different types of bacterial colonies were observed.

4.4 *IN VITRO* EVALUATION OF ANTAGONISTS AGAINST PATHOGENS

The fungal antagonists isolated from SMS in previous studies conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara *viz.*, *T. hamatum*, *T. viride* (SMS) along with *T. viride* (reference culture) and bacterial antagonists *viz. B. subtilis* (SMS) along with reference culture *P. fluorescens* were evaluated against soil borne pathogens *viz. P. aphanidermatum*, *P. palmivora*, *F. oxysporum*, *R. solani*, *S. rolfsii* and *R. solanacearum* under *in vitro* condition.

4.4.1 *In vitro* evaluation of fungal antagonists against fungal pathogens

The *in vitro* evaluation of fungal antagonists against fungal pathogens was carried out by dual culture method and per cent inhibition was calculated.

4.4.1.1 *In vitro* evaluation of fungal antagonists against *P. aphanidermatum*

The fungal antagonists *viz.*, *T. viride* (SMS), *T. hamatum* and *T. viride* (reference culture) were evaluated against the major damping off pathogen *P. aphanidermatum* by dual culture method. The efficiency of antagonism and per cent inhibition are presented in the Table 3.

The pathogen was found to be virulent and fast growing, so it took three days to complete full growth in monoculture, while all the antagonists took four days to complete full growth in monoculture. Among the antagonists, variation in growth was noticed on the third day in monoculture with maximum growth in *T. viride* (reference culture, 80.5 mm). In dual culture, the variation in growth was noticed from second day for both the pathogen and antagonists. On the second day, the lowest growth of the pathogen was obtained from dual culture with *T. hamatum* (47.6 mm) followed by 51.6 mm in *T. viride* (SMS) and 52.3 mm in *T. viride* (reference culture). On the third day, the antagonist *T. viride* (SMS) gave maximum per cent inhibition (50.77 per cent) over control followed by *T. hamatum* and *T. viride* (reference culture) which gave 48.88 per cent inhibition over control. A prominent clear zone was noticed in the dual culture with *T. viride* (SMS) compared to other two.

But, from fourth day onwards brown metabolite production was noticed in all the dual cultures. The growth of the pathogen in dual culture was 41.6 mm in *T. viride* (SMS), 43.0 mm in *T. viride* (reference culture) and 44.6 mm in *T. hamatum*, while the growth of antagonists were 51 mm for *T. viride* (SMS), 49.3 mm for *T. hamatum* and 46.0 mm for *T. viride* (reference culture)

On fifth day, *T. hamatum* gave maximum per cent inhibition (60.00 per cent) in dual culture and it produced dark yellowish metabolite over the entire pathogen. In

Table 3: *In vitro* evaluation of fungal antagonists against *P. aphanidermatum*

Antagonists		Colony diameter of *P. aphanidermatum*(mm)										PIOC (%)
		Days after incubation										
		1		2		3		4		5		
		P	A	P	A	P	A	P	A	P	A	
Trichoderma viride (isolate from SMS)	D	38.5	15.3	51.6	38.0	44.3	48.3	41.6	51.0	39.0	51.0	56.66
	M	38.6	14.0	69.3	43.0	90.0	63.0	90.0	90.0	90.0	90.0	
T. hamatum (isolate from SMS)	D	40.6	19.3	47.6	36.0	46.0	45.0	44.6	49.3	36.0	49.3	60.00
	M	38.6	21.0	69.3	53.0	90.0	77.6	90.0	90.0	90.0	90.0	
T. viride (Reference culture)	D	41.6	20.0	52.3	38.3	46.0	45.0	43.0	46.0	40.3	46.0	55.22
	M	38.6	20.0	69.3	53.3	90.0	80.5	90.0	90.0	90.0	90.0	

PIOC – Per cent inhibition over control

P- Pathogen A- Antagonist M- Monoculture D- Dual culture

case of *T. viride* (SMS), it gave 56.66 per cent inhibition followed by *T. viride* (reference culture) showed 55.22 per cent inhibition and the metabolite produced was light yellowish orange coloured (Plate 2).

4.4.1.2 *In vitro* evaluation of fungal antagonists against *P. palmivora*

The antagonists *viz. T. viride* (SMS), *T. hamatum* and *T. viride* (reference culture) were compared for their efficacy against *P. palmivora*. The antagonistic efficiency and per cent inhibition are presented in Table 4.

The pathogen took six days to complete full growth in monoculture. The initial growth of the pathogen in dual culture was 17.3 mm, 17.6 mm and 18.6 mm with *T. hamatum*, *T. viride* (reference culture) and *T. viride* (SMS), while on second day the growth of the pathogen was enhanced to 27.3 mm, 30.0 mm and 30.0 mm for *T. hamatum*, *T. viride* (SMS) and *T. viride* (reference culture) respectively.

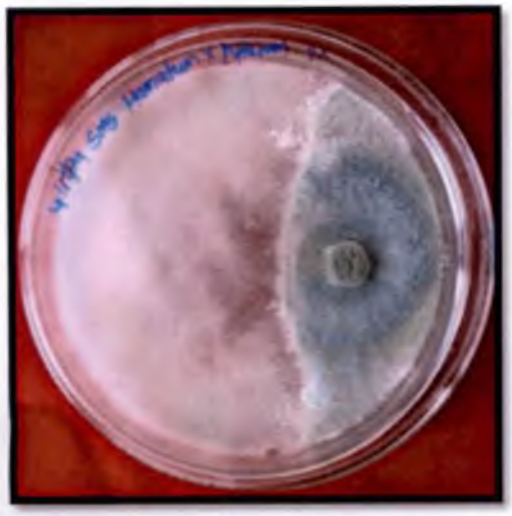
The growth of the pathogen was arrested on third day to 34.0 mm *T. hamatum*, and 40.0 mm recorded for both *T. viride* (reference culture) and *T. viride* (SMS). From fourth day onwards all the antagonists started to over grew the pathogen. On sixth day all the antagonists attained cent per cent inhibition over control. The mycelial growth of the pathogen became sparsed and growth was suppressed on the same day (Plate 3).

4.4.1.3 *In vitro* evaluation of fungal antagonists against *F. oxysporum*

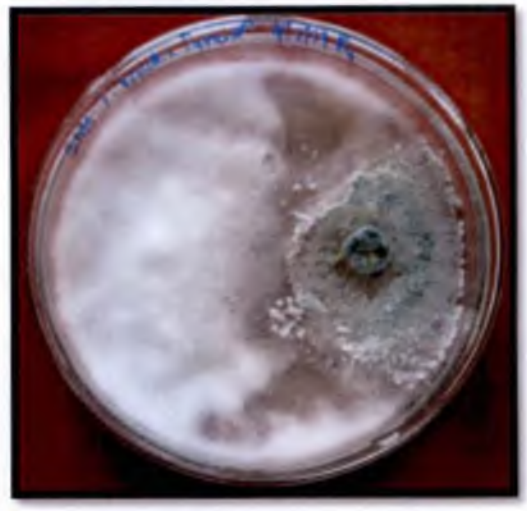
The fungal antagonists were evaluated against *F. oxysporum*. The efficiency of antagonism and per cent inhibition were worked out and presented in Table 5.

Plate 2: *In vitro* evaluation of fungal antagonists against *P. aphanidermatum*

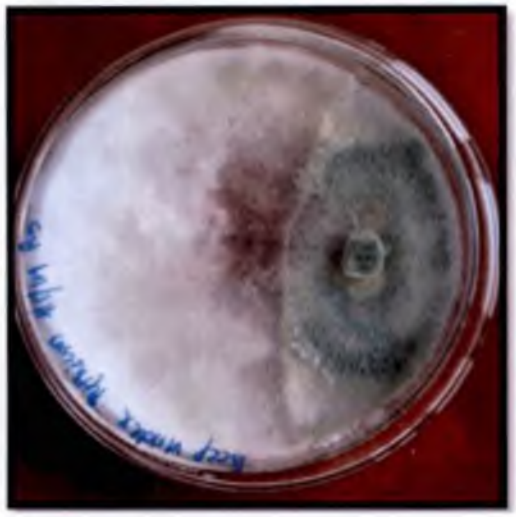
(on 5th day)



T.hamatum × *P.aphanidermatum*



T.viride × *P.aphanidermatum*



T.viride (reference culture) × *P.aphanidermatum*

P.aphanidermatum

Table 4: *In vitro* evaluation of fungal antagonists against *P. palmivora*

Antagonists		Colony diameter of *P. palmivora* (mm)												PIOC (%)
		Days after incubation												
		1		2		3		4		5		6		
		P	A	P	A	P	A	P	A	P	A	P	A	
Trichoderma viride (isolate from SMS)	D	18.6	22.6	30.0	32.0	40.0	50.3	40.0	64.3	40.0	79.0	40.0	90.0	100
	M	23.0	14.0	39.0	43.0	52.0	69.3	67.0	90.0	81.0	90.0	90.0	90.0	
T. hamatum (isolate from SMS)	D	17.3	23.3	27.3	33.3	34.0	52.3	34.0	65.0	34.0	81.6	34.0	90.0	100
	M	23.0	21.0	39.0	53.0	52.0	77.3	67.0	90.0	81.0	90.0	90.0	90.0	
T. viride (Reference culture)	D	17.6	21.3	30.0	31.3	40.0	48.3	40.0	65.0	40.0	81.3	40.0	90.0	100
	M	23.0	20.0	39.0	53.3	52.0	80.5	67.0	90.0	81.0	90.0	90.0	90.0	

PIOC – Per cent inhibition over control

P- Pathogen A- Antagonist M- Monoculture D- Dual culture

Plate 3: *In vitro* evaluation of fungal antagonists against *P.* palmivora (on 6th day)

T.hamatum × *P. palmivora*

T.viride × *P.palmivora*

T.viride (Reference culture) × *P. palmivora*

P. palmivora

Table 5: *In vitro* evaluation of fungal antagonists against *F. oxysporum*

Antagonists		Colony diameter of *F. oxysporum* (mm)																PIOC (%)
		Days after incubation																
		1		2		3		4		5		6		7		8		
		P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	
Trichoderma viride (isolate from SMS)	D	20.0	16.3	29.0	34.2	38.6	41.0	50.0	54.0	50.0	64.0	50.0	67.0	50.0	86.0	50.0	90.0	100
	M	23.3	20.0	31.6	53.3	40.3	80.5	53.3	90.0	67.3	90.0	77.6	90.0	84.6	90.0	90.0	90.0	
T. hamatum (isolate from SMS)	D	18.0	18.3	26.3	30.6	44.0	59.0	44.0	72.0	44.0	89.3	44.0	90.0	44.0	90.0	44.0	90.0	100
	M	23.3	21.0	31.6	53.0	40.3	77.6	53.3	90.0	67.3	90.0	77.6	90.0	84.6	90.0	90.0	90.0	
T. viride (Reference culture)	D	21.6	20.6	29.6	30.6	37.3	38.6	45.3	48.3	50.3	57.3	50.3	69.3	50.3	80.0	50.3	90.0	100
	M	23.3	24.0	31.6	43.0	40.3	69.3	53.3	90.0	67.3	90.0	77.6	90.0	84.6	90.0	90.0	90.0	

PIOC – Per cent inhibition over control

P- Pathogen A- Antagonist M- Monoculture D- Dual culture

The pathogen attained complete growth in monoculture by eighth day after inoculation. The variation in growth of pathogen in dual culture on second day was 26.3 mm with *T. hamatum*, 29.0 mm with *T. viride* (SMS) and 29.6 mm with *T. viride* (reference culture). The growth of pathogen in dual culture on third day was 37.3 mm for *T. viride* (reference culture), 38.6 mm for *T. viride* (SMS) and 44.0 mm for *T. hamatum*. The growth of pathogen was arrested from third day onwards. The antagonists started to over grew the pathogen from fourth day onwards. All the antagonists were recorded 100 per cent inhibition over control on 8th day after inoculation (Plate 4).

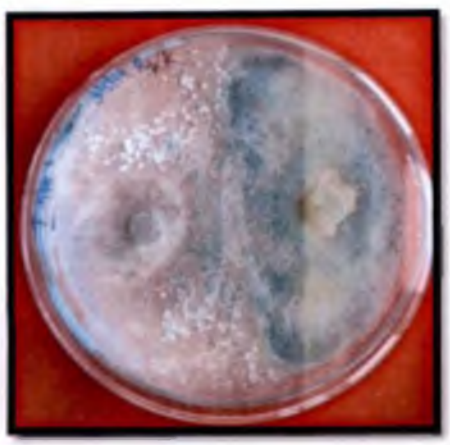
4.4.1.4 *In vitro* evaluation of fungal antagonists against *R. solani*

The antagonistic efficiency of *T. viride* (SMS), *T. hamatum* and *T. viride* (reference culture) were tested on *R. solani* and the results are presented in Table 6.

It took four days for *R. solani* to obtain complete growth in monoculture. In dual culture, the growth of Rhizoctonia was slow as compared to monoculture. On second day after inoculation the growth of *R. solani* in dual culture was 45.0 mm with *T. hamatum*, 47.0 mm with *T. viride* (reference culture) and 48.6 mm with *T. viride* (SMS). The same trend was noticed for growth of *R. solani* on third day with 41.0 mm, 43.6 mm and 46.3 mm for *T. hamatum*, *T. viride* (reference culture) and *T. viride* (SMS) respectively. A brown coloured metabolite production as well as cessation of growth of pathogen was noticed on the same day.

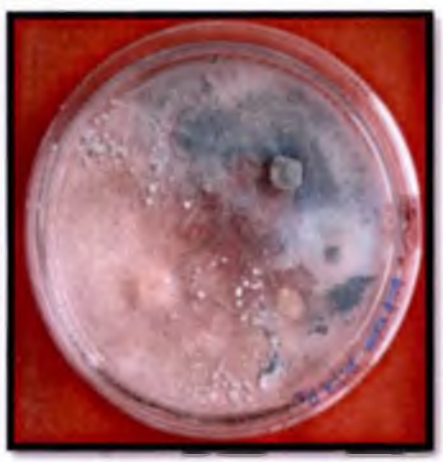
The maximum inhibition of the pathogen was achieved in *T. hamatum* (57.44 per cent) followed by 53.77 per cent with *T. viride* (reference culture) and 51.88 per cent by *T. viride* (SMS). On the fifth day in dual culture, growth of antagonists was

Plate 4: *In vitro* evaluation of fungal antagonists against *F. oxysporum* (on 8th day)



T.hamatum × *F. oxysporum*

T.viride × *F. oxysporum*



T.viride (reference) × *F. oxysporum*

F. oxysporum

Table 6: *In vitro* evaluation of fungal antagonists against *R. solani*

Antagonists		Colony diameter of *R. solani* (mm)										PIOC (%)
		Days after incubation										
		1		2		3		4		5		
		P	A	P	A	P	A	P	A	P	A	
Trichoderma viride (isolate from SMS)	D	23.3	14.6	48.6	42.0	46.3	45.0	43.3	46.3	40.0	46.3	51.88
	M	52.3	20.0	69.0	48.3	80.6	61.6	90.0	90.0	90.0	90.0	
T. hamatum (isolate from SMS)	D	22.6	20.0	45.0	44.0	41.0	47.3	38.3	49.3	37.0	54.0	57.44
	M	52.3	20.0	69.0	49.6	80.6	62.3	90.0	90.0	90.0	90.0	
T. viride (Reference culture)	D	23.0	18.0	47.0	44.0	43.6	46.3	41.6	47.3	40.3	49.3	53.77
	M	52.3	20.0	69.0	49.3	80.6	64.0	90.0	90.0	90.0	90.0	

PIOC – Per cent inhibition over control

P- Pathogen A- Antagonist M- Monoculture D- Dual culture

maximum in *T. hamatum* (54.0 mm) followed by *T. viride* (reference culture, 49.3 mm and *T. viride* (SMS, 46.3 mm). A prominent brown coloured metabolite production was noticed in all the dual cultures on fifth day (Plate 5).

4.4.1.5 *In vitro* evaluation of fungal antagonists against *S. rolfsii*

An *in vitro* evaluation was carried out to find out the antagonistic efficiency of antagonists *viz. T. viride* (SMS), *T. hamatum* and *T. viride* (reference culture) against *S. rolfsii* by dual culture method. The results are presented in Table 7.

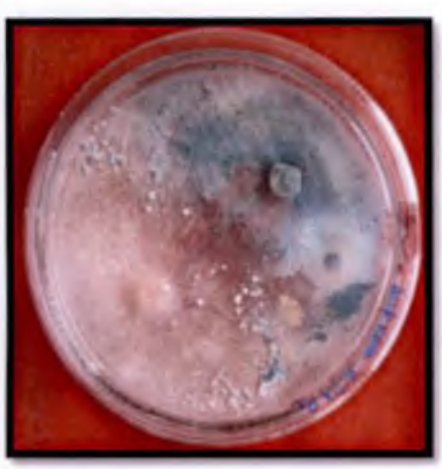
The pathogen was found to be virulent and aggressive, so it took only three days to attain complete growth in monoculture. Initially the growth of the pathogen in all the dual cultures was found to be uniform. But the variation in growth was observed from second day onwards in dual cultures, which recorded 42.0 mm for *T. viride* (reference culture), 43.6 mm for *T. viride* (SMS) and 45.3 mm for *T. hamatum*, while in case of antagonists, a growth of 47.0 mm, 45.6 mm and 42.3 mm for *T. viride* (SMS), *T. viride* (reference culture) and *T. hamatum* respectively were recorded.

