EXPLOITATION OF SPENT MUSHROOM SUBSTRATE AS MULCH FOR THE MANAGEMENT OF RHIZOME ROT COMPLEX DISEASE OF GINGER

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THESIS

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Kerala Agricultural University, Thrissur

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KERALA, INDIA

2012

DECLARATION

I hereby declare that the thesis entitled **"Exploitation of spent mushroom substrate as mulch for the management of rhizome rot complex disease of ginger"** is a bonafide record of research work done by me during the course of research and that it 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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Certified that thesis entitled **"Exploitation of spent mushroom substrate as mulch for the management of rhizome rot complex disease of ginger"** is a bonafide record of research work done by Ms. Remya J. S. under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Remya J. S.** (2010-11-123), a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Pathology**, agree that the thesis entitled **"Exploitation of spent mushroom substrate as mulch for the management of rhizome rot complex disease of ginger"** may be submitted by **Ms. Remya J. S. (2010-11-123)**, in partial fulfillment of the requirement for the degree.

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Introduction

1. INTRODUCTION

Mushroom is an important vegetable for its nutritive and medicinal values. Cultivation of mushrooms, a profitable and eco-friendly enterprise, involves bioconversion of cellulose waste into edible biomass. Spent mushroom substrate (SMS) is the composted organic materials remained after the harvest of a mushroom crop, found to be rich in plant nutrients including minerals and are used as manure in different crops including ginger. Additionally about 5 kg spent mushroom compost is produced as a by-product in producing one kg mushroom (Uzun, 2004). After mushroom cultivation, a considerable amount of spent substrate remains as residual material. It creates various environmental problems including ground water contamination and nuisance if not handled properly. Amongst various edible fungi, oyster mushrooms (*Pleurotus* spp.) have a broad adaptability due to wide range of suitable substrates, simple cultivation technique, minimal cultural requirements and increased acceptability in the market. It degrades most of the lignocellulosic agro-wastes and is an efficient means for the conversion of worthless wastes into valuable proteins.

Role of SMS for the production of agricultural crops is very promising. It has been found to be a good growing medium for majority of the vegetables and field crops, and has shown multifaceted utilities in improving the yield and quality of the crop, and management of diseases, which is really encouraging for the mushroom industry. The other utilities of spent mushroom substrate, like in vermicomposting, bioremediation and as organic-mineral fertilizer are boon to the country's farming system. Application of SMS increases the organic and nutrient status of soil and resulted strongest correlations between soil nutrient levels and plant yield (Courtney and Mullen, 2008). The actinomycetes, bacteria and fungi inhabiting the SMS not only play role in its further decomposition but also exert some antagonism to the normal pathogens surviving and multiplying in the soil eco-system. The SMS may have variable chemical and physical properties due to variability of ingredients and processing; however, it is generally regarded as a neutral soil amendment. According to Ahlawat and Sagar (2007), the phosphorus and potassium requirements of the crop plants can be fulfilled by incorporating 5% of SMS by volume, while nitrogen requirement can be fully met by 25% of SMS by volume. The SMS will add micronutrients to the soil, which is a

marketable feature when compared to inorganic soil amendments typically comprised of exclusively NPK. Addition of SMS will add organic matter to the soil. Regular addition of organic matter to soils is an important aspect of sustainable soil management and can have positive effects on the chemical, physical and biological properties of the soil. Well degraded spent mushroom substrate can act as a better conditioner for harbouring growth promoting and antagonistic microorganisms in the rhizosphere.

India being the world's largest producer, consumer and exporter of ginger, has a predominant position in the global market and accounts for 50 per cent of world's total production. Ginger is obtained from the underground stems or rhizome of *Zingiber officinale,* a herbaceous tropical perennial belonging to the family Zingiberaceae. It is usually grown as an annual. It is being cultivated in India for fresh vegetable and as dried spice. The whole plant is refreshingly aromatic, but the underground rhizome is valued as spice. Its medicinal value is increasingly being recognized nowadays.

One of the important factors limiting the production of ginger is the occurrence of diseases. Soft rot, incited by *Pythium aphanidermatum* and bacterial wilt by *Ralstonia solanacearum* Yabuuchi (Smith) are at present considered as very serious threats in most of the ginger growing areas. Since both pathogens occur simultaneously with similar symptoms in same crop, this problem is termed as 'rhizome rot complex disease'. It causes serious destruction to ginger crop, since the control measures are not that much effective especially under hot, humid conditions of Kerala. Because of its destructive potential to various crops, the control of *P. aphanidermatum* and *R. solanacearum* have attracted much research attention. As ginger is a remunerative crop, farmers usually adopt all available remedial measures to increase the productivity, where plant protection plays an important role. Even after adopting various cultural and chemical measures, bacterial wilt and soft rot of ginger continues to be a complex disease. However, every effort has to be made to reduce the losses due to the disease by adopting a suitable management strategy. Sustainable agriculture necessitates the use of eco-friendly technologies for the production as well as protection of crops. So far disease management use of eco-friendly measures like bioagents and organic amendments should be given more importance.

The effect of various organic amendments in reducing severity of diseases caused by soil borne pathogens has been widely observed. Although several organic materials have been found useful in controlling diseases caused by fungi and bacteria, no work has been carried out against rhizome rot complex disease of ginger using spent mushroom substrate of *Pleurotus* sp. With due consideration of the above facts, the study entitled "Exploitation of spent mushroom substrate as mulch for the management of rhizome rot complex disease of ginger" was taken up with the following objectives.

1) To isolate different microorganisms from different growth stages of mushroom

2) To study the *in vitro* effect of isolated organisms against the pathogens

3) To develop a microbial consortium from SMS for the management of rhizome rot complex disease of ginger

4) To study the effectiveness of spent mushroom substrate as mulch for ginger

5) To evaluate its efficacy in the management of rhizome rot complex disease

Review of Literature

2. REVIEW OF LITERATURE

Ginger is one of the earliest known oriental spices and is being cultivated in India for both as fresh vegetable and as a dried spice, since time immemorial. Ginger is obtained from the rhizomes of *Zingiber officinale*. The ginger family is a tropical group, especially abundant in Indo-Malaysian region, consisting of more than 1200 plant species in 53 genera. In India, during the year 2010, the area under cultivation is 107.5 thousand ha and the total production is 385.3 thousand metric tons.

A scanning of literature on diseases of ginger showed that rhizome rot complex disease is the major constraint in the production of ginger. It is caused by a multitude of pathogens either alone or in combination and distributed worldwide, wherever, ginger cultivation is persuading intensively. Rhizome rot as the name indicates results ultimately in rotting of rhizome, which is economically important part of ginger. The term rhizome rot is loosely used for all the diseases affecting the rhizome irrespective of the pathogens involved. The major pathogens involved in rhizome rot are *Pythium aphanidermatum* causing soft rot and *Ralstonia solanacearum* (Smith) Yabuuchi causing bacterial wilt. Pathogens infecting in the field also may cause decay of rhizomes in storage as well.

2.1 RHIZOME ROT (SOFT ROT)

Rhizome rot is the most destructive disease of ginger in India. The disease is caused by *Pythium aphanidermatum* (Edson) Fitzp. The pathogen infects roots, collar and succulent parts of the rhizome. In Kerala losses can be as high as 90 per cent during years of heavy incidence (Rajan and Agnihotri, 1989). Dake and Edison (1989) reported that 19.8 per cent of rhizome rot of ginger was associated with *P. aphanidermatum.* Crop loss depends on the stage of crop growth at which infection starts. If it occurs early, total loss of affected clump results whereas the crop loss is partial if affected at a later stage (Sarma, 1994)

2.1.1 Symptomatology

Symptoms have been described by many authors. Similar symptoms are seen both in ginger and turmeric. The important development in symptomatology and epidemiology of rhizome rot are the recent recognition of the role of root infection in rhizome rot. The following description of symptoms is based on that of Iyer, (1987) and Sarma (1994). Ginger is susceptible to infection throughout its life. But sprouts, roots, developing rhizome and collar region of the pseudostem are vulnerable to infection.

In mature plants, the leaves become pale and tips of leaf turn yellow. Yellowing spreads along the margins of the leaves from tip downwards. Lower most leaf (oldest) is affected first. Later yellowing spreads to all leaves of affected pseudostem from bottom upwards. Tender tissues of the collar region turn soft, watery and rot and the plant topples. The rotten parts emit foul smell owing to decay. Rotting is followed by invasion of opportunistic organisms like fungi, bacteria, insects and other saprophytes.

2.1.2 causal agent

Several species of *Pythium* have been reported as causal agents of soft rot in different parts of the world. Six species were recorded in different parts of India (excluding synonymous taxa). Among these, *P. aphanidermatum* and *P. myriotylum* are widely distributed, whereas the other species are restricted to certain pockets. High incidence of *Pythium* induced rhizome rot have been reported during wet years (Rajan and Agnihotri, 1989) implying the role of high soil water content on disease onset and spread. The disease is generally less in well-drained fields (Sarma, 1994).

Pythium species are capable of saprophytic survival on plant debris. They are normal inhabitants of many soils. They produce oospores, the perennating structures of the fungi and may reproduce on non host crops and weeds. Saprophytic survival of the fungus in soil is influenced by environmental factors, soil temperatures, moisture and presence of other microbes. Severity of *Pythium* disease is more in areas where rainfall is high or heavy clay soils where drainage is impede (Rajan and Singh, 1973).

2.2 BACTERIAL WILT

The disease is endemic in majority of the ginger growing areas *viz*., Kerala, Sikkim and many other north eastern regions of the country causes yield loss up to 100 per cent under conducive conditions (Mathew *et al*., 1979; Dohroo, 1991).

The disease was first reported from Madras state (Thomas, 1941). Later, it was reported from Kerala (Sarma *et al*., 1978), Bihar (Ojha *et al*., 1986) and Himachal Pradesh (Dohroo, 1991). Once disease cited, the spread would be fast and incidence as high as (50- 60 per cent) was recorded resulting in severe loss of affected clumps.

2.2.1 symptomatology

Water-soaked patches or linear streaks on the collar region of the pseudostems. Later leaves become flaccid with intense yellowish bronze colour and droop. The leaves roll up and the whole plant dries. Pseudostems come off easily with a gentle pull. Milky bacterial exudate oozes out on pressing the rhizome gently (Sarma, 1994).

2.2.2 causal agent

The *Ralstonia solanacearum* (Smith) Yabuuchi has been identified as the causal agent of the disease (Sarma *et al*., 1978). The disease is typically seed and soil borne. Generally noticed during June - July and maximum during August- September, ensuring high soil moisture and relative humidity and low temperature (Sarma *et al*., 1978). The disease spread is along the gradient and spreads through soil water.

2.3 SPENT MUSHROOM SUBSTRATE

Mushroom growing is an eco-friendly activity as it utilizes the waste from agriculture and in turn produces fruit bodies with excellent and unique nutritional and medicinal attributes. The production of spent mushroom substrate after crop harvest is a matter of concern because it creates various environmental problems including ground water contamination and nuisance if not handled properly.

Male (1981) reported that SMS improves the environment for plant root growth by decreasing soil bulk density, increasing aggregate stability, reducing cold and surface crust formation, increasing the infiltration rate and increasing the water content of the soil. Study by Iswandi *et al.,* (1987) has shown that isolates of *P. aeruginosa* from spent mushroom substrate are effective root colonizers. In addition *P. aeruginosa* strains have been shown to protect plants against several pathogens such as damping off caused by *Pythium splendens. Pseudomonas* and *Bacillus* species in spent mushroom composts are effective in suppressing a number of root and soil borne pathogens (Phae and Shoda, 1990; Phae *et al*., 1990; Sugimoto *et al*., 1990). Harris (1992) reported that spent mushroom substrate application to potato crop soil improved the moisture holding capacity of the soil and this resulted in increased plant uptake of nutrients.

Fahy *et al*., (1994) incorporated SMS into a corn field having poor quality soil. Corn yields were significantly higher in SMS amended plots and the nitrogen content of both grain and stover was significantly higher than the control. The corn grown on SMS amended plots never showed symptoms of nutrient deficiency while corn in control plots, and other plots at the farm displayed periodic nutrient deficiency symptoms.

According to Maher (1994) there is a very high level of soluble salts in SMS and that high EC levels are a factor limiting the proportion that can be used in a substrate. NPK experiments show that SMS behaves very differently as a source of N compared with P and K. The five per cent level is adequate to produce a good size plant when the SMS is supplying either P or K, however SMS is not an effective N source. Any higher ratio of application and EC becomes a damaging factor.

Maynards (1994) and Wang *et al*. (1994) reported that vegetable production could be sustained using spent mushroom compost. According to Steffen (1994), soil properties associated with greater crop productivity, such as increased water holding capacity and decreased bulk density, have been shown to improve with addition of SMS. The addition of organic matter to the soil and the use of a straw mulch dramatically increased yields of tomato when compared to a system that was unamended and mulched with black polyethylene.

Rhodas and Olson (1995) suggested that optimum SMC was 44.8 ton ha^{-1} for many crops and they postulated that increased yield was due to the nitrogen availability which was resulted with SMC treatments. Steward (1995) and Steward *et al.* (1997) observed that spent mushroom substrate application to the soil resulted in increased yield of potato.

According to Craft and Nelson (1996) compost stimulates a natural defence system in plants. The disease suppressiveness of SMS against damping off disease (*Pythium ultimum)* was evaluated and addition of SMS @ 25-100% to perlite significantly provided effective control of damping off disease in tomato. The lowest wilt disease incidence and the highest yield of aubergine has been recorded by Yohalem *et al.* (1996) from the plots applied with SMS+paddy straw (1 ton/ ha + 2 tons/ ha) along with the recommended doze of NPK as compared to FYM (10 tons/ ha) and poultry manure (1.4 tons/ ha).

Li *et al*. (1998) found that crop yield dramatically increased in SMC and vermiculite ratio of 2 to 1 in tomato and cucumber in a comparison to that of the control. According to Steward *et al.* (1998), SMC treatments positively affected crop yield and plant nutrition uptake. LaMondia *et al*. (1999) reported that soil amendment with spent mushroom compost may be a means of reducing the effects of potato early dying and increasing tuber yield when one or both the pathogens are present.

Hampton *et al.* (2001) studied about the composted spent mushroom substrate as mulch for biological control of weeds in vegetable crops. Shibuya and Minami (2001) reported that carbohydrate and protein elicitors that induce defence mechanism in plants are released from the mycelia of fungal pathogens. Therefore, the mycelia of mushrooms that are prevalent in SMS are abundant sources of elicitors and thus application of SMS to plants may be useful for the control of plant diseases.

Viji *et al*. (2003) obtained a total of 849 bacterial isolates from two spent mushroom substrate samples and tested for antagonism against the gray leaf spot pathogen. Thirty two of the 849 unidentified bacterial isolates significantly inhibited the mycelial growth of *P. grisea in vitro.* The six isolates were identified by fatty acid profile analysis as *Pseudomonas aeruginosa* (Schroeter) Migula. *P. aeruginosa* is one of the most predominant bacteria in fresh SMS tested.

According to Uzun (2004) after mushrooms have been harvested, the SMS can be used in organic farming to improve soil water infiltration, water holding capacity, permeability and aeration. SMS is more expensive than mineral fertilizers in terms of the nutrient content per unit weight, but provides many benefits that mineral fertilizers cannot. SMS improves the biological, physical and chemical characteristics of the soil. For this reason, SMS should be applied once every few years in sustainable fruit growing. According to Davis *et al.* (2005) spent mushroom substrate exhibits suppressive characteristics against various fungi as well as against plant diseases caused by fungi. Therefore, aged mushroom compost can be used as a biological control agent for artillery fungi.

Ahlawat *et al.* (2006) studied the effects of spent mushroom substrate (SMS) recomposted by different methods and mixed with arable soil on the vegetative growth, yield and quality attributes of cauliflower (*Brassica oleracea* var. botrytis cv. Pusa Snowball-1). Mixing of anaerobically recomposted SMS at 2.5 kg/m + chemical fertilizers (N, P and K at 12.5, 7.5 and 6.5 g/m^2, respectively) significantly enhanced vegetative growth during curd harvesting, gross and net yields, and quality attributes, and reduced the incidence of black rot disease and larval infestation. The mortality of plants during seedling transplanting was also lower under anaerobic SMS treatments. Thus, the use of anaerobically recomposted SMS with chemical fertilizers for cauliflower cultivation was found to be a better option of SMS disposal and raising high-quality vegetable crops.

Suess *et al.* (2006) reported that SMS has the potential to play an important role as a soil conditioner for agricultural production. SMS contains a diverse range of soil microorganisms. This has been 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 will enhance and accelerate regular soil processes such as nutrient mobilization and aggregate formation. Organic Matter is one of the most desirable components of SMS. Addition of SMS will add organic matter to the soil. When SMS is applied as the soil mulch, it acts as a natural barrier to weeds and disease. It also regulates soil temperature and humidity. 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. Research has been conducted on aqueous extracts of SMS, (otherwise known as compost tea), in order to suppress plant diseases. Compost tea is a liquid extract of SMS that contains nutrients and microorganisms.

Paredes *et al.* (2007) carried out a study involving the analysis of different organic matter (OM) fractions in the two types of SMS obtained from 19 mushroom industries in Spain. In this experiment, 40 different types of SMS [19 spent substrates of *Agaricus bisporus* (SMS-AB) and 21 spent substrates of *Pleurotus* (SMS-P)] were evaluated for OM; soluble polyphenols; organic nitrogen (Norg); total organic carbon (Corg); water-soluble carbon (Cw); cation exchange capacity (CEC); Corg/Nt, Cw/Norg, and CEC/Corg ratios; humic acid-like carbon (Cha); fulvic acid-like carbon (Cfa); 0.1 M sodium hydroxide (NaOH)-extractable organic carbon (Cex); humification ratio (HR) (Cex/Corg × 100); humification index (HI) (Cha/Corg × 100), percentage of humic acid-like carbon (Pha) (Cha/Cex × 100) and Cha/Cfa ratio. The study of the different fractions of OM showed that SMS-P had higher contents of Corg, Cw, and soluble polyphenols than SMS-AB. However, the fraction of organic N was significantly higher in SMS-AB than in SMS-P.

Poczik (2007) conducted a trial in order to examine the possibility of using spent mushroom composts of button mushroom in horticultural production. The mediums tested included the control and those containing 100%, 50% and 25% spent compost respectively. According to the results obtained up to now those mediums containing 25% and 50% spent compost were superior to the control both in terms of yield and crop quality. The substrate containing 100% spent compost did not show any significant differences in yield as compared to the control, in crop quality, on the other hand, the 100% treatment provided more favourable results.

According to Kaviyarasan and Siva (2007) isolation and identification of effective local isolate of *T. harzianum* and formulation based on the *Pleurotus* spent compost produced more chitinase, indicate that compost is a suitable carrier, not only to supply nutrients but a good substrate to support the survival time in the soil. Castro *et al.* (2008) reported that after growing 29 days on *Pleurotus* spent mushroom substrate, *Salvia officinalis* plants showed a marked increase in biomass and some minerals

compared to the control. This improvement can be due to the higher air porosity and content of certain essential mineral nutrients to the substrate.

Youngll *et al.* (2008) conducted a study to isolate and identify xylanase- and cellulase-producing thermophilic bacteria from stacked spent mushroom substrates and to determine the optimal medium conditions for their growth. Bacteria with the highest xylanase and CMCase activities were strain 3 and 201-7. Both of them were identified as *Bacillus* spp. and named *B. subtilis* KU3 and *B. subtilis* KU201-7.

Ahlawat *et al.* (2009) evaluated spent mushroom substrate (SMS) of different weathering age and recomposted by different methods was used as manure for nutritionally poor soil and its effects were studied on vegetative growth, yield and quality of tomato. Results of the study revealed that amending of arable soil with 6 to 24 months old naturally weathered SMS @ 25 tonnes/ha, enhanced the vegetative growth, yield and quality of tomato over FYM and control treatments. Soils manured with anaerobically recomposted SMS, also reduced the incidence of leaf curl virus, fruit rotting microbes and fruit borer both on plants and tomatoes. The use of anaerobically recomposted SMS for tomato cultivation was found to be a better option of spent mushroom substrate disposal and raising crops organically.

Medina *et al.* (2009) reported the possibility of using spent mushroom substrate (SMS) in the production of horticultural seedlings replacing part of the peat in the growing media. Three vegetable species with different salt sensitivities, the less sensitive being tomato (*Lycopersicon esculentum* var. Muchamiel), the moderately salt-sensitive being courgette (*Cucurbita pepo* L. var. Afrodite F1) and the most salt-sensitive being pepper (*Capsicum annum* L. var. Lamuyo F1) were grown in 12 media containing SMS of two types of mushroom (*Agaricus bisporus* (SMS-AB) and *Pleurotus ostreatus* (SMS-PO) or a mixture of both 50% (v/v) (SMS-50), as well as peat in various ratios. Regarding the most suitable SMS-based substrates for plant growth, any substrate could be used for tomato seedling production. However, all SMS-AB-based substrates and the media containing low dose of SMS-PO and SMS-50 were adequate for growth of courgette and pepper.

Polat *et al.* (2009) reported the effect of spent mushroom compost on quality and productivity of cucumber grown in green houses. All of the spent mushroom compost

treatments resulted in higher yield than control treatment. Sagar *et al.* (2009) conducted a study on the indigenous technical knowledge on the use of spent mushroom substrate (SMS) for agriculture in Himachal Pradesh, Haryana, Punjab, Uttaranchal, Uttar Pradesh and Delhi. The mushroom growers and researchers observed yield enhancement and lower incidence of diseases in field and horticultural crops like ginger, capsicum, tomato, cauliflower, pea, potato, garlic, wheat, paddy, maize and apple, along with changes in soil physical conditions, when SMS was used as an amendment. Majority of the farmers (38.89%) noticed decrease in incidence of insect, pests and diseases in the crops manured with SMS. The application of SMS as soil amendments promotes a population of antagonistic microorganisms, which interfere with the activities of pathogenic fungi. In the case of ginger and onion, the 12-18 months old aerobically recomposted SMS gave superior fruit yield as well as quality along with lower incidence of rotting the bulbs and rhizomes.

Kadiri and Mustapha (2010) studied the effects of spent mushroom substrate on the vegetative growth and yields of cowpea and tomato. Autoclaved and unautoclaved spent mushroom substrates, mixed with loamy soil in ratio 1:9 were utilized with both composted and uncomposted spent mushroom substrates. Composted spent mushroom substrate mixed with loamy soil produced greater vegetative growth and yields of cowpea and tomato than uncomposted spent mushroom substrate, which in turn gave better results than loamy soil controls

The effect of SMS on disease suppression (damping off caused by *Pythium aphanidermatum*) and plant growth promotion using SMS were evaluated by Sanam and Gokulapalan (2010). Resident fungi were isolated from SMS using serial dilution plate technique. The fungal isolates isolated from SMS were tested for their antagonism to *Pythium aphanidermatum.* These included species of *Trichoderma, Aspergillus* and *Rhizopus* and they exhibited varying levels of antagonism towards *P. aphanidermatum. In vivo* studies for the disease suppression and growth promotion in tomato seedling were carried using various concentrations of SMS (0%, 25%, 50%, 75%, 100%) on volume basis, in combination with sand. There was no disease incidence in plants growing in media amended with 75% SMS. The plant growth studies showed improvement in plant growth using 25%, 50% and 75% SMS. Plant growth was comparatively poor in 100% SMS.

Eudoxie and Alexander (2011) evaluated the quality and effectiveness of substrate mushroom compost (SMS) as a complete substitute for promix (PM) in the germination, growth and development of tomato seedling. Germination percentage was >95% for both SMS fine (SMSF) and PM treatments. SMS treated seedlings were taller (32%) and possessed a greater number of leaves (12%) at 5 weeks after seeding (WAS) than PM seedlings at 6 WAS. SMS was shown to be a better media for tomato seedling production.

Godwin-Egein *et al.* (2011) carried out an investigation on the effect of spent mushroom substrate on the wilt disease of tomato when the spent substrate is used as soil amendment. Stem diameter, plant height, number of leaves, disease incidence and disease severity were evaluated. Two tomato varieties tropimech and tomato U82B at 4 to 6 weeks old stage 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. The ratio 1:4 was observed to have the most positive effect on the disease, while the ratio 1:5 had the least effect on the disease.

Parada *et al.* (2011) studied about the protective effect of fresh spent mushroom substrate (SMS) of hatakeshimeji (*Lyophyllum decastes* Sing.), a popular culinary-medicinal mushroom, and its water extract against anthracnose of cucumber. Plants were treated with water extract from SMS or autoclaved water extract by spraying the whole plant or by dipping the first true leaf, and inoculated with *Colletotrichum orbiculare* seven days later. Plants treated with either of the extracts showed a significant reduction of necrotic lesions.

SuoXia *et al*. (2011) evaluated the spent mushroom compost and the garden wastes compost as components of substrates in partial substitution of peat for seedlings production. Then its effects on cucumber (*Cucumis sativus* L.) seedling quality were investigated comparing with the control (commercial substrates). Plant height, stem diameter, dry matter accumulation and seedling index and other indicators of cucumber seedling cultivated on substrate were significantly excelled that of the control treatment.

Szulc *et al*. (2011) conducted three-year field experiment to study the effect of raw and composted spent mushroom substrate on maize yield and soil properties. The investigations shown that fertilization with spent mushroom substrates and composts

made of this substrate increased yields of maize as compared to the control treatment. These fertilizers have also favourable effect on the soil chemical properties in terms of raising soil pH and increasing the contents of available forms of P, K and Mg and improving the sorption properties of the soil. Among the studied fertilizers compost made from spent mushroom substrate and sewage sludge showed the best impact on yield of maize and improving the soil chemical properties.

2.4 EFFECT OF ISOLATED ORGANISMS ON THE PATHOGENS

Attempts have been made by many scientists to test the *in vitro* effect of antagonistic organisms against fungal and bacterial pathogens.

2.4.1 Fungal antagonists against fungal pathogen

D'Ercole *et al.* (1984) tested 60 isolates of *T. viride, T. harzianum, T. koningii, T. hamatum, T. polysporum, T. pseudokoningii* and *T.* spp. on PDA against *Rhizoctonia solani, R. fragariae, Fusarium moniliforme, Pythium ultimum* and *Verticillium dahliae.* Four types of antagonism were found: relief formation in the contact zone between the fungi, lytic phenomena, complete covering of the pathogen by the antagonist and variable behaviour. Dennis and Webster (1986) assessed the effectiveness of volatile and non volatile metabolites produced by *T. viride* on two ginger rot pathogens.

Bhardwaj *et al.* (1988) reported that rhizome rot of ginger, caused by *Pythium aphanidermatum* and *Fusarium equiseti*, was controlled during storage by 3 *Trichoderma* spp. to varying degrees. Volatile substances produced by *T. viride* seemed to be effective against *P. myriotylum* as the growth of this pathogen was completely inhibited by these substances (Rathore *et al.,* 1990).

Pot culture experiments established the efficacy of *T. harzianum* for control of rhizome rot of ginger. Suppression of *Pythium aphanidermatum* and rhizome rot of ginger by *Aspergillus niger, A. terreus, penicillium* spp. and *Absidia cylinrospora* is reported by Balakrishnan *et al.* (1996).

Shanmugam and Varma (1999) isolated native microorganisms from the rhizosphere of healthy ginger plants among rhizome rot affected plants in diseased fields and screened *in vitro* for their antagonistic effects against the rhizome rot pathogen *Pythium aphanidermatum* by dual culture and cell free culture filtrate studies. *Aspergillus niger, A. fumigatus, A. flavus* and *Trichoderma viride* were found to be potential antagonists.

According to Shanmugham *et al.* (2000) dual culture of the antagonistic organisms with *P. aphanidermatum* indicated that only *T. harzianum* and *T. viride* were potential antagonists. Rajan *et al*. (2002) reported that a biocontrol agent, *T. harzianum*, isolated from Sikkim was found effective in control of ginger diseases substantially.

Tong and LiangHao (2002) reported that some *Trichoderma* strains are promising biological control agents of plant disease. They observed that chitinolytic enzymes of *Trichoderma* play an important role in mycoparasitism, which is considered as one of the mechanisms involved in the antagonism of *Trichoderma* against plant fungal pathogens (including *Rhizoctonia, Fusarium, Alternaria, Ustilago, Venturia, Pythium, Phytophthora* and *Botrytis*). The induced production, the physical and chemical characteristics of chitinases from *Trichoderma* strains and their antagonism against plant fungal pathogens, and the potential use of the chitinases and chitinase genes from *Trichoderma* for biological control is also discussed.

According to Szekeres *et al.* (2004) several *Trichoderma* strains have been reported to be effective in controlling plant diseases, and the action of fungal hydrolytic enzymes is considered as the main mechanism involved in the antagonistic process. Strain *Trichoderma harzianum* T334 is a potential biocontrol agent against plant pathogenic fungi with the ability to produce low levels of proteases constitutively. To improve its fungal antagonistic capacity, mutagenetic program was undertaken for the construction of protease overproducing derivates.

Sala *et al.* (2007) reported the effectiveness of *Trichoderma* as biocontrol agent against a number of plant pathogens based on several mechanisms, such as antibiosis, mycoparasitism, induction of defence responses and other adjunct mechanisms, such as growth promotion. Such isolates were previously selected for *in vitro* antagonism

towards *Pythium* sp., *Rhizoctonia solani* and *Sclerotium rolfsii,* on Potato Dextrose Agar amended with *Trichoderma* culture filtrates.

Sharma (2008) conducted dual culture experiment with the biocontrol agents: *Trichoderma harzianum, T. viride, Gliocladium virens* and *Absidia cylindrospora* and plant extracts from *Agave americana, Azadirachta indica, Cassia fistula, Eucalyptus terticornis* and *Vitex negundo* against the causal fungi of yellows and rhizome rot of ginger. *T. harzianum, T. viride, A. indica* and *A. americana* were the most effective in reducing mycelial growth of *Fusarium oxysporum* f.sp. *zingiberi* and *Pythium aphanidermatum,* causal agents of yellows and rhizome rot of ginger, respectively.

Pandey *et al.* (2010), selected 12 *Trichoderma* isolates as biocontrol agents against a panel of soil borne pathogens as *Pythium aphanidermatum*, *Rhizoctonia solani* and *Pythium ultimum.* Dual culture method and food poisoned technique were used to test the antagonistic effect of *Trichoderma* spp. isolates against fungal pathogens. Culture filtrate of Tv7 at 80% conc., and culture filtrate of Th4 at 90% & 100% conc. have shown maximum inhibition against *Pythium aphanidermatum*.

Mishra (2010) screened ten strains of *Trichoderma* species against *Pythium aphanidermatum* by dual culture method. Efficacy of culture filtrates of the strains was also determined. Since mycoparasitism plays important role in antagonism mechanism of *Trichoderma* species, extracellular enzymatic activity of the strains was assayed. Among the strains tested, *T. viride* 1433 was found most effective against *P. aphanidermatum.*

According to Zembek *et al.* (2011) antagonism of *Trichoderma* spp. against phytopathogenic fungi is widely exploited for biocontrol of plant diseases. A crucial role in the biocontrol mechanism is attributed to cell-wall-degrading enzymes secreted by *Trichoderma* spp. Therefore, more efficient production and secretion of the enzymes should elevate the biocontrol abilities of *Trichoderma* spp. Because the majority of secretory hydrolases are glycoproteins, it has been postulated that the posttranslational modification of these proteins could constitute a bottleneck in their production and secretion.

Muthukumar *et al.* (2011) evaluated the effect of eight isolates of *Trichoderma* species (from chilli rhizosphere) were tested against *P. aphanidermatum.*

All the *Trichoderma* species had varied antagonistic effects against the pathogen. Among them, TVC_3 recorded maximum growth inhibition of *P. aphanidermatum* and produced more amounts of volatile and non-volatile metabolites. The culture filtrate of the *Trichoderma* isolate TVC_3 recorded complete inhibition on the mycelial growth of pathogen at 15% concentration.

According to Maheswari and Sirchabai (2011) *in vitro* studies on biocontrol agents against turmeric rhizome rot pathogen *Pythium aphanidermatum* revealed that fungal bioagent was inhibiting the growth of pathogen. *Trichoderma viride* (79 mm) was best inhibiting the colony growth of *Pythium aphanidermatum* (11 mm) in 288 hours P=<0.001 statistically significant difference. Experts recommend that rhizome treatment with biofungicide like *Trichoderma* spp., can effectively control of this disease.

2.4.2 Bacterial antagonists against fungal pathogen

Heungens *et al.* (1992) *P. aeruginosa* str. 7NSK2, a bacterium beneficial to plant growth, produces 2 siderophores, pyoverdin and pyochelin, under iron-limiting conditions. Mutants deficient in pyoverdin and pyoverdin + pyochelin were obtained by mutagenesis. *In vitro* antibiosis of 7NSK2 and the 2 mutants against a plant pathogenic *Pythium* sp. was tested in iron-limiting conditions. In non-sterile pot soil artificially infested with *Pythium sporangia*, seed (but not soil) inoculation with all 3 *P. aeruginosa* isolates significantly (P<0.01) protected tomato seedlings against pre-emergence damping off, whereas only 7NSK2 and the pyoverdin-deficient mutant also gave protection against post-emergence damping off. Results suggested that siderophore production is involved in the antagonistic effect of 7NSK2 against *Pythium*, particularly in control of post-emergence damping off.

BingGan *et al.* (2002) evaluated the efficacy of different bacterial strains as biological control agents in controlling *Pythium ultimum* (strain ONCUR01) and *P. spinosum* (strain MP43419) causing seedling damping off in cucumber in pot and commercial vegetable field experiments. In pot experiments, the biological control

efficacy of *Pseudomonas aeruginosa* strain CR56 against *Pythium ultimum* and *P. spinosum* was 94.4 and 51.4% respectively.

Singh *et al.* (2003) reported that two plant growth-promoting rhizobacteria (PGPR), *viz., Pseudomonas fluorescens* strain Pf4 and *P. aeruginosa* strain Pag, protected chickpea (*Cicer arietinum*) plants from *Sclerotium rolfsii* infection when applied singly or in combination as seed treatment. The two PGPR strains induced the synthesis of specific phenolic acids, salicylic acid (SA), as well as total phenolics at different growth stages of chickpea seedlings with varied amount. The maximum amount of total phenolics was recorded in all the aerial parts of 4-week-old plants.

According to Parneel *et al.* (2004) cocoyam (*Xanthosoma sagittifolium*), an important staple food for millions of people in the tropics, suffers from the root rot disease caused by *Pythium myriotylum*. Two phenazine-producing *Pseudomonas aeruginosa* strains, isolated from the rhizosphere of healthy cocoyams, were very effective against *Pythium myriotylum in vitro*. Greenhouse experiments demonstrated that compost, prepared from locally available organic matter inhibited the infection of *Pythium myriotylum* on cocoyam.

Al-Hinai *et al.* (2010) conducted a study to isolate and characterize bacterial strains from greenhouse soils with potential for suppression of *Pythium* damping-off of cucumber. Out of 100 bacterial isolates collected from greenhouse soils, 20 isolates were found to be antagonistic to *Pythium aphanidermatum* in *in vitro* studies. Identification of these bacterial isolates to the species level using API20NE as well as sequences of the 16S ribosomal RNA confirmed their identity as *Pseudomonas aeruginosa*. This is the first report of occurrence of *P. aeruginosa* with antagonistic activity against *P. aphanidermatum* in greenhouse soils in Oman.

2.4.3 Fungal antagonists against bacterial pathogen

Guijing *et al.* (1998) observed that conidial extracts of *Trichoderma koningii* B4-88 completely inhibited the growth of *R. solanacearum* and recovery rates of the antibiotic substance extracted by alcohol and NaOH were 0.89 per cent and 0.82 per cent respectively. Manimala (2003) observed that among *Trichoderma* sp., *T, viride* and

T. pseudokoningii were the promising ones by recording a maximum antagonism index value of 6000 and showed complete inhibition of all the six isolates of *R. solanacearum* by its lysis and over growth type of antagonistic property.

Das and Bora (2000) reported that the biological control of bacterial wilt in tomato by *Ralstonia solanacearum* was performed with few established biological control agents, *Pseudomonas fluorescens, Bacillus subtilis, Trichoderma harzianum, T. viride, T. koningii, Aspergillus terreus* and *Gliocladium virens*. The antagonists were evaluated *in vitro* for their inhibitory action against the wilt pathogen, *Ralstonia solanacearum*, using agar plate assays in dual culture. Manimala (2003) reported that with regard to fungal culture filtrates, better inhibitory effect on *R. solanacearum* was observed with *Trichoderma virens* and *T. pseudokoninji*.

Ramesh and Korikanthimath (2004) evaluated two species of *Trichoderma* and two isolates of *Pseudomonas fluorescens* against *Pythium aphanidermatum,* causal agent of damping off, and *Ralstonia solanacearum,* a wilt pathogen, in aubergine. In case of wilt, all antagonist treatments proved effective in reducing the disease incidence and increasing yield under field conditions. The growth parameters and vigour index recorded in the nursery were high in *Pseudomonas fluorescens* treatments followed by *Trichoderma* treatment.

According to Priya *et al*. (2005) carried out an *in vitro* evaluation to find out the efficiency of bacterial and fungal antagonists in checking the growth of pathogens of Kacholam. Among the two fungal antagonists tested *Trichoderma viride* was more efficient than *Aspergillus niger* because of its faster growth over the pathogen. However, both the antagonists showed complete overgrowth and dense sporulation on the area where the pathogen was streaked.

Hussain and Bora (2008) conducted an experiment with half recommended dose each of carbofuran 3G, neem cake, streptocycline and full dose of *Trichoderma harzianum* against *Meloidogyne incognita* and *Ralstonia solanacearum* complex in brinjal under field conditions. The treatment effectively improved all the plant growth parameters and yield of the crop with corresponding decrease in the nematode reproductive rate. The treatment also produced minimum final bacterial population in the soil along with less percent wilt incidence (PWI).

Maketon *et al.* (2010) evaluated seventy-eight bacterial isolates and two commercially available microorganisms for control of *Ralstonia solanacearum* , wilt disease pathogen of ginger in Thailand. Two bacteria, *Bacillus subtilis* K1 and *Pseudomonas fluorescence* PS12 and the commercially available fungus *Trichoderma harzianum* AP-001 (TrisanTM) provided the best disease control.

2.4.4 Bacterial antagonists against bacterial pathogen

Suresh and Ravi (1992) observed inhibition zones when *Pseudomonas purpurescens* was tested on *P. solanacearum.* Silveira *et al.* (1996) evaluated a number of microorganisms including *Streptomyces griseochemogenus, S. Griseus, P. fluorescens, T. pseudokoningii, P. aeruginosa, B. coagulens, B. megatherium* and *B. cereus* against *R. solanacearum*, and were effective under *in vitro* condition.

Anith *et al*. (2000) observed that seed treatment with *P. fluorescens* strain EM 85 along with soil solerization decreased the wilt incidence in ginger to 7.42 per cent and increased the yield to 29.42 t/ha compared to 19.5 t/ha in control plots.

Chao-hui and Jin-fei (2011) collected diseased ginger and the surrounding soils, and the high-virulence strains of the pathogen of ginger blast (*Ralstonia solanacearum*) were isolated, *Bacillus* was used to carry out antagonistic test. Three strains LW -4, LW-7 and LW-32 had strong antagonistic effect against *R. solanacearum*, the area of their inhibition zone was larger than other strains. The study provided theoretical basis for the control of ginger blast (*Ralstonia solanacearum*) with *Bacillus* spp.

2.5 PREPARATION OF CONSORTIUM

Potential benefits of application of a single biocontrol agent has been demonstrated in many studies, inconsistent performance may occur when only a single organism was used because a single biocontrol agent is not likely to be acive in all kinds of soil environment and agricultural ecosystem. The possibility of introduction of a consortium of inoculants may provide an ever wider spectrum of disease suppression.

The use of combined inoculation of fungal and bacterial antagonists has already been reported as an effective disease management method (Pierson and Weller, 1994).

Duffy *et al*. (1996) demonstrated in field trials that the combinations of *Trichoderma koningii* and *Pseudomonas fluorescens* Q29Z-80 increased the yield compared to *P. fluorescens* Q29Z-80 alone, but not different from *T. koningii* alone. According to Mishra *et al.* (2002) the use of mixtures of biocontrol agents would be a reliable means for management of plant diseases. Chaube and Sharma (2002) reported that *T. harzianum* or *T. viride* and *P. fluorescens* were compatibile and improved plant growth and suppressed seedling disease of cabbage, brinjal and tomato significantly when treatments were combined and integrated with solarisation.

Kumar *et al.* (2004) conducted an experiment during 2002-04 in Tamil Nadu, India, coffee pulp compost prepared by using a microbial consortium containing *Trichoderma reesi* and *Pseudomonas fluorescens* was applied to a bhindi crop growing on a clay loam soil and receiving different fertilizer treatments. The application of coffee pulp compost at 10 t ha^{-1} with 75% of the recommended NPK rate resulted in the highest fruit yield of 17.00 t ha^{-1}.

Shahida (2007) found that *Trichoderma* sp. and *P. fluorescens* were compatible with each other. According to Ojo and Oso (2008) native microbial consortium of a wastewater ecosystem found to utilize detergent components were characterized using standard and conventional methods. The organisms identified were *Enterococcus majodoratus, Klebsiella liquefasciens, Enterobacter liquefasciens, Klebsiella aerogenes, Escherichia coli, Enterobacter agglomerans, Staphylococcus albus, Pseudomonas aeruginosa, Proteus* sp., *Klebsiella oxytoca, Brevibacterium* sp., *Myceliophthora thermophila, Geomyces* sp., *Alternaria alternata, Verticillium alboatrum, Aspergillus flavus, Trichoderma* sp. and *Aspergillus oryzae*. The biodegradation of the synthetic detergent components that occurs in wastewater, sewage treatment plants and in the ultimate open-water receiving ecosystems is primarily the result of microbial activities.

Jain *et al*. (2010) selected *Pseudomonas aeruginosa* PJHU15, *Trichoderma harzianum* TNHU27 and *Bacillus subtilis* BHHU100 from rhizospheric soils based on compatibility, antagonistic and plant growth promotion activities. The microbes were

used as consortia to assess their ability to trigger the phenylpropanoid and antioxidant activities and accumulation of proline, total phenol and pathogenesis-related (PR) proteins in pea under the challenge of the soft-rot pathogen *Sclerotinia sclerotiorum*. The compatible microbial consortia triggered defence responses in an enhanced level in pea than the microbes alone and provided better protection against Sclerotinia rot. Rhizosphere microbes in consortium can enhance protection in pea against the soft-rot pathogen through augmented elicitation of host defence responses.

2.6 EFFECT AQUEOUS EXTRACT OF SMS

According to Yohalem *et al.* (1994) anaerobically fermented aqueous extract of spent mushroom substrate was most effective for the inhibition of germination of conidia of *Venturia inequalis,* causal agent of apple scab. The material was most inhibitory to conidial germination after 5 to 9 days incubation in ratios from 2:1 to 4:1, water:SMS (volume). Two genera of bacteria were consistently isolated from the extract, a pseudomonad and a *Bacillus*.

Yohalem *et al*. (1996) reported that aqueous extracts from 2 sources of spent mushroom substrate (SMS) were anaerobically fermented for 7 days and amended with spreader-sticker. These were then applied at weekly intervals to apple trees (cv. McIntosh) from green-tip to petal-fall and biweekly thereafter to investigate the effect on apple scab caused by *Venturia inaequalis*. Disease incidence decreased but to a lesser extent. Higher populations of bacteria were detected on leaves treated with the extracts. No differences occurred in total numbers of fungi. Inhibitory activity of extracts under different conditions was assessed *in vitro* by inhibition of *V. inaequalis* conidia germination.

According to Guo *et al.* (2001), spent mushroom substrate leachate contains the inorganic cations K^+, Na^+, $Ca^{2+,}$ and Mg^{2+} with concentrations ranging from 72 to 316, 21 to 76, 2 to 116, and 27 to 86 mmol/ L respectively and inorganic anions chloride, SO_4^{2-} , and NO^{3-} with concentrations ranging from 27 to 196, 79 to 324 and 0.03 to 118 mmol/ L respectively. Uzun (2004), Cronin (1996) and Andrews *et al.* (1994) reported the effectiveness of SMS at controlling apple scab. Compost tea is a liquid extract of

SMS prepared by mixing one part of compost with four parts of water. It contains soluble nutrients and beneficial microorganisms, including bacteria, fungi, protozoa and nematodes. It can be used to control apple scab disease (*Venturia inaequalis*). Compost tea reduces conidia germination in *V. inaequalis* by 98% probably due to heat stable compounds produced by anaerobic microorganisms during the incubation of the compost slurry.

Mansour *et al*. (2011) used clarified compost tea of three different plant sources of slurries of spent mushroom substrate (SMS), path and/or rice straw, as inhibitors for different foliar and soil borne pathogens. In greenhouse trails, soil amendment with compost tea (SMS) showed high effect in reducing root rot incidence caused by *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolinae* at pre-emergence damping-off stage. The same treatment reduced root rot disease after 45 days caused by the previous three pathogens. Seed treatment with compost tea reduced root rot diseases at the pre- and post emergence stages. Meanwhile, coating seeds with compost tea had a good effect in reducing root rot incidence under field conditions. It possessed a strong antifungal active effect against soil borne pathogens. It is worth to recommend the practical use of compost tea or seed treatments to control soil borne plant pathogens as a substitute of chemical fungicides without any risk to human, animal and environment.

Materials and Methods

3. MATERIALS AND METHODS

The present study on 'Exploitation of spent mushroom substrate for the management of rhizome rot complex disease of ginger' was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the period 2010-2012. The details of the materials used and the techniques adopted for the investigation are described below.

3.1 ISOLATION OF PATHOGENS ASSOCIATED WITH THE DISEASES

Isolation of *Pythium aphanidermatum* associated with the rhizome rot disease was done by taking small bits of infected rhizome along with some healthy tissues, surface sterilized with one percent sodium hypochlorite, washed in three changes of sterile distilled water and placed in Petri dishes mediated with Potato Dextrose Agar (PDA). All the dishes were incubated for 2-3 days under cool condition and observed for the growth of pathogen (Plate 1A).

For the isolation of bacterial pathogen *Ralstonia solanacearum*, ginger plants showing different types of symptoms on foliage and rhizomes were collected from farmer's field and brought to the laboratory. Samples were then washed under tap water. The wilted plants were subjected to ooze test to confirm the presence of bacteria. The infected rhizomes were cut into small bits, surface sterilized and placed on a clean glass slide with a drop of sterile water. The bacterial ooze streaming out from xylem vessels were streaked on Triphenyl Tetrazolium Chloride (TZC) medium and incubated for 48 hour at room temperature (Plate 1B).

3.1.1 Purification of pathogens

The fungi, *P. aphanidermatum* grown on the PDA medium was purified by hyphal tip method, subcultured and maintained on PDA slants for future investigations.

Plate 1 : Symptomatology of rhizome rot complex disease of ginger

A. Soft rot

B. Bacterial wilt

In the case of *R. solanacearum*, circular, fluidal, slimy, white or creamy white colonies with light pink centres which characterize virulent colonies were selected after incubation at 30 ± 2^0C for 24 to 48 h and were then purified by repeated streaking on the TZC medium. Permanent stock cultures of the bacterium was maintained in vials by preparing bacterial suspensions containing 2 to 3 loopful of pure cultures of bacteria in 5ml of sterile distilled water. The suspensions were stored at 5^0C under refrigeration and also under room temperature. The cultures were tested periodically for virulence and purified by streaking on TZC medium.

3.2 PRODUCTION OF SPENT MUSHROOM SUBSTRATE

Spent mushroom substrate (SMS) is the composted organic material remaining after a crop of mushroom is harvested. For the production of SMS, *Pleurotus florida* and *P. sajor-caju* were the mushroom species selected and they were produced in paddy straw, saw dust and neopeat (Plate 2).

3.2.1 Isolation and pure culturing of mushrooms

Tissue culture technique was used for isolation of different species of oyster mushrooms *viz., Pleurotus florida* and *Pleurotus sajor-caju*. Medium sized sporocarps having medium maturity were selected for tissue culture. The selected mushroom was surface sterilized using ethanol under aseptic conditions, split lengthwise into two equal halves from pileus to stipe and small bits of tissue from the junction of stipe and pileus was scooped out using a sterile scalpel. The tissue was transferred into Petri dish plated with PDA medium under aseptic condition. The inoculated Petri dishes were incubated at room temperature for seven days and observed for mycelial growth. The mycelial mat obtained from each mushroom was subcultured periodically and maintained as pure culture for further studies.

3.2.2 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 grains were thoroughly washed with tap water and cooked for 30 minutes in boiling water till it become soft. The cooked grains were decanted through sieve to remove excess water and were

evenly spread on a clean muslin cloth for cooling. To the cooled grains, calcium carbonate was added @ 40g/ kg of seeds, thoroughly mixed, filled in polypropylene bags upto 2/3rd capacity, plugged with cotton and autoclaved at 1.05kg/cm^2 for 2 hours 30 minutes. After cooling the sterilized grains were inoculated aseptically with actively growing pure cultures of *Pleurotus florida* and *P. sajor-caju* 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.