The maximum per cent inhibition over control was recorded for *T. viride* (reference culture, 43.33 per cent) followed by *T. viride* (SMS, 40.77 per cent) and *T. hamatum* (31.55 per cent). A thick white line was noticed in all the dual cultures on the third day after inoculation. But fourth day onwards over growth of pathogen was noticed in all the dual cultures except *T. viride* (reference culture). The same trend was observed on fifth day also. The growth of the pathogen in dual culture was 63.3 mm for *T. viride* (reference culture), 79.3 mm for *T. hamatum* and 85.0 mm for *T. viride* (SMS) (Plate 6).

Plate 4: *In vitro* evaluation of fungal antagonists against *F. oxysporum* (on 8th day)

T.hamatum × *F. oxysporum*

T.viride × *F. oxysporum*



T.viride (reference) × *F. oxysporum*

F. oxysporum

maximum in *T. hamatum* (54.0 mm) followed by *T. viride* (reference culture, 49.3 mm and *T. viride* (SMS, 46.3 mm). A prominent brown coloured metabolite production was noticed in all the dual cultures on fifth day (Plate 5).

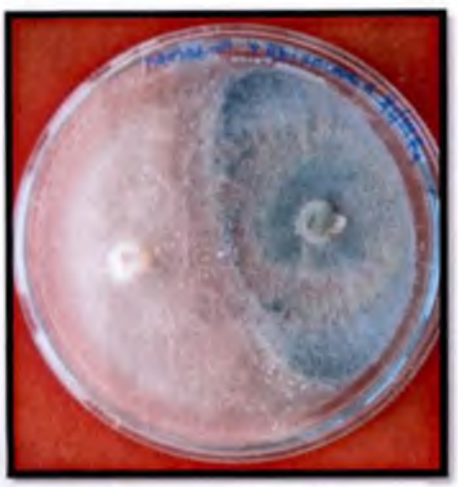
4.4.1.5 *In vitro* evaluation of fungal antagonists against *S. rolfsii*

An *in vitro* evaluation was carried out to find out the antagonistic efficiency of antagonists *viz. T. viride* (SMS), *T. hamatum* and *T. viride* (reference culture) against *S. rolfsii* by dual culture method. The results are presented in Table 7.

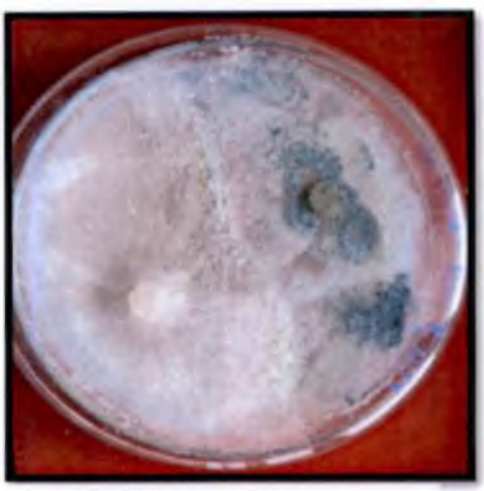
The pathogen was found to be virulent and aggressive, so it took only three days to attain complete growth in monoculture. Initially the growth of the pathogen in all the dual cultures was found to be uniform. But the variation in growth was observed from second day onwards in dual cultures, which recorded 42.0 mm for *T. viride* (reference culture), 43.6 mm for *T. viride* (SMS) and 45.3 mm for *T. hamatum*, while in case of antagonists, a growth of 47.0 mm, 45.6 mm and 42.3 mm for *T. viride* (SMS), *T. viride* (reference culture) and *T. hamatum* respectively were recorded.

The maximum per cent inhibition over control was recorded for *T. viride* (reference culture, 43.33 per cent) followed by *T. viride* (SMS, 40.77 per cent) and *T. hamatum* (31.55 per cent). A thick white line was noticed in all the dual cultures on the third day after inoculation. But fourth day onwards over growth of pathogen was noticed in all the dual cultures except *T. viride* (reference culture). The same trend was observed on fifth day also. The growth of the pathogen in dual culture was 63.3 mm for *T. viride* (reference culture), 79.3 mm for *T. hamatum* and 85.0 mm for *T. viride* (SMS) (Plate 6).

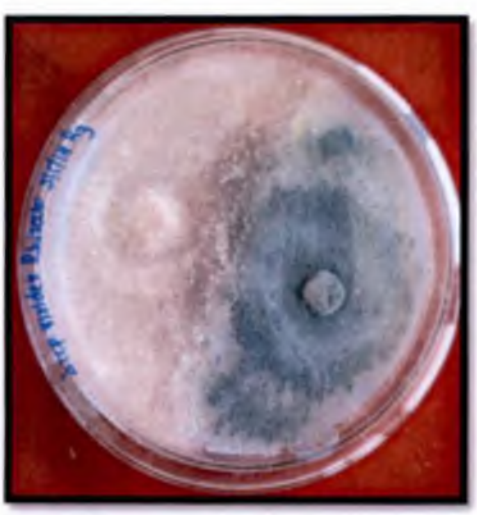
Plate 5: *In vitro* evaluation of fungal antagonists against *R. solani* (on 4th day)



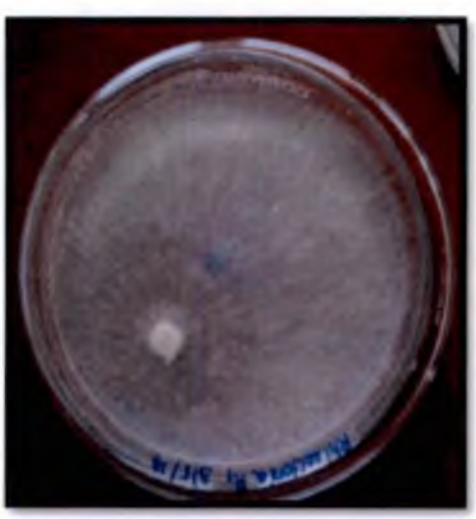
T.hamatum × *R. solani*



T.viride × *R. solani*



T.viride (reference culture) × *R. solani*



R. solani

Table 7: *In vitro* evaluation of fungal antagonists against *S. rolfsii*

Antagonists		Colony diameter of *S. rolfsii* (mm)										PIOC (%)
		Days after incubation										
		1		2		3		4		5		
		P	A	P	A	P	A	P	A	P	A	
Trichoderma viride (isolate from SMS)	D	17.3	14.3	43.6	47.0	53.3	46.6	61.6	45.0	85.0	45.0	40.77
	M	21.5	20.3	53.0	54.7	90.0	76.6	90.0	90.0	90.0	90.0	
T. hamatum (isolate from SMS)	D	17.6	14.6	45.3	42.3	61.6	45.0	77.3	45.0	79.3	45.0	31.55
	M	21.5	20.7	53.0	52.5	90.0	77.6	90.0	90.0	90.0	90.0	
T. viride (Reference culture)	D	18.0	22.6	42.0	45.6	51.0	45.0	55.0	45.0	63.3	45.0	43.33
	M	21.5	20.0	53.0	52.5	90.0	72.2	90.0	90.0	90.0	90.0	

PIOC – Per cent inhibition over control

P- Pathogen A- Antagonist M- Monoculture D- Dual culture

Plate 6: *In vitro* evaluation of fungal antagonists against *S. rolfsii* (on 3rd day)

T.hamatum × *S. rolfsii*

T.viride × *S. rolfsii*

T.viride (reference culture) × *S. rolfsii*

S. rolfsii

4.4.2. *In vitro* evaluation of bacterial antagonists against fungal pathogens

The bacterial antagonists procured from previous studies *viz. Bacillus subtilis* and reference culture of Kerala Agricultural University *i.e., P. fluorescens* were evaluated against *P. aphanidermatum*, *P. palmivora, F. oxysporum*, *R. solani*, *S. rolfsii* and *R. solanacearum* under *in vitro* condition.

4.4.2.1 *In vitro* evaluation of bacterial antagonists against *P. aphanidermatum*

The bacterial antagonists *viz. B. subtilis* and *P. fluorescens* were evaluated against the major damping off causing pathogen *P. aphanidermatum*. The results are presented in Table 8.

The antagonist *P. fluorescens* gave maximum per cent inhibition over control. The pathogen showed complete growth in monoculture on third day after inoculation. On the first day growth of pathogen in dual culture was low as compared to monoculture. In dual culture growth was 30.0 mm with *B. subtilis* and 32.0 mm with *P. fluorescens*. The pathogen started to over grew the *B. subtilis* on second day after inoculation, while with *P. fluorescens* the growth of the pathogen was enhanced to 43.6 mm and then the growth was arrested giving 51.55 per cent inhibition over control. The bacterial antagonist *B. subtilis* was not at all effective against *P. aphanidermatum* (Plate 7).

4.4.2.2 *In vitro* evaluation of bacterial antagonists against *P. palmivora*

The bacterial antagonists *viz. B.subtilis* and *P. flourescens* were evaluated against *P. palmivora*. The results are presented in Table 9.

Both the antagonists recorded inhibition of growth of pathogen in dual culture. On the first day after inoculation, the growth of pathogen was 23.5 mm with

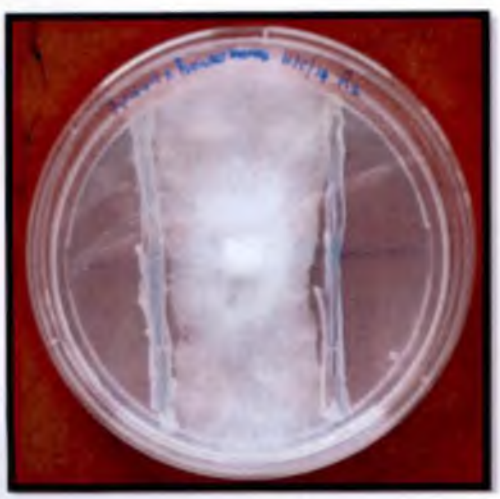
Table 8: *In vitro* evaluation of bacterial antagonists against *P. aphanidermatum*

Antagonists		Colony diameter of *P. aphanidermatum* (mm)				PIOC (%)
		Days after inoculation				
		1	2	3	4	
Bacillus subtilis (isolated from SMS)	D	30.0	68.0	90.0	90.0	0.00
	M	48.6	63.0	90.0	90.0	
Pseudomonas fluorescens (reference culture)	D	32.0	43.6	43.6	43.6	51.55
	M	48.6	63.0	90.0	90.0	

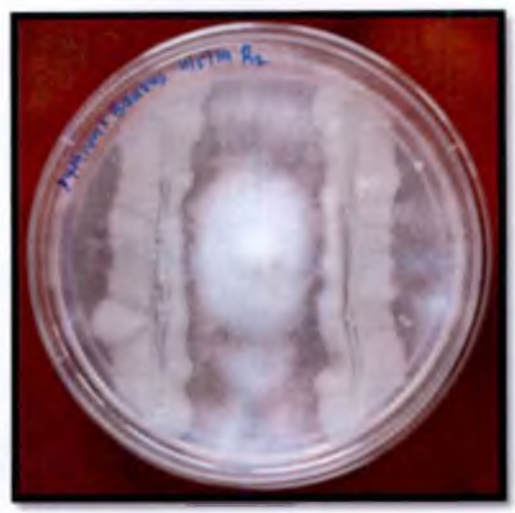
PIOC – Per cent inhibition over control

M- Monoculture D- Dual culture

Plate 7: *In vitro* evaluation of bacterial antagonists against *P.aphanidermatum* (on 2nd day)

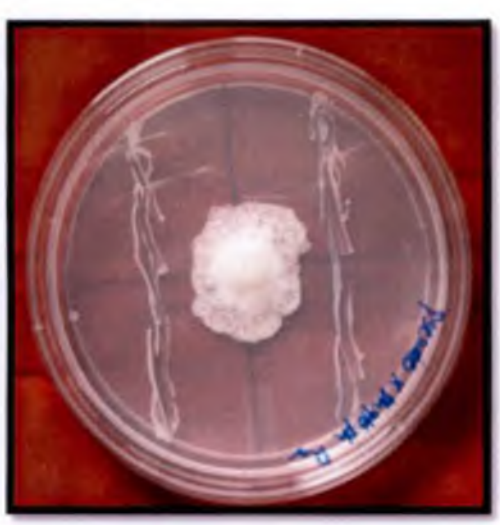


P.fluorescens × *P. aphanidermatum*



B.subtilis × *P. aphanidermatum*

Plate 8: *In vitro* evaluation of bacterial antagonists against *P. palmivora* (on 2nd day)



P. fluorescens × *P. palmivora*

B.subtilis × *P. palmivora*

Table 9: *In vitro* evaluation of bacterial antagonists against *P. palmivora*

Antagonists		Colony diameter of *P. palmivora* (mm)						PIOC (%)
		Days after inoculation						
		1	2	3	4	5	6	
Bacillus subtilis (isolated from SMS)	D	23.5	26.5	25.0	25.0	25.0	25.0	72.22
	M	30.5	41.0	51.0	69.0	80.0	90.0	
P. fluorescens (reference culture)	D	26.0	29.0	33.0	33.0	33.0	33.0	63.33
	M	30.5	41.0	51.0	69.0	80.0	90.0	

PIOC – Per cent inhibition over control

M- Monoculture D- Dual culture

B. subtilis and 26.0 mm with *P. fluorescens*, while in monoculture 30.5 mm growth was recorded. From third day onwards growth of the pathogen was completely arrested to 25.0 mm by *B. subtilis* and 33.0 mm by *P. fluorescens*. The pathogen took six days to complete 90 mm growth. The antagonist *B. subtilis* gave maximum inhibition of 72.22 per cent over control, but aversion type inhibition was also observed. The *P. fluorescens* gave 63.33 per cent inhibition over control (Plate 8).

4.4.2.3 *In vitro* evaluation of bacterial antagonists against *F. oxysporum*

The antagonistic efficiency of *B. subtilis* and *P. fluorescens* were evaluated against *F. oxysporum*. The results are presented in Table 10.

Both the antagonists inhibited the growth of pathogen in dual culture. The initial growth of the pathogen in monoculture as well as in dual culture was found to be almost uniform on the first day after inoculation. But on second day *F. oxysporum* have 21.0 mm with *P. fluorescens* and 24.0 mm with *B. subtilis* and the pathogen growth was enhanaced upto 24.0 mm with *P. fluorescens* and 28.0 mm with *B. subtilis*, thereafter the growth of pathogen seem to be arrested in both the dual cultures. The antagonist *P. fluorescens* recorded maximum inhibition of 73.33 per cent, while *B. subtilis* showed 68.88 per cent inhibition over control (Plate 9).

4.4.2.4 *In vitro* evaluation of bacterial antagonists against *R. solani*.

The two bacterial antagonists were evaluated against *R. solani*. The per cent inhibition was recorded and the results are presented in Table 11.

A reduction in growth of *Rhizoctonia* was recorded in dual culture with *B. subtilis*. The growth of the pathogen was low in case of dual culture with *B. subtilis* as compared to monoculture. While the pathogen showed increased growth in dual

Table 10: *In vitro* evaluation of bacterial antagonists against *F. oxysporum*

Antagonists		Colony diameter of *F. oxysporum* (mm)							PIOC (%)
		Days after inoculation							
		1	2	3	4	5	6	7	
Bacillus subtilis (isolated from SMS)	D	13.0	24.0	28.0	28.0	28.0	28.0	28.0	68.88
	M	14.0	35.0	41.0	58.0	69.0	81.0	90.0	
P. fluorescens (reference culture)	D	12.1	21.0	24.0	24.0	24.0	24.0	24.0	73.33
	M	14.0	35.0	41.0	58.0	69.0	81.0	90.0	

PIOC – Per cent inhibition over control

M- Monoculture D- Dual culture

Plate 9: *In vitro* evaluation of bacterial antagonists against *F. oxysporum* (on 2nd day)

P. fluorescens × *F. oxysporum*

B. subtilis × *F. oxysporum*

Table 11: *In vitro* evaluation of bacterial antagonists against *R. solani*

Antagonists		Colony diameter of *R. solani* (mm)				PIOC (%)
		Days after inoculation				
		1	2	3	4	
Bacillus subtilis (isolated from SMS)	D	25.0	30.0	30.0	30.0	66.66
	M	30.0	62.0	80.0	90.0	
P. fluorescens (reference culture)	D	32.6	45.3	80.0	90.0	0.00
	M	30.0	62.0	80.0	90.0	

PIOC – Per cent inhibition over control

M- Monoculture D- Dual culture

culture with *P. fluorescens* even on the first day after inoculation. The same trend was observed on second day also.

From third day onwards, the growth of the pathogen was arrested (30.0 mm) in the dual culture with *B. subtilis*, while the pathogen over grew *P. fluorescens*. But in dual culture, aversion type of inhibition was noticed with *B. subtilis*. Pathogen attained 90.0 mm in monoculture on fourth day as well as dual culture with *P. fluorescens*. The antagonist *B. subtilis* gave 66.66 per cent inhibition over control, while *P. fluorescens* has no effect in suppressing the pathogen (Plate 10).

4.4.2.5 *In vitro* evaluation of bacterial antagonists against *S. rolfsii*

The bacterial antagonists were evaluated against *S. rolfsii*. The per cent inhibition was calculated and the results are presented in Table 12.

There was a reduction in growth of *S. rolfsii* in dual culture with bacterial antagonists on the first day after inoculation. From second day onwards the growth of the pathogen in the dual culture with *B. subtilis* was arrested whereas the growth of the pathogen with *P. fluorescens* showed an over growth over the antagonist. The pathogen attained 90.0 mm growth on fourth day after inoculation in dual culture with *P. fluorescens* as in monoculture. The antagonist *B. subtilis* recorded 77.77 per cent inhibition on the growth of pathogen, whereas *P. fluorescens* did not show any effect on pathogen growth (Plate 11).

4.4.3 *In vitro* evaluation of fungal antagonists against *R. solanacearum*

The bacterial pathogen was evaluated under *in vitro* condition against *T. hamatum*, *T. viride* (SMS) and *T. viride* (reference culture). The per cent inhibition was calculated and the results are presented in Table 13. All the three fungal

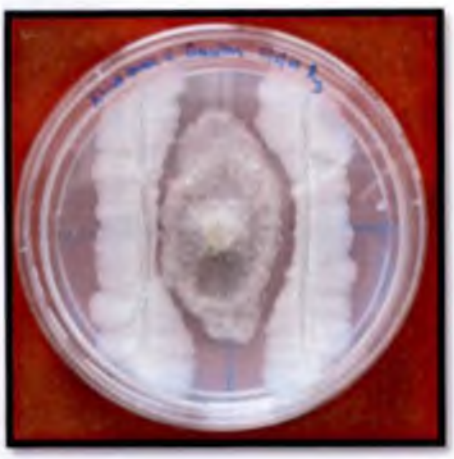
Table 12: *In vitro* evaluation of bacterial antagonists against *S. rolfsii*

Antagonists		Colony diameter of *S. rolfsii* (mm)				PIOC (%)
		Days after inoculation				
		1	2	3	4	
Bacillus subtilis (isolated from SMS)	D	16.6	20.0	20.0	20.0	77.77
	M	30.0	72.0	80.0	90.0	
P. fluorescens (reference culture)	D	25.3	50.3.0	80.0	90.0	0.00
	M	30.0	72.0	80.0	90.0	

PIOC – Per cent inhibition Over Control

M- Monoculture D- Dual culture

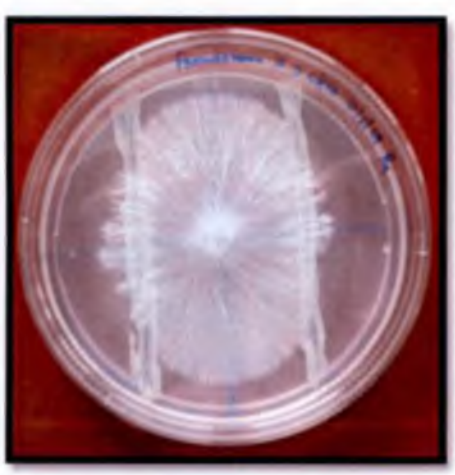
Plate 10: *In vitro* evaluation of bacterial antagonists against *R. solani* (on 2nd day)



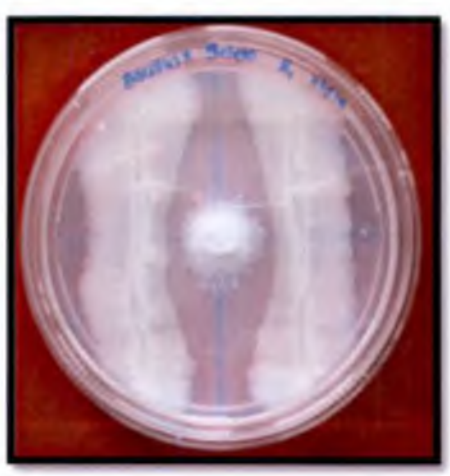
B.subtilis × *R. solani*

P. fluorescens × *R. solani*

Plate 11: *In vitro* evaluation of bacterial antagonists against *S. rolfsii* (on 2nd day)



P.fluorescens × *S. rolfsii*



B.subtilis × *S.rolfsii*

Table 13: *In vitro* evaluation of fungal antagonists against *R. solanacearum*

Antagonists		Colony diameter of fungal antagonists (mm)				PIOC (%)
		Days after inoculation				
		1	2	3	4	
T. viride (isolated from SMS)	D	11.3	22.3	53.3	90.0	100
	M	22.3	50.3	75.3	90.0	
T. hamatum (isolated from SMS)	D	12.0	40.3	64.3	90.0	100
	M	22.3	55.0	82.3	90.0	
T. viride (reference culture)	D	12.3	38.0	51.6	90.0	100
	M	20.0	50.6	74.3	90.0	

PIOC – Per cent inhibition over control D – Dual culture M – Monoculture

antagonists were found to be effective against *R. solanacearum*. In all the dual cultures it gave 100 per cent inhibition over pathogen. The fungal antagonist *T. hamatum* sporulated over the entire pathogen and light yellow coloured metabolite production was also observed. *T. viride* (SMS) also recorded 100 per cent inhibition and sporulated over the entire pathogen. A prominent yellow coloured metabolite production was noticed. In case of *T. viride* (reference culture) also, it sporulated over the entire pathogen and recorded 100 per cent inhibition. The sporulation was uniform and an yellow coloured metabolite production was noticed. Among the three, *T. hamatum* recorded maximum growth on third day after inoculation (Plate 12).

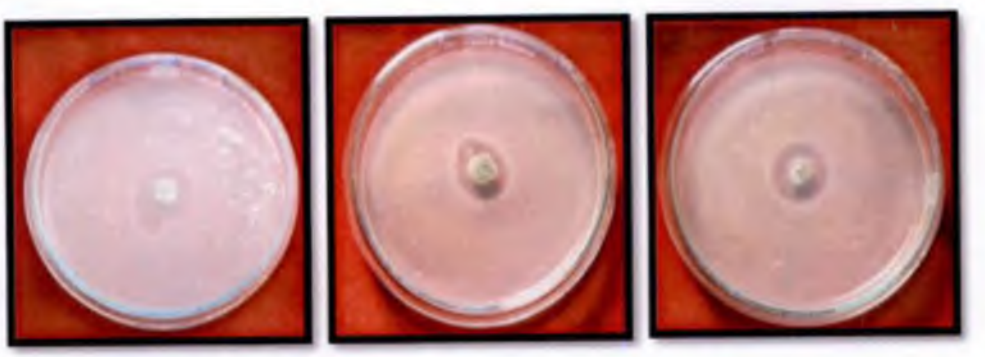
4.4.4 *In vitro* evaluation of bacterial antagonists against *R. solanacearum*

The bacterial antagonists *viz. B. subtilis* and *P. fluorescens* were evaluated against *R. solanacearum*. The reference culture *P. fluorescens* produced maximum inhibition zone of 31.3 mm, while *B. subtilis* produced an inhibition zone of 22 mm. The maximum inhibition was achieved with *P. fluorescens* (Plate 13).

4.5 MUTUAL COMPATIBILITY OF SELECTED ANTAGONISTS

Compatibility studies were conducted to find out the best compatible fungal and bacterial combination of antagonists. Among the bacterial antagonists *P. fluorescens* was found to be compatible with all the three fungal antagonists *viz. T. hamatum, T. viride* (SMS) and *T. viride* (reference culture). In the case of *B. subtilis* a reduction in the growth of all three fungal antagonists were noticed on first day itself. Whereas with *P. fluorescens* the growth pattern of fungal antagonists was same as in monoculture. Mutual inhibition of all the three fungal antagonists and *B. subtilis* was noticed on second day after inoculation.

Plate 12: *In vitro* evaluation of fungal antagonists against *R.solanacearum* (on 2nd day)



T.hamatum | *T.viride* | *T.viride* (Reference culture)

Plate 13: *In vitro* evaluation of bacterial antagonists against *R.solanacearum* (on 2nd day)

R. solanacearum × *P. fluorescens* | *R. solanacearum* × *B.subtilis*

Metabolite production was also noticed in dual culture of fungal antagonists *viz.*, *T. hamatum* and *T. viride* (SMS) with *B. subtilis*.

A brown coloured metabolite was produced in dual culture of *T. hamatum* with *B. subtilis*, whereas in case of *T. viride* (SMS) it produced a dark brown coloured metabolite. No metabolite production was noticed in dual culture of *T. viride* (reference culture) with *B. subtilis*. But in the bacterial culture *P. fluorescens* (reference culture) the fungal antagonist *T. hamatum* was more compatibile than other two *Trichoderma* isolates. In case of *T. viride* (SMS) and *T. viride* (reference culture), the mycelial growth was slightly thickened with *P. fluorescens* (Plate 14).

Based on *in vitro* studies, *T. hamatum* (from SMS), *P. fluorescens* (reference culture) and their consortium were selected for further studies.

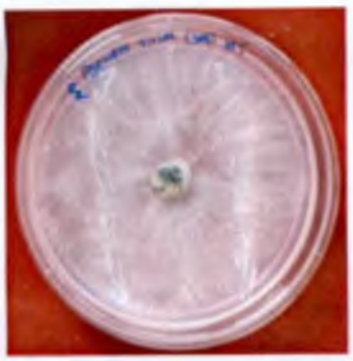
4.6 BIOSOFTENING OF PADDY STRAW SMS

Biosoftening of paddy straw SMS with selected microorganisms were carried out. The SMS was fortified with different selected antagonists *viz. T. hamatum, P. fluorescens*, and microbial consortium of *T. hamatum* and *P. fluorescens*. The raw SMS served as control. The observations like microbial population of treated SMS, visual observations, physico-chemical parameters and nutritional status were analyzed.

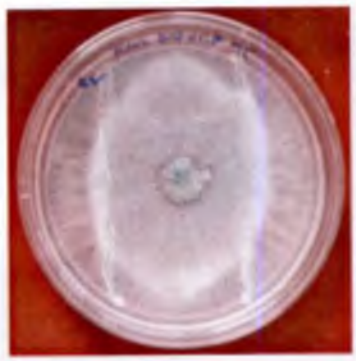
4.6.1 Enumeration of microorganisms at monthly intervals

The microbial population of fortified SMS as well as control was analyzed at monthly intervals from the time of fortification to sixty days after fortification. The microbial population was found to be decreased as the time interval increases.

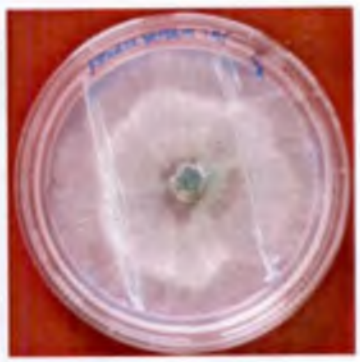
Plate 14: *In vitro* evaluation of mutual compatibility among antagonists (on 2nd day)



P. fluorescens × *T. viride*

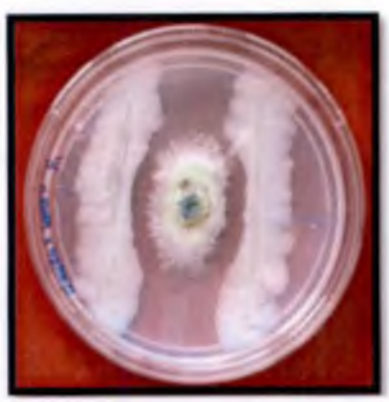


P. fluorescens × *T. viride* (Reference culture)

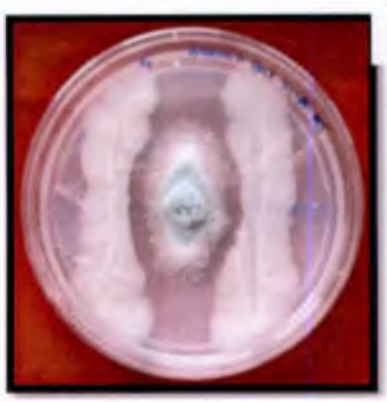


P. fluorescens × *T.hamatum*

B. subtilis × *T. viride*



B. subtilis × *T. hamatum*



B. subtilis × *T. viride* (reference culture)

The microbial population of both fungi and bacteria were maximum at the time of fortification. The results are presented in Table 14.

The fungal population in the treatment of SMS fortified with *T. hamatum* recorded 56.75× 10^7 cfu / g of substrate, which was significantly superior to others. This was followed by SMS fortified with consortium which recorded 34.50× 10^7 cfu/ g of substrate and SMS fortified with *P. fluorescens* giving 25.25×10^7 cfu / g of substrate.

After thirty days of fortification, the microbial population was decreased considerably. In the treatment SMS fortified with *T. hamatum* the fungal population was 32.75× 10^7 cfu/ g of substrate followed by SMS fortified with consortium (15× 10^7 cfu/ g of substrate). In control, the fungal population was 8.75×10^7 cfu/ g of substrate, whereas SMS fortified with *P. fluorescens* recorded a fungal population of 5.75× 10^7 cfu/ g of substrate. There was further decrease in fungal population at sixty days after fortification. The SMS fortified with *T. hamatum* recorded maximum population of 25× 10^7 cfu/ g of substrate was significantly superior and followed by SMS fortified with consortium (6.25× 10^7 cfu/ g of substrate). In control the population was 3.25× 10^7 cfu/ g of substrate, while SMS fortified with *P. fluorescens* recorded 2.75× 10^7 cfu/ g of substrate.

In case of bacterial population the same trend was noticed and the treatment SMS fortified with *P. fluorescens* was significantly superior by giving maximum population at all intervals. The bacterial population was high at immediately after fortification and afterwards it was found to be decreased. At the time of fortification the populataion in the treatment SMS fortified with *P. fluorescens* was 80.75× 10^8 cfu/ g of substrate followed by SMS fortified with consortium which recorded 51×

Table 14: Enumeration of microorganisms from substrate at monthly intervals

Treatments	Fungal population ($\times 10^7$ cfu/g of substrate)			Bacterial population (10^8 cfu/g of substrate)		
	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF
SMS fortified with *P. fluorescens*	25.25^{bc}	5.75^{b}	2.75^{b}	80.75^{a}	59.25^{a}	40.00^{a}
SMS fortified with *P. fluorescens* + *T. hamatum*	34.50^{b}	15.00^{b}	6.25^{b}	51.00^{b}	39.00^{b}	21.50^{b}
SMS fortified with *T. hamatum*	56.75^{a}	32.75^{a}	25.00^{a}	13.50^{c}	1.50^{c}	0.75^{c}
Control (SMS as such)	16.00^{c}	8.75^{b}	3.25^{b}	26.50^{c}	4.75^{c}	1.75^{c}
CV	10.11	19.83	39.24	12.17	15.65	15.78
CD (0.05)	11.11	10.25	7.86	17.44	13.57	8.38

0 DAF: At the time of fortification

30 DAF: Thirty days after fortification

60 DAF: Sixty days after fortification

10^8 cfu/ g of substrate and SMS fortified with *T. hamatum* recorded 13.50× 10^8 cfu/ g of substrate, whereas in control it was 26.50× 10^8 cfu/ g of substrate.

The bacterial population at thirty days after fortification was again declined in all the treatments including control. The treatment SMS fortified with *P. fluorescens* gave 59.25× 10^8 cfu/ g of substrate and SMS fortified with consortium recorded 39× 10^8 cfu/ g of substrate, but in control the population was 4.75 × 10^8 cfu/ g of substrate and SMS fortified with *T. hamatum* gave 1.50× 10^8 cfu/ g of substrate. After sixty days of fortification the treatment SMS fortified with *P. fluorescens* recorded 40× 10^8 cfu/ g of substrate and the treatment of SMS with consortium contains 21.50× 10^8 cfu/ g of substrate, whereas in control the population was 1.75× 10^8 cfu/ g of substrate and SMS fortified with *T. hamatum* recorded 0.75× 10^8 cfu/ g of substrate.

4.6.2 Effect of fortification on visual parameters

The visual observations like changes occurred in colour, texture and volume were recorded. The results are presented in Table 15.

Thirty days after fortification a slight variation in colour was noticed only in the treatment SMS fortified with *P. fluorescens*. The initial golden yellow colour was changed to light brown. The same trend was also observed after sixty days of fortification and there was no change in colour for other treatments during the same period.

Initially in all treatments the texture was found to be rough. But thirty days after fortification the SMS fortified with *P. fluorescens* was softened while no changes were observed in other treatments. But sixty days after fortification, the treatment of fortification of SMS with *T. hamatum* became softened just like

P. fluorescens. While the treatments SMS fortified with consortium and control, a slight variation in texture was noticed.A slight variation in volume of SMS was noticed in all the treatments including control after sixty days of fortification. After thirty days of fortification, a slight reduction in volume was noticed only in the treatment fortified with *P. fluorescens.*

4.6.3 Effect of treatments on physico – chemical parameters

The effect of treatments on physico-chemical parameters *viz.* pH, EC and moisture content were analysed at monthly intervals. The results are presented in Table 16.

The pH at the time of fortification was found to be almost neutral. The SMS fortified with consortium has recorded a pH range of 7.4 followed by SMS fortified with *P. fluorescens* (7.3), 7.0 for SMS treated with *T. hamatum* and in control it was 6.7. But thirty days after fortification, a slight reduction in pH was recorded. SMS fortified with consortium and with *P. fluorescens* were found to be on par with each other. A pH range of 7.0 was recorded for SMS fortified with *T. hamatum.* The control maintained the same pH as that at the initial level. After sixty days of fortification, further slight reduction was noticed. All the treatments except control were on par with each other. In case of control, a slight reduction was noticed (Fig. 1).

The electrical conductivity was recorded at monthly intervals. The results are given in Table 16.