3.2.3 Preparation of substrates

Paddy straw, saw dust and neopeat were used as substrates for mushroom production using *P. florida* and *P. sajor-caju* spawn.

3.2.3.1 Paddy straw

Good quality paddy straw was chopped into bits of four to five cm length and used for mushroom cultivation.

3.2.3.2 Saw dust

Fresh saw dust of soft wood trees collected from a saw mill in Thrissur (dist.) was used. It was filled in gunny bags after removing large pieces of wood and large pieces of inert materials.

3.2.3.3 Neopeat

Neopeat bricks were placed in suitable containers, poured 20-30 litres water/brick and left the bricks to soak in water. Then stirred the expanded bricks until it becomes light and fluffy. From one brick, 65-70 litres of coirpith was got and used for the study.

3.2.4 Sterilization of substrates

Chemical sterilization was followed for sterilizing different substrates. The substrates were transferred to gunny bags, steeped in a solution made of carbendazim (75ppm) and formaldehyde (500ppm) for 18h. The excess water drained off and spread

Plate 2 : Substrates and the Oyster mushroom species used for the study

A. Substrates used

Paddy straw

Saw dust

Neopeat

B. Mushroom species used

Pleurotus florida

Pleurotus sajor-caju

on a clean floor for drying. The moisture content of the substrate was maintained at optimum level and was used for bed preparation.

3.2.5 Preparation of beds

The standard compact poly bag method described by Bhaskaran *et al.* (1978) was used for bed preparation using different substrates. For the preparation of mushroom beds poly bags of size 30x60cm with 150-200 gauges thickness was used. About 30 holes of 0.5mm size were made on each polythene bag and the bottom was tied with a twine. Then the perforated bags were filled upto five cm height with sterilized substrate and pressed with hand for making it even. Then 20-25g 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 four times. Finally the bag was 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 25-28^{0}C and 80-90 per cent respectively by spraying water inside the room. After spawn run, the beds were transferred to cropping room, where the temperature and RH were maintained at 25 to 26^{0}C and 80 to 90 per cent respectively.

3.2.6 Observations

Observations on the nature of mycelial growth, time taken for spawn run, number of days taken for mushroom production, weight of sporocarps, and the microbial population of substrate at different growth stages were recorded.

3.3 ISOLATION OF MICROORGANISMS FROM DIFFERENT SMS

Quantitative estimation of bacteria, fungi and actinomycetes from different SMS was carried out on Nutrient Agar medium, Rose Bengal Agar medium and Kenknights medium respectively by using serial dilution method (Johnson and Curl, 1972). For that

samples were collected at the time of bed preparation, spawn running period (15^{th} day), during harvest (30^{th} day) and after harvest (50^{th} day).

Ten grams of each sample was suspended in conical flask containing 90ml sterile water to obtain 10^{-1} dilution. The flasks were shaken for 15 minutes and serial dilutions were made to obtain the final dilution of 10^{-8}. Dilutions of 10^{-3}, 10^{-4} and 10^{-8} were used for actinomycetes, fungi and bacteria respectively. One ml from the required dilution was poured aseptically into a sterile Petri dish to which cooled molten media was poured and gently rotated. The inoculated Petri dishes were incubated at room temperature.

Number of fungal, bacterial and actinomycetes colonies from each sample was recorded and pure cultures of the predominant microorganisms were maintained on appropriate media for further study.

3.4 *In vitro* EFFECT OF ISOLATED ORGANISMS ON THE PATHOGENS

The antagonistic effect of isolated organisms against *R. solanacearum* and *P. aphanidermatum* were tested under laboratory conditions using dual culture method as suggested by Skidmore and Dickinson (1976). Inhibitory effect of these isolated organisms was compared with the reference cultures *Pseudomonas fluorescens* and *Trichoderma viride.*

3.4.1 *In vitro* evaluation of bacterial antagonists against *Pythium aphanidermatum*

The antagonistic efficiency of bacterial isolates against *P. aphanidermatum* was tested under *in vitro* condition by two methods, streaking the bacterial isolates on one side and both sides of pathogen. Three replications were kept for each antagonist and the monoculture of *P. aphanidermatum* was maintained as control.

3.4.1.1 Streaking on both sides

A mycelial disc of pathogen of 6mm 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.

3.4.1.2 Streaking on one side

A mycelial disc of pathogen of 6mm size was inoculated at the centre of one half of Petri dish with PDA medium. The bacterial isolate was inoculated as a line of streak at the centre of other half of Petri dish. The Petri dishes were incubated at room temperature and observations on growth of pathogen were taken at regular intervals. The per cent inhibition of growth over control was calculated by the formula suggested by Vincent (1927).

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

C = Growth of fungus in control (mm)

T = Growth of fungus in treatment (mm)

3.4.2 *In vitro* evaluation of fungal antagonists against *Pythium aphanidermatum*

A mycelial disc of 6mm 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 on the same day. 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 24h of inoculation. The per cent inhibition of mycelia growth of pathogen over control was calculated as mentioned in 3.4.1.2.

The nature of antagonistic reaction against pathogen was assessed by following method of 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

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

The following methods of inoculation were 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 kept for each antagonist.

3.4.3.1 Cross streaking method

Both the test and the indicator organism were streaked perpendicular to each other on the plates mediated with nutrient agar medium. The plates were observed daily for the lysis at the juncture of the pathogen and the antagonists.

3.4.3.2 Point inoculation of the antagonistic organism

A loopful of antagonistic organism 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.

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

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

3.4.4.1 Streaking on both sides

A mycelial disc of antagonists of 6mm size was cut from the actively growing culture and inoculated at the centre of Petri dish mediated with PDA medium. The bacterial pathogen was inoculated as a line of streak on either side of the antagonist on the same day.

3.4.2.2 Streaking on one side

A mycelial disc of antagonists of 6mm size was inoculated at the centre of one half of Petri dish. On the centre of other half of same Petri dish, the bacterial pathogen was streaked.

The Petri dishes were incubated at room temperature (26 ± 2^0C) and observations on growth of antagonists were recorded upto and when the growth in the control plates fully covered the 90mm growth. The per cent inhibition of mycelial growth of pathogen over control was calculated as mentioned in 3.4.1.2.

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

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

3.5.1 *In vitro* evaluation of mutual compatibility among fungal antagonists

Compatibility among fungi was tested by employing dual culture technique as mentioned in 3.4.4.

3.5.2 *In vitro* evaluation of mutual compatibility among bacterial antagonists

The mutual compatibility among bacteria was tested by cross streaking technique. Bacterial isolate of 48h old was streaked perpendicular to each other on the Petri dishes containing nutrient agar. The plates were then incubated at room

temperature and observed daily for the lysis of growth at the juncture point of two isolates for 72h. Lysis at the juncture point indicated incompatibility.

3.5.3 *In vitro* evaluation of mutual compatibility between fungal and bacterial antagonists

A mycelial disc of 10mm size of the fungal antagonist was inoculated at the centre of the Petri dish plated with PDA medium. On the next day bacterial isolates were inoculated as a line of streak on either side of the disc, leaving 2.25cm 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 PREPARATION OF MICROBIAL CONSORTIUM

Based on the results of the compatibility study of selected microorganisms, an attempt was made to prepare a consortium of microorganisms isolated from spent mushroom substrate. Three types of consortia were prepared using the selected isolates. Fungal and bacterial consortia were prepared separately to study their effects on the pathogens.

The fungal consortium was prepared using five day old cultures of the selected fungal antagonists grown separately on PDA medium. The fungal mycelia on PDA medium was scrapped out and mixed with sterile water. These fungal mycelia in sterile water were blended using a mixer grinder. The colony forming units of the fungal isolate was made adjusted to 10^6 cfu per ml. For preparing two litres of consortia, two plates of fungal culture were required to make the concentration as 10^6 cfu per ml.

A bacterial consortium was prepared using 48h old culture of the selected bacteria grown in NA medium. Bacterial cells were harvested by scrapping the surface growth with a sterile microscopic slide and then mixed in sterile water. The cell count of the bacterial isolate was made adjusted to 10^8 cfu/ml. For preparing two litres of consortia, one plate of bacterial culture was required to make the cell count 10^8 cfu/ml.

A combined consortium of selected fungal and bacterial antagonists was prepared in the same manner as mentioned above. The total volume was made up to two

litres using sterile water. The cell count of the each isolate was made adjusted to 10^6 spores per ml for fungi and 10^8 cfu/ml for bacteria.

3.7 IDENTIFICATION OF SELECTED ANTAGONISTS

Based on the efficacy of antagonistic activity against pathogen, the most efficient isolates were selected and an attempt was made to identify them.

3.7.1 Identification of fungal antagonist

The fungal antagonist was identified based on the cultural and morphological characters.

3.7.1.1 Cultural characters

Cultural characters of the antagonists such as colour, shape, texture of fungal colony, growth rate and sporulation on the PDA medium were studied in detail.

3.7.1.2 Morphological characters

Morphological characters of fungal isolates in pure culture were studied. Permanent slides were prepared from the pure culture by slide culture technique. Using micrometry measurements on the size of hyphae was recorded. Photomicrographs of the hyphae and conidia were also made. These characters were compared with the characters given in CMI descriptions of pathogenic fungi and bacteria. For further confirmation, the cultures were sent to 'National Centre for Fungal Taxonomy', in New Delhi.

3.7.2 Identification of bacterial antagonists

Characterization of different bacterial isolates was done based on cultural and morphological characters. They were identified by 16S rDNA sequence analysis. The identification got confirmed by biochemical tests. For each test 24 - 48h old cultures were used.

3.7.2.1 Cultural characters

The bacterial isolates were streaked on nutrient agar medium in Petri dishes. After incubation period of 24h, the colonies were observed for its shape, elevation and margin.

3.7.2.2 Morphological characters

For morphological studies 24h old culture of bacteria was used. Gram staining was employed to study Gram reaction. Pigment production was also tested.

3.7.2.2.1 **Gram's staining -** Gram's staining was done as per the procedure described by Hucker and Conn (1923).

3.7.2.2.2 **Pigment production -** The bacterial cultures were streaked on King's B medium and incubated at room temperature for 48h. The dishes were observed under ultra violet light for fluorescent pigment production around the colonies.

3.7.2.3 16S rDNA sequence analysis of isolated antagonistic bacteria

After the cultural and morphological study, the antagonistic bacteria were identified by 16S rDNA sequence analysis.

3.7.2.3.1 **Amplification of 16S rDNA genes -** Bacterial colony was mixed with 10µl sterile water and kept at 98^0C for two minutes to denature. After a brief centrifugation to sediment the bacterial cell constituents, 1µl of supernatant was taken and used as a template for amplification of 16S rDNA gene. The details of the primer used are given below.

Primer details	**Sequence 5'-3'**	**Length (Base pairs)**
27f	AGAGTTTGATCCTGGCTCAG	20
1492r	TACGGTACCTTGTTACGACTT	20

Polymerase chain reaction was carried out in Eppendorf Master Cycler. The composition of the reaction mixture for PCR is as follows.

Component	Per reaction volume required
Template	1.0 µl
10X Taq buffer A	2.5 µl
dNTP mix (10mM)	1.0 µl
Forward primer (10 picomoles)	1.0 µl
Reverse primer (10 picomoles)	1.0 µl
Taq DNA polymerase (0.3U)	2.0 µl
Dislled water	16.5 µl
TOTAL	25 µl

The reaction was set in 200µl microfuge tube chilled over ice flakes. A momentary spin was given to mix completely all reagents and set in thermal cycler for amplification. The details of the thermal cycler programme are as follows-

No.	Step	Temperature (^{0}C)	Time (Min)
1	Initial denaturation	95	3.00
2	Denaturation	94	1.30
3	Annealing	55	0.40
4	Primer extension	72	1.30
5	Step 2-4	34 cycles	-
6	Final extension	72	20.00

3.7.2.3.2 **Agarose gel electrophoresis** - The quality of isolated DNA was evaluated through agarose gel electrophoresis (Sambrook *et al*., 1989). 1X TAE buffer was prepared from the 50X TAE (pH 8.0) stock solutions. Agarose (0.9%) was weighed and dissolved in TAE buffer by boiling. Ethidium bromide prepared from a stock of 10mg/ ml was added to it at a concentration of 0.5 µg/ml and mixed well. The open end of gel casting tray was sealed with cellotape, the comb was placed properly and dissolved agarose was poured into the tray. The gel was placed in the electrophoresis unit after 30 minutes with the well side directed towards the cathode. 1X TAE buffer was added to the buffer tank so as to cover the well with a few mm of buffer. Then 5µl DNA sample was mixed with 1µl tracking dye (6X) and carefully loaded into the wells

using a micropipette. The λ DNA / Eco RI / Hind III Double Digest (Genei, Banglore) were used as the molecular weight marker. The cathode and the anode of the electrophoresis unit were connected to the power pack (Hoefer, USA) and the gel was run at constant voltage of 100V. The power was turned off when the tracking dye reached at about 3cm from the anode end.

3.7.2.3.3 **Gel documentation -** The DNA bands separated by electrophoresis were viewed and photographed using Vision Works LS software and UVP Gel Doc-IT^{TM} imaging system.

3.7.2.3.4 **Sequencing of the product -** The product was purified and sequenced at Scigenome Cochin, using the universal primers 27 f and 1492 r.

3.7.2.3.5 **Nucleotide sequence analysis -** The Blastn programme (http://www.ncbi.nlm.nhm.gov/blast/) was used to find out the homology of the nucleotide sequences.

3.7.2.4 Biochemical characters

The identification of bacterial antagonists by 16s rDNA sequence analysis was confirmed by biochemical characterization. The following tests were done for the biochemical characterization of the isolates.

3.7.2.4.1 **Catalase test -** A few drops of three per cent hydrogen peroxide were placed at the centre of a sterile glass slide and a loopful of bacterial isolate was agitated in the solution. Formation of effervescence indicated the positive reaction (Cappucino and Sherman, 1992).

3.7.2.4.2 **Oxidase test** – A loopful of bacterial isolate was rubbed on the oxidase disc. Purple colour development will be there if the reaction is positive.

3.7.2.4.3 **Starch hydrolysis test -** A loopful of bacterial isolate was spot inoculated on Petri dish containing NA medium with 0.2 per cent soluble starch (Appendix I). Starch hydrolysis was tested after 48h of inoculation by flooding the agar surface with Lugol's iodine solution. A colourless zone in contrast to the blue background indicated positive starch hydrolysis (Cappucino and Sherman, 1992).

3.7.2.4.4 **Levan production from sucrose** - Peptone beef extract medium containing five per cent sucrose (Appendix I) was used for this test. The bacterial isolates were streaked over the medium in sterilized Petri dish and the growth characters were observed after 48h. Presence of large, white domed and mucoid colonies indicated the production of levan from sucrose (Hayward, 1964).

3.7.2.4.5 **Gelatin liquefaction** – Sterilized nutrient gelatine medium (Appendix I) was spot inoculated with 48h old culture of bacterium. After incubation of seven days agar surface was flooded with 0.2 per cent $HgCl_2$ solution in dilute HCl and observed for the clear zone around the bacterial growth.

3.7.2.4.6 **Urease test** - Christensens urea agar (Appendix I) (Christensen,1946) was used in this test. Ninety ml of aliquots of the medium were dispensed in 250ml conical flask and autoclaved. To each flask 10ml of 20 per cent sterilized urea solution was added and dispensed in sterilized test tubes in 5 ml quantities and slants were prepared. The test tubes were inoculated with isolates and observations recorded periodically. A change in colour of medium from yellow to pink or red indicated urease production.

3.7.2.4.7 **Mode of utilization of glucose** - To determine whether the bacterium utilized glucose only under aerobic condition or both under aerobic and anaerobic condition, one percent glucose was added to the prepared Hayward's semi solid medium (Appendix I) and dispensed in tubes upto 4cm. The medium was sterilized by tindalization and inoculated in duplicate by stabbing with straight inoculation needle charged with bacterial growth. In one of the tubes the medium was sealed with 1cm layer of sterilized liquid paraffin. The tubes were inoculated at room temperature and observation on change in colour was taken at regular intervals upto15 days.

3.7.2.4.8 **Nitrate reduction** - Nitrate agar medium (Appendix I) was dispensed in test tubes, autoclaved and incubated with 24 h old cultures of bacteria. The test tubes were then incubated at room temperature and tested for the reduction of nitrate at regular intervals upto 15 days. The test was performed by adding a few drops of Griess Llosvays reagent (Appendix I) consisting of sulphanilic acid (0.8% in 5M acetic acid) and dimethyl alpha naphthyl amine (0.5% on 5M acetic acid) to the nitrate broth culture. Absence of pink or red colour development indicated the presence of nitrate as such or reduced to ammonia and free nitrogen.

3.7.2.4.9 **Arginine dihydrolase reaction** - For this test Thornley semi solid medium (Appendix I) (Thornley, 1960) was used. About 5ml quantities of the medium were dispensed in 15ml capacity screw capped test tubes and sterilized in autoclave at 121^0C. The medium was inoculated by stabbing with a loopful of bacterial isolate. The surface of the medium was sealed with 3ml molten one per cent agar cooled to 45^0C and the tubes were incubated for seven days at room temperature. Any colour change of the medium to pink or red was recorded at regular intervals for a period of seven days.

3.7.2.4.10 **Production of hydrogen sulphide** - Ability of bacterium to liberate H_2S was tested using peptone water medium (Appendix I). Five ml of medium was dispensed in test tubes and autoclaved. Lead acetate paper stripes of 5 x 50cm size were prepared by soaking them in super saturated solution of lead acetate. The strips were dried, autoclaved and again dried. The tubes were inoculated with bacterial isolates and lead acetate strips were inserted aseptically by the side of the plug in the tube. The tubes were incubated at room temperature and observations were recorded at regular intervals upto 14 days for blackening of test strips.

3.7.2.4.11 **Growth at 4^0C and 41^0C** - Bacterial culture were streaked in NA medium and incubated at 4^0C and 41^0C and observed the growth after 24 and 48 hours.

3.8 MECHANISM OF ANTAGONISM

The various mechanisms involved in the antagonistic reaction of selected bacterial and fungal antagonists against *P. aphanidermatum* and *R. solanacearum* were studied under lab condition by the following methods.

3.8.1 Mechanism of antagonism of selected fungal antagonist against the pathogens

The mechanism of antagonism of selected fungal isolates against the pathogens was studied by the methods like production of volatile metabolites, production of non volatile metabolites and mycoparasitism.

3.8.1.1 Production of volatile metabolites

To study the effect of volatile metabolites released by the fungal antagonists against fungal pathogens, discs of 6mm diameter were excised with a cork borer from the leading edge of an actively growing culture of the pathogen and were placed at the centre of Petri dish. Discs from the actively growing antagonist were excised and were placed similarly in another Petri dish with PDA medium.

The plate containing the pathogen was placed over the plate with the antagonist after removing the lids of the plates. The two plates were held together by adhesive tapes. Three replications were maintained for each antagonist. The control plates include the pathogen paired with uninoculated plate with PDA medium alone. The plates were incubated and diameter of growth of colony of the pathogen was recorded upto when the control plates were fully covered with fungal pathogen growth.

3.8.1.2 Production of non volatile metabolites

Effect of non volatile metabolites against the fungal and bacterial pathogens were studied by two methods, *viz*., cellophane paper method and culture filtrate method. Culture filtrate method was used in the case of both fungal and bacterial pathogens.

3.8.1.2.1 **Cellophane paper method** - To study the effect of non volatile metabolites released by the fungal antagonists against fungal pathogens, sterilized cellophane paper was placed over the surface of solidified agar medium aseptically and incubated the plates for 24h. Discs from actively growing antagonist were excised and were placed at the centre of the cellophane paper. The plates were incubated at room temperature for 7 days. Plates maintained without inoculation of antagonistic fungus served as control.

After 7 days incubation cellophane paper was removed along with the fungal mat of the antagonist. Then a mycelial disc of 8mm diameter was cut from the culture of *P. aphanidermatum* and was inoculated at the centre of Petri plate and incubated. Plates incubated earlier only with the cellophane paper without antagonistic fungus served as control. Next day onwards, the mycelial growth of the pathogen was recorded.

3.8.1.2.2 **Culture filtrate method** - To study the effect of non volatile metabolites released by the fungal antagonists against fungal pathogens, 6mm disc was cut from 4 day culture of potential fungal antagonist and placed in 100ml conical flask containing 25ml potato dextrose broth and incubated for 7 days at room temperature. The culture filtrates were filtered twice through double layered filter paper and was sterilized. The sterilized culture filtrates were used to test antagonistic property against both fungal and bacterial pathogens.

For *in vitro* evaluation of culture filtrates against the fungal pathogens, 1ml of sterilized culture filtrates were poured into each Petri dish and mixed with the media and allowed to solidify. Mycelial discs of 6mm diameter were cut from actively growing 7 day old culture of fungal pathogen and placed at the centre of each Petri dish containing the medium with filtrate. Three replications were maintained for each filtrate. Media without culture filtrate served as control. The inoculated Petri dishes were incubated at 28 ± 2^{0}C. The diameter of fungal colony was recorded upto and when the growth in the control plates fully covered 90mm growth in Petri dish. Per cent inhibition was calculated as mentioned in 3.4.1.2.

For *in vitro* evaluation of culture filtrates against the bacterial pathogen, the pathogen was seeded on Nutrient Agar medium. Sterilized filter paper discs dipped in the fungal culture filtrate were placed at the centre of each Petri plate. Three replications were maintained. Observations on zone of inhibition were recorded 48h after inoculation. Paper discs dipped in sterile water served as control.

3.8.1.3 Mycoparasitism

Mycoparasitism of the selected fungal antagonist against the fungal pathogen was studied under *in vitro* condition on plain agar medium by cellophane paper method. Plain agar medium was prepared and was poured into sterile Petri plates. Sterilized cellophane paper was placed aseptically over the surface of solidified agar medium. Discs from actively growing fungal antagonists and fungal pathogen were excised and were placed on the cellophane paper of same Petri dish at 2cm from the periphery of the dish. All the plates were incubated under room temperature and observed for the mechanism of antagonism.

When the mycelium of both the antagonists and pathogen touched each other, that portion was cut along with the cellophane paper using a sterile blade, and observed under microscope.

3.8.2 Mechanism of antagonism of selected bacterial antagonists against the pathogens

The mechanisms involved in the antagonism of selected bacterial isolates against the pathogens were studied by the following methods.

3.8.2.1 Production of non volatile metabolites

Effect of non volatile metabolites against the fungal and bacterial pathogens was studied by culture filtrate method. A loopful of bacterial antagonists from 48h old culture was inoculated into Nutrient Agar broth, and incubated for four days at room temperature. The culture filtrates were filtered twice through double layered filter paper and was sterilized. The sterilized culture filtrates were used to test antagonistic property against bacterial pathogens.

For *in vitro* evaluation of bacterial culture filtrates against the bacterial pathogen, filter paper discs of 5mm diameter was autoclaved, dried and soaked in the culture filtrates, drained the excess filtrate and placed on the medium seeded with *R. solanacearum* at two centimetre from the periphery of the plate. The disc dipped in sterile distilled water served as control. Three replications were kept for each filtrate. The inhibition zone developed around the filter paper was measured at 24-48 hours after incubation.

In vitro evaluation of bacterial culture filtrate against fungal pathogen was also studied by poison food technique as described in 3.8.1.2.2

3.8.2.2 Production of siderophores

The potential antagonistic bacteria were tested for the production of iron chelating siderophores by CAS assay.

First step was the preparation of CAS indicator solution. For this initially 60.5mg of chrome azurol S was dissolved in 50ml of Millipore water. After that, 10ml of Fe III solution (27mg $FeCl_3$. $6H_2O$ and 83.3µl concentrated HCl in 100ml Milli pore water) was added, along with 72.9mg hexadecyltrimethyl ammonium bromide (HDTMA) dissolved in 40ml Milli pore water. The HDTMA solution was added slowly while stirring, resulting in a dark blue solution (100ml total volume), which was then autoclaved.

Second step involved preparation of succinate medium (Appendix I). The pH of the solution was adjusted to 7.0 using 6M NaOH. The solution was then autoclaved.

The third step was the preparation of CAS agar plates. Here, the autoclaved succinate medium was cooled to 50^0C on a water bath. The CAS indicator solution was also cooled to 50^0C. Once cooled, 10ml of CAS indicator solution was added to the succinate medium with constant stirring, which was added carefully and slowly along with the walls of the flask with constant stirring, but at a speed so as not to generate any bubbles. Once mixed thoroughly, the resulting solution 100ml was poured into sterile Petri plates, each plate receiving approximately 25ml of blue agar. The antagonistic bacteria were streaked on this in a zig zag manner and the plates were incubated at room temperature. Under minimal iron conditions, siderophore is produced and released into the culture medium. Siderophore was detected by the presence of an orange halo around the inoculated area initially and then it spread on the entire medium.