At the time of fortification, SMS fortified with consortium recorded the highest EC value (0.143 dS/m) followed by SMS with *T. hamatum* (0.131 dS/m),

Table 16: Effect of treatments on physico - chemical parameters of SMS at different stages of fortification

Treatments	pH			EC (ds/m)			Moisture content (%)		
	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF
SMS fortified with *P. fluorescens*	7.3^{ab}	7.2^{a}	7.2^{a}	0.111^{bc}	0.110^{bc}	0.090^{a}	53.00^{a}	49.50^{a}	48.50^{a}
SMS fortified with *P. fluorescens* +*T. hamatum*	7.4^{a}	7.3^{a}	7.2^{a}	0.143^{a}	0.139^{a}	0.012^{b}	52.50^{a}	48.50^{a}	48.00^{a}
SMS fortified with *T. hamatum*	7.0^{b}	7.0^{b}	7.0^{a}	0.131^{ab}	0.130^{ab}	0.011^{b}	49.50^{a}	47.50^{a}	46.50^{a}
Control (SMS as such)	6.7^{c}	6.7^{c}	6.6^{b}	0.108^{c}	0.103^{c}	0.090^{a}	40.50^{b}	38.50^{b}	38.50^{b}
CV	1.32	0.87	1.34	6.02	7.28	1.56	4.02	4.61	4.61
CD (0.05)	0.26	0.17	0.26	0.02	0.02	0.02	5.46	5.88	5.80

0 DAF: At the time of fortification

30 DAF: Thirty days after fortification

60 DAF: Sixty days after fortification

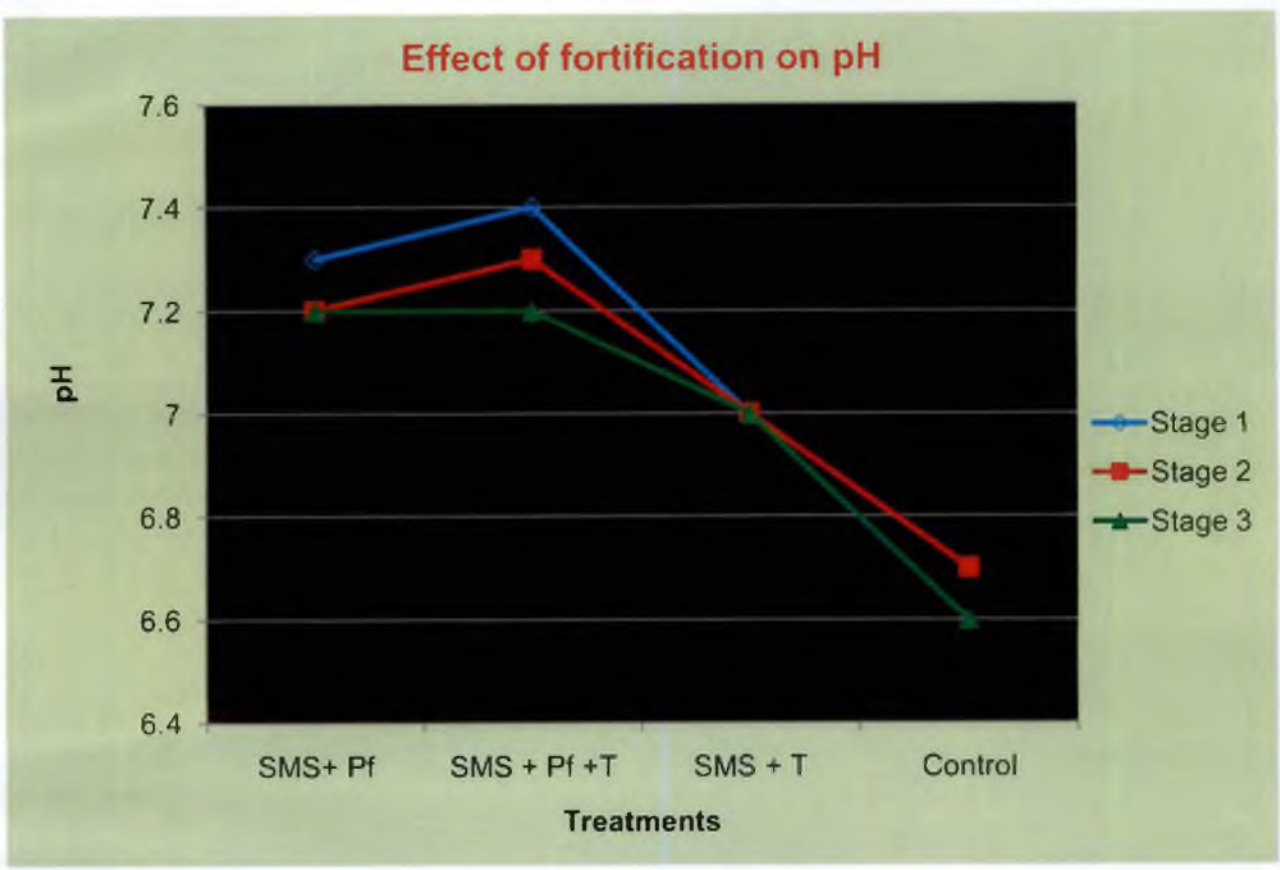


Fig. 1 Effect of fortification on pH

SMS with *P. fluorescens* (0.111 dS/m) and control (0.108 dS/m). Thirty days after fortification, a slight reduction was observed in all the treatments including control. SMS fortified with consortium recorded an EC of 0.139 dS/m, followed by SMS with *T. hamatum* (0.130 dS/m), SMS with *P. fluorescens* (0.110 dS/m) and control (0.103 dS/m). After sixty days of fortification, a gradual reduction in EC level was observed.

The treatment SMS fortified with *P.fluorescens* and control recorded an EC of 0.090 dS/m and were found to be on par, while SMS fortified with consortium and with *T.hamatum* recorded an EC of 0.012 dS/m and 0.011 dS/m respectively and were on par (Fig. 2).

The initial moisture level was found to be uniform in all the treatments except in control (40.50%). The same trend was also noticed at thirty as well sixty days after fortification. A reduction in the moisture level was observed at monthly intervals. Sixty days after fortification, a moisture content of 48.50 per cent, 48.00 per cent, 46.50 per cent and 38.50 per cent were recorded in SMS with *P. fluorescens,* SMS with consortium, SMS with *T. hamatum* and control, respectively (Fig. 3).

4.7 EFFECT OF BIOSOFTENING ON NUTRIENT STATUS OF SMS

The SMS was fortified with *T. hamatum, P. fluorescens* and consortium of *T. hamatum* and *P. fluorescens*. The nutrient content of each treatment was analyzed at zero, thirty and sixty days after fortification.

4.7.1 Effect of biosoftening on primary nutrient content

The primary nutrients like Carbon, Nitrogen, Phosphorus and Potassium were analyzed and C:N ratio was calculated. The results are presented in Table 17.

Carbon content:

The 'C' content of all the treatments was analyzed at zero, thirty and sixty days after fortification intervals. At each month, 'C' content was found to be increased in all the treatments. At the time of fortification the C content was 24.73 per cent, 24.68 per cent

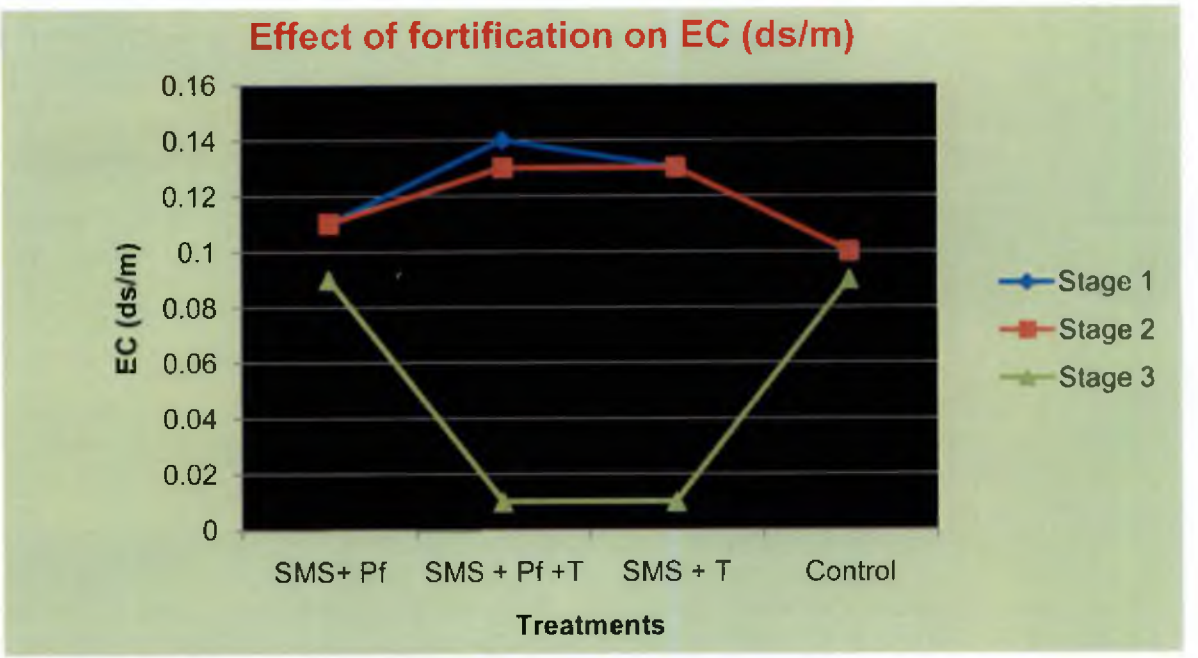


Fig. 2 Effect of fortification on EC (ds/m)

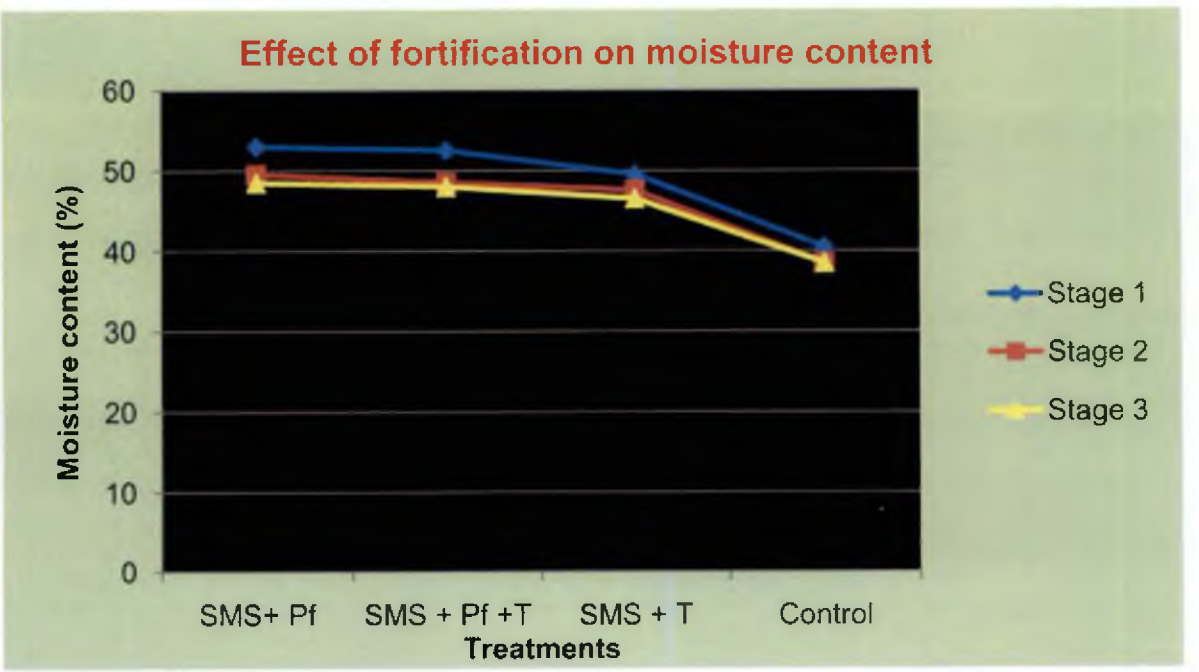


Fig. 3 Effect of fortification on moisture content

Table 17: The contents of primary nutrients of SMS at different stages of fortification

Treatments	C (%)			N (%)			P (%)			K (%)			C:N ratio		
	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF
SMS fortified with *P. fluorescens*	22.59[b]	28.00	33.50	1.20[b]	1.05	0.81	0.90[a]	0.78[a]	0.66[a]	1.23[b]	1.05[b]	0.95	18.82	26.66	41.11
SMS fortified with *P. fluorescens* + *T. hamatum*	24.73[a]	26.55	33.50	2.00[a]	1.35	0.75	0.81[a]	0.56[b]	0.66[a]	1.72[a]	1.68[a]	1.37	12.36	19.66	44.66
SMS fortified with *T. hamatum*	24.68[a]	27.63	30.50	1.85[a]	1.05	0.58	0.58[b]	0.47[bc]	0.42[b]	1.59[a]	1.52[a]	1.18	13.34	26.31	52.58
Control (SMS as such)	21.73[b]	28.50	35.50	1.30[b]	1.04	0.76	0.44[b]	0.34[c]	0.34[b]	1.27[b]	1.06[b]	1.00	16.71	27.40	46.71
CV	2.74	3.22	7.05	8.03	14.24	15.85	9.48	10.43	9.32	0.31	6.71	11.17			
CD (0.05)	1.78	NS	NS	0.35	NS	NS	0.18	0.15	0.13	7.78	0.24	NS			

0 DAF: At the time of fortification

30 DAF: Thirty days after fortification

60 DAF: Sixty days after fortification

NS: Non significant

22.59 per cent and 21.73 per cent for SMS with consortium, SMS with *T. hamatum,* SMS with *P. fluorescens* and control respectively. There was no significant difference among the treatments on thirty days and sixty days after fortification.

Nitrogen content:

The nitrogen content of the samples was analyzed at zero, thirty and sixty days after fortification. SMS fortified with consortium recorded a maximum of 2.00 per cent, followed by 1.85 per cent in SMS with *T. hamatum,* 1.30 per cent in control and 1.20 per cent in SMS with *P. fluorescens.*

The 'N' content was found to be decreasing over advanced stages of fortification. However, there was no significant difference in 'N' content among the treatments at thirty and sixty days after fortification. In the final stage, SMS fortified with *P. fluorescens* recorded maximum 'N' (0.81 per cent) followed by control (0.76 per cent), 0.75 per cent in SMS with consortium and 0.58 per cent in SMS with *T. hamatum.*

Phosphorus content:

The 'P' content was analyzed at zero, thirty and sixty days after fortification and was found to be decreased at advanced stages. The highest 'P' content was recorded in SMS treated with *P. fluorescens* at initial and thirty days after fortification. Initially, 0.90 per cent of P, 0.78 per cent at thirty days after fortification and 0.66 per cent at sixty days after fortification were recorded. The 'P' content at sixty days after fortification was 0.66 per cent, 0.66 per cent, 0.42 per cent and 0.34 per cent in SMS with consortium, SMS with *P. fluorescens,* SMS with *T. hamatum* and control, respectively.

Potassium content:

The 'K' content of the different treatments was analyzed. It was found to be decreasing at monthly intervals. Maximum 'K' content was recorded in SMS treated with consortium in all the stages. The lowest 'K' content was recorded in SMS with *P. fluorescens.*

C:N ratio:

The C:N ratio of the SMS was analyzed. At the time of fortification, the lowest C:N ratio recorded in the treatment SMS with consortium (12.36 per cent), the maximum C:N ratio was recorded in SMS with *P. fluorescens* (18.82 per cent) and at each stage C:N ratio was found to be increased.

4.7.2 Effect of biosoftening on secondary nutrient content

The secondary nutrients *viz.* Calcium and Magnesium content were analyzed at zero, thirty and sixty days after fortification. The results are presented in Table 18.

Calcium content:

All the treatments were found to be significantly different. A slight reduction in Ca content was observed in all the treatments at sixty days after fortification. The highest Ca content was recorded in control in all the stages, 73.17 mg kg^{-1}, at zero and thirty days after fortification and at sixty days after fortification it was 73.02 mg kg^{-1}. SMS fortified with *P. fluorescens* was found to be on par with the control. The least Ca content was recorded in SMS fortified with *T. hamatum*, at zero and thirty days after fortification. The Ca content at zero and thirty days after fortification was 61.87 mg kg^{-1} and at sixty days after fortification it was 61.67 mg kg^{-1}.

Table 18: The contents of secondary nutrients of SMS at different stages of fortification

Treatments	Ca (mg kg^{-1})			Mg (mg kg^{-1})		
	0 DAF	**30 DAF**	**60 DAF**	**0 DAF**	**30 DAF**	**60 DAF**
SMS fortified with *P. fluorescens*	71.49[a]	71.49[a]	71.17[a]	4.69	4.72	4.76
SMS fortified with *P. fluorescens* + *T. hamatum*	64.95[b]	64.95[b]	64.91[b]	4.46	4.51	4.53
SMS fortified with *T. hamatum*	61.87[c]	61.87[c]	61.67[c]	4.76	4.79	4.78
Control (SMS as such)	73.17[a]	73.17[a]	73.02[a]	4.67	4.72	4.70
CV	1.27	1.27	1.49	4.96	4.77	4.89
CD (0.05)	2.39	2.39	2.81	NS	NS	NS

0 DAF: At the time of fortification

30 DAF: Thirty days after fortification

60 DAF: Sixty days after fortification

NS: Non significant

Magnesium content:

There is no significant variation in Mg content among the treatments due to fortification. However maximum Mg content of 4.76 mg kg^{-1} was recorded in SMS fortified with *T. hamatum* at the time of fortification. The Mg content was 4.79 mg kg^{-1} and 4.78 mg kg^{-1} at thirty days after fortification and sixty days after fortification respectively.

4.7.3 Effect of biosoftening on micro nutrient content

The micro nutrients *viz*. Zinc, Iron, Magnanese and Copper were analyzed at zero, thirty and sixty days after fortification. The results are presented in Table 19.

Zinc content:

The 'Zn' content of all the treatments was found to be increased at zero, thirty and sixty days after fortification. The maximum 'Zn' content was recorded in SMS with *T. hamatum* at the time of fortification (95.65 mg kg^{-1}), thirty days after fortification (96.60 mg kg^{-1}) and sixty days after fortification (97.46 mg kg^{-1}).

The 'Zn' content of SMS with consortium was 88.75 mg kg^{-1} at the time of fortification, 89.60 mg kg^{-1} thirty days after fortification and 90.04 mg kg^{-1} sixty days after fortification. The least 'Zn' content was recorded in SMS with *P. fluorescens i.e.*, 72.95 mg kg^{-1} at the time of fortification, 73.61 mg kg^{-1} at thirty days after fortification and 74.23 mg kg^{-1} at sixty days after fortification.

Iron content:

A slight increase in 'Fe' content was observed in all the treatments. There was no significant difference among the treatments.

Table 19: The contents of micro nutrients of SMS at different stages of fortification

Treatments	Zn (mg kg^{-1})			Fe (mg kg^{-1})			Mn (mg kg^{-1})			Cu (mg kg^{-1})		
	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF	0 DAF	30 DAF	60 DAF
SMS fortified with *P. fluorescens*	72.95^{d}	73.61^{c}	74.23^{c}	592.45	593.20	593.70	1.54	1.82	1.92	5.50^{a}	4.50	3.85
SMS fortified with *P. fluorescens* + *T. hamatum*	88.75^{b}	89.60^{b}	90.04^{b}	594.90	595.85	596.60	1.64	1.90	2.04	6.25^{a}	5.20	4.35
SMS fortified with *T. hamatum*	95.65^{a}	96.60^{a}	97.46^{a}	596.50	597.15	597.95	1.53	1.82	1.95	4.40^{b}	3.85	3.00
Control (SMS as such)	85.55^{c}	86.55^{b}	86.97^{b}	594.00	594.70	595.50	1.54	1.83	2.04	4.45^{b}	4.00	3.15
CV	2.79	1.44	1.35	0.27	0.27	0.28	2.80	1.52	3.16	5.57	9.29	12.42
CD (0.05)	1.17	3.48	3.27	NS	NS	NS	NS	NS	NS	0.79	NS	NS

0 DAF: At the time of fortification

30 DAF: Thirty days after fortification

60 DAF: Sixty days after fortification

NS: Non significant

Sixty days after fortification, maximum 'Fe' content of 597.95 mg kg^{-1} was recorded in SMS with *T. hamatum,* followed by SMS with consortium (596.60 mg kg^{-1}) and control (595.50 mg kg^{-1}). The least 'Fe' content was recorded in SMS with *P. fluorescens* (593.70 mg kg^{-1})

Manganese content

There was no significant difference in the Mn content observed among treatments at all the stages. However, SMS treated with consortium as well as control recorded maximum Mn content. A slight increase in Mn content was observed in all the treatments. The consortium recorded 1.64 mg kg^{-1}, 1.90 mg kg^{-1} and 2.04 mg kg^{-1}, at the time of fortification, thirty days after fortification and sixty days after fortification respectively. While, control recorded 1.54 mg kg^{-1}, 1.83 mg kg^{-1} and 2.04 mg kg^{-1} at the time of fortification, thirty days after fortification and sixty days after fortification respectively.

Copper content

The treatments were found to be significantly different at the time of fortification. A slight decrease in Cu content was recorded in all the treatments at advanced stages. At the time of fortification SMS treated with consortium recorded maximum Cu content (6.25 mg kg^{-1}). The same trend was noticed at thirty days and sixty days after fortification.

4.8 EFFECT OF FORTIFIED SMS AGAINST SOIL BORNE DISEASES OF TOMATO

The potential of fortified SMS for the management of soil borne diseases of tomato *viz.* damping off in nursery and bacterial wilt were evaluated under two pot culture experiments. Since the selected efficient bacterial antagonist is *P. fluorescens* (reference culture), the number of treatments in pot culture experiments were eleven

and twelve for the management of damping off disease and management of bacterial wilt disease of tomato respectively.

4.8.1 Management of damping off of tomato (Experiment 1)

All the treatments were applied at the time of sowing. The effect of treatments on per cent germination was recorded at different intervals like 7, 14 and 21 days after sowing. Biometric observations and disease incidence were recorded at 45 days after sowing. The results are detailed below.

4.8.1.1 Effect of treatments on per cent germination

The per cent germination was recorded at 7, 14 and 21 days after sowing. The results are presented in Table 20.

The germination per cent varied considerably at different intervals. At 7 days after sowing significant difference was not observed between treatments. However highest germination per cent was recorded in T5 (potting mixture + soil drenching with *P. fluorescens*, 38.22 per cent) followed by T4 (potting mixture + soil drenching with *T. hamatum*, 37.33 per cent) and T6 (potting mixture + soil drenching with consortium, 37.33 per cent). At 14 days after sowing also, all treatments were on par with maximum germination in T5 (potting mixture + soil drenching with *P. fluorescens*, 54.21 per cent) followed by T6 ((potting mixture + soil drenching with consortium, 52.44 per cent) and T9 (potting mixture + SMS, 52.44 per cent). But at 21 days after sowing a considerable variation was observed between treatments on germination per cent. The treatment T3 (potting mixture + SMS fortified with consortium) was significantly superior to all other treatments in giving highest seed germination (83.99 per cent), followed by T2 (potting mixture + SMS fortified with

P. fluorescens, 75.99 per cent) and T4 ((potting mixture + soil drenching with *T. hamatum*, 72.88 per cent). The lowest germination per cent was recorded in absolute control T11 (44.44 per cent). All the treatments *viz.* T1 (potting mixture + SMS fortified with *T. hamatum*), T4 (potting mixture + soil drenching with *T. hamatum*) and T9 (potting mixture + SMS) were on par with the best treatment T2 (potting mixture + SMS fortified with *P. fluorescens*) (Fig. 4).

4.8.1.2 Effect of treatments on per cent disease incidence of tomato damping off caused by *P. apahanidermatum*

The challenge inoculation of *P. aphanidermatum* was given at 21 days after sowing. The per cent disease incidence for each treatment was recorded separately upto 45 days after sowing. All the treatments were found to be significantly different statistically. The results are presented in Table 21.

The treatment T3 (potting mixture + SMS fortified with consortium) was found to be significantly superior, with lowest disease incidence (23.30 per cent) followed by T6 (potting mixture + soil drenching with consortium), T5 (potting mixture + soil drenching with *P. fluorescens*), T7 (potting mixture + soil drenching with *T. viride* (reference culture)) and T4 (potting mixture + soil drenching with *T. hamatum*) and recorded 27.33, 34.25, 35.17 and 35.25 per cent disease incidence respectively. The treatments T1 (potting mixture + SMS fortified with *T. hamatum*) and T2 (potting mixture + SMS fortified with *P. fluorescens*) were on par. The treatment T10 (control) found to be significantly inferior to other treatments, which recorded highest disease incidence (68.27 per cent) (Fig.5).

Table 21: Effect of treatments on per cent disease incidence in the management of damping off

Treatments	Treatment details	Per cent disease incidence
T1	PM amended with 50% fortified SMS with *T. hamatum*	36.37 (-0.56)^{bcd}
T2	PM amended with 50% fortified SMS with *P. fluorescens*	35.77 (-0.59)^{bcd}
T3	PM amended with 50% fortified SMS with *T. hamatum*+*P. fluorescens*	23.30 (-1.22)^{d}
T4	PM + soil drenching of *T. hamatum*	35.25 (-0.61)^{cd}
T5	PM + soil drenching of *P. fluorescens*	34.25 (-0.65)^{cd}
T6	PM + soil drenching of *T. hamatum*+*P. fluorescens*	27.33 (-1.01)^{cd}
T7	PM +soil drenching with Reference culture *T. viride*	35.17 (-0.61)^{cd}
T8	PM +fungicide drenching using copper hydroxide (2 g/lit)	40.06 (-0.40)^{bc}
T9	PM +50 % of raw SMS	38.73(-0.47)^{bc}
T10	PM alone (control)	68.27 (0.79)^{a}
T11	Absolute control	0.00 *
	CV	20.72
	CD(0.05)	13.31

* Not included in statistical analysis

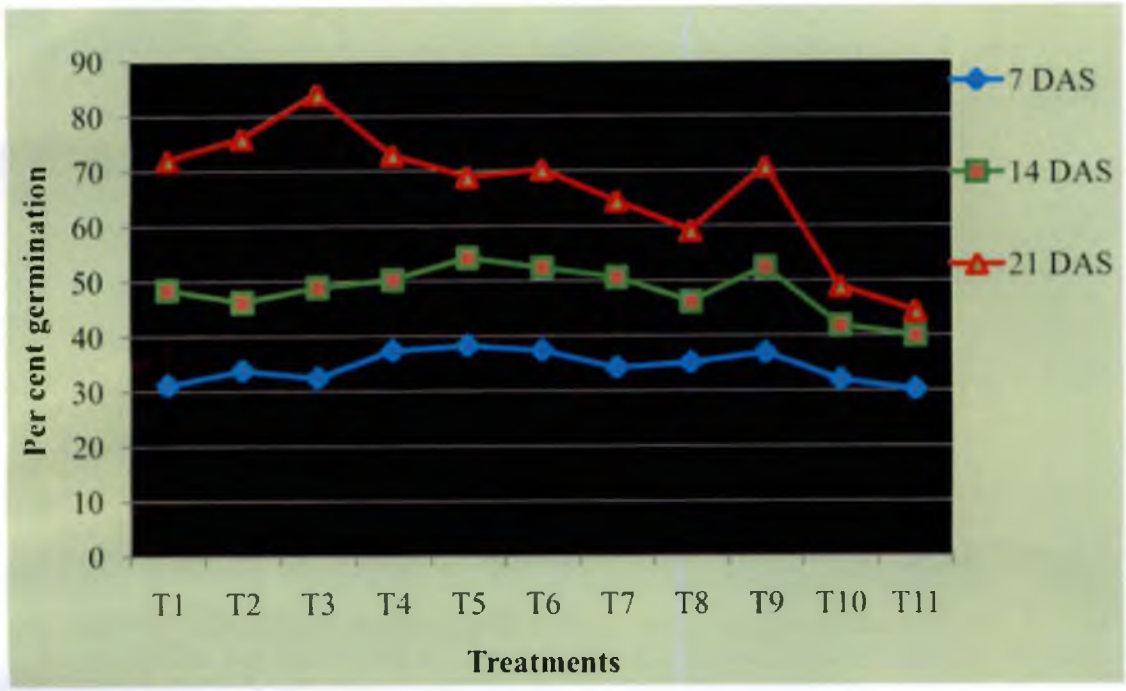


Fig. 4 Effect of fortified SMS on seed germination

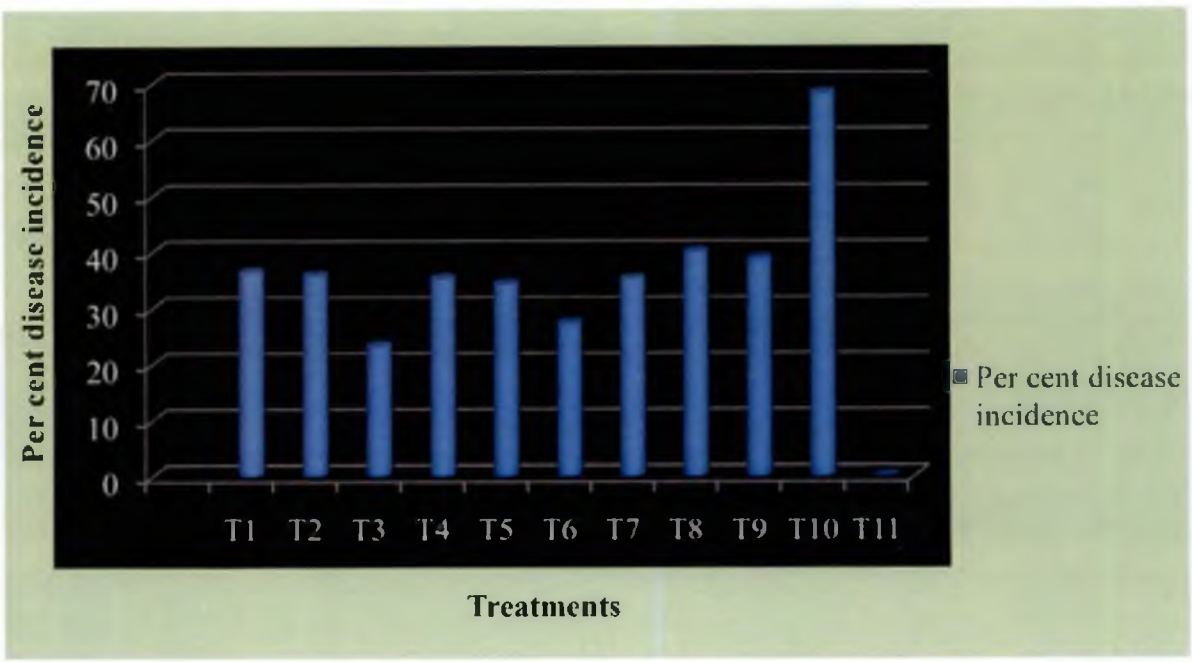


Fig. 5 Effect of treatments on per cent disease incidence of tomato damping off

4.8.1.3 Effect of treatments on biometric characters

The effect of treatments on biometric characters like root length, shoot length and plant vigour index were observed at 45 days after sowing. The results are presented in Table 22 (Plate 15).

Root length:

The treatments were found to be significantly different and T6 (potting mixture + soil drenching with consortium) was significantly superior to all other treatments in giving maximum root length (15.77 cm) followed by T9 (potting mixture + SMS, 12.70 cm). The treatments T5 (potting mixture + soil drenching with *P. fluorescens*) and T7 (potting mixture + soil drenching with *T. viride* (reference culture)) were on par. The lowest root length was recorded by T11 absolute control (4.28 cm).

Shoot length:

The observations on shoot length of seedlings were taken on 45 days after sowing. All the treatments were found to be significantly different. Among the treatments T6 (potting mixture + soil drenching with consortium) with a shoot length of 35.72 cm, T5 (potting mixture + soil drenching with *P. fluorescens*) with 35.39 cm and T7 (potting mixture +soil drenching with *T. viride* (reference culture) with 35.25 cm were found to be superior to other treatments. The treatments T2 (28.00 cm), T9 (27.73 cm), T8 (27.43 cm) and T1 (27.17 cm) were on par. The lowest shoot length of 10.38 cm was recorded in T11 (absolute control).

Table 22: Effect of treatments on biometric characters of plants in the management of tomato damping off

Treatments	Treatment details	Mean root length (cm)	Mean shoot length (cm)	Plant vigour index (PVI) †
T1	PM amended with 50% fortified SMS with *T. hamatum*	9.007^{f}	27.17^{d}	2604
T2	PM amended with 50% fortified SMS with *P.fluorescens*	8.76^{f}	28.00^{d}	2795
T3	PM amended with 50% fortified SMS with *T. hamatum*+*P. fluorescens*	11.19^{cd}	30.92^{c}	3538
T4	PM + soil drenching of *T. hamatum*	10.72^{d}	33.51^{b}	3224
T5	PM + soil drenching of *P. fluorescens*	11.50^{c}	35.39^{a}	3231
T6	PM + soil drenching of *T. hamatum*+*P. fluorescens*	15.77^{a}	35.72^{a}	3617
T7	PM +soil drenching with Reference culture *T. viride*	11.44^{c}	35.25^{a}	3009
T8	PM +fungicide drenching using copper hydroxide (2 g/lit)	8.84^{f}	27.43^{d}	2145
T9	PM +50 % of raw SMS	12.70^{b}	27.73^{d}	2857
T10	PM alone (control)	4.57^{h}	11.80^{f}	801
T11	Absolute control	4.28^{h}	10.38^{g}	652
CV		3.15	2.67	
CD (0.05)		0.50	1.20	

† Not included in statistical analysis

Plate 15: Effect of treatments on biometric characters in the management of damping off

Plant vigour index:

The plant vigour index was also calculated at 45 days after sowing. Among the treatments T6 (potting mixture + soil drenching with consortium, 3617) gave maximum plant vigour index followed by T3 (potting mixture + SMS fortified with consortium, 3538), T5 (potting mixture + soil drenching with *P. fluorescens*, 3231), T4 (potting mixture + soil drenching with *T. hamatum*, 3224) and T7 (potting mixture + soil drenching with *T. viride* (reference culture), 3009). The minimum plant vigour index was recorded in T11 (absolute control, 652) (plate 16).

4.8.2 Management of bacterial wilt of tomato (Experiment 2)

A pot culture experiment was conducted using 30 days old seedlings. The observations *viz.* shoot length, root length, fresh weight of shoot and root, per cent disease incidence and yield were recorded.

4.8.2.1 Effect of treatments on biometric characters in the management of bacterial wilt

The biometric parameters *viz.* shoot length, root length, fresh weight of root and shoot and yield were recorded.

Shoot length:

The shoot length was recorded at 30, 45 and 60 days after transplanting. The results are presented in Table 23.

At 30 days after transplanting significant difference in shoot length was observed among treatments. The treatment T3 (potting mixture + SMS fortified with consortium, 37.11 cm) found to be significantly superior to others followed by T9 (potting mixture + SMS, 36. 58 cm). There was no significant difference among the

Plate 16: Comparison of SMS treatments with control in the management of damping off

Table 23: Effect of treatments on shoot length in the management of bacterial wilt

Treatments	Treatment details	Shoot length (cm)		
		30 DAT	45 DAT	60 DAT
T1	PM amended with 50% fortified SMS with *T. hamatum*	33.50^{abc}	60.58	79.16^{ab}
T2	PM amended with 50% fortified SMS with *P. fluorescens*	31.75^{abcde}	51.91	80.87^{ab}
T3	PM amended with 50% fortified SMS with *T.hamatum+P. fluorescens*	37.11^{a}	53.85	88.25^{a}
T4	PM + soil drenching of *T. hamatum*	32.58^{abcd}	52.83	70.69^{bcd}
T5	PM + soil drenching of *P. fluorescens*	29.41^{abcde}	44.58	76.50^{abc}
T6	PM + soil drenching of *T. hamatum+P. fluorescens*	30.33^{abcde}	52.58	77.41^{ab}
T7	PM +soil drenching with Reference culture *T. viride*	25.75^{bcde}	49.66	72.91^{abcd}
T8	PM +fungicide drenching using copper hydroxide (2 g/lit)	31.00^{abcde}	41.91	72.16^{abcd}
T9	PM +50 % of raw SMS	36.58^{ab}	67.16	79.75^{ab}
T10	PM alone (control)	22.16^{de}	45.50	60.16^{cd}
T11	Absolute control	21.50^{e}	33.66	59.58^{cd}
T12	Control (with POP recommendation)	21.83^{de}	41.25	72.41^{abcd}
CV		22.31	24.70	13.90
CD(0.05)		10.84	NS	17.01

DAT- Days after transplanting

treatments T1, T2 and T8. The least shoot length was recorded in T11 (absolute control, 21.50 cm).