3.8.2.3 Production of hydrogen cyanide

Production of hydrogen cyanide (HCN) by the potential bacterial isolates was detected by the following method of Wei *et al*. (1991). Log phase of bacterial culture were inoculated to 25 ml of Kings B broth supplemented with 4.4 g/l of glycine taken in a sterile Petri plate. Sterile filter paper strips soaked in picric acid solution (2.5g picric acid + 12.5g Na_2CO_3 in 1000ml of water) were placed in the lid of each plate. Petri

dishes were sealed with parafilm and incubated for 72h in a slow shaking platform. Change in colour of filter paper strips from yellow to brown and then to red indicates the production of hydrogen cyanide. The reaction was scored on a 1-4 scale depending on the colour generation.

3.9 *In vitro* EFFECT OF SELECTED ANTAGONISTS AGAINST MUSHROOM SPECIES

The antagonistic effect of selected fungal and bacterial isolates against *Pleurotus florida* and *P. sajor-caju* were tested under laboratory condition using dual culture method.

3.9.1 ***In vitro* evaluation of bacterial antagonists against *P. florida* and *P. sajor-caju***

A mycelial disc of *Pleurotus* sp. of 6mm 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 the mushroom fungus on the same day. The Petri dishes were incubated at room temperature and observations on growth of mushroom species were taken at regular intervals. Petri dishes inoculated with the *Pleurotus* species alone were kept as control.

3.9.2 ***In vitro* evaluation of fungal antagonists against *P. florida* and *P. sajor-caju***

A mycelial disc of 6mm diameter was cut from actively growing culture of mushroom species and was 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 on the same day. Three replications were maintained. The mushroom fungus and the antagonist grown as monoculture served as control. The growth measurements were taken at regular intervals after 24h of inoculation.

3.10 *In vitro* EFFECT OF AQUEOUS EXTRACTS OF DIFFERENT SMS

Inhibitory effects of concentrated filtered aqueous extracts of paddy straw, saw dust and neopeat SMS of *P. florida* and *P. sajor-caju* on both the pathogens were studied. This was carried out by poisoned food technique for *P. aphanidermatum* and by inhibition zone technique for *R. solanacearum.*

Ten grams of spent mushroom substrate was mixed with sterile water in 1:4 ratio on volume basis. The flasks were shaken for 15 minutes and filtered through muslin cloth to get the aqueous extract. It was then filter sterilized and used to check the inhibitory effects on the pathogens by poisoned food technique and inhibition zone technique.

3.10.1 *In vitro* effect of aqueous extracts against *P. aphanidermatum*

For *in vitro* evaluation of aqueous extracts of SMS against *P. aphanidermatum,* 100ml PDA was taken in 250ml conical flask and sterilized at 1.05 kg/cm^2 pressure for 20 minutes. One ml of aqueous extract was added into sterile Petri dish. Then medium was added with that @ 25ml per plate, mixed well and allowed to solidify. Mycelial discs of 6mm diameter were cut from actively growing four day old culture of *P. aphanidermatum* and placed at the centre of each Petri dish containing poisoned medium. Three replications were maintained for each treatment. Media without aqueous extract served as control. The inoculated Petri dishes were incubated at 28 ± 2^0C. The diameter of fungal colony was recorded up to and when the growth in the control plates fully covered the medium. The per cent inhibition of mycelial growth of pathogen over control was calculated as mentioned in 3.4.1.2.

3.10.2 *In vitro* effect of aqueous extracts against *R. solanacearum*

For *in vitro* evaluation of aqueous extracts of SMS against *R. solanacearum*, the bacterial isolate were seeded on Nutrient Agar medium. Sterilized filter paper discs dipped in aqueous extracts of paddy straw, saw dust and neopeat SMS of *P. florida* and *P. sajor-caju* were placed separately at the centre of each Petri plate. Three replications

were maintained for each aqueous extract. Observations on zone of inhibition were recorded 48h after inoculation. Paper discs dipped in sterile water served as control.

3.11 EVALUATION OF SMS UNDER POT CULTURE CONDITION

Evaluation of SMS against rhizome rot complex disease of ginger was conducted under pot culture condition (Plate 3). Challenge inoculation of the pathogens, *viz., Pythium aphanidermatum* and *Ralstonia solanacearum* were done at 45 days after planting. Spent mushroom substrate was used as mulch at the time of planting, 60 DAP and 120 DAP as recommended in POP. The details of the pot culture experiment are as follows:-

Design - CRD

Replication - Three

Number of treatments - 14

Number of plants/ treatment - 12

Variety - Himachal

The treatment details of the experiment are given below :-

Experiment no. I :- Management of *Pythium aphanidermatum*

T1 – Paddy straw SMS of *Pleurotus florida*

T2 – Paddy straw SMS of *P. sajor-caju*

T3 – Saw dust SMS of *P. florida*

T4 – Saw dust SMS of *P. sajor-caju*

T5 – Neopeat SMS of *P. florida*

T6 – Neopeat SMS of *P. sajor-caju*

T7 – Fungal- bacterial consortium from SMS

T8 – Soil drenching with Copper hydroxide 0.2% at 7 DAI

T9 – *Trichoderma viride* (Reference culture) @ 10g/ pot

T10 – Best fungal antagonist

T11 –Best bacterial antagonist

T12 – Best fungal antagonist + Reference culture *T. viride*

(As fungal consortium)

T13 – Control (With pathogen)

T14 –Absolute control (Without pathogen)

(For treatments T7 to T14 dried paddy straw were used as mulch)

Experiment no. II :- Management of *Ralstonia solanacearum*

T1 – Paddy straw SMS of *Pleurotus florida*

T2 – Paddy straw SMS of *P. sajor-caju*

T3 – Saw dust SMS of *P. florida*

T4 – Saw dust SMS of *P. sajor-caju*

T5 – Neopeat SMS of *P. florida*

T6 – Neopeat SMS of *P. sajor-caju*

T7 – Fungal- bacterial consortium from SMS

T8 – Soil drenching with Copper hydroxide 0.2% at 7 DAI

T9 – *Pseudomonas fluorescens* (Reference culture) @ 10g/ pot

T10 – Best fungal antagonist

T11 –Best bacterial antagonist

T12 –Best bacterial antagonist + Reference culture *P. fluorescens*

(As bacterial consortium)

T13 –Control (With pathogen)

T14 – Absolute control (Without pathogen)

3.11.1 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 filled in the earthern pots of size 12 x 12 inch. Ginger variety Himachal procured from Wadakkancherry area of Palakkad (dt.) was used for the study. Seed rhizomes weighing 15-20gm were planted in pot and mulched with spent mushroom substrate. All the cultural operations except fungicidal application were carried out as per the Package of Practices Recommendations, Crops 2007 (KAU, 2007). The consortium was prepared in sterile distilled water and was given as soil drench at the time of planting, 60 DAP and 120 DAP. Two hundred ml of the consortium was used for soil drenching. Best fungal and bacterial antagonist against each pathogens were also given separately.

3.11.2 Observations recorded

Observations on the germination percentage, plant biometric characters and yield at different intervals were recorded.

3.11.2.1 Per cent disease incidence

Challenge inoculation of pathogens was done at 45 days after planting. Then per cent disease incidence was recorded for each treatment separately.

3.11.2.2 Germination percentage

The number of rhizomes germinated in each treatment was counted 30 days after planting to calculate the germination percentage.

3.11.2.3 Biometric observations

The following observations were taken at monthly intervals at two to five months after planting.

Plate 3: Over view of pot culture experiment

- Number of tillers – Number of tillers were recorded by counting the fully emerged ones.
- Number of leaves/ tiller – Number of leaves were recorded by counting the fully opened leaves in each tiller.
- Height of tiller – Distance from the base of the tiller to the tip of plant was taken as the height and expressed in centimetres.

3.11.2.4 Yield of rhizomes

The fresh rhizome yield per pot from each treatment was recorded at the final harvest and was expressed in g/pot.

3.11.2.5 Pest incidence

Observations on pest incidence (Rhizome maggot) were recorded by preparing a score chart as follows.

Score	% damage
0	0
1	1 – 20 %
2	21 – 40 %
3	41 – 60 %
4	61 – 80 %
5	81 – 100 %

3.11.3 *In vitro* evaluation of fungal and bacterial antagonists against insecticides

The compatibility between selected antagonists and the insecticides *viz.*, Chlorpyrifos (2.5ml/L), Confidor (0.5ml/L) and Rogor (1.5ml/L) were evaluated by poisoned food technique and inhibition zone technique.

In the case of fungal antagonists, PDA medium was added with the insecticides with recommended dosage, mixed well and allowed to solidify. Mycelial discs of 6mm diameter were cut from actively growing culture of fungal antagonists and placed at the centre of each Petri dish containing poisoned medium. Three replications were maintained for each treatment. Media without insecticides served as control. Fungal

antagonists which are having growth on the poisoned medium with insecticides were selected as compatible. If the growth is inhibited, they were considered as incompatible.

For *in vitro* evaluation of insecticides against bacterial antagonists, inhibition zone technique was followed. Bacterial antagonists were seeded on Nutrient Agar medium. Sterilized filter paper disc dipped in recommended dosage of insecticide was kept at the centre of each Petri dish. Three replications were maintained. Filter paper disc dipped in sterile water served as control. Observations on zone of inhibition were recorded 48h after inoculation. The bacteria with no inhibition zone around the filter paper were selected as compatible with insecticides and if the inhibition zone is formed, they were considered as incompatible.

3.12 STATISTICAL ANALYSIS

Analysis of variance was done on the data collected using the statistical package MSTAT (Freed, 1986). Multiple comparisons among the treatment means were done using DMRT (Duncan's Multiple Range Test).

Results

4. RESULTS

The present investigation was carried out to find out the efficacy of spent mushroom substrate as mulch for the management of rhizome rot complex disease of ginger. For this, studies were conducted on isolation of pathogens associated with the disease, production of spent mushroom substrate, isolation of microorganism from SMS, *in vitro* effect of isolated organisms against pathogens, mutual compatibility among them, mechanism of antagonism, identification of selected antagonists and pot culture experiment. The results of the study are presented below.

4.1 ISOLATION OF PATHOGENS ASSOCIATED WITH THE DISEASES

Diseased specimens of rhizome rot of ginger were collected from the farmer's field for the isolation of fungal pathogen, *Pythium aphanidermatum*. The affected plants showed pale leaves with yellow tips. Yellowing spreads along the margin of the leaves from tip downwards. Tender tissues of the collar region first became soft, watery and rot and then the plant toppled down. The rotten parts emitted foul smell owing to decay. Isolation was done on PDA medium and incubated for 2-3 days under cool condition. The pathogen was purified by hyphal tip method, subcultured and maintained on PDA slants for future investigations.

Ginger plants showing typical wilt symptoms *viz.,* drooped and rolled up leaves with intense yellowish bronze colour, water soaked patches on the collar region of the pseudostems, and having milky bacterial ooze were collected from the farmer's field. Isolation of the pathogenic bacterium, *Ralstonia solanacearum* was done from the infected rhizomes in TZC agar medium and yielded typical colonies. The pathogen was identified based on shape, size and colour of colonies as circular, fluidal, slimy white with light pink pin point centre. The cultures were purified by repeated cycles of streaking. Single colonies were selected, subcultured and maintained in Nutrient Agar slants and also in sterile distilled water.

4.2 PRODUCTION OF SPENT MUSHROOM SUBSTRATE

The isolation of oyster mushrooms *viz.*, *Pleurotus florida* and *P. sajor-caju* was done as per the standard tissue culture technique. White mycelial growth was observed from the second day onwards and it took seven days to complete full growth in 9cm Petri dish. The cultures were maintained on PDA slants by periodical sub culturing and were used for mushroom production.

Spawn was prepared in sorghum grains as described under 3.2. After 15 days of incubation the inoculated sorghum grains became a hard mass, completely impregnated with white mycelium.

Paddy straw, saw dust and neopeat were sterilized as mentioned in 3.2.4 and were used as substrates for the production of mushrooms like *P. florida* and *P. sajor-caju*, under optimum moisture condition.

4.3 EVALUATION OF DIFFERENT SUBSTRATES ON GROWTH AND YIELD OF OYSTER MUSHROOMS

The selected substrates *viz*., paddy straw, saw dust and neopeat were evaluated for their efficacy on the growth and production of *P. florida* and *P. sajor-caju.* The performances of mushrooms were estimated by recording the nature of mycelial growth, time taken for spawn run, time taken for mushroom production and weight of the sporocarps.

4.3.1 Effect of different substrates on nature of mycelial growth

Data on the effect of different substrates on the nature of mycelial growth of *P. florida* and *P. sajor-caju* are given in Table 1.

In paddy straw (T_1) and saw dust (T_2), *P. florida* and *P. sajor-caju* showed very good mycelial growth. But in neopeat (T_3) the growth was thickened at the point of inoculation and mycelial spread was very sparse, in the case of both the species.

4.3.2 Effect of different substrates on the time for spawn run

The time taken for spawn run for *P. florida* and *P. sajor-caju* on different substrates like paddy straw, saw dust and neopeat are given in Table 2.

The two species of oyster mushrooms *viz., P. florida* and *P. sajor-caju* recorded less time for spawn run in paddy straw (T_1). *P. florida* took 15.2 days and *P. sajor-caju* took 14 days in paddy straw. Among the different substrates tested, the days for spawn run were maximum in saw dust for both the species. Neopeat showed very sparse mycelial growth. In both the species, growth was not complete also. When the species were compared for their performance on each substrate, *P. sajor-caju* took minimum days for spawn run compared to *P. florida*. Among the treatments, T_1 (paddy straw) was superior to all others, which took a mean minimum time (14.6 days) for spawn run irrespective of species.

4.3.3 Effect of different substrates on the time taken for first harvest of mushrooms

The number of days taken for first harvest of mushroom in *P. florida* and *P. sajor-caju* varied with different substrates (Table 3).

In *P. florida* and *P. sajor-caju*, an earlier harvest (23.6 days and 21.2 days respectively) was noticed in T_1 (paddy straw). It was followed by T_2 (saw dust) and maximum time was recorded by neopeat. In neopeat, *P. florida* and *P. sajor-caju* took 24.8 days and 24.4 days respectively. Among the different species studied *P. sajor-caju* took minimum time for the first harvest of mushroom (22.5 days) compared to *P. florida*, which took 24.3 days. Among the substrates T_1 (paddy straw) took minimum time for mushroom production (22.4 days) irrespective of species (Fig.1).

4.3.4 Effect of different substrates on yield of mushrooms

Effect of different substrates on the yield of sporocarps of *P. florida* and *P. sajor-caju* was studied and the results are furnished in Table-4. The data revealed that, there was significant difference among the treatments and species (Fig.2).

Table 1: Effect of different substrates on the nature of mycelial growth of mushrooms

Tr.No	Treatments	*P. florida*	*P. sajor-caju*
T_1	Paddy straw	+ + +	+ + +
T_2	Saw dust	+ + +	+ + +
T_3	Neopeat	+	+

+ + + : Very good, + : Very sparse

Table 2: Effect of different substrates on the time taken for spawn run of mushrooms

Tr.No	Treatments	*Days for spawn run		Mean
		P. florida	*P. sajor-caju*	
T_1	Paddy straw	15.2	14	14.6
T_2	Saw dust	16	15.4	15.6
T_3	Neopeat	Very sparse	Very sparse	-
	Mean	15.6	14.7	

*Mean of five replications

Table 3: Effect of different substrates on the days taken for first harvest of mushrooms

Tr. No	Treatments	*Days for first harvest		Mean
		P. florida	***P. sajor-caju***	
T_1	Paddy straw	23.6	21.2	22.4
T_2	Saw dust	24.4	21.8	23.1
T_3	Neopeat	24.8	24.4	24.6
	Mean	24.3	22.5	

*Mean of five replications

Table 4: Effect of different substrates on the yield of mushrooms

Tr. No	Treatments	*Yield (g/bed)		Mean
		P. florida	***P. sajor-caju***	
T_1	Paddy straw	252.60^{b}	230.80^{b}	241.7^{b}
T_2	Saw dust	551.80^{a}	332.60^{a}	442.2^{a}
T_3	Neopeat	122.80^{c}	120.60^{c}	121.7^{c}
	Mean	309.07^{a}	228^{b}	

*Mean of five replications

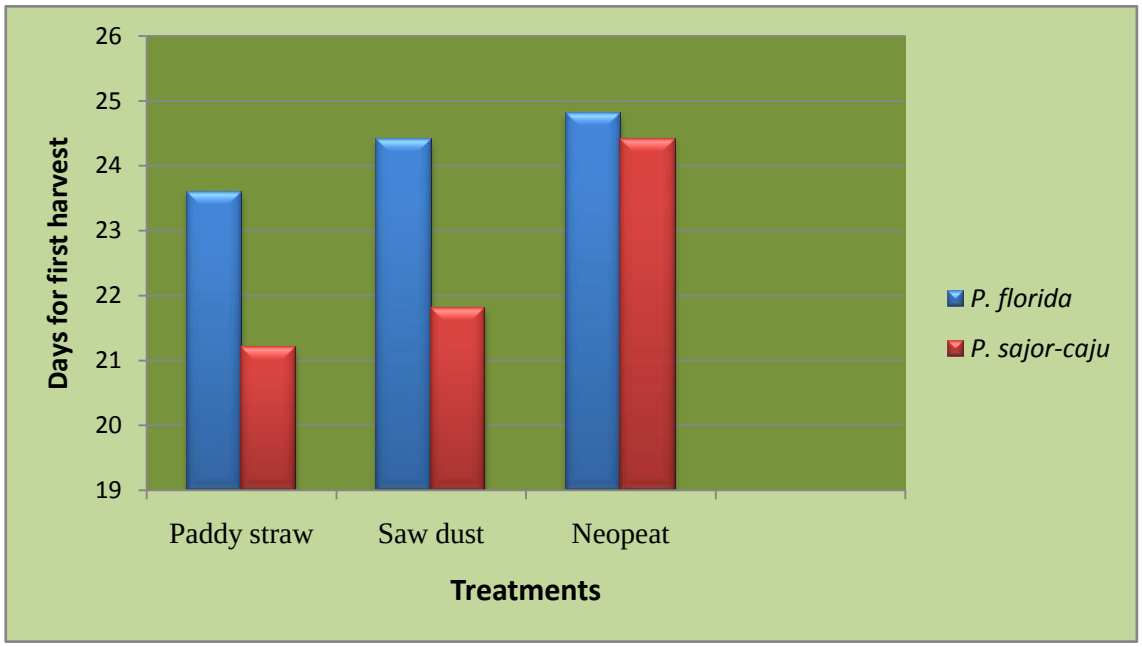


Fig. 1. Effect of different substrates on days taken for first harvest of mushrooms

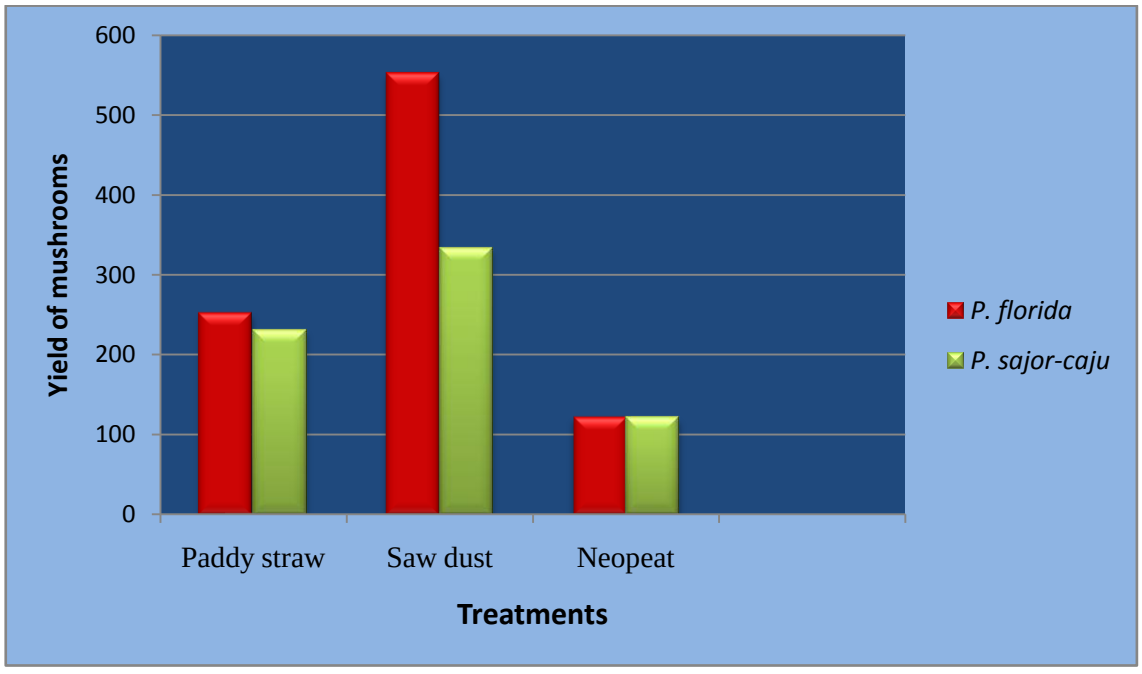


Fig. 2. Effect of different substrates on yield of mushrooms

Saw dust (T_2) gave significantly higher yield in both the case, *P. florida* (551.80g/bed) and *P. sajor-caju* (332.60g/bed). The mushrooms were large in size and were very robust compared to that of others. Among the different substrates, the lowest yield was recorded in neopeat by *P. florida* (122.80g/bed) and *P. sajor-caju* (120.60g/bed). The mushrooms produced in neopeat were very thin and small in size.

In all treatments *P. florida* recorded maximum yield (309.07g/bed) and was significantly superior to *P. sajor-caju* (228g/bed). Among the treatments, T_2 (saw dust) recorded an average yield of 442.2g, and were significantly superior to other treatments. In neopeat (T_3) yield was very poor (121.7g) (Plate 4).

4.4 ISOLATION OF MICROORGANISMS FROM DIFFERENT SMS

The microbial population associated with different substrates at the time of bed preparation, spawn running period (15^{th} day), during harvest (30^{th} day) and after harvest (50^{th} day) were evaluated by serial dilution technique and the data are given in Table 5 and 6. In all the treatments, the number of fungal and bacterial colonies were found to be varied from each stage. The actinomycetes colonies were not observed in Ken knights medium from any of the samples. Microbes isolated during different growth stages of mushroom are listed in Table 7.

In the case of both *P. florida* and *P. sajor-caju*, the fungal population in all the treatments were less at the time of spawn run (S_2), compared to S_1 (during bed preparation). But during harvest (S_3), the population started increasing and showed highest number after harvest (S_4). The mean number of fungi in *P. florida* paddy straw beds at the time of bed preparation (S_1) was 35.67, which decreased to 26.67 during S_2 and at S_3 and S_4, the values were 67.33 and 72.67 respectively. The same trend was noticed in the case of saw dust and neopeat. Maximum number of fungal colonies was noticed in T_1 (paddy straw) irrespective of the time of sampling, whereas it was minimum in T_3 (neopeat). Among the two species, the fungal population was higher in *P. florida* beds compared to *P. sajor-caju.* Three types of fungi were observed in plates. They were named as FA-1, FA-2 and FA-3.

Plate 4 : Performance of Oyster mushrooms in different substrates

P. florida in Saw dust

P. florida in Paddy straw

P. florida in Neopeat

Table 5: Enumeration of fungal population from substrates at different stages of cropping

Tr. No.	Treatments	*Fungal population (x10^4cfu/g of substrate)							
		P.florida				*P. sajor-caju*			
		S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4
1	Paddy straw	35.67	26.67	67.33	72.67	35.67	23.00	38.33	47.67
2	Saw dust	30.00	11.67	43.67	56.67	30.00	12.33	30.67	43.67
3	Neopeat	9.33	4.33	12.33	15.67	9.33	8.00	14.00	19.33
	CD value	6.70				5.44			

*mean of three replications

S_1 – At the time of bed preparation S_2 – Spawn running period

S_3 – During harvest of mushroom S_4 -After harvest of mushroom

Table 6: Enumeration of bacterial population from substrates at different stages of cropping

Tr. No.	Treatments	*Bacterial population (x10^8cfu/g of substrate)							
		P.florida				*P. sajor-caju*			
		S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4
1	Paddy straw	29.00	137.0	15.33	9.67	29.00	86.33	13.67	7.33
2	Saw dust	17.33	72.67	9.33	7.33	17.33	58.33	14.67	9.00
3	Neopeat	9.33	39.00	30.33	15.67	9.33	37.67	7.33	7.67
	CD value	8.45				6.44			

*mean of three replications

S_1 – At the time of bed preparation S_2 – Spawn running period

S_3 – During harvest of mushroom S_4 -After harvest of mushroom

The maximum population of bacteria was recorded at the time of spawn run (S_2) in both *P. florida* and *P. sajor-caju*. But during harvest (S_3) it starts decreasing and was minimum after harvest (S_4). During harvest (S_3) and after harvest (S_4) a fast growing and spreading type bacterium (BA-3) was observed and it inhibited all the other bacterial colonies developed in the dish. In the case of paddy straw, the mean number of bacterial colonies in *P. florida* beds at the time of bed preparation (S_1) was 29, which increased to 137 during S_2 and at S_3 and S_4, the values were 15.33 and 9.67 respectively. The same trend was noticed in saw dust and neopeat. Maximum number of bacterial colonies was noticed in T_1 (paddy straw), whereas it was minimum in T_3 (neopeat). From four different growth stages of mushroom, five different types of bacteria were isolated. They were named as BA-1, BA-2, BA-3, BA-4 and BA-5.

Table 7 : List of microbes isolated during different growth stages of mushroom

	At the time of bed preparation **(S_1)**	Spawn running period **(S_2)**	During mushroom harvest **(S_3)**	After mushroom harvest **(S_4)**
FUNGI	FA-2 FA-3	FA-2	FA-2 FA-3	FA-1 FA-2 FA-3
BACTERIA	BA-2 BA-5	BA-1 BA-2 BA-5	BA-3 BA-5	BA-3 BA-4 BA-5

4.5 *In vitro* EFFECT OF ISOLATED ORGANISMS AGAINST THE PATHOGENS

The antagonistic activity of three fungal and five bacterial antagonists was evaluated against *P. aphanidermatum* and *R. solanacearum* under *in vitro* condition.

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

The results of the experiment are presented in Table 8. It is found that BA-4, BA-5 and *P. fluorescens* were effective against *P. aphanidermatum*. The isolate BA-4 gave 79.7 per cent inhibition on second day itself. Whereas BA-5 gave 73.7 per cent inhibition on third day and *P. fluorescens* gave 74.4 per cent inhibition on fourth day. But BA-1, BA-2 and BA-3 did not show any inhibition against *P. aphanidermatum*. Pathogen had over grown on these antagonists from second day onwards (Plate 5A).

4.5.2 *In vitro* evaluation of fungal antagonists against *Pythium aphanidermatum*

The efficacy of isolated fungal antagonists against *P. aphanidermatum* was studied by dual culture method and per cent inhibition was calculated. The results are presented in Table 9 (Plate 5B).

It was found that all the fungal isolates and reference culture *Trichoderma viride* gave cent per cent inhibition against *P. aphanidermatum* on sixth day of incubation. The initial growth rate of antagonist was almost same in both mono and dual culture. The growth of antagonist was faster as compared to the pathogen in dual culture. In the case of FA-1, on the second day of incubation average growth of pathogen was 45.3mm. On third day of incubation, FA-1 started over growing the pathogen. Then the growth of pathogen against FA-1 was 40mm. But FA-2, FA-3 and *T. viride*, started over growing the pathogen on the fourth day. On third day, the growth of pathogen against FA-2, F-3 and *T. viride* was 42.6mm, 44.3mm and 41.3mm respectively. But on fourth day, the growth was 27.7mm, 28.3mm and 30.7mm respectively. All the isolates took six days for complete over growth of the pathogen. But FA-1 inhibited the pathogen faster than others. Greater sporulation of the isolate FA-1 was noticed when it over grew the pathogen. The type of antagonism noticed in FA-1 was cessation of growth and over

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

Antagonists		*Colony diameter of *P. aphanidermatum* (mm) Days after incubation					PIOC
		1	2	3	4	5	
BA-1	D	30	47.7	69.7	90		0
	M	31	54	79	90		
BA-2	D	34.7	53	74	90		0
	M	31	54	79	90		
BA-3	D	23.3	41.7	59.7	74.7	90	0
	M	31	54	79	90		
BA-4	D	17.7	18.3	18.3	18.3		79.7
	M	31	54	79	90		
BA-5	D	21	22.3	23.7	23.7		73.7
	M	31	54	79	90		
P. fluorescens	D	17.7	20.3	22.3	23		74.4
	M	31	54	79	90		

*Mean of three replications

D- Dual culture

M- Monoculture

PIOC – Per cent Inhibition Over Control

Table 9: *In vitro* evaluation of fungal antagonists against *P. aphanidermatum*

Antagonists		*Colony diameter of fungal antagonists and *P. aphanidermatum* (mm)												Type of antagonism
		Days after incubation												
		1		2		3		4		5		6		PIOC
		T	A	T	A	T	A	T	A	T	A	T	A	
FA-1	D	22	35.3	45.3	44.7	40	50	24.6	65.3	10.3	79.7	0	90	100	III & II
	M	31	36	54	49	79	61	90	77	90	88	90	90		
FA-2	D	22.7	31	36.7	41.3	42.6	47.4	27.7	62.3	17.7	72.3	0	90	100	II
	M	31	32	54	42	79	55	90	72	90	85	90	90		
FA-3	D	28.3	32.3	32.7	36.3	44.3	45.7	28.3	61.7	16.7	73.3	0	90	100	II
	M	31	33	54	46	79	58	90	73	90	84	90	90		
T. viride	D	19.7	23.7	41	42.3	41.3	48.7	30.7	59.3	18	72	0	90	100	II
	M	31	22	54	45	79	59	90	69	90	86	90	90		

*Mean of three replications

T- Test organism D- Dual culture I – Homogeneous III – Cessation of growth

A- Antagonist M- Monoculture II – Over growth IV – Aversion

PIOC – Per cent Inhibition Over Control

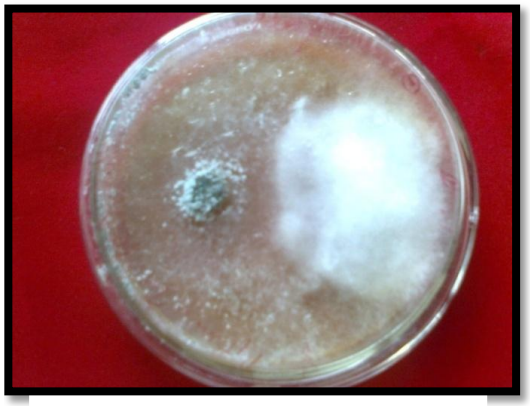
Plate 5: *In vitro* evaluation of bacterial and fungal antagonists against *P. aphanidermatum*

A. Bacterial antagonists against *P. aphanidermatum* (2nd day)

P. aphanidermatum x BA-4

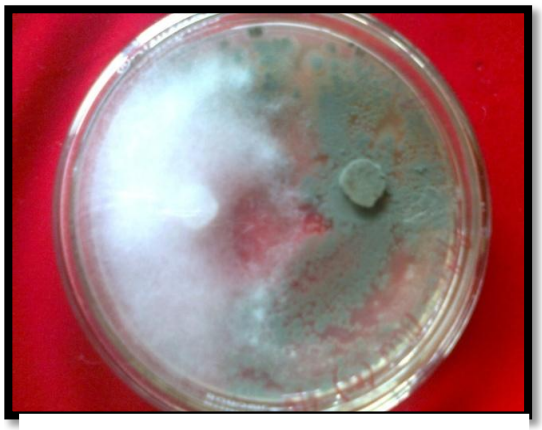
P. aphanidermatum x BA-5

B. Fungal antagonists against *P. aphanidermatum* (4th day)



P. aphanidermatum x FA-1

P. aphanidermatum x *T. viride*



P. aphanidermatum x FA-2

P. aphanidermatum x FA-3

growth on the pathogen. But in all the other isolates and *T. viride,* only over growth was noticed.

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

The antagonistic efficiency of five bacterial antagonists were evaluated by two methods *viz.,* point inoculation of bacterial antagonists and cross streaking of pathogen and antagonists. The bacterial antagonist, BA-5 and *P. fluorescens* showed lysis at the juncture after 48h of inoculation in cross streaking method. In point inoculation method, BA-5 produced an inhibition zone of 26mm against *R. solanacearum*, whereas the reference culture *P. fluorescens* showed an inhibition zone of 24mm (Plate 6A).

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

Among the two methods adopted (streaking on one side and streaking on both sides), streaking on both sides was found more effective in testing the antagonistic property of four fungal antagonists against *R. solanacearum.* The results of the experiment are presented in Table 10 (Plate 6B).

From the data furnished in the table, it was found that all the isolated fungi and reference culture *Trichoderma viride* gave 100 per cent inhibition against *R. solanacearum.* Among the antagonists, FA-1 was found to be more effective, because of the faster growth over the pathogen and production of a dark brown coloured metabolite. All the isolates took six days for complete over growth on the pathogen. But FA-1 inhibited the pathogen at faster rate compared to others. On 1st day, the average growth was highest in FA-1 (36mm), followed by FA-3 (33mm). On 5th day also the growth was highest in FA-1 (88mm), but it was followed by *T. viride* (86mm). Reference culture, *T. viride* also showed complete over growth on sixth day. But sporulation was less on the area where the pathogen was streaked. Metabolite production was not observed in reference culture. Sporulation was found to be more in FA-1, over the area where the pathogen was streaked.

Table 10: *In vitro* evaluation of fungal antagonists against *R. solanacearum*

Antagonists		*Colony diameter of fungal antagonists (mm)						PIOC
		Days after incubation						
		1	2	3	4	5	6	
FA-1	D	33.7	46.3	59.7	72.7	83.3	90	100
	M	36	49	61	77	88	90	
FA-2	D	27.7	40.7	56	70.3	82.3	90	100
	M	30	44	58	72	85	90	
FA-3	D	29.7	41.3	54	68	80	90	100
	M	33	46	58	73	84	90	
T. viride	D	31.3	43.7	54	65.7	81	90	100
	M	32	45	59	69	86	90	

*Mean of three replications

D- Dual culture

M- Monoculture

PIOC – Per cent Inhibition Over Control

Plate 6: *In vitro* evaluation of bacterial and fungal antagonists against *R. solanacearum*

A. Bacterial antagonist against *R. solanacearum* (2nd day)

R. solanacearum x BA-5

B. Fungal antagonists against *R. solanacearum* (6th day)

R. solanacearum x FA-1

R. solanacearum x *T. viride*

R. solanacearum x FA-2

R. solanacearum x FA-3

4.6 *In vitro* EVALUATION OF MUTUAL COMPATIBILITY OF THE SELECTED FUNGAL AND BACTERIAL ISOLATES

Based on the inhibitory effect of isolated organisms on the pathogens, the effective antagonists against the pathogens were selected and others were discarded. The effective antagonists were listed in Table 11.

Table 11: Effective antagonists against *P. aphanidermatum* and *R. solanacearum*

	Fungal antagonists	**Bacterial antagonists**
P. aphanidermatum	FA-1 FA-2 FA-3	BA-4 BA-5
R. solanacearum	FA-1 FA-2 FA-3	BA-5

Then the compatibility between these antagonistic organisms was studied by dual culture technique. It was tested for the preparation of consortium, which was given as a treatment in pot culture experiment. Compatibility was tested among fungal antagonists, bacterial antagonists and between fungal and bacterial antagonists. The reactions of various combinations of antagonists were listed in Table 12.

Table 12: Compatibility reactions of various combinations of effective antagonists

Sl. No.	**Isolates**	**Observations recorded**	**Type of reaction**
1	FA-1 × FA-2	Intermingling of hyphae	I
2	FA-1 × FA-3	Overgrowth of FA-1 on FA-3	O
3	FA-1 × BA-4	Mutual inhibition with thick mycelial strand	MT

4	FA-1 × BA-5	Intermingling of mycelial growth with bacterial colony	I
5	FA-1 × *T. viride*	Intermingling of hyphae	I
6	FA-1 × *P. fluorescens*	Overgrowth of FA-1 on *P. fluorescens*	O
7	FA-2 × FA-3	Intermingling of hyphae	I
8	FA-2 × BA-4	Intermingling of mycelial growth with bacterial colony	I
9	FA-2 × BA-5	Overgrowth of FA-2 on BA-5	O
10	FA-2 × *T. viride*	Mutual inhibition with a clear zone (0.9cm)	MC
11	FA-2 × *P. fluorescens*	Overgrowth of FA-2 on *P. fluorescens*	O
12	FA-3 × BA-4	Intermingling of mycelial growth with bacterial colony	I
13	FA-3 × BA-5	Intermingling of mycelial growth with bacterial colony	I
14	FA-3 × *T. viride*	Overgrowth of *T. viride* on FA-3	O
15	FA-3 × *P. fluorescens*	Intermingling of mycelial growth with bacterial colony	I
16	BA-4 × BA-5	Mutual inhibition with a clear zone (0.5cm)	MC
17	BA-4 × *T. viride*	Mutual inhibition with a clear zone (0.3cm)	MC
18	BA-4 × *P. fluorescens*	Intermingling of bacterial growth without lysis	I

19	BA-5 × *T. viride*	Overgrowth of *T. viride* on BA-5	O
20	BA-5 × *P. fluorescens*	Intermingling of bacterial growth without lysis	I

I – Intermingling of hyphae

O – Over growth

MC – Mutual inhibition with clear zone

MT – Mutual inhibition with thick mycelial strand

No inhibition on the growth of two antagonistic fungi in dual culture method indicated the mutual compatibility between the fungal antagonists. No lysis at the juncture point of two bacterial isolates indicated that two bacterial isolates are compatible to each other. In the case of fungal-bacterial pairs, if both the antagonists had grown together in the Petri dish, then they were selected as compatible pairs. The selected compatible pairs were listed in Table 13.

Table 13: Selected compatible pairs among the effective isolated antagonists

Fungal pairs	Fungal bacterial pairs	Bacterial pairs
FA-1 × FA-2	FA-1 × BA-5	BA-4 × *P. fluorescens*
FA-2 × FA-3	FA-2 × BA-4	BA-5 × *P. fluorescens*
FA-1 × *T. Viride*	FA-3 × *P. fluorescens*	
	FA-3 × BA-4	
	FA-3 × BA-5	

4.7 IDENTIFICATION OF SELECTED ANTAGONISTS

The selected fungal and bacterial antagonists were identified based on the cultural, morphological and biochemical characters. In the case of bacteria 16S rDNA sequence analysis was also carried out for the identification.

4.7.1 Identification of fungal antagonist

Cultural and morphological characters of the fungal antagonist (FA-1) was studied in detail on PDA medium and based on that it was identified (Plate 7).

4.7.1.1 Cultural characters

Colonies - Fast growing, smooth surface, become hairy and colour change from whitish green to dark green.

4.7.1.2 Morphological characters

Mycelium – Hyaline, smooth, septate, branched and of 2-4µm wide.

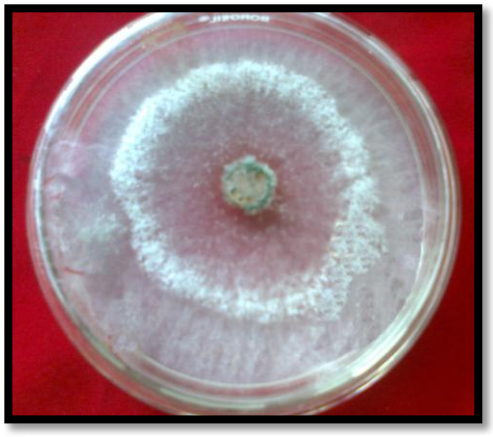
Conidiophores – Long and slender without sterile hyphae, side branches long, arise in compact tuft and all branches stand at wide angle.

Phialides - Slender, not crowded, 8-12µm long, arise in groups of more than 2-3 number. They are curved, pin shaped, narrower at the base widening above the middle and attenuated into long neck.

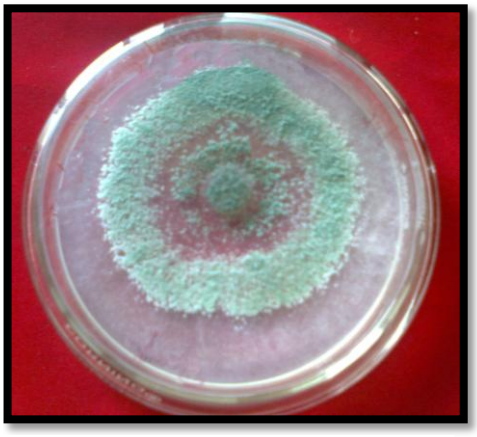
Phialospore - Globose, rough walled and of the size 2-4µm.

Based on these characters, this isolate was identified as *Trichoderma viride*. The identification got confirmed from National Centre for Fungal Taxonomy (NCFT), New Delhi with their identification number 4605.11.

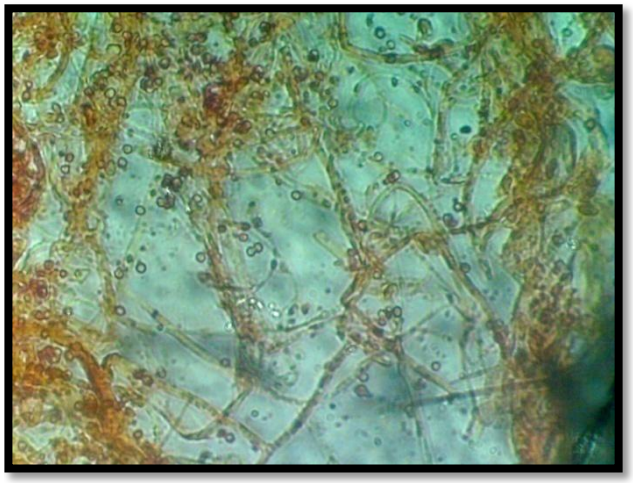
Plate 7: Promising *Trichoderma viride* isolates against pathogens



T. viride from SMS



T. viride (Reference culture)



T. viride from SMS

4.7.2 Identification of bacterial antagonists

Cultural and morphological characters of the two selected bacterial antagonists were studied and they were identified by 16S rDNA sequence analysis. The identification got confirmed by biochemical tests.

4.7.2.1 Cultural characters

The cultural characters of the bacterial antagonists BA-4 and BA-5 were studied by streaking on nutrient agar medium. After the incubation period of 24h, the colonies were observed as circular, convex, smooth and slimy in nature. The isolate BA-5 was white in colour. But the isolate BA-4 is light brown in colour initially and then turns to dark brown on maturity. The isolate BA-5 was more slimy in nature.

4.7.2.2 Morphological characters

For morphological studies 24h old culture of bacteria was used. Gram staining was employed to study Gram reaction. Isolates BA-4 and BA-5 were found to be gram negative, short rods. Pigment production was not observed in BA-5. But BA-4 produced fluorescent, diffusible pigment in Kings B medium, which was observed under UV light. It was positive in the case of diffusible, non fluorescent pigment also. The pigment is diffusible to the medium when it is kept for five days.

4.7.2.3 16S rDNA sequence analysis of isolated antagonistic bacteria

After evaluating the cultural and morphological characters of the bacterial antagonists, they were identified by 16S rDNA sequence analysis using Polymerase Chain Reaction.

4.7.2.3.1 **Amplification of 16S rDNA gene -** Amplification of 16S rDNA gene was carried out by colony PCR. The PCR product was checked on 0.9% (w/v) agarose gel and documented.

4.7.2.3.2 **Direct purification of PCR product -** Since only a single band was obtained, PCR product was directly purified.

4.7.2.3.3 **Sequencing of 16S rDNA gene -** Purified product of 1500bp from BA-4 and BA-5 were sequenced.

4.7.2.3.4 **Nucleotide sequence analysis -** Homology search of nucleotide sequences obtained from the Isolates BA-4 and BA-5 with other reported 16S rDNA gene sequences were carried out. The BA-4 showed homology with *Pseudomonas aeruginosa* and the BA-5 showed homology with *Klebsiella pneumoniae.*

4.7.2.4 Biochemical characters

The identification of bacterial antagonists using 16S rDNA sequence analysis was confirmed by biochemical tests. Biochemical characterization of BA-4 and BA-5 were studied and presented in Table 14.

4.7.2.4.1 **Catalase test -** Positive catalase reaction was shown by the BA-5 by the production of effervescence upon addition of a few drops of hydrogen peroxide. But no effervescence production was observed in BA-4.

4.7.2.4.2 **Oxidase test -** A loopful of each isolate was taken and rubbed on oxidase disc. BA-4 showed purple colour development, proving that the isolates were positive for oxidase test. Colour change was not observed in BA-5.

4.7.2.4.3 **Starch hydrolysis test -** None of the isolates were able to hydrolyse starch as evidenced by the absence of a colourless zone around the bacterial growth in contrast to the blue background.

4.7.2.4.4 **Levan production from sucrose -** Both the isolates failed to produce levan from sucrose as indicated by the absence of raised convex colonies on the media supplemented with five per cent sucrose.

4.7.2.4.5 **Gelatin liquefaction -** Both the isolates were not capable of liquefying gelatine. Clear zone formation around the bacterial colony was absent in both cases.

4.7.2.4.6 **Urease test** - The test was done on Christensen's Urea Agar medium. BA-5 gave positive result in urease test as indicated by the colour change of Christensen's Urea Agar medium from yellow to pink within five days of inoculation. It took 4 days to give positive result. No colour change was noticed in the case of BA-4.

4.7.2.4.7 **Mode of utilization of glucose** - In the case of BA-4, utilization of glucose showed positive results. The colour change was noticed from 7^{th} day onwards. BA-5 showed no colour change.

4.7.2.4.8 **Nitrate reduction** - The test was done on Nitrate agar medium. BA-5 showed the development of pink colour, which indicate that it has the ability to reduce nitrate. But in BA-4, pink colour was absent.

4.7.2.4.9 **Arginine dihydrolase reaction –** The inoculation was done on Thornley semi solid medium. BA-4 gave pink colour to the medium on fourth day indicated their ability to hydrolyse arginine. But BA-5 showed negative result.

4.7.2.4.10 **Production of hydrogen sulphide –** Both the isolates showed negative result towards the production of hydrogen sulphide. In both the cases lead acetate paper stripes were not turned to black in colour.

4.7.2.4.11 **Growth at 4^0C and 41^0C** - In the case of BA-4, growth was there at 41^0C and it didn't grow at 4^0C. Optimum temperature for BA-5 is 25^0C and that of BA-4 is 30^0C, but a temperature of 37^0C favoured the bacterial antagonistic property.

From the biochemical characterization, BA-4 was confirmed as *Pseudomonas aeruginosa* and BA-5 was confirmed as *Klebsiella pneumoniae*.

Among the effective fungal antagonists, the most effective antagonist, FA-1 alone was selected for the further study. Since FA-2 and FA-3 were identified as *Aspergillus* sp., there is a possibility that it may act as pathogen against ginger in future. So they were not selected for field level application.

Table 14: Biochemical characterization of bacterial isolates

Sl. No.	Characters	Observations	
		BA-4	BA-5
1	Gram staining	-	-
2	Fluorescent, diffusible pigment	+	-
3	Diffusible, non fluorescent pigment	+	-
4	Non diffusible, non fluorescent pigment	+	-
5	Catalase test	-	+
6	Oxidase test	+	-
7	Starch hydrolysis test	-	-
8	Levan production from sucrose	-	-
9	Gelatin liquefaction	-	-
10	Urease test	-	+
11	Mode of utilization of glucose	+	-
12	Nitrate reduction	-	+
13	Arginine dihydrolase reaction	+	-
14	Production of hydrogen sulphide	-	-
15	Growth at 41^0C	+	-

4.8 MECHANISM OF ANTAGONISM

The various mechanisms involved in the antagonistic reaction of fungal and bacterial antagonists against *P. aphanidermatum* and *R. solanacearum* were studied by the methods described in 3.8.

4.8.1 Mechanism of antagonism of selected fungal antagonist (*T. viride*) against the pathogens

The mechanism of antagonism of selected fungal isolates against the pathogens was studied and the results are presented below.

4.8.1.1 Production of volatile metabolites

The effect of volatiles produced by fungal antagonist, *T. viride* against *P. aphanidermatum* was studied. Results showed that there was no effect of the volatile metabolites of *T viride* on the growth of the pathogen. In the plates which are held together as described in 3.8.1.1, the growth of *Pythium* was same as in control.

4.8.1.2 Production of non volatile metabolites

Effect of non volatile metabolites against the fungal and bacterial pathogens was studied by two methods. Cellophane paper method was used in the case of fungal pathogens. But culture filtrate method was used in the case of both fungal and bacterial pathogens.