At 45 days after transplanting no significant differences were found among the treatments. However the treatment T9 (potting mixture + SMS) recorded maximum shoot length of 67.16 cm, followed by the treatments T1 (potting mixture + SMS fortified with *T. hamatum*, 60.58 cm) then T3 (potting mixture + SMS fortified with consortium, 53.85 cm). The treatment T11 (absolute control) recorded the lowest shoot length of 33.66 cm.

But 60 days after transplanting, significant difference in shoot length was obtained among treatments. The treatment T3 (potting mixture + SMS fortified with consortium) was found to be superior to other treatments and recorded a shoot length of 88.25 cm, followed by T2 (potting mixture + SMS fortified with *P. fluorescens*) with 80.87 cm, T9 (potting mixture + SMS) with 79.75 cm and T1 (potting mixture + SMS fortified with *T. hamatum*) with79.16 cm. The lowest shoot length of 59.58 cm was recorded in T11 (absolute control).

Root length:

The root length was recorded after harvest and the results are presented in Table 24. A significant difference among treatments on root length was observed. The result revealed that treatment T6 (potting mixture + soil drenching with consortium, 31.22 cm) and T2 (potting mixture + SMS fortified with *P. fluorescens*, 31.16 cm) were found to be significantly superior. The treatments T9 (potting mixture + SMS, 30.88 cm), T1 (potting mixture + SMS fortified with *T. hamatum*, 30.66 cm) and T3 (potting mixture + SMS fortified with consortium, 29.88 cm) were on par. The lowest root length was recorded in T10 (control, 23.44 cm).

Table 24: Effect of treatments on plant biometric characters in the management of bacterial wilt

Treatments	Treatment details	Root length (cm)	Fresh weight of root (g)	Fresh weight of shoot (g)	Yield (g/ plant)
T1	PM amended with 50% fortified SMS with *T. hamatum*	30.66^{ab}	7.33^{bcd}	82.33^{bcde}	178.33^{abcd}
T2	PM amended with 50% fortified SMS with *P. fluorescens*	31.16^{a}	9.00^{ab}	118.00^{a}	239.66^{ab}
T3	PM amended with 50% fortified SMS with *T. hamatum+P. fluorescens*	29.88^{ab}	7.33^{bcd}	111.33^{ab}	223.00^{ab}
T4	PM + soil drenching of *T. hamatum*	27.16^{c}	6.33^{cd}	58.66^{ef}	112.0^{bcde}
T5	PM + soil drenching of *P. fluorescens*	29.44^{abc}	5.66^{d}	67.33^{def}	61.33^{cde}
T6	PM + soil drenching of *T. hamatum+P. fluorescens*	31.22^{a}	7.00^{bcd}	103.00^{abc}	177.00^{abcd}
T7	PM +soil drenching with Reference culture *T. viride*	28.99^{abc}	7.66^{bcd}	61.33^{ef}	126.66^{bcde}
T8	PM +fungicide drenching using copper hydroxide (2 g/lit)	27.22^{c}	8.00^{bc}	84.00^{bcde}	235.33^{ab}
T9	PM +50 % of raw SMS	30.88^{ab}	10.33^{a}	92.33^{abcd}	272.66^{a}
T10	PM alone (control)	23.44^{d}	5.66^{d}	48.00^{f}	35.00^{e}
T11	Absolute control	24.42^{d}	6.00^{cd}	58.00^{ef}	56.66^{de}
T12	Control (with POP recommendation)	29.16^{abc}	6.33^{cd}	75.00^{cdef}	193.33^{abc}
CV		4.75	16.83	22.67	52.36
CD(0.05)		2.28	2.02	29.93	134.46

Fresh weight of roots:

The results of effect of treatments on fresh weight of roots are presented in Table 24. The treatments were found to be significantly different and the treatment T9 (potting mixture + SMS) was superior to other treatments and recorded a fresh weight of 10.33 g followed by T2 (potting mixture + SMS fortified with *P. fluorescens,* 9.00 g). The treatments T7 (potting mixture + soil drenching with *T. viride* (reference culture), 7.66 g), T1 (potting mixture + SMS fortified with *T. hamatum*, 7.33 g), T3 (potting mixture + SMS fortified with consortium, 7.33 g) and T6 (potting mixture + soil drenching with consortium, 7.0 g) were on par. The least root weight was recorded in T10 (control, 5.66 g).

Fresh weight of shoots:

The results on effect of treatments on fresh weight of shoots are presented in Table 24. The treatments were significantly different. Among the treatments, T2 (potting mixture + SMS fortified with *P. fluorescens*, 118.0 g) was significantly superior to others followed by T3 (potting mixture + SMS fortified with consortium, 111.33 g). The lowest shoot weight was recorded in T10 (control, 48.0 g).

Yield:

The results on the effect of treatments on yield are presented in Table 24. Treatments were significantly different. Among the treatments T9 (potting mixture + SMS) was found to be significantly superior (272.66 g) followed by T2 (potting mixture + SMS fortified with *P. fluorescens*, 239.66 g) and T3 (potting mixture + SMS fortified with consortium, 223.0 g). The treatments T1 ((potting mixture + SMS fortified with *T. hamatum*) and T6 (potting mixture + soil drenching with consortium)

were found to be on par. The lowest yield was recorded in T10 (control, 35.0 g) (Fig. 6).

4.8.2.2 Effect of treatments on per cent disease incidence caused by *R. solanacearum*

The effect of treatments on per cent disease incidence of bacterial wilt was recorded at weekly intervals. The results are presented in Table 25 (Fig 7).

There was no wilt incidence in the plants even at 30 days after transplanting. Hence challenge inoculation was given at 30 days after transplanting. Observations on disease incidence were recorded at weekly intervals after challenge inoculation. At one week after inoculation, there was no disease incidence in T2 (potting mixture + SMS fortified with *P. fluorescens*), T3 (potting mixture + SMS fortified with consortium) and T9 (potting mixture + SMS). In T11 (absolute control) also no disease was recorded. However, minimum disease incidence was recorded in T1 (3.70 per cent), T6 (5.55 per cent) and T4 (8.33 per cent). The maximum disease incidence was observed in T10 (control, 66.66 per cent) followed by T8 (potting mixture + soil drenching with Kocide, 46.66 per cent).

The treatments were found to be significantly different at 2 weeks after inoculation also. There was no wilt incidence recorded in the treatments T9 (potting mixture + SMS) and T11 (absolute control). However minimum incidence was observed in T2 (4.76 per cent) (potting mixture + SMS fortified with *P. fluorescens*) followed by T6 (5.55 per cent) (potting mixture + soil drenching with consortium). The treatments T1, T3, T5 and T7 were found to be on par. Hundred per cent wilt incidence was recorded in T10 (control).

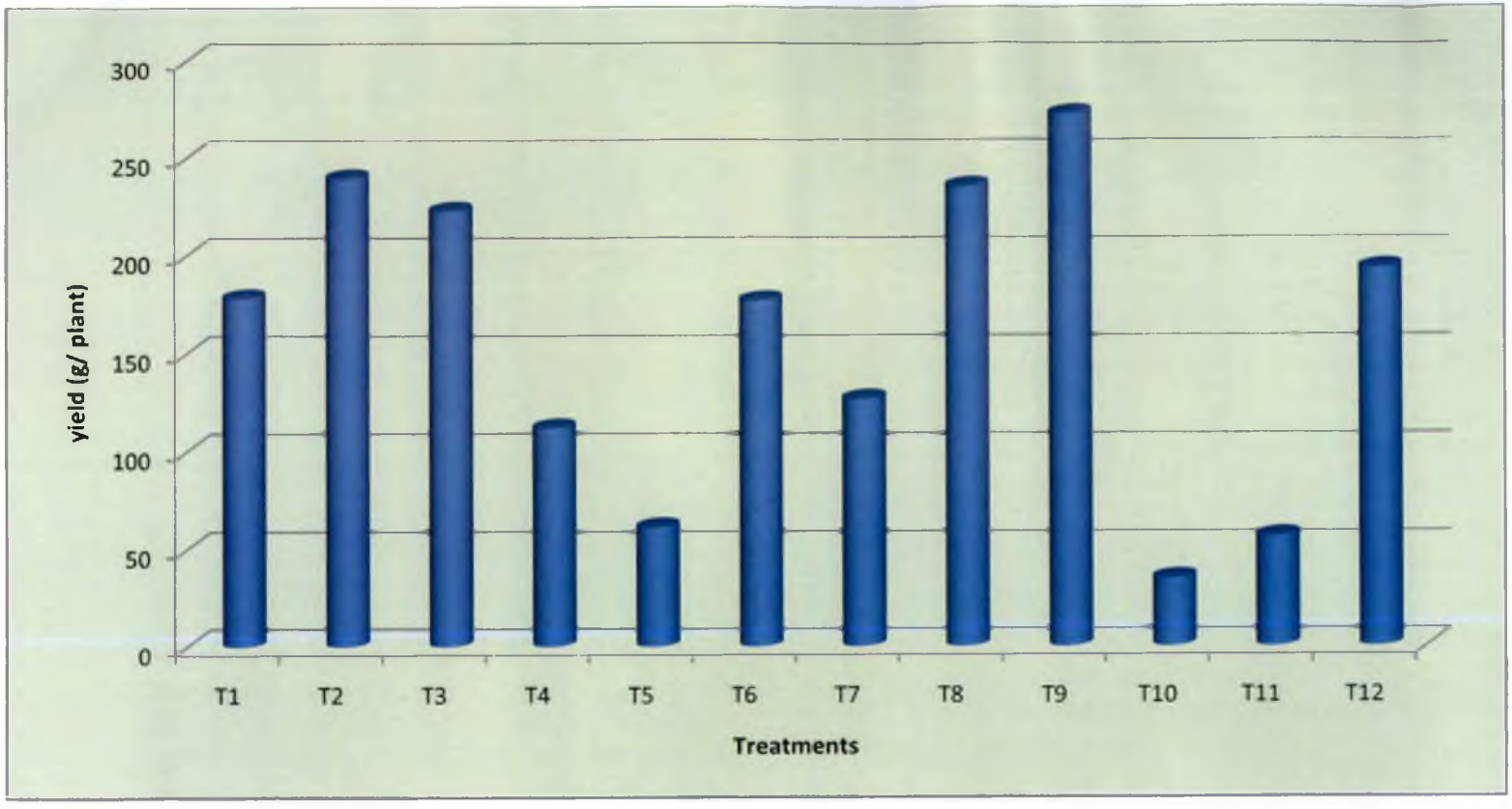


Fig. 6 Effect of treatments on yield in tomato

Table 25: Effect of treatments on disease incidence at different intervals in the management of bacterial wilt

Treatments	Treatment details	1 WAI	2 WAI	3WAI	At harvest
T1	PM amended with 50% fortified SMS with *T. hamatum*	3.70 ^{c}	12.16 ^{bcd}	20.50^{cde}	43.12 (0.71)^{a}
T2	PM amended with 50% fortified SMS with *P. fluorescens*	0.00 ^{c}	4.76 ^{cd}	21.42 ^{cde}	37.30 (0.65) ^{a}
T3	PM amended with 50% fortified SMS with *T. hamatum+P. fluorescens*	0.00 ^{c}	20.00 ^{bcd}	20.00 ^{cde}	27.50 (0.50) ^{a}
T4	PM + soil drenching of *T. hamatum*	8.33 ^{c}	47.22 ^{b}	47.22^{bcd}	63.88 (0.92) ^{a}
T5	PM + soil drenching of *P. fluorescens*	13.33^{bc}	30.00^{bcd}	63.33^{ab}	76.66 (1.06) ^{a}
T6	PM + soil drenching of *T. hamatum+P. fluorescens*	5.55^{c}	5.55 ^{cd}	13.88 ^{de}	34.16 (0.57) ^{a}
T7	PM +soil drenching with Reference culture *T. viride*	13.33^{bc}	40.00^{bc}	53.33^{bc}	60.00 (0.88) ^{a}
T8	PM +fungicide drenching using copper hydroxide (2 g/lit)	46.66^{ab}	46.66 ^{b}	46.66^{bcd}	53.33(0.81) ^{a}
T9	PM +50 % of raw SMS	0.00^{c}	0.00^{d}	0.00 ^{c}	34.16(0.57) ^{a}
T10	PM alone (control)	66.66 ^{c}	100.00*	100.00*	100.00*
T11	Absolute control	0.00*	0.00*	0.00*	0.00*
T12	Control (with POP recommendation)	30.55^{abc}	47.22^{b}	47.22^{bcd}	71.38 (1.05) ^{a}
CV		147.14	76.53	60.48	36.82
CD(0.05)		37.01	37.52	37.94	Non significant

* Not included in statistical analysis WAI – Week after inoculation

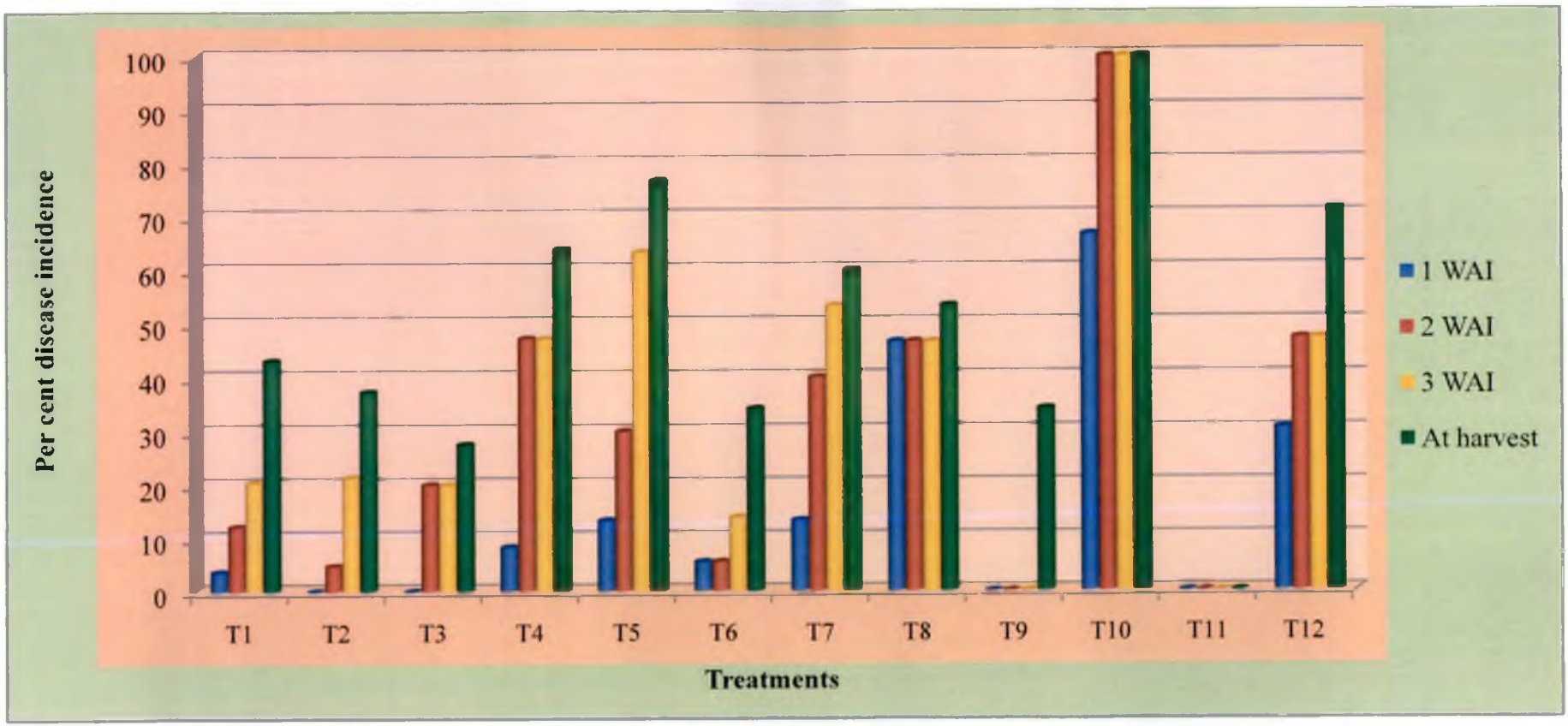


Fig. 7 Effect of treatments on per cent disease incidence caused by *R. solanacearum*

The treatments were found to be significantly different at three weeks after challenge inoculation. The results revealed that in treatments T9 and T11, no wilt incidence was recorded. However, 13.88 per cent incidence was recorded in T6 (potting mixture + soil drenching with consortium). The treatments *viz.* T3 (potting mixture + SMS fortified with consortium, 20 per cent), T1 (potting mixture + SMS fortified with *T. hamatum*, 20.50 per cent) and T2 (potting mixture + SMS fortified with *P. fluorescens*, 21.42 per cent) were on par. The maximum incidence of 100 per cent was recorded in T10 (control).

The treatments were found to be non significant at harvest. The minimum wilt incidence was recorded in T3 (potting mixture + SMS fortified with consortium, 27.50 per cent) followed by T9 (potting mixture + SMS, 34.16 per cent) and T2 (potting mixture + SMS fortified with *P. fluorescens*, 37.30 per cent). The 100 per cent incidence was recorded in T10 (control) followed by T12 (control with POP recommendation, 71.38 per cent) (Plate 17).

Plate 17: Best treatment for the management of bacterial wilt

Control

T3- PM + SMS fortified
with consortium

DISCUSSION

5. DISCUSSION

Spent Mushroom Substrate (SMS) is the growing material left at the end of several mushroom harvests, which is considered as 'spent'. These residual materials are rich with many potential antagonists, which makes them an ideal source to use in plant disease management. Apart from disease management, the SMS also serves as a component in potting mixtures used for horticultural crops, because SMS of *Pleurotus* contains high per cent of primary nutrients (N, P and K), as well as secondary and micro nutrients. Evaluation of fresh SMS against rhizome complex disease of ginger and nursery disease of pepper have been carried out previously in the department of Plant Pathology. So far no prior attempt has been undertaken to evaluate the antagonists for fortification to soften the SMS, and fortified SMS as potting mixture against soil borne diseases of tomato. Hence a study was undertaken to know the potential of fortified SMS of oyster mushroom in the management of soil borne diseases of tomato.

The main soil borne diseases in tomato cultivation are damping off and bacterial wilt. The damping off disease caused by the major pathogen *P. aphanidermatum,* and other pathogens associated with the disease are *P. palmivora*, *R. solani*, *S. rolfsii* and *F. oxysporum* (Asaka and Shoda (1996); Tsahouriduo and Tanasoulopouls (2001); Jayaraj *et al.* (2006); Haque and Nandkar (2012)). The fungal pathogens were isolated from collected specimens showing typical rotting symptom and incubated on PDA. After purification the pure culture of all the pathogens were maintained in PDA slants.

The other dreadful disease in tomato is bacterial wilt, caused by *R. solanacearum*. The pathogen was isolated on TZC agar medium. Colonies appeared as smooth, fluidal, slimy and creamy white with light pink centre. After purification, the pure culture was maintained on NA slants.

The most commonly cultivated mushroom species *P. florida* was used for the study and grown on paddy straw for the production of SMS.

The microbial population at different stages of cropping was enumerated. An increasing trend was noticed in the case of fungal population. The fungal population at the time of spawn running was low. The population tends to be increased during harvest as well as after harvest. This may be due to the active growth of mushroom fungus during the initial period, which resulted in lesser population. Whereas in later stages when the growth of mushroom fungus weakened, colonization by saprophytic fungi may be increased. The similar results were also reported by Remya (2012) and Roshna (2013).

The bacterial population at the time of spawn running was high, but it started decreasing during the time of harvest. This may be due to the enhanced colonization of other saprophytic fast growing and spreading bacteria. Remya (2012) and Roshna (2013), also observed similar results.

The antagonists used in this study *viz., T. hamatum*, *T. viride* and *B. subtilis* are isolates from SMS, obtained in previous studies along with the reference cultures of KAU, *T.viride* and *P. fluorescens*. They were evaluated against soil borne pathogens of tomato (*P. aphanidermatum*, *P. palmivora*, *R. solani*, *S. rolfsii*, *F. oxysporum* and *R. solanacearum*).

The fungal antagonists were evaluated against *P. aphanidermatum* by dual culture method. On 3rd day after inoculation in dual culture with *T. viride* (SMS), the growth of pathogen was less and growth of antagonist *T. viride* (SMS) was high compared to the dual culture with *T. hamatum*. But on 5th day the growth of pathogen in dual culture with *T. hamatum* was lower compared to that of *T. viride* (SMS). This may be due to the production of dark yellowish metabolite by *T. hamatum* compared to *T. viride* (SMS), which produced light yellowish orange coloured metabolite. Bello *et al.* (1997) reported the production of volatile compounds by *T. hamatum* against

wide range of fungal pathogens, which might be one possible mechanism of biological control. According to Singh *et al.* (2014), among the four isolates of *Trichoderma* sp., *T. harzianum* recorded maximum growth inhibition (60.38 per cent) against *P. apahanidermatum* and produced more amounts of volatile and non volatile metabolites as well as complete inhibition of mycelial growth of the pathogen.

The *in vitro* evaluation of fungal antagonists against *P. palmivora* showed 100 per cent inhibition of the pathogen by three antagonists. On the 6th day antagonists over grew the pathogen. Mpika *et al.* (2009) evaluated 43 isolates of *Trichoderma* sp. against *P. palmivora*. Among them *T. virens* was the best which reduced the mycelial growth upto 97.90 per cent. Roshna (2013) reported *T. hamatum* as effective against *P. capsici* due to its over growth and metabolite production over the pathogen.

The antagonistic efficiency of fungal antagonists against *F. oxysporum* revealed that on 8th day after inoculation all the antagonists gave 100 per cent inhibition and all of them over grown the pathogen. This result supports the earlier report on the better antagonistic effiency of *T. virens* against *F. oxysporum.* f. sp. *lycopercisi* (Haque and Nandkar, 2012).

The three fungal antagonists were evaluated against *R. solani*. The results revealed that *T. hamatum* was found to be best and gave 57.44 per cent inhibition. The over growth of the antagonists were observed from 5th day and sparse growth of mycelia was noticed in case of pathogen. Here also a brown coloured metabolite production was observed from 3rd day onwards. According to Bello *et al.* (1997), the inhibition of *R. solani* and other fungal pathogens in *in vitro* evaluation with *T. hamatum* might be due to volatile metabolites produced by the antagonist.

The fungal antagonists *viz.*, *T. hamatum*, *T. viride* and reference culture *T. viride* were evaluated against *S. rolfsii*. Among the antagonists, *T. viride* (reference culture) gave 43.33 per cent inhibition. In all the dual cultures a thick white line was

noticed at the joining point of antagonist and pathogen. This may be due to coiling of hyphae. The coiling and lysis of hyphae of *S. rolfsii* by *T. harzianum* and *T. longibrachiatum* was reported by Yuquab and Shahazad (2005). But Roshna (2013) reported *T. hamatum* as more effective than *T. viride* in inhibiting the mycelial growth under *in vitro* conditions.

The bacterial antagonists *viz., B. subtilis* and *P. fluorescens* were also evaluated against all the fungal pathogens. The bacterial antagonist *P. fluorescens* gave maximum per cent inhibition against *P. aphanidermatum*, whereas *B. subtilis* has no effect. Hultberg *et al.* (2000) studied *in vitro* and *in vivo* interactions between different strains (5.014 and its mutant 5-2/4) of *P. fluorescens* and *P. ultimum* in tomato seedlings. Strain 5.014 inhibited *P. ultimum* to a larger extent than the mutant 5-2/4 on King's medium B, a medium that promotes siderophore production. On yeast–malt medium, mutant strain 5-2/4 inhibited the growth of *P. ultimum* to a larger extent than 5.014 and produced a high amount of the antibiotic 2, 4-diacetylphloroglucinol.

The antagonistic efficiency of bacterial antagonists were also evaluated against *P. palmivora*. The *B. subtilis* recorded 72.22 per cent inhibition, while *P.fluorescens* gave 63.33 per cent inhibition only. Roshna (2013) conducted *in vitro* evaluation of *P. aeruginosa* and *B. subtilis* against *P. capsici* and found that both of them were effective.

The *in vitro* evaluation of bacterial antagonists against *F. oxysporum* revealed that *P. fluorescens* was more effective (73.33 per cent), while *B. subtilis* gave 68.88 per cent inhibition. According to Baysal *et al.* (2008), among the two strains of *B. subtilis* against *F. oxysporum.* f. sp. *redicis- lycopersici,* the strain EU07 has showed more suppression on growth of the pathogen compared to the strain QST 713. Rini and Sulochana (2007) evaluated 56 isolates of *P. fluorescens* from Kerala

against *F. oxysporum* and *R. solani.* They found that the isolates P20 and P28 were most effective against *F. oxysporum.*

The *in vitro* studies against *R. solani* revealed that *B. subtilis* expressed more antagonistic activity (66.66%), while *P. fluorescens* has no effect. But aversion type of inhibition was noticed for *B. subtilis* in the dual culture. According to Haung *et al.* (2012), in the dual culture with *B. subtilis* (SQR – N43) and *R. solani*, at 48 hr after inoculation hyphal deformation, enlargement of cytoplasmic vacuoles and cytoplasm leakage were observed. An *in vitro* study using 41 bacterial isolates of *P. fluorescens* was conducted by Ahmadzadeh and Tehrani (2009) and reported 6 isolates as having highest level of antagonistic activity against *R. solani.* According to Seema and Devaki (2012), *in vitro* evaluation of *B. subtilis* gave 50 per cent inhibition of *R. solani* and on 5th day the colour of the media turned to brown at the antagonized portion. This may be due to the presence of antibiotic like inturin A and surfactin produced by *B. subtilis.*

Among the two bacterial antagonists tested against *S. rolfsii*, the bacterial antagonist *B. subtilis* gave 77.77 per cent inhibition, while *P. fluorescens* has no effect. Nalisha *et al.* (2006) found that *B. subtilis* produced an antifungal substance which has inhibitory effect on wide range of fungi, including *S. rolfsii.*

Evaluations of fungal and bacterial antagonists were also conducted under *in vitro* condition against bacterial pathogen *R. solanacearum.* All the fungal antagonists showed 100 per cent inhibition by 4th day after incubation against *R. solanacearum.* In dual culture with *T. hamatum* the fungus over grew the pathogen on 2nd day onwards and an inhibition zone was also noticed around the fungal disc, whereas the other antagonists started overgrowth on the pathogen from 3rd day onwards. According to Remya (2012), among the fungal antagonists tested, *T. viride* was found

to be efficient than others and reference culture due to fast growth over the pathogen and its capability to produce metabolite.

The bacterial antagonists were also evaluated against *R. solanacearum*, among them *P. fluorescens* gave inhibition zone of 31 mm, while *B. subtilis* gave 22 mm only. Aliye *et al.* (2008) screened one hundred and twenty rhizosphere bacterial isolates against virulent strain of *Ralstonia solanacearum* (PPRC-Rs) and among the bacterial isolates, *Bacillus subtilis* PFMRI, *Paenibacillus macerans* BS-DFS, *Paenibacillus macerans* PF9 and *P. fluorescens* PF20 were found to inhibit the growth of the pathogen significantly.

The mutual compatibility among fungal and bacterial antagonists were also tested and found *P. fluorescens* and *T. hamatum* more compatible whereas *B. subtilis* was found to be incompatible with all the three fungal antagonists. An aversion type of inhibition was also noticed in case of dual culture with *B. subtilis*. Rini and Sulochana (2007) reported the compatibility between *Trichoderma* sp. and *P. fluorescens*.

Biosoftening of SMS with the selected organisms was carried out in order to soften the SMS without deteriorating its qualities. Here the SMS was fortified with *T. hamatum*, *P. fluorescens* and a microbial consortium of these two organisms. The microbial population, visual parameters, physico-chemical parameters and nutritional status were analyzed at 0 day, 30^{th} day and 60^{th} day after fortification.

The microbial population of fortified samples was enumerated at different intervals. The fungal population at the time of fortification was high in samples fortified with *T. hamatum*. Afterwards, a decreasing trend was noticed at 30 days and 60 days after fortification. This may be due to the fact that the time period required

for the growth and proliferation of added fungus is less than 30 days, and further decrease in population may be due to lesser moisture content in the samples on prolonged storage. Singh *et al.* (2012) used SMS as a carrier for mass multiplication of *T. viride* and *T. atroviride* and found maximum population at 10 days after incubation and these were viable upto 30 days. Similar result was also obtained by Shitole *et al.* (2014), where SMS was used as a carrier for multiplication of *T. viride.* The population was highest at 30 days, further decrease was noticed thereafter.

In case of bacterial population also the same trend was noticed. Initially, the population was high but the population was decreased afterwards. This may be due to the decreased moisture content during storage. According to Weber *et al.* (2001), the bacterial community on rice straw under anoxic condition was relatively stable after 15 days of incubation. Therefore assume that the colonization process is mainly driven by multiplication of initial colonizers and the cells lose activity with age.

The visual parameters like colour, texture and volume of different treatments and control were recorded at 0, 30 and 60 days after fortification. The SMS fortified with *P. fluorescens* showed a slight enhancement in colour, reduction in volume as well as softened to certain level. According to Rajan *et al.* (2005), to obtain good quality arecanut fibre, biosoftening with the fungus *Phanerochete* strain required only 7 days for improvement in colour and softness.

The physico-chemical parameters like pH, EC and moisture content of different treatments were analyzed at different intervals. A slight reduction in pH was noticed in all the treatments at 30 days and 60 days after fortification. The pH was neutral in all the treatments at different storage level except control where it was slightly lower than neutral point. This may be because of the lower microbial activity in control due to lesser moisture content. The pH is an important parameter which

controls the availability of nutrients like P, Fe and Zn. According to Kumar *et al.* (2007) fungal inoculation in paddy straw compost lowered the pH and pH range of 6.3 to 7.5 was acceptable value for the final product. Ahlawat *et al.* (2007) also noticed decrease in pH from initial to final storage period on weathering of button mushroom spent.

The electrical conductivity was also found to be decreased as the storage period increases in all the treatments. EC is a measure of dissolved salts and the EC value below 3 mS is considered as safe limit. According to Kumar *et al.* (2007), addition of inoculum like *Aspergillus nidulans, Scytalidium thermophilum* and *Humicola* sp. reduced the electrical conductivity of paddy straw.

The moisture content was also found to be decreased during storage and this might have resulted in the decrease in microbial population and other parameters during storage. According to Bhanu *et al.* (2014) the optimum moisture level of 60-65 per cent has to be maintained by turning at weekly interval for SMS of button mushroom to be a supporting medium for *T. harzianum* (10^4 to 8.72×10^6).

The primary nutrients, secondary nutrients and micro nutrients were also analyzed at different intervals of 0, 30 and 60 days after fortification. The primary nutrients N, P, K and C content were analyzed. There was a considerable decrease in N content, which might be due to temporary locking of nitrogen by microbes *i.e.*, immobilization by microbes. A decrease in nitrogen content during composting was reported by Viji and Neelanarayana (2015).

The Phosphorus content was also decreased, which might be due to leaching of P. Similar results were reported by Viji and Neelanarayan (2015) during composting.

There was a significant decrease in Potassium content also due to storage of SMS at different intervals. This might be due to leaching loss of K. Ahlawat (2007) also reported decreasing K content from 8 to 16 months after weathering. But Viji and Neelanarayan (2015) reported an increase in K content after composting. Potassium plays an important role in plant growth. According to Polat *et al.* (2009) keeping of spent mushroom compost (SMC) of *A. bisporus* outside for longer time resulted in loss of 94 per cent K, 33 per cent P and 15 per cent N.

The Carbon content was found to be increased during storage. This might be due to loss of moisture which resist degradation and resulted in the presence of undecomposed material. According to Goyal and Sindhu (2011), 11-27 per cent of the total C is lost during the initial stage of active composting and about 62- 66 per cent during whole composting time. This reduction may be due to intense activity of microbiota present in the compost.

The C: N ratio at each time interval was found to be increased. The increase in total C content at 60 days after fortification might be due to decrease in moisture content. This indicates that decomposition was incomplete. The fortified SMS with *T. hamatum* showed decreased C:N ratio at initial stage. According to Kumar *et al.* (2007), initial low C:N ratio caused fast degradation of cellulose and hemicelluloses, while high initial C:N ratio resulted in low degradation of both cellulose and hemicelluloses. Goyal and Sindhu (2011) found maximum decrease in C:N ratio of paddy straw compostable material obtained with fungal consortium. The decreased C:N ratio at the end of composting process is an indication of maturity of compost. Romaine and Holcomb (2000) reported that the SMS product has a C:N ratio well below 30:1. The C:N ratio is an important indicator of N_2 availability. If C: N ratio is above 35:1, soil microorganisms can immobilize nitrogen.

SMS is a rich source of secondary nutrients *viz*., Ca and Mg and micro nutrients *viz*., Zn, Fe, Mn and Cu etc., which makes them different from other organic substrates. There was slight increase of nutrients like Zn, Fe, Mg and Mn contents during storage. While Ca and Cu contents were found to be decreased. According to Ahlawat (2007), weathering of button mushroom spent (8 to 16 months) leads to increase in Zn, Fe, Mn, Mg, Ca and Cu contents.

Storage upto sixty days could not completely degrade the fortified SMS and raw SMS and this might be due to insufficient moisture content in the treatments.

The potential of fortified spent mushroom substrate for the management of damping off disease of tomato was evaluated by pot culture experiment. The seed germination was recorded at 7, 14 and 21 days after sowing. The maximum seed germination was recorded in the treatment of potting mixture with soil drenching of *P. fluorescens* at 7 and 14 days after sowing. Soil drenching with bioagents might have induced fast germination which in turn produces healthy seedlings. But 21 days after sowing, the treatment in which potting mixture amended with 50 per cent SMS fortified with microbial consortium of *P. fluorescens* and *T. hamatum* recorded the highest seedling emergence. The initial inhibition of seed germination in the SMS amended treatments might be due to coarse texture of *Pleurotus* paddy straw SMS as well as high aeration porosity and low water holding capacity which leads to dryness. The highest germination at 21 days may be due to the fact that SMS improved the physical properties of potting mixture with slow release of nutrients, along with the presence of microbial population which enhances the speed of germination and vigour of seedlings. Romaine and Holocomb (2000) found that tomato seeds did not germinate well in perlite mixture with unseived or coarse SMS. Because unsieved and coarse SMS had low water holding capacity and hence the media dried rapidly

and inhibited seed germination. According to Shitole *et al.* (2013), 75 per cent SMS in potting media gave effective germination of tomato at 21 days after sowing. Siddant and Sign (2009) found that *Spinacia oleraceae* took shorter time for germination with 5 per cent SMS supplemented treatments.