4.8.1.2.1 **Cellophane paper method -** The effect of non volatile metabolites of the fungal antagonist, *T. viride* against *P. aphanidermatum* was studied by using a cellophane paper as described in 3.8.1.2.1. Results showed that non volatile metabolites produced 100 per cent inhibition against *P. aphanidermatum*.

4.8.1.2.2 **Culture filtrate method -** This method was followed for *in vitro* evaluation of culture filtrate of *T. viride* against *P. aphanidermatum* and *R.*

solanacearum. The culture filtrate, showed 100 per cent inhibition against *P. aphanidermatum*. But in the case of *R. solanacearum* it showed zero per cent inhibition.

4.8.1.3 Mycoparasitism

The isolated *T. viride* and *P. aphanidermatum* were inoculated on the cellophane paper kept over the PDA medium and when the mycelium of the fungal antagonist (FA-1) and pathogen touched each other, that portion was cut along with the cellophane paper using a sterile blade and observed under microscope. From the microscopic study, it was observed that the type of mycoparasitism between *T. viride* and *P. aphanidermatum* is coiling. The mycelia of *T. viride* coiled around the mycelia of *P. aphanidermatum* (Plate 8A)

4.8.2 Mechanism of antagonism of selected bacterial antagonists against the pathogens

The mechanisms involved in the antagonism of selected bacterial isolates against the pathogens were studied and the results are presented below.

4.8.2.1 Effect of bacterial culture filtrate against the pathogens

For *in vitro* evaluation of culture filtrates of bacterial antagonists (*P. aeruginosa* and *K. pneumoniae*) against *R. solanacearum*, filter paper disc method was followed. The sterilized culture filtrates were used to test antagonistic property against both fungal and bacterial pathogens. No effect was observed in the case of bacterial culture filtrates against *R. solanacearum.*

In vitro evaluation of bacterial culture filtrate against fungal pathogen was also studied by poisoned food technique. The results showed that bacterial culture filtrate had no effect against *P. aphanidermatum*. But the fluffiness of pathogen was less compared to the control.

4.8.2.2 Production of siderophores

Siderophore production of bacterial antagonists (*P. aeruginosa* and *K. pneumonia*e) was tested as described in 3.8.5. Siderophore production was observed on *P. aeruginosa*. It produced a zone of orange colouration of 2.4 mm width after 48 hours of incubation and then it spread on the entire medium within five days. But the colour change in the medium was not observed in *K. pneumoniae* (Plate 8B).

4.8.2.3 Production of hydrogen cyanide

The bacterial isolates (*P. aeruginosa* and *K. pneumonia*e) were tested for their ability to produce hydrogen cyanide (HCN) following the method of picric acid assay. None of the isolates were found to be cyanogenic in nature as evidenced by no change in the colour of filter paper from yellow to brown or to red.

4.9 *In vitro* EFFECT OF SELECTED ANTAGONISTS AGAINST MUSHROOM SPECIES

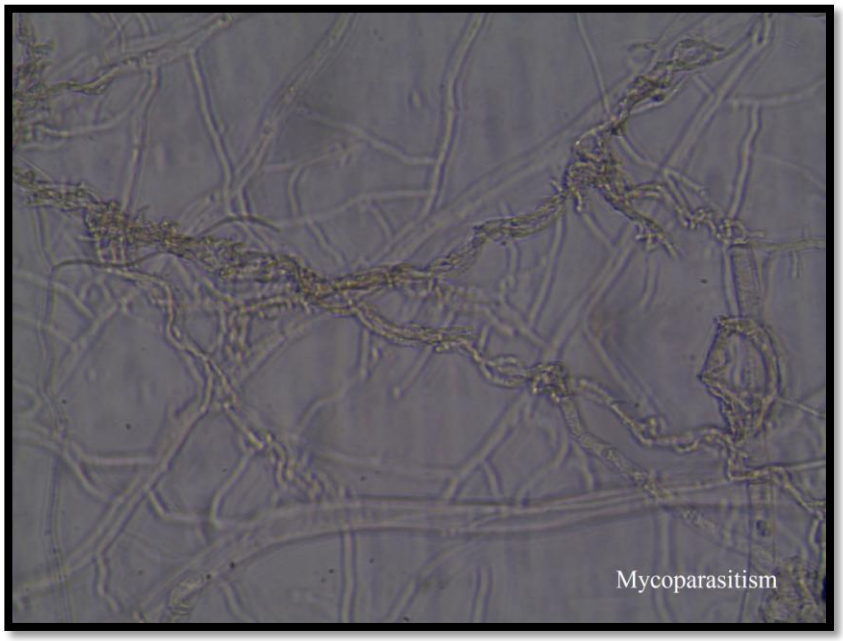
In vitro effect of selected fungal antagonist, *Trichoderma viride* from SMS (FA-1) and bacterial antagonists, *Pseudomonas aeruginosa* (BA-4) and *Klebsiella pneumoniae* (BA-5) against *Pleurotus florida* and *P. sajor-caju* was tested by dual culture method.

4.9.1 ***In vitro* evaluation of bacterial antagonists against *P. florida* and *P. sajor-caju***

The results of the experiment are presented in Table 15 and 16. It was found that bacterial antagonists, *P. aeruginosa* and *K. pneumoniae* were not inhibiting the mushroom fungi. During initial three days, the growth of both *Pleurotus* sp. in dual culture was less compared to monoculture. From the fourth day onwards, growth started increasing in dual culture than the monoculture. Complete growth in Petri dishes was noticed on seventh day in both mono and dual culture. When *P. aeruginosa* was tested

Plate 8: Mechanism of antagonism of fungal and bacterial antagonists

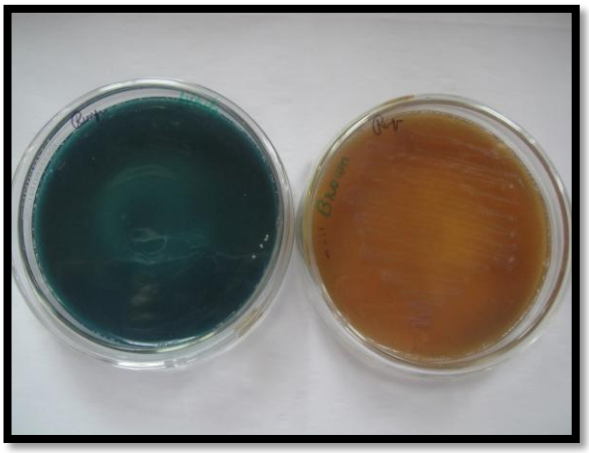
A. Mycoparasitism of *T. viride* (FA-1) on *P. aphanidermatum* (40X)



B. Siderophore production of *P. aeruginosa* (BA-4)

Two days after incubation

(Zone of orange colouration due to siderophore production)



Five days after incubation

a. Without bacterial inoculation
b. With bacterial inoculation

Table 15 : ***In vitro*** **evaluation of bacterial antagonists against** ***Pleurotus florida***

Antagonists		*Colony diameter of *Pleurotus florida* (mm)							Percent inhibition
		Days after inoculation							
		1	2	3	4	5	6	7	
Pseudomonas aeruginosa (BA-4)	D	12.67	28.8	39.2	59.7	74.9	83.5	90	0
	M	13	29	40	58	73	81	90	
Klebsiella pneumoniae (BA-5)	D	13.1	28.4	39.8	60.1	75.3	84.2	90	0
	M	13	29	40	58	73	81	90	

*mean of three replications

D- Dual culture M- Monoculture

Table 16 : ***In vitro*** **evaluation of bacterial antagonists against** ***Pleurotus sajor-caju***

Antagonists		*Colony diameter of *Pleurotus sajor-caju* (mm)							Percent inhibition
		Days after inoculation							
		1	2	3	4	5	6	7	
Pseudomonas aeruginosa (BA-4)	D	16.8	29.5	42.7	65.3	79.1	83.9	90	0
	M	17	31	44	62	76	84	90	
Klebsiella pneumoniae (BA-5)	D	17.3	30.4	43.1	66.8	80.2	85.1	90	0
	M	17	31	44	62	76	84	90	

*mean of three replications

D- Dual culture M- Monoculture

against *P. florida*, on fourth day the growth of *P. florida* in monoculture was 58mm, whereas the growth in dual culture was 59.7mm. When *K. pneumoniae* was tested, the growth of *P. florida* on fourth day was 60.1mm. The same trend was noticed in *P. sajor-caju* also.

4.9.2 *In vitro* evaluation of fungal antagonist (FA-1) against *P. florida* and *P. sajor-caju*

The efficiency of the selected fungal antagonist, *Trichoderma viride* from SMS (FA-1) against mushroom fungi was assessed by dual culture method and per cent inhibition was calculated. The *Trichoderma viride* was isolated from the SMS sample taken after the production of mushroom (S_4). The results of the experiment are presented in Table 17. It was found that *T. viride* gave cent per cent inhibition against both the mushroom species. It started overgrowing the mushroom species on fourth day and the complete over growth was observed on sixth day. Growth of *P. florida* against *T. viride* on third day was 32.6mm. But on the fourth day it became 24.3mm. Same trend was noticed in the case of *P. sajor-caju* also.

4.10 *In vitro* EFFECT OF AQUEOUS EXTRACTS OF DIFFERENT SMS

Inhibitory effects of concentrated filtered aqueous extracts of SMS on both the pathogens were studied. This was carried out by poisoned food technique for *P. aphanidermatum* and by inhibition zone technique for *R. solanacearum.*

4.10.1 *In vitro* effect of aqueous extracts against *R. solanacearum*

Filter sterilized aqueous extracts of paddy straw, saw dust and neopeat SMS of *P. florida* and *P. sajor-caju* were evaluated for their inhibitory effect against *R. solanacearum*. Out of this only the aqueous extract of paddy straw SMS of *P. sajor-caju* was found to be effective against *R. solanacearum.* An inhibition zone of 26mm was developed around the filter paper disc dipped in aqueous extract of paddy straw

Table 17 : ***In vitro*** **evaluation of fungal antagonist (FA-1) against *P. florida* and *P. sajor-caju***

Mushroom species		*Colony diameter of FA-1 and mushroom species (mm)														PI
		Days after incubation														
		1		2		3		4		5		6		7		
		T	A	T	A	T	A	T	A	T	A	T	A	T	A	
P. florida	D	12.5	34.2	27.9	46.7	32.6	57.4	24.3	65.7	10.1	79.9	0	90			100
	M	13	36	29	49	40	61	58	77	73	88	81	90	90	90	
P. sajor-caju	D	16.4	35.3	29.5	47.1	36.4	53.6	29.7	60.3	12.8	77.2	0	90			100
	M	17	36	31	49	44	61	62	77	76	88	84	90	90	90	

*Mean of three replications

T- Test organism D- Dual culture

A- Antagonist M- Monoculture PI - Per cent Inhibition

SMS of *P. sajor-caju.* In the case of all other treatments, no inhibition zone was observed.

4.10.2 *In vitro* effect of aqueous extracts against *P. aphanidermatum*

Filter sterilized aqueous extracts of paddy straw, saw dust and neopeat SMS of *P. florida* and *P. sajor-caju* were evaluated for their inhibitory effect against *P. aphanidermatum* by poisoned food technique. Results showed that none of the treatments were effective against *P. aphanidermatum*. The pathogen attained full growth on all the treatments. But the fluffiness of the pathogen was less in plates treated with aqueous extract compared to the control.

4.11 EVALUATION OF SMS UNDER POT CULTURE CONDITION

The effectiveness of SMS and the isolated antagonistic organisms to manage the rhizome rot complex disease of ginger was studied by two pot culture experiments. Separate experiments were conducted for the management of *Pythium aphanidermatum* and *Ralstonia solanacearum*. Observations on germination percentage, and other growth parameters *viz.*, number of tillers, number of leaves per tiller and height of tillers were recorded at different intervals. The yield of ginger rhizomes was also recorded. After 45 days of planting, challenge inoculation of the pathogen was done and per cent disease incidence was calculated.

All the treatments were applied at three times, at the time of planting, 60 days after planting and 120 days after planting. From the *in vitro* study it is clear that *T. viride* from SMS is most effective against *P. aphanidermatum*. The isolated *T. viride* is compatible with reference culture *Trichoderma viride*. So *T. viride* from SMS + *T. viride* (reference culture) was selected as a fungal consortium treatment against *P. aphanidermatum.* In the case of *Ralstonia solanacearum, K. pneumoniae* is most effective. It is compatible with reference culture *Pseudomonas fluorescens*. So *K. pneumoniae* + *P. fluorescens* (reference culture) was selected as a bacterial consortium treatment against *R. solanacearum.* For fungal-bacterial consortium, *T. viride* from SMS + *K. pneumoniae* was selected and it was given as a consortium against both *P.*

aphanidermatum and *R. solanacearum*. Apart from this, effectiveness of *T. viride* from SMS alone against *P. aphanidermatum* and *K. pneumoniae* alone against *R. solanacearum* were also tested in pot culture experiment.

4.11.1 Management of *Pythium aphanidermatum* (Experiment no.1)

The effect of each treatment on per cent disease incidence by *Pythium aphanidermatum*, germination percentage, number of tillers, number of leaves per tiller and height of tillers are presented below.

4.11.1.1 Per cent disease incidence

Challenge inoculation of pathogens was done at 45 days after planting. The per cent disease incidence was recorded for each treatment at four months after planting. Significant difference was observed between treatments. The results are presented in Table 18.

The results revealed that the plants which received paddy straw SMS of *P. sajor-caju* as mulch (T_2) and the antagonist *T. viride* from SMS (T_{10}) were completely free of disease. These treatments were followed by T_{12} (*T. viride* from SMS + Reference culture *T. viride*) with 2.67 per cent disease incidence giving 97.33 per cent decrease over control. The treatments T_7 (*T. viride* from SMS + *K. pneumoniae*) and T_{11} (*P. aeruginosa*) also showed lesser disease incidence of 5.67 per cent with 94.33 per cent decrease over control. Disease incidence in other biological treatments T_3 (Saw dust SMS of *P. florida*), T_4 (Saw sust SMS of *P. sajor-caju*), T_6 (Neopeat SMS of *P. sajor- caju*), T_5 (Neopeat SMS of *P. florida*) and T_9 (Reference culture *T. viride*) ranged from 14 to 36 per cent, whereas those plants treated with fungicide copper hydroxide (0.2 per cent) showed disease incidence of 53 per cent with 47 per cent decrease over control. Maximum disease incidence of 100 per cent was noticed in control plots (T_{13}) (Fig. 3).

Table 18: Effect of treatments on per cent disease incidence in the management of *P. aphanidermatum*

Treatment no.	Treatments	*Per cent disease incidence	Per cent +/- over control
T_1	Paddy straw SMS of *P. florida*	8.33 (0.29)^{def}	- 91.67
T_2	Paddy straw SMS of *P. sajor-caju*	0.00 (0.14)^{f}	-100.00
T_3	Saw dust SMS of *P. florida*	19.67 (0.46)^{cd}	-80.33
T_4	Saw dust SMS of *P. sajor-caju*	14.00 (0.38)^{de}	-86.00
T_5	Neopeat SMS of *P. florida*	36.00 (0.64)^{bc}	-64.00
T_6	Neopeat SMS of *P. sajor-caju*	22.00 (0.47)^{cd}	-78.00
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	5.67 (0.24)^{ef}	-94.33
T_8	Copper hydroxide 0.2%	53.00 (0.81)^{b}	-47.00
T_9	Reference culture *T. viride* @10g/pot	36.00 (0.64)^{bc}	-64.00
T_{10}	*T. viride* from SMS	0.00 (0.14)^{f}	-100.00
T_{11}	*Pseudomonas aeruginosa*	5.67 (0.24)^{ef}	-94.33
T_{12}	*T. viride* from SMS + Reference culture *T. viride*	2.67 (0.19)^{ef}	-97.33
T_{13}	Control	100.00 (1.43)^{a}	-
T_{14}	Absolute control	94.33 (1.33)^{a}	-

*mean of three replications

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

The figures in the parenthesis are arc sine transformed values

4.11.1.2 Germination percentage

The results of germination percentage are presented in Table 19. Among the various treatments, T_{12} (*T. viride* from SMS + Reference culture *T. viride)* recorded cent per cent germination followed by T_{10} (*T. viride* from SMS) with 97.23 per cent germination. The treatments T_{12} and T_{10} showed 16.67 per cent and 13.9 per cent increased efficiency over control. The treatment T_{11} (*Pseudomonas aeruginosa*) showed 94 per cent germination and was on par with T_2 (paddy straw SMS of *P. sajor-caju* as mulch). They showed 11.14 per cent increased efficiency over control. Among the treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* (94.47%) and saw dust SMS of *P. sajor-caju* (91.67%) showed better germination percentage. The least germination percentage of 69.47 was recorded in T_8 (soil drenching with copper hydroxide). Rhizomes in control and absolute control showed per cent germination of 83.33 and 72.23 respectively (Fig. 4).

4.11.1.3 Number of tillers

Number of tillers recorded after two, three, four and five months of planting showed a significant difference among the treatments. The results are presented in Table 20. All treatments had a positive effect in increasing the number of tillers compared to control. After two months of planting, maximum number of tillers are in T_{10} (*T. viride* from SMS) and T_{12} (*T. viride* from SMS + Reference culture *T. viride*). The average number of tillers in T_{10} and T_{12} at 2MAP was 7.11. The least number was noticed in T_{14} (Absolute control). Among the treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* showed better result (6.33). After three months of planting, maximum number of tillers was recorded again in T_{10} (*T. viride* from SMS) (Plate 9). The average number of tillers observed in T_{10} at 3MAP was 8.78. It was followed by T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) with an average of 8.00 tillers. The least number was noticed in T_{13} (control).

Four months after planting, the number of tillers ranged from 0.33 in T_{13} (Control) to a maximum of 9.79 in T_{10} (*T. viride* from SMS). The treatment T_{10} was followed by T_{12} (*T. viride* from SMS + Reference culture *T. viride*), T_2 (Paddy straw SMS of *P. sajor-caju* as mulch), and T_{11} (*Pseudomonas aeruginosa*). After five months of

Table 19: Effect of treatments on germination percentage in the management of *P. aphanidermatum*

Treatment no.	Treatments	*Germination % (30 DAP)	Per cent +/- over control
T_1	Paddy straw SMS of *P. florida*	86.10 (9.26)^{abcde}	+ 2.77
T_2	Paddy straw SMS of *P. sajor-caju*	94.47 (9.72)^{abc}	+ 11.14
T_3	Saw dust SMS of *P. florida*	88.87 (9.42)^{abcd}	+ 5.54
T_4	Saw dust SMS of *P. sajor-caju*	91.67 (9.57)^{abcd}	+8.34
T_5	Neopeat SMS of *P. florida*	75.00 (8.65)^{cde}	- 8.33
T_6	Neopeat SMS of *P. sajor-caju*	72.23 (8.49)^{de}	- 11.1
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	91.67 (9.57)^{abcd}	+ 8.34
T_8	Copper hydroxide 0.2%	69.47 (8.27)^{e}	-13.86
T_9	Reference culture *T. viride* @10g/pot	77.80 (8.80)^{bcde}	-5.53
T_{10}	*T. viride* from SMS	97.23 (9.86)^{ab}	+ 13.9
T_{11}	*Pseudomonas aeruginosa*	94.43 (9.71)^{abc}	+ 11.14
T_{12}	*T. viride* from SMS + Reference culture *T. viride*	100.00(10.00)^{a}	+ 16.67
T_{13}	Control	83.33 (9.12)^{abcde}	-
T_{14}	Absolute control	72.23 (8.50)^{de}	-

*mean of three replications

DAP- Days after planting

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

The figures in the parenthesis are square root transformed values

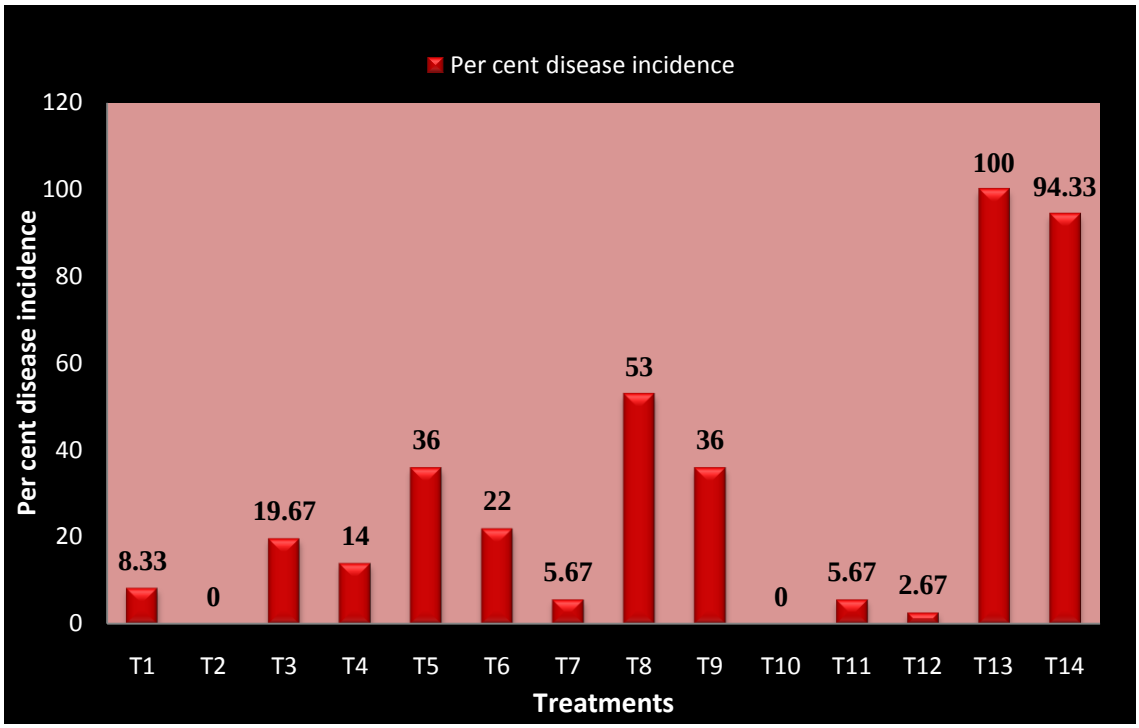


Fig. 3 Effect of different treatments on per cent disease incidence in the management of *P. aphanidermatum*

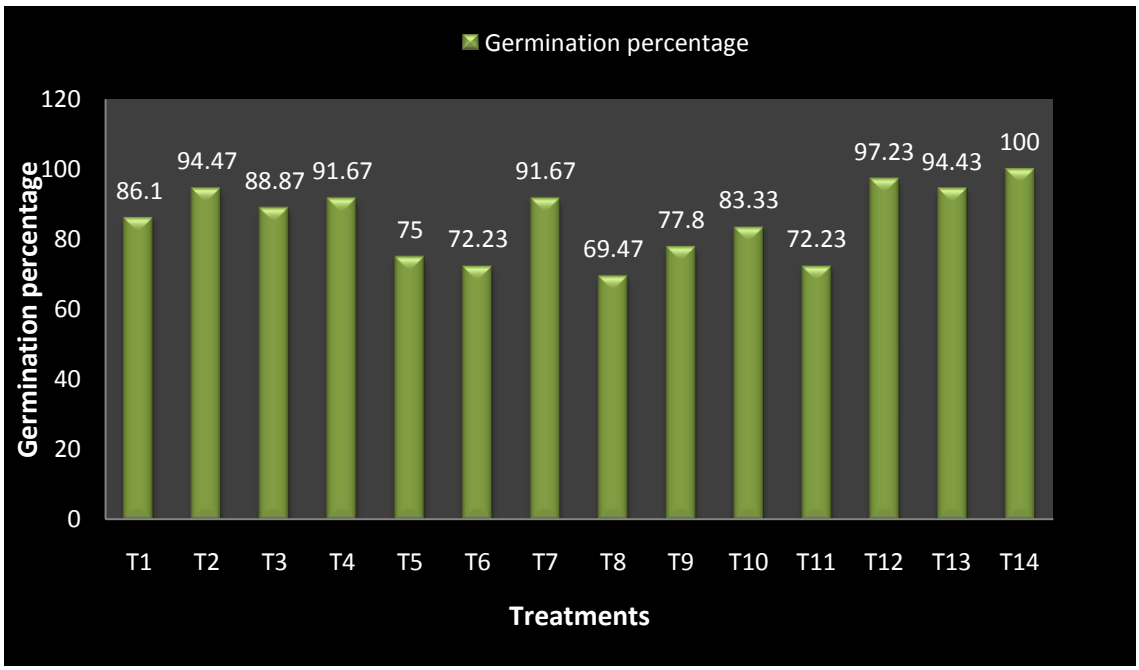


Fig. 4 Effect of different treatments on germination percentage in the management of *P. aphanidermatum*

Table 20: Effect of treatments on number of tillers in the management of *P. aphanidermatum*

Treatment no.	Treatments	*Number of tillers			
		2 MAP	3 MAP	4 MAP	5 MAP
T_1	Paddy straw SMS of *P. florida*	3.22^{cd}	4.89^{cd}	6.00^{d}	5.44^{fg}
T_2	Paddy straw SMS of *P. sajor-caju*	6.33^{a}	8.00^{ab}	8.55^{b}	10.56^{ab}
T_3	Saw dust SMS of *P. florida*	3.00^{cd}	5.56^{c}	5.55^{de}	6.33^{ef}
T_4	Saw dust SMS of *P. sajor-caju*	2.78^{cd}	4.33^{de}	5.67^{de}	7.22^{de}
T_5	Neopeat SMS of *P. florida*	3.11^{cd}	4.33^{de}	4.11^{f}	4.67^{g}
T_6	Neopeat SMS of *P. sajor-caju*	2.78^{cd}	3.44^{ef}	4.67^{ef}	4.89^{g}
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	4.44^{bc}	5.77^{c}	7.00^{c}	8.00^{d}
T_8	Copper hydroxide 0.2%	2.11^{d}	2.44^{fg}	2.22^{g}	2.22^{h}
T_9	Reference culture *T. viride* @10g/pot	2.22^{d}	3.44^{ef}	4.67^{ef}	5.11^{g}
T_{10}	*T. viride* from SMS	7.11^{a}	8.78^{a}	9.79^{a}	11.20^{a}
T_{11}	*Pseudomonas aeruginosa*	5.55^{ab}	7.00^{b}	7.99^{c}	9.22^{c}
T_{12}	*T. viride* from SMS + Reference culture *T. viride*	7.11^{a}	7.78^{ab}	8.78^{b}	10.01^{b}
T_{13}	Control	2.00^{d}	1.56^{g}	0.33^{h}	-
T_{14}	Absolute control	1.55^{d}	1.78^{g}	2.11^{g}	0.33^{i}

*mean of three replications

MAP- Months after planting

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

planting also the maximum number of tillers was observed in T_{10} (*T. viride* from SMS) followed by T_2 and T_{12}, and the least in T_{13} (Control).

4.11.1.4 Number of leaves per tiller

The data on the number of leaves revealed significant difference among the treatments. The results are presented in Table 21. Maximum number of leaves of 14.13 was observed in T_{10} (*T. viride* from SMS) at two months after planting. It was followed by T_{12} (*T. viride* from SMS + Reference culture *T. viride)* and T_{11} (*Pseudomonas aeruginosa*). The same trend was noticed at three months after planting (Plate 9). Four months after planting also there was significant difference among the treatments. The number of leaves ranged from 1.89 to 21.23 with the minimum in T_{13} (Control) and the maximum in T_{10} (*T. viride* from SMS). After five months of planting also the maximum number of leaves was noticed in T_{10} and the least in control. In the case of treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* showed highest number of leaves per tiller during all months, which was followed by saw dust SMS of *P. sajor-caju.* In control and absolute control after three months of planting, the number of leaves starts decreasing.

4.11.1.5 Height of tillers

Observations on height of tillers were recorded at two, three, four and five months after planting. The results are presented in Table 22. All treatments had a positive effect in increasing the height of tillers compared to control. There were significant differences among treatments on height of tillers at two months after planting. A maximum height of 38.9cm was observed in T_{10} (*T. viride* from SMS) followed by 36.23 in T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) and 34.67 in T_{11} (*Pseudomonas aeruginosa*). At three months after planting the same trend was noticed, the height of plants ranged from 23.13cm (T_{13}) to 42.67cm (T_{10}). After four months also, plants in T_{10} (*T. viride* from SMS) showed maximum height and the least in control. At 5MAP maximum height was observed in T_{10} (49.13cm), followed by T_{12} (*T. viride* from SMS + reference culture *T. viride*) (Plate 9).

Plate 9: Comparison of isolated *T. viride* with reference culture *T. viride,* and their combined effect on plant growth characters

Reference culture *T. viride*

T. viride from SMS

T. viride from SMS + Reference culture *T. viride*

Table 21: Effect of treatments on number of leaves/ tiller in the management of *P. aphanidermatum*

Treatment no.	Treatments	*Number of leaves/tillers			
		2 MAP	3 MAP	4 MAP	5 MAP
T_1	Paddy straw SMS of *P. florida*	10.32^{cde}	12.70^{cde}	15.33^{cd}	17.20^{cde}
T_2	Paddy straw SMS of *P. sajor-caju*	12.20^{c}	16.00^{b}	19.00^{b}	21.23^{b}
T_3	Saw dust SMS of *P. florida*	8.44^{fg}	10.54^{f}	12.77^{e}	14.90^{f}
T_4	Saw dust SMS of *P. sajor-caju*	10.77^{cd}	13.77^{c}	16.90^{c}	18.10^{cd}
T_5	Neopeat SMS of *P. florida*	10.67^{cd}	12.90^{cd}	14.77^{d}	16.77^{def}
T_6	Neopeat SMS of *P. sajor-caju*	9.56^{def}	11.70^{def}	13.80^{de}	15.10^{ef}
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	12.10^{c}	13.57^{c}	17.10^{c}	19.10^{c}
T_8	Copper hydroxide 0.2%	8.67^{efg}	11.00^{ef}	12.87^{e}	14.87^{f}
T_9	Reference culture *T. viride* @10g/pot	6.44^{h}	8.56^{g}	9.67^{f}	15.10^{ef}
T_{10}	*T. viride* from SMS	14.13^{a}	18.33^{a}	21.23^{a}	23.90^{a}
T_{11}	*P. aeruginosa*	13.23^{ab}	17.00^{ab}	19.43^{ab}	21.77^{b}
T_{12}	*T. viride* from SMS + Reference culture *T. viride*	13.87^{ab}	18.30^{a}	20.80^{ab}	22.57^{ab}
T_{13}	Control	7.33^{gh}	6.78^{h}	1.89^{h}	-
T_{14}	Absolute control	7.22^{gh}	8.56^{g}	7.44^{g}	1.89^{g}

*mean of three replications

MAP- Months after platning

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

Table 22: Effect of treatments on height of tillers in the management of *P. aphanidermatum*

Treatment no.	Treatments	*Height of tillers			
		2 MAP	3 MAP	4 MAP	5 MAP
T_1	Paddy straw SMS of *P. florida*	23.57^{de}	25.90^{efg}	31.00^{defg}	33.70^{def}
T_2	Paddy straw SMS of *P. sajor-caju*	36.23^{b}	41.00^{ab}	44.77^{a}	45.77^{ab}
T_3	Saw dust SMS of *P. florida*	22.47^{de}	25.00^{efg}	28.77^{efg}	30.77^{ef}
T_4	Saw dust SMS of *P. sajor-caju*	27.47^{cd}	31.23^{de}	34.77^{cde}	38.03^{b}
T_5	Neopeat SMS of *P. florida*	27.70^{cd}	30.33^{def}	33.67^{def}	36.10^{cdef}
T_6	Neopeat SMS of *P. sajor-caju*	30.67^{bc}	33.57^{cd}	34.30^{de}	38.87^{cd}
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	31.20^{bc}	35.47^{cd}	36.20^{cd}	38.90^{cd}
T_8	Copper hydroxide 0.2%	22.00^{de}	24.47^{fg}	27.33^{fg}	29.77^{f}
T_9	Reference culture *T. viride* @10g/pot	20.00^{e}	23.57^{g}	26.90^{fg}	30.10^{ef}
T_{10}	*T. viride* from SMS	38.90^{a}	42.67^{a}	45.67^{a}	49.13^{a}
T_{11}	*P. aeruginosa*	34.67^{ab}	38.67^{ab}	42.00^{ab}	45.57^{ab}
T_{12}	*T. viride* from SMS + Reference culture *T. viride*	34.37^{ab}	38.20^{ab}	41.13^{ab}	45.90^{ab}
T_{13}	Control	22.56^{de}	23.43^{g}	8.89^{h}	-
T_{14}	Absolute control	21.56^{de}	23.13^{g}	25.10^{g}	8.22^{g}

*mean of three replications

MAP- Months after planting

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

4.11.1.6 Yield of rhizome

The data on the average yield of ginger rhizomes per plant are presented in Table 23. Analysis of the data revealed significant differences among the treatments. The treatment T_{10} (*T. viride* from SMS) produced the maximum yield of 323.20g followed by T_{12} (*T. viride* from SMS + Reference culture *T. viride)*, T_{11} (*Pseudomonas aeruginosa*), T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) and T_7 (*T. viride* from SMS + *Klebsiella pneumonia)* which were on par with each other. The lowest yield was in T_{13} (Control). All the treatments were having more than cent per cent increase over control. The treatment, T_{10} showed 286.56 per cent increase over control. Among the treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* (316.03g) showed highest yield, followed by saw dust SMS of *P. sajor-caju* (252.14 g) (Fig. 5).

4.11.2 Management of *Ralstonia solanacearum* (Experiment no.2)

The effect of each treatment on per cent disease incidence by *R. solanacearum,* germination percentage, number of tillers, number of leaves per tiller and height of tillers were studied and the results were presented in Table 24 to 29.

4.11.2.1 Per cent disease incidence

The plants were challenge inoculated with *R. solanacearum* at 45 days after planting and observations on per cent disease incidence was recorded at four months after planting. The results are presented in Table 24. The treatments T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) and T_{10} (*T. viride* from SMS) were significantly superior to all other treatments and the plants in these treatments did not show any wilt disease incidence. These treatments were followed by T_7 (*T. viride* from SMS + *Klebsiella pneumoniae*) and T_{11} (*Klebsiella pneumoniae)* which recorded 4.05 per cent disease incidence with 95.95 per cent decrease over control (Fig. 6).

The plants receiving *K. pneumoniae* + reference culture *P. fluorescens* (T_{12}) and saw dust SMS of *P. sajor-caju* (T_5) also found to be promising in controlling bacterial wilt incidence with lesser percentage incidence of 4.05 and 7.05 respectively. These treatments showed 90.28 and 92.95 per cent decrease over control respectively.

Table 23: Effect of treatments on yield of rhizome in the management of *P. aphanidermatum*

Treatment no.	Treatments	*Yield (gm/pot)	Per cent +/- over control
T_1	Paddy straw SMS of *P. florida*	205.07^{cd}	+ 145.28
T_2	Paddy straw SMS of *P. sajor-caju*	316.03^{a}	+ 277.98
T_3	Saw dust SMS of *P. florida*	207.93^{c}	+ 148.69
T_4	Saw dust SMS of *P. sajor-caju*	252.14^{b}	+ 201.56
T_5	Neopeat SMS of *P. florida*	225.25^{c}	+ 169.40
T_6	Neopeat SMS of *P. sajor-caju*	214.52^{c}	+ 156.57
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	311.90^{a}	+ 273.04
T_8	Copper hydroxide 0.2%	176.23^{e}	+ 110.77
T_9	Reference culture *T. viride* @10g/pot	187.97^{de}	+ 124.81
T_{10}	*T. viride* from SMS	323.20^{a}	+ 286.56
T_{11}	*Pseudomonas aeruginosa*	319.50^{a}	+ 282.13
T_{12}	*T. viride* from SMS + Reference culture *T. viride*	322.36^{a}	+ 285.55
T_{13}	Control	83.61^{f}	-
T_{14}	Absolute control	86.62^{f}	-

*mean of three replications

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

Fig. 5 Effect of different treatments on yield of rhizome in the management of *P. aphanidermatum*

Table 24: Effect of treatments on per cent disease incidence in the management of *R. solanacearum*

Treatment no.	Treatments	*Per cent disease incidence	Per cent +/- over control
T_1	Paddy straw SMS of *P. florida*	9.03 (0.29)[c]	-90.97
T_2	Paddy straw SMS of *P. sajor-caju*	0.00 (0.14)[c]	-100.00
T_3	Saw dust SMS of *P. florida*	11.00 (0.33)[c]	-89.00
T_4	Saw dust SMS of *P. sajor-caju*	7.05 (0.23)[c]	-92.95
T_5	Neopeat SMS of *P. florida*	27.67 (0.55)[b]	-72.33
T_6	Neopeat SMS of *P. sajor-caju*	11.00 (0.33)[c]	-89.00
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	4.05 (0.19)[c]	-95.95
T_8	Copper hydroxide 0.2%	44.33 (0.73)[b]	-55.67
T_9	Reference culture *P. fluorescens* @10g/pot	27.67 (0.55)[b]	-72.33
T_{10}	*T. viride* from SMS	0.00 (0.14)[c]	-100.00
T_{11}	*Klebsiella pneumonia*	4.05 (0.19)[c]	-95.95
T_{12}	*Klebsiella pneumoniae* + Reference culture *P. fluorescens*	9.72 (0.27)[c]	-90.28
T_{13}	Control	100.00 (1.43)[a]	-
T_{14}	Absolute control	95.95 (1.38)[a]	-

*mean of three replications

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

The figures in the parenthesis are arc sine transformed values

The other biological treatments T_3 (Saw dust SMS of *P. florida*), T_6 (Neopeat SMS of *P. sajor-caju*, T_5 (Neopeat SMS of *P. florida*) and T_9 (Reference culture *P. fluorescens*) recorded disease incidence ranging from 11 per cent to 27.67 per cent, with per cent decrease over control ranging from 89 to 72.33. The plants treated with fungicide copper hydroxide (0.2 per cent) recorded 44.33 per cent disease incidence with 55.67 per cent decrease over control. The control plants (T_{13}) recorded 100 per cent wilt incidence.

4.11.2.2 Germination percentage

The results of germination percentage are presented in Table 25. Among the various treatments, 100 per cent germination was recorded with T_{11} (*Klebsiella pneumoniae*). It showed 49.99 per cent increased efficiency over control. The treatment T_{10} (*T. viride* from SMS) showed 94.43 per cent germination, which was on par with T_3 (saw dust SMS of *P. florida* as mulch). They were having 41.64 per cent more efficiency over control. The least germination percentage of 66.67 was recorded in T_8 (soil drenching with copper hydroxide) and T_{13} (control) (Fig. 7).

4.11.2.3 Number of tillers

Number of tillers after two, three, four and five months of planting showed a significant difference among the treatments. The results are presented in Table 26. All treatments had a positive effect in increasing the number of tillers compared to control.

After two months of planting maximum number of tillers (7.33) was observed in T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) followed by T_1 (Paddy straw SMS of *P. florida)* (5.89) and T_{10} (*T. viride* from SMS) (5.67) and the least in T_{13} (Control) (2.00) and T_{14} (Absolute control) (2.00). After three months, maximum number of tillers was recorded again in T_2 (8.11), which was followed by T_{10} (*T. viride* from SMS) (7.34) and the least in control. Four months after planting, the number of tillers ranged from 0.22 in T_{13} (Control) to a maximum of 9.22 in T_2 (Paddy straw SMS of *P. sajor-caju* as mulch). The treatment T_2 was followed by T_{10} (*T. viride* from SMS). After five months of planting also the maximum number of tillers was observed in T_2 (10.9) followed by T_{10}

Table 25: Effect of treatments on germination percentage in the management of *R. solanacearum*

Treatment no.	Treatments	*Germination % (30 DAP)	Per cent +/- over control
T_1	Paddy straw SMS of *P. florida*	88.90 (9.42)^{abcd}	+ 22.23
T_2	Paddy straw SMS of *P. sajor-caju*	91.67 (9.58)^{abc}	+ 25
T_3	Saw dust SMS of *P. florida*	94.43 (9.73)^{ab}	+ 27.76
T_4	Saw dust SMS of *P. sajor-caju*	83.33 (9.13)^{abcde}	+ 16.66
T_5	Neopeat SMS of *P. florida*	75.00 (8.68)^{bcde}	+ 8.33
T_6	Neopeat SMS of *P. sajor-caju*	83.33 (9.15)^{abcde}	+ 16.66
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	86.10 (9.29)^{abcde}	+ 19.43
T_8	Copper hydroxide 0.2%	66.67 (8.18)^{e}	0
T_9	Reference culture *P. fluorescens* @10g/pot	69.47 (8.36)^{de}	+ 2.8
T_{10}	*T. viride* from SMS	94.43 (9.73)^{ab}	+ 27.76
T_{11}	*Klebsiella pneumoniae*	100.00 (10.03)^{a}	+ 33.33
T_{12}	*Klebsiella pneumoniae* + Reference culture *P. fluorescens*	91.67 (9.59)^{abc}	+ 25
T_{13}	Control	66.67 (8.18)^{e}	-
T_{14}	Absolute control	72.23 (8.53)^{cde}	-

*mean of three replications

DAP- Days after planting

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

The figures in the parenthesis are square root transformed values

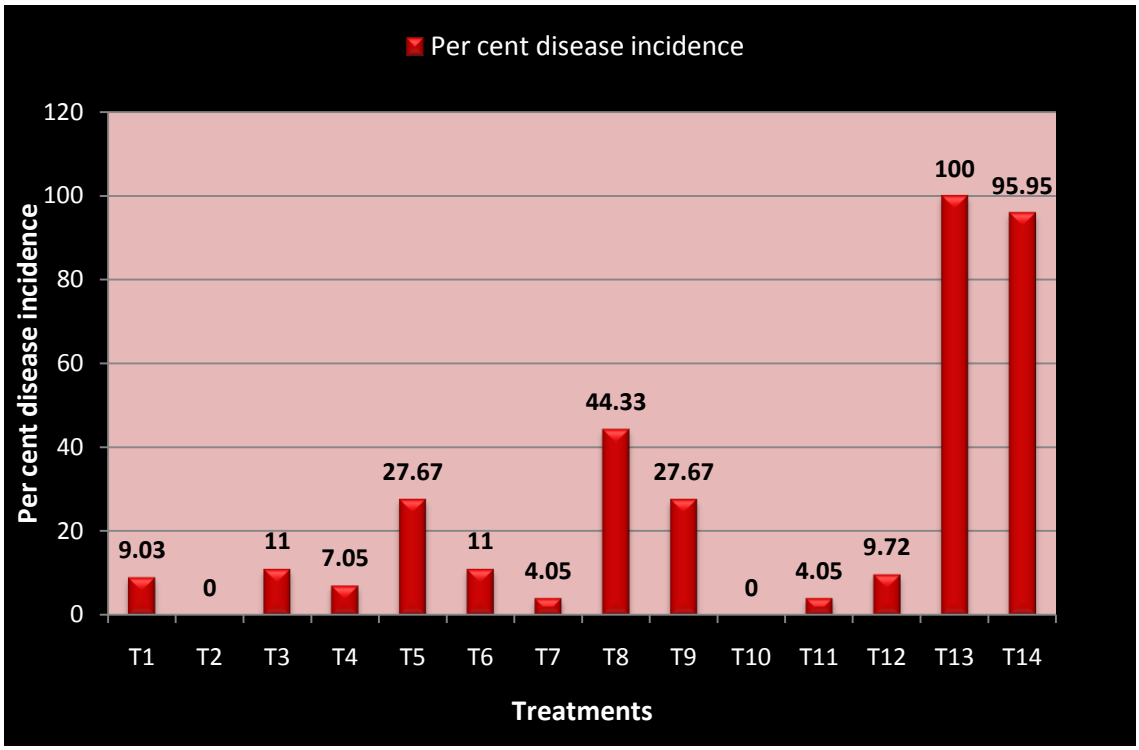


Fig. 6 Effect of different treatments on per cent disease incidence in the management of *R. solanacearum*

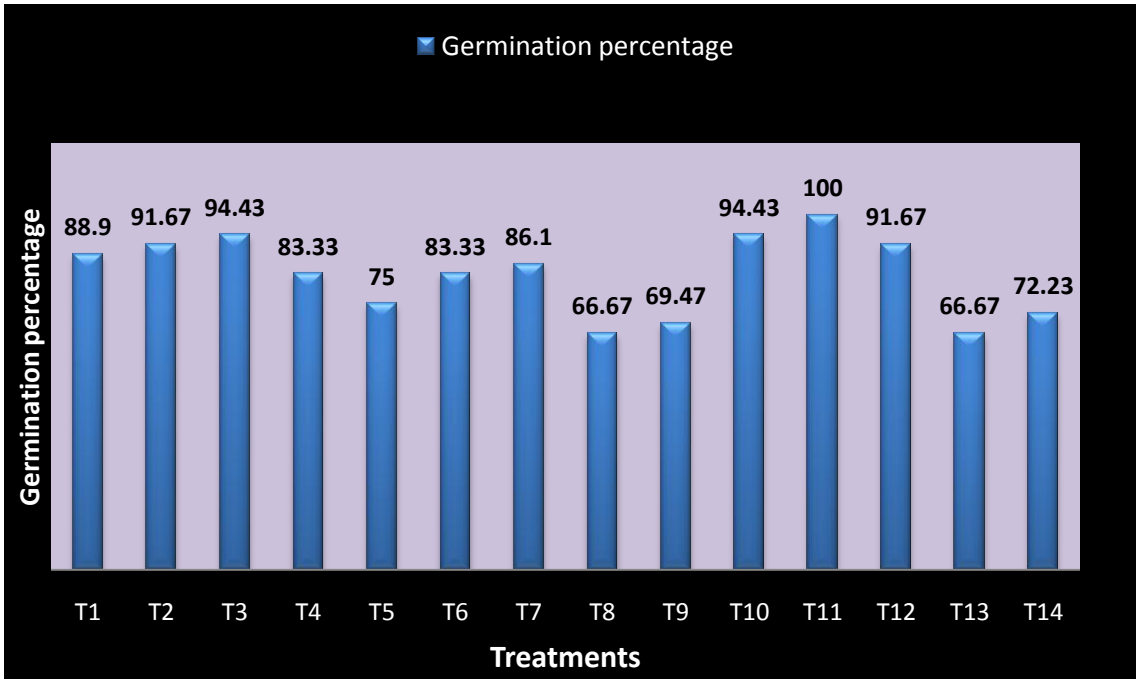


Fig. 7 Effect of different treatments on germination percentage in the management of *R. solanacearum*

Table 26: Effect of treatments on number of tillers in the management of *R. solanacearum*

Treatment no.	Treatments	*Number of tillers			
		2 MAP	3 MAP	4 MAP	5 MAP
T_1	Paddy straw SMS of *P. florida*	5.89^{b}	6.56^{de}	7.00^{cd}	7.78^{ef}
T_2	Paddy straw SMS of *P. sajor-caju*	7.33^{a}	8.11^{a}	9.22^{a}	10.9^{a}
T_3	Saw dust SMS of *P. florida*	4.78^{c}	4.78^{ef}	5.22^{e}	7.56^{ef}
T_4	Saw dust SMS of *P. sajor-caju*	4.22^{cd}	5.33^{de}	6.67^{d}	7.44^{f}
T_5	Neopeat SMS of *P. florida*	3.00^{de}	3.89^{fg}	5.00^{e}	5.67^{gh}
T_6	Neopeat SMS of *P. sajor-caju*	3.11^{de}	4.45^{efg}	5.00^{e}	6.44^{g}
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	5.56^{bc}	6.78^{bc}	7.89^{c}	8.44^{e}
T_8	Copper hydroxide 0.2%	2.67^{e}	3.55^{fg}	2.78^{f}	3.00^{i}
T_9	Reference culture *P. fluorescens* @10g/pot	2.33^{e}	3.33^{gh}	4.44^{e}	5.00^{h}
T_{10}	*T. viride* from SMS	5.67^{b}	7.34^{ab}	8.22^{ab}	9.67^{b}
T_{11}	*Klebsiella pneumoniae*	5.33^{bc}	6.55^{bc}	7.67^{b}	9.00^{bc}
T_{12}	*Klebsiella pneumoniae* + Reference culture *P. fluorescens*	5.22^{bc}	6.22^{bc}	7.33^{b}	8.56^{cd}
T_{13}	Control	2.00^{e}	1.44^{i}	0.22^{g}	-
T_{14}	Absolute control	2.00^{e}	2.22^{hi}	2.44^{f}	0.22^{j}

*mean of three replications

MAP- Months after planting

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

and T_{11} and minimum in T_{13} (Control). In the case of treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* showed highest number of tillers.

4.11.2.4 Number of leaves per tiller

The data on the number of leaves revealed significant difference among the treatments. The results are presented in Table 27. Two months after planting, maximum number of leaves (16.33) was noticed in T_{12} *(Klebsiella pneumoniae* + Reference culture *P. fluorescens)* which was on par with T_{10} (*T. viride* from SMS) (15.53) and T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) (15.1). Least number was observed in control (7.33). Three months after planting, the same trend was noticed with maximum number of leaves recorded in $T_{12,}$ followed by T_{10} and T_2. But four months after planting, the number of leaves ranged from 1.33 to 23.00 with the maximum in T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) and the minimum in T_{13} (Control). Almost same trend was noticed at 5MAP with maximum number of leaves in T_2 and the least in control and absolute control.

From the Table 27, it was clear that after two and three months of planting the maximum number of leaves per tiller was observed in T_{12} *(Klebsiella pneumoniae* + Reference culture *P. fluorescens)* and after four and five months of planting, T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) recorded maximum number of leaves.

4.11.2.5 Height of tillers

Observations on height of tillers were recorded two, three, four and five months after planting. The results are presented in Table 28. All treatments had a positive effect in increasing the height of tillers compared to control.

After two months of planting maximum height of tillers (41.57) was observed in T_{10} (*T. viride* from SMS) and was followed by T_{11}, T_2 and T_{12}. But after three months, maximum height of tillers (46.1) was recorded in T_2 (Paddy straw SMS of *P. sajor-caju* as mulch), which was followed by T_{10} (*T. viride* from SMS) and the least in control. Four months after planting, the height of tillers ranged from 6.00 in T_{13} (Control) to a maximum of 48.77 in T_{10} (*T. viride* from SMS). The treatment T_{10} was followed by T_2

Table 27: Effect of treatments on number of leaves/ tiller in the management of *R. solanacearum*

Treatment no.	Treatments	*Number of leaves/tillers			
		2 MAP	3 MAP	4 MAP	5 MAP
T_1	Paddy straw SMS of *P. florida*	12.67^{bc}	13.77^{cd}	16.20^{c}	18.90^{cd}
T_2	Paddy straw SMS of *P. sajor-caju*	15.10^{a}	19.67^{a}	23.00^{a}	25.87^{a}
T_3	Saw dust SMS of *P. florida*	11.77^{c}	14.20^{c}	16.53^{bc}	18.53^{cd}
T_4	Saw dust SMS of *P. sajor-caju*	11.57^{c}	16.23^{b}	18.47^{b}	17.90^{d}
T_5	Neopeat SMS of *P. florida*	9.10^{d}	11.97^{d}	13.53^{ef}	15.20^{e}
T_6	Neopeat SMS of *P. sajor-caju*	11.33^{c}	13.00^{cd}	15.00^{de}	17.10^{d}
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	12.57^{bc}	16.13^{b}	18.23^{bc}	20.00^{c}
T_8	Copper hydroxide 0.2%	8.00^{d}	9.46^{e}	9.67^{g}	10.77^{f}
T_9	Reference culture *P. fluorescens* @10g/pot	7.67^{d}	9.34^{e}	11.77^{f}	13.90^{e}
T_{10}	*T. viride* from SMS	15.53^{a}	19.03^{a}	21.67^{a}	23.80^{b}
T_{11}	*Klebsiella pneumonia*	14.67^{ab}	18.47^{a}	21.23^{a}	23.10^{b}
T_{12}	*K. pneumoniae* + Reference culture *P. fluorescens*	16.33^{a}	19.87^{a}	22.47^{a}	24.47^{ab}
T_{13}	Control	7.33^{d}	7.67^{e}	1.33^{i}	-
T_{14}	Absolute control	7.67^{d}	8.11^{e}	7.00^{h}	1.22^{g}

*mean of three replications

MAP- Months after planting

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

Table 28: Effect of treatments on height of tillers in the management of *R. solanacearum*

Treatment no.	Treatments	*Height of tillers			
		2 MAP	3 MAP	4 MAP	5 MAP
T_1	Paddy straw SMS of *P. florida*	32.43^{cd}	30.80^{cde}	31.87^{d}	42.20^{c}
T_2	Paddy straw SMS of *P. sajor-caju*	39.57^{abc}	46.10^{a}	46.87^{ab}	50.53^{ab}
T_3	Saw dust SMS of *P. florida*	29.67^{de}	32.67^{cd}	35.10^{cd}	38.33^{cd}
T_4	Saw dust SMS of *P. sajor-caju*	28.00^{de}	32.13^{cde}	35.67^{cd}	39.00^{cd}
T_5	Neopeat SMS of *P. florida*	24.10^{e}	26.57^{de}	29.33^{d}	33.10^{d}
T_6	Neopeat SMS of *P. sajor-caju*	28.23^{de}	30.97^{cde}	34.10^{cd}	37.43^{cd}
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	32.87^{bcd}	36.80^{bc}	40.10^{bc}	43.77^{bc}
T_8	Copper hydroxide 0.2%	22.67^{e}	24.77^{de}	28.77^{d}	31.77^{d}
T_9	Reference culture *P. fluorescens* @10g/pot	22.33^{e}	25.43^{de}	29.20^{d}	32.90^{d}
T_{10}	*T. viride* from SMS	41.57^{a}	45.67^{a}	48.77^{a}	51.90^{a}
T_{11}	*Klebsiella pneumoniae*	40.10^{ab}	43.90^{ab}	46.70^{ab}	50.00^{ab}
T_{12}	*K. pneumoniae* + Reference culture *P. fluorescens*	39.47^{abc}	43.33^{ab}	46.80^{ab}	49.67^{ab}
T_{13}	Control	22.47^{e}	24.23^{e}	6.00^{e}	-
T_{14}	Absolute control	23.97^{e}	25.90^{de}	27.43^{d}	6.79^{e}

*mean of three replications

MAP- Months after planting

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

(Paddy straw SMS of *P. sajor-caju* as mulch) (46.87). After five months of planting also the maximum height of tillers was observed in T_{10} (51.90) followed by T_2, T_{11} and T_{12}, and the least in T_{13} (Control).

4.11.2.6 Yield of rhizome

The data on the average yield of ginger rhizomes per plant are presented in Table 29. Analysis of the data revealed significant differences among the treatments. The treatment T_2 (Paddy straw SMS of *P. sajor-caju* as mulch) produced the maximum yield of 318.64g followed by T_{10} (*T. viride* from SMS) having average yield of 301.35g and the lowest yield was in T_{13} (Control). All the treatments recorded more than cent per cent increase in yield over control. The treatment T_2 showed 330.83 per cent increase over control (Fig. 8). Better performance was noticed in all the treatments which received SMS as mulch. (Plate 10)

4.11.3 Pest Incidence

After five months of planting, rhizome maggot infestation was observed on plants in both the experiments. This resulted in a general reduction in yield of rhizome. Observations on pest incidence were recorded by preparing a score chart, based on the severity as described in Materials and Methods (3.11.2.5). The results are presented in Table 30. In Experiment no.1 (Management of *P. aphanidermatum*), pest incidence was minimum in T_{10} (*T. viride* from SMS) followed by T_2, T_{11} and T_{12}. Therefore a better stand of crop was noticed in these treatments. In Experiment no.2 (Management of R. solanacearum), minimum pest incidence was noticed in both T_{10} (*T. viride* from SMS) and T_2 (Paddy straw SMS of *P. sajor-caju* as mulch). Both treatments showed better result. Maximum pest incidence was noticed in T_{13} (Control) and T_{14} (Absolute control), followed by T_8 (Soil drenching with Copper hydroxide 0.2% at 7 DAI) and T_9 (Reference culture @ 10g/ pot) in both the experiments.

Plate 10: Comparison of SMS as mulch treatments with Control (T_{13})

Table 29: Effect of treatments on yield of rhizome in the management of *R. solanacearum*

Treatment no.	Treatments	*Yield (gm/pot)	Per cent +/- over control
T_1	Paddy straw SMS of *P. florida*	222.15[d]	+ 200.37
T_2	Paddy straw SMS of *P. sajor-caju*	318.64[a]	+ 330.83
T_3	Saw dust SMS of *P. florida*	227.26[d]	+ 207.27
T_4	Saw dust SMS of *P. sajor-caju*	266.09[c]	+ 259.78
T_5	Neopeat SMS of *P. florida*	215.54[d]	+ 191.42
T_6	Neopeat SMS of *P. sajor-caju*	218.72[d]	+ 195.72
T_7	*T. viride* from SMS + *Klebsiella pneumoniae*	285.75[b]	+ 286.36
T_8	Copper hydroxide 0.2%	176.06[e]	+ 138.04
T_9	Reference culture *P. fluorescens* @10g/pot	186.13[e]	+ 151.66
T_{10}	*T. viride* from SMS	301.35[ab]	+ 307.45
T_{11}	*Klebsiella pneumoniae*	296.95[b]	+ 301.50
T_{12}	*Klebsiella pneumoniae* + Reference culture *P. fluorescens*	299.62[b]	+ 305.11
T_{13}	Control	73.96[f]	-
T_{14}	Absolute control	85.66[f]	-

*mean of three replications

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

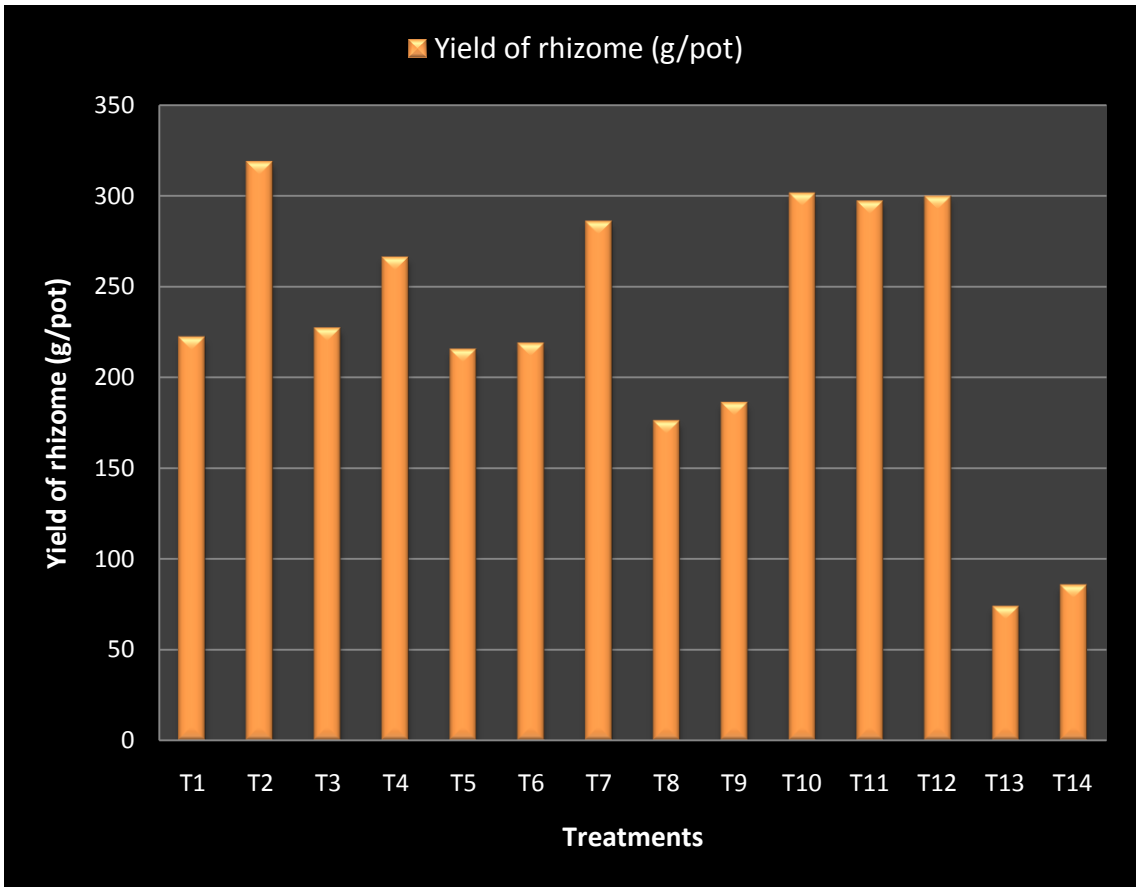


Fig. 8 Effect of different treatments on yield of rhizome in the management of *R. solanacearum*

Table 30: Effect of various treatments on pest incidence (Rhizome maggot)

Treatment no.	Treatments	*Score	
		Management of *P.aphanidermatum*	**Management of *R.solanacearum***
T_1	Paddy straw SMS of *P. florida*	3.66	2.33
T_2	Paddy straw SMS of *P. sajor-caju*	1.33	1.33
T_3	Saw dust SMS of *P. florida*	2.33	2.33
T_4	Saw dust SMS of *P. sajor-caju*	1.67	1.67
T_5	Neopeat SMS of *P. florida*	2.33	3.00
T_6	Neopeat SMS of *P. sajor-caju*	3.33	3.00
T_7	*T. viride* from SMS + *K. pneumoniae*	1.67	1.67
T_8	Copper hydroxide 0.2%	4.67	4.67
T_9	*T. viride* (Ex. No.1) / *P. fluorescens* (Ex. No.2) @10g/pot	4.67	4.33
T_{10}	*T. viride* from SMS	1.00	1.33
T_{11}	*Pseudomonas aeruginosa* (Ex. No.1)/ *Klebsiella pneumoniae* (Ex. No.2)	1.33	1.66
T_{12}	*T. viride* from SMS + Reference culture *T. viride* (Ex. No.1)/ *K. pneumoniae* + Reference culture *P. fluorescens* (Ex. No.2)	1.33	1.66
T_{13}	Control	5.00	5.00
T_{14}	Absolute control	5.00	5.00

*mean of three replications

Compatibility between the selected antagonists and the insecticides *viz.*, Chlorpyrifos (2.5ml/L), Confidor (0.5ml/L) and Rogor (1.5ml/L) were evaluated as described in 3.11.3. The results are presented in Table 31.

Table 31: Compatibility between selected antagonists and the insecticides

Antagonists	**Chlorpyrifos (2.5ml/L)**	**Confidor (0.5ml/L)**	**Rogor (1.5ml/L)**
Trichoderma viride from SMS (FA-1)	IC	C	C
Trichoderma viride (Reference culture)	IC	C	C
Pseudomonas aeruginosa (BA-4)	C	C	C
Klebsiella pneumoniae (BA-5)	C	C	C
Pseudomonas fluorescens (Reference culture)	C	IC	C

IC – Incompatible

C – Compatible

Since all the selected fungal and bacterial isolates were compatible with the insecticide Rogor (1.5ml/L), it was selected for soil drenching against rhizome maggot. It was applied at the rate of 100ml per pot in all the treatments including control. Better result was noticed after 24 hours.

Plate 11:Best treatments for the management of rhizome rot complex disease of ginger

T. viride **from SMS (T_{10}) with control (T_{13})**
(Best treatment for the management of *P. aphanidermatum*)

Paddy straw SMS of *P. sajor-caju* **(T_2) with control (T_{13})**
(Best treatment for the management of *R. solanacearum*)

Discussion

5. DISCUSSION

Mushroom growing is an eco-friendly activity as it utilizes the wastes from agriculture and in turn produces fruit bodies with excellent and unique nutritional and medicinal attributes. Spent mushroom substrate (SMS) is the composted organic material remained after the harvest of a crop of mushroom. It is rich in plant nutrients including minerals and was used as manure in different crops. Recent findings illustrate that substrate after one cycle cultivation of mushroom can be used for disease management. Sanam and Gokulapalan (2010) reported the effect of SMS on disease suppression (damping off caused by *Pythium aphanidermatum*) and plant growth promotion in tomato. The lowest wilt disease incidence and the highest yield of aubergine had been recorded by Yohalem *et al.,* (1996) from the plots applied with SMS + paddy straw. Similar reports are there on the effectiveness of SMS against fungal and bacterial diseases of various crops by several workers (Harris, 1992; Craft and Nelson, 1996; Viji *et al*., 2003; Ahlawat *et al.,* 2006). So far no attempt has been made on the use of spent mushroom substrate from oyster mushroom, against rhizome rot complex disease of ginger. Under these circumstances, a study was conducted to know the effectiveness of oyster mushroom SMS to control the rhizome rot complex disease of ginger.

Soft rot by *Pythium aphanidermatum* and bacterial wilt, caused by *Ralstonia solanacearum* and are the two common rhizome rot diseases of ginger. Both exhibit almost similar symptoms during rainy season and can occur in the same crop simultaneously. Hence, this problem is termed as rhizome rot complex disease. Ginger plants showing typical wilt symptoms and soft rot symptoms were collected from the farmer's field. The fungal pathogen *Pythium aphanidermatum* was isolated from the rhizomes showing typical soft rot symptoms. It was identified by white, coenocytic, fluffy mycelium and lobbed sporangium. After purification, pure cultures of both the pathogens were maintained. The bacterial wilt pathogen isolated from wilted ginger plants on TZC agar medium showed circular, smooth, convex, fluidal, slimy and creamy white colonies with light pink centre after 48 hours of incubation indicating its identity as *Ralstonia solanacearum*.

Since *Pleurotus florida* and *P. sajor-caju* are the most suitable mushroom species in Kerala, they were selected for the production of spent mushroom substrate. The substrate used were the agricultural waste materials like paddy straw, saw dust and neopeat. Among these substrates, paddy straw and saw dust were equally good by producing very good mycelial growth compared to neopeat. The time for spawn run and harvest were found minimum for paddy straw compared to saw dust and this might be due to the high cellulose content in paddy straw. But in neopeat, apart from this, there is high lignin content which is responsible for poor mycelial colonization by the oyster mushroom species. According to Geetha and Sivaprakasam (1998) oyster mushroom species prefer substrates rich in cellulose content. The substrates rich in lignin supported minimum growth of oyster mushrooms. Lignin was found to adversely affect the active production of cellulases.

Among the two mushroom species, *P. sajor-caju* took minimum time for complete spawn run in both the substrates, paddy straw and saw dust. The time taken for first harvest of mushroom was also minimum for *P. sajor-caju* compared to *P. florida*. Das *et al*. (2000), Chandrasekhar *et al*. (2001), Maheswari *et al.* (2004) and Elizabeth (2009) also observed faster rate of spawn run of *P. sajor-caju* in paddy straw.

Among the different substrates tested, significantly higher yield was obtained in saw dust, followed by paddy straw, by both the mushroom species. This may be due to the increased quantity of substrate in a saw dust bed compared to paddy straw bed. Thomas *et al.* (1998) reported that fruit body formation by individual species of *Pleurotus* was governed by extent of production of carbohydrate on substrate and climatic factors like relative humidity and temperature. Shah *et al.* (2004) reported maximum number and weight of sporophores of *P. ostreatus* grown on sawdust. According to Geetha and Sivaprakasam (1998), substrates with less lignin favour cellulase activity and increased the growth and yield of *P. djamor* and *P. citrinopileatus*. In neopeat yield was very poor similar to the growth parameters. This is due to the high content of lignin. According to Thomas *et al.* (1998), the yield of mushroom was directly related to the spread of mycelium into the substrate. Similar reports were also made by Geetha and Sivaprakasam (1998), Owseph *et al.* (2001),

Shashirekha and Rajarathnam, (2007) and Elizebeth (2009). Average yield of *P. sajor-caju* was less compared to *P. florida.* This may be due to the fact that weight of the individual sporocarp of *P. sajor-caju* is less than that of *P. florida*.

The microbial interaction during the growth of mushroom in different substrates was studied by enumerating microorganisms *viz*., fungi and bacteria in different growth stages of mushroom. In the case of *P. florida* and *P. sajor-caju,* the fungal population in all the treatments were less at the time of spawn run compared to that at the time of bed preparation. It may be due to the fast growth and colonization of mushroom fungus. But during harvest, the fungal population again starts increasing and it reached maximum after harvest. This indicates the involvement of fungi in degradation process. After harvest stage population of *Trichoderma* (FA-1) was more. This may be due to the fact that *Trichoderma* sp. is having higher cellulase and hemicellulase activity leading to better degradation process. Maximum number of fungal colonies was noticed in paddy straw, whereas it was minimum in neopeat. Similar results were reported by Elizabeth (2009) in the case of *P. sajor-caju* on paddy straw. The fungi associated with the substrates were tentatively identified as *Aspergillus* sp. (FA-2 and FA-3) and *Trichoderma* sp. (FA-1).

In the case of bacterial population, maximum population was obtained at the time of spawn run, in both *P. florida* and *P. sajor-caju*. But during harvest, it started decreasing and was minimum after harvest. This is because of the presence of a fast growing and spreading type bacterium (BA-3), which inhibited all the other developing bacterial colonies. Maximum number of bacterial colonies was noticed in paddy straw, whereas it was minimum in neopeat. From four different stages of mushroom growth, five different types of bacteria *viz*., BA-1, BA-2, BA-3, BA-4 and BA-5 were obtained. The isolate BA-5 appeared in all different stages of mushroom growth, but BA-4 was observed only after the harvest of mushroom.

An *in vitro* evaluation was conducted to find out the efficiency of isolated organisms against the pathogens, *P. aphanidermatum* and *R. solanacearum*. The three

different fungal antagonists and five different bacterial isolates were evaluated *in vitro* against the two pathogens. Among the bacterial isolates tested under *in vitro* against *P. aphanidermatum*, the isolates BA-4, BA-5 and *P. fluorescens* were found to be effective. The antagonist BA-4 showed 79.7 per cent inhibition, whereas BA-5 and *P. fluorescens* showed 73.7 and 74.4 per cent inhibition respectively. BingGan *et al.* (2002) reported that the biological control efficacy of *Pseudomonas aeruginosa* strain CR56 against *Pythium ultimum* and *P. spinosum* was 94.4 and 51.4% respectively.

Among the three isolated fungal antagonists and reference culture *T. viride*, tested against *P. aphanidermatum*, FA-1 (*Trichoderma* sp.) was found to be more effective than others, because of its faster growth rate and have completely overgrown the pathogen within six days after incubation. All the isolates showed 100 per cent inhibition. But on the third day after incubation, FA-1 started over growing the pathogen, whereas *T. viride*, FA-2, and FA-3 started over growing on the fourth day. The type of antagonism in FA-1 was cessation of growth and then over growth. But in all the other isolates only overgrowth was noticed. Dense sporulation was observed over the pathogen after over growth in the case of FA-1, FA-2 and FA-3. According to Shanmugham *et al.* (2000) dual culture of antagonistic organisms with *P. aphanidermatum*, only *T. harzianum* and *T. viride* were the potential antagonists. Bhardwaj *et al.* (1988) reported that rhizome rot of ginger, caused by *Pythium aphanidermatum* and *Fusarium equiseti*, was controlled by *Trichoderma* spp. to varying degrees.

Among the bacterial isolates tested against *R. solanacearum*, only BA-5 was found effective against the pathogen. On comparing with the efficacy of reference culture *P. fluorescens*, by point inoculation method, BA-5 produced an inhibition zone of 26mm, whereas *P. fluorescens* produced an inhibition zone of 24mm. Anith *et al.* (2000) observed that seed treatment with *P. fluorescens* strain EM 85 along with soil solarization decreased the wilt incidence in ginger to 7.42 per cent with an increased yield of 29.42 t/ha over control.

All the fungal isolates were also found effective against *R. solanacearum*. Among the fungal isolates, FA-1 (*Trichoderma* sp.) was found to be more efficient than all other isolates and reference culture due to its faster growth over the pathogen and its capacity for metabolite production. It took two days and all the others took three days to over grow the pathogen. Similarly, complete over growth of pathogen by *Trichoderma* was observed by D'Ercole *et al.* (1984). Manimala (2003) also reported complete inhibition of *R. solanacearum* by *T. viride* and *T. pseudokoningii*. Weindling (1934) reported that biocontrol activity of strain of *Trichoderma virens* was due to production of lethal principle, Gliotoxin. However, an interesting thing noticed was that compared to the reference culture *T. viride*, the antagonist FA-1 produced maximum thick overgrowth and sporulation on the area where the pathogen was streaked. The antagonists might be utilizing the degraded products of the pathogen.

Based on the *in vitro* tests on the antagonistic property of fungal and bacterial isolates against the pathogens, five organisms were selected to develop an effective microbial consortium against rhizome rot complex disease of ginger. The fungal antagonists FA-1, FA-2 and FA-3 were effective against both the pathogens. In the case of bacterial antagonists, BA-4 was effective against *P. aphanidermatum*, whereas BA-5 was effective against both the pathogens. So these five antagonists were selected and the compatibility between these organisms was studied by dual culture technique. Compatibility was tested among fungal antagonists, bacterial antagonists and between fungal and bacterial antagonists. Intermingling was the type of reaction noticed in the case of all compatible pairs. Similar compatibility among *Trichoderma*, *Pseudomonas* and *Bacillus* were reported by several workers (Duffy *et al.*, 1996; Manoranjitham and Prakasam, 2000; Jisha *et al.*, 2002). Compatibility studies between *P. fluorescens*, *Bacillus