The challenge inoculation with *P. aphanidermatum*, the important damping off pathogen was given at 21 days after sowing. The treatment in which potting mixture amended with 50 per cent fortified SMS with microbial consortium of *T. hamataum* and *P.fluorescens* recorded minimum disease incidence. The similar findings were recorded by Shitole *et al.* (2013), where 75 per cent SMS with consortium of *T. viride* and *P. fluorescens* was effective in controlling pre and post emergence damping off disease caused by *P. aphanidermatum* and also reported that 50 per cent SMS provided effective disease control. According to Davis *et al.* (2005), the 50 per cent SMS or greater levels provides effective disease control in tomato seedlings against *Pythium* sp. Romaine and Holomb (2000) showed that addition of 25 per cent SMS to perlite significantly increased tomato seedling survival from 25 to 59 per cent against damping off by *P. ultimum*. According to Egein *et al.* (2011), SMS:soil @ 1:4 ratio was highly effective in controlling fusarial wilt of tomato. The suppression of colonization of fungus is exclusion by competition or by nutrient depletion indicating the establishment of the rhizosphere by biocontrol organism (Hultberg *et al.*, 2000).

The biometric observations were recorded at 45 days after sowing. The treatment soil drenching with consortium of *T. hamatum* and *P. fluorescens* recorded maximum root length, shoot length and plant vigour followed by the treatment potting mixture with 50 per cent SMS. Shitole *et al.* (2013) recorded maximum root and shoot length at 30 days after sowing in the treatments *T. viride* + *P. fluorescens* along with 75 per cent SMS combination. Jain *et al.* (2013) reported that plants

showed increased growth when treated with consortium of *B. subtilis* + *P. aeruginosa* + *T. harzianum*. Similar results were also reported by Roshna (2013), highest biometric parameters in pepper cuttings when treated with microbial consortium of *T. hamatum* and *P. aeruginosa*.

A pot culture experiment was conducted to evaluate the effectiveness of fortified SMS for the management of bacterial wilt of tomato caused by *R. solanacearum*. Maximum shoot length was recorded by the treatment potting mixture amended with 50 per cent fortified SMS with microbial consortium of *T. hamatum* and *P. fluorescens*, at 30, 45 and 60 days after transplanting followed by other SMS treatments. This might be due to the availability of rich source of nutrients and other molecules present in SMS, which makes it well suited for supporting luxuriant plant growth. According to Egein *et al.* (2011), 1:4 ratio of SMS:soil has shown a significant difference on plant height of tomato. Fresh SMS is a rich source of Nitrogen, Carbon and other elements, which provides luxuriant plant growth as well as the nutrients being released from the SMS as it decomposes benefited the plants (Romaine and Holocomb, 2000).

The maximum root length and fresh weight of shoot were recorded in the treatment potting mixture amended with 50 per cent fortified SMS with *P. fluorescens*, while maximum fresh weight of root and yield were obtained in the treatment potting mixture amended with 50 per cent fresh SMS. The enhanced growth parameters noticed in SMS treatments might be due to greater nutrient composition as well as other molecules which were present in the SMS that influenced growth and development at early stages. According to Medina *et al.* (2012) addition of SMS increased the soil enzyme activity especially phosphatase activity. These biological catalysts are good markers of soil fertility. Mushroom compost has good physical and chemical characteristics that make it ideal for

blending with landscape as mulch to enhance growth of horticultural plants (Davis *et al.*, 2005). Siddant and Sigh (2009) reported that during growth on straw, *Pleurotus* releases humic acid like fractions which when added to soil increases its fertility. In addition, humic acid substances may affect the plant biochemical process. Viji *et al.* (2012) reported that fluorescent siderophore pyroverdin produced by *P. aeruginosa* strain, not only supplies the cell with iron but also keeps other microbes harmful to the plant at bay and plays important role in growth stimulation. According to Polat *et al.* (2009), addition of 4 tonnes per hectare of SMS increased the yield of cucumber in green house. Siddant and Sigh (2009) reported the positive effect of SMS on yield of *Spinacia oleracea* where its higher proportion showed significant production of crop. This may be because, the SMS improved the physical property of soil by decreasing soil bulk density, increasing aggregate stability, reducing surface crust formation and diurnal temperature changes, increasing the infiltration rate, aeration and water retaining capacity of the soil. It is well known that physical properties of soil were directly related to crop yield.

The challenge inoculation with *R. solanacearum* was given at 30 days after transplanting. The minimum disease incidence was noticed in the treatment potting mixture amended with 50 per cent fortified SMS with *T. hamatum* and *P.fluorescens*. This might be due to antagonistic activity of the bio -agents as well as disease suppression mechanism of SMS. Remya (2012) reported reduction of rhizome rot complex disease caused by *R. solanacearum* in ginger by addition of SMS as mulch. Wei *et al.* (2011) also reported that *Bacillus* fortified organic fertilizer controlled bacterial wilt of tomato. According to Parada *et al.* (2011), the mycelium prevalent in SMS is abundant source of elicitors which helped them to control diseases. The water extracts as well as autoclaved water extracts from SMS contained water soluble and heat stable elicitors for inducing systemic acquired resistance in cucumber plants against anthracnose disease. According to Egein *et al.* (2011), the bio-control

mechanism for plant pathogens include competition, hyper parasitism, parasitism, predation and production of extra cellular metabolites such as antibiotics, hydrogen cyanide and siderophores.

The *in vitro* studies revealed that, among the three fungal antagonists *T. hamatum* was found to be best. Among the bacterial antagonists evaluated, *P. fluorescens* was selected. Compatibility studies also showed that *T. hamatum* and *P.fluorescens* were compatible. These oraganisms along with microbial consortium were selected for biosoftening studies and pot culture evaluation. The biosoftening experiment with the selected organisms upto 60 days proved that the treatment with *P.fluorescens* was effective in giving reduction in volume, softeness and colour change to a certain level. A significant decrease was noticed in physico-chemical properties, nutrient content and microbial population during storage, which might be due to the insufficient moisture content in the treatments. In the pot culture experiments also potting mixture amended with 50 per cent fortified SMS using a microbial consortium of *T. hamatum* and *P. fluorescens* gave minimum disease incidence against damping off and bacterial wilt. All the SMS supplemented treatments showed an enhanced plant growth, may be due to the unique property of oyster SMS in imparting Systemic Acquired Resistance (SAR) to the plants, thereby improving plant health, which requires further investigation.

SUMMARY

SUMMARY

The substrate left after the harvest of mushroom is termed as spent mushroom substrate. These waste materials have many potential uses. Among them the disease controlling property is quite interesting. SMS is rich with many potential antagonists. The present investigation was carried out to study the potential of fortified SMS against soil borne diseases of tomato.

The major soil borne diseases associated with the tomato is damping off and bacterial wilt diseases. The diseased specimens of damping off and bacterial wilt were collected and pathogens *viz. P. aphanidermatum*, *P. palmivora*, *F. oxysporum*, *R. solani* and *S. rolfsii* associated with damping off and *R. solanacearum* associated with bacterial wilt were isolated. The pure cultures of the pathogens were maintained for further studies.

The antagonists *viz.*, *T. hamatum*, *T. viride* and *B. subtilis* obtained from oyster SMS in the previous studies along with reference culture of KAU, *T. viride* and *P. fluorescens* were evaluated against soil borne pathogens of tomato. An *in vitro* study was conducted to evaluate the antagonistic efficiency of three fungal antagonists against *P. aphanidermatum*. On the 3rd day *T. viride* (SMS) was found to be effective in showing maximum inhibition of pathogen, but on the 5th day after inoculation *T. hamatum* showed maximum inhibition, by producing more metabolites.

The *in vitro* evaluation against *P. palmivora* showed that *T. hamatum* was best on 3rd day after inoculation by complete inhibition of the growth of pathogen. From 4th day onwards all the antagonists started to over grew the pathogen. On 6th day after

inoculation all the fungal antagonists were found to be effective and all gave 100 per cent inhibition over control.

The fungal antagonists *viz.*, *T. hamatum*, *T. viride* and reference culture *T. viride* were evaluated against *F. oxysporum*. On the 4th day *T. hamatum* was found to be best by giving maximum inhibition of the pathogen. The pathogen attained complete growth in the monoculture on 8th day after inoculation. At that time the antagonists showed 100 per cent inhibition of the pathogen in all the dual cultures.

The antagonistic effiency was evaluated against *R. solani* by dual culture method. The maximum inhibition (57.44 per cent) was recorded in the dual culture with *T. hamatum*. A brown coloured metabolite production and cessation in mycelial growth were also noticed in dual culture with *T. hamataum* on 3rd day after inoculation.

The fungal antagonists were evaluated against *S. rolfsii*, among them *T. viride* (reference culture) gave 43.33 per cent inhibition on 3rd day after inoculation. A thick white line was observed at the joining point between fungal pathogen and antagonist.

The bacterial antagonists *viz.*, *B. subtilis* and *P. fluorescens* were evaluated against *P. aphanidermatum*. The maximum per cent inhibition was recorded with *P. fluorescens*, while *B. subtilis* have no effect. The *in vitro* studies against *P. palmivora* showed that *B. subtilis* was found to be best and gave 72.22 per cent inhibition. But aversion type inhibition was noticed. The antagonistic effiency of bacterial antagonists were tested against *F. oxysporum*. Among them the *P. fluorescens* recorded highest per cent inhibition. The *in vitro* studies against *R. solani* and *S. rolfsii* revealed that *B. subtilis* was found to be best to inhibit the growth of both the pathogens.

A compatibility study was carried out with fungal and bacterial antagonist. *P. fluorescens* and *T. hamatum* were found to be mutually more compatible than others, while *B. subtilis* was found to be incompatible with all the fungal antagonists.

The selected antagonists *viz.*, *T. hamatum* and *P. fluorescens* and a microbial consortium of these two organisms were applied @ 300 ml/ kg of SMS for biosoftening of SMS. The treated SMS and control were kept for 60 days. The microbial population, physico- chemical parameters, visual changes and nutrient contents were analyzed at different intervals of 0, 30 and 60 days after fortification.

The microbial population was analysed at different intervals *viz.*, at the time of fortification, and thirty as well as sixty days after fortification. Initially the fungal and bacterial population was high in the fortified samples, but afterwards the population decreased considerably.

The treatment with *P. fluorescens* showed slight colour change, reduction in volume and softened the SMS to certain level. The physico-chemical parameters *viz.* pH, EC and moisture content were analyzed. A significant reduction was noticed in all the parameters due to storage. This might be due to the insufficient moisture content in all the treatments during the storage period.

The nutrient contents of fortified SMS and raw SMS were analyzed. The primary nutrients *viz.*, Nitrogen, Potassium, Phosphorus and Carbon were estimated. All the nutrients except C were found to be decreased, while C content increased. The C:N ratio of fresh SMS was below 18.82:1 at the time of fortification. The C:N ratio also found to be high at thirty as well as sixty days after fortification. This may be due to lower moisture content of the samples during storage period.

The secondary nutrient Calcium and micro nutrient Copper were found to be decreased during the storage. While the nutrients *viz.*, Magnesium, Zinc, Iron and Manganese were found be increased.

The pot culture evaluation for the management of tomato damping off revealed that the maximum seed germination, at 7 and 14 days after fortification, was recorded in the treatment potting mixture + soil drenching with *P. fluorescens.* But 21 days after sowing the maximum seedling emergence was recorded in the treatment potting mixture amended with 50 per cent SMS fortified with microbial consortium of *T. hamatum* and *P. fluorescens.* The initial inhibition in germination of seeds in the SMS supplemented treatments may be due to the coarse texture of SMS which is not favourable for seed germination. The challenge inoculation was given at 21 days sowing. The minimum disease incidence was recorded in the treatment potting mixture amended with fortified SMS with microbial consortium of *T. hamatum* and *P. fluorescens*. The highest biometric characters *viz.*, root length, shoot length and plant vigour index were also recorded in the treatment potting mixture + soil drenching with microbial consortium of *T. hamatum* and *P. fluorescens*.

Potential of fortified SMS was evaluated against bacterial wilt disease by pot culture experiment. The highest biometric characters were recorded on SMS supplemented treatments at different intervals. The maximum shoot length was recorded in the treatment potting mixture amended with 50 per cent SMS fortified with a microbial consortium of *T. hamatum* and *P. fluorescens*. The maximum yield and fresh weight of root were recorded in the treatment potting mixture amended with fresh SMS, while maximum shoot weight and root length were recorded in the treatment potting mixture amended with 50 per cent SMS fortified with *P. fluorescens*. The challenge inoculation was given at 30 days after transplanting. The minimum disease incidence was recorded in the treatment SMS with microbial

consortium of *T. hamatum* and *P. fluorescens*. The other SMS supplemented treatments also showed reduced disease incidence.

From this study it is clear that the fortified SMS opens a new way in the plant disease management. Apart from the disease management property, it also provides plant growth promotion. This may be due to the active molecules and potential antagonists present in the SMS.

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APPENDIX

Nutrient Agar Medium

Beef extract	- 1g
Peptone	- 5g
Sodium chloride	- 5g
Agar	- 15g
Distilled water	-1000ml

Kenknight's Agar medium (KAM)

Glucose	-1g
KH_2PO_4	- 0.1g
$NaNO_3$	- 0.1g
KCl	- 0.1g
$MgSO_4$. &H_2O	- 1.5g
Agar	- 20g
Distilled water	- 1000ml

King's B medium

Peptone	-20g
Glycerol	-10ml
KH_2PO_4	-10g
$MgSO_4.7H_2O$	-1.5g

APPENDIX -1

MEDIA COMPOSITION

Potato Dextrose Agar (PDA)

Potato	- 200g
Dextros	- 20g
Agar	- 20g
Distilled water	- 1000ml
pH	- 7.0

Martin Rose Bengal Agar (MRBA)

KH_2PO_4	- 1g
$MgSO_4.\ 7H_2O$	- 0.5g
Peptone	- 5g
Dextrose	- 10g
Rose Bengal	- 0.03g
Streptomycin	- 30g
Agar	- 20g
Distilled water	- 1000ml

Nutrient Agar Medium

Beef extract	- 1g
Peptone	- 5g
Sodium chloride	- 5g
Agar	- 15g
Distilled water	-1000ml

Kenknight's Agar medium (KAM)

Glucose	-1g
KH_2PO_4	- 0.1g
$NaNO_3$	- 0.1g
KCl	- 0.1g
$MgSO_4$. &H_2O	- 1.5g
Agar	- 20g
Distilled water	- 1000ml

King's B medium

Peptone	-20g
Glycerol	-10ml
KH_2PO_4	-10g
$MgSO_4.7H_2O$	-1.5g

Agar	- 20g
Distilled water	- 1000ml
pH	- 7.2 7.4

Triphenyl Tetrazolium Chloride (TZC) medium

TZC	- 1%
Peptone	- 10 g
Casein hydrolysate	- 1g
Glucose	- 5g
Agar	-20g
Distilled water	-1000ml
pH	- 6.8

POTENTIAL OF FORTIFIED SPENT MUSHROOM SUBSTRATE FOR THE MANAGEMENT OF SOIL BORNE DISEASES OF TOMATO

By

ARATHIKRISHNA V. K.

(2013-11-138)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement

for the degree of

MASTER OF SCIENCE IN AGRICULTURE

(PLANT PATHOLOGY)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

Department of Plant Pathology

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

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ABSTRACT

Mushrooms are produced on natural materials taken from agricultural waste. SMS is the substrate left after harvesting of mushroom fruit bodies. SMS contains a diverse range of soil microorganisms. This is proven by its disease suppressing properties and its effectiveness in bioremediation. Additions of microorganisms to soil ultimately enhance and accelerate regular soil process such as nutrient mobilization. Among the beneficial uses of SMS the disease controlling property is quite interesting.

Tomato is one of the most widely used vegetable. The bacterial wilt and damping off are the two serious soil borne diseases of this crop. The management of diseases using chemicals is not safer to environment due to residual problem. Increasing concern regarding food safety and environmental pollution has generated an interest in eco friendly practices like soil amendment and application of biocontrol agents to manage the plant diseases. Under these circumstances this study was taken up to assess nutritional and disease management aspects of fortified SMS in tomato

The antagonists used for this study like *Trichoderma hamatum*, *T. viride* and *Bacillus subtilis* are the isolates from SMS obtained from the previous studies conducted in the Department of Plant Pathology along with reference cultures of Kerala Agricultural University *viz. T. viride* and *Pseudomonas fluorescens*. They were evaluated against major soil borne fungal pathogens like *Pythium aphanidermatum*, *Phytophthora palmivora, Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* and bacterial pathogen *Ralstonia solanacearum* of tomato.

The *in vitro* evaluation showed that *T. hamatum* was the best among three fungal antagonists. While in case of bacteria *P. fluorescens* was the best. A microbial consortium of these two organisms was also prepared.

The selected best antagonists and consortium were applied to SMS @ 300 ml per kg and kept for biosoftening for 60 days. The fortified SMS with *P. fluorescens* softened the SMS to a certain level. The primary nutrients like N, P, K, secondary nutrient Ca and micro nutrient Cu were found to be decreased as the time increases.

In the pot culture experiment for the management of damping off, the treatment SMS fortified with consortium gave maximum per cent inhibition against the disease. In the management of bacterial wilt also SMS fortified with consortium found to be best in disease suppression as well as plant growth promotion.

All the treatments with SMS were found to posses disease management property and enhance the plant growth. From this study it is clear that fortified SMS paves a new way in disease management. For confirmative result, elaborative field study has to be conducted and the quality of SMS has to be worked out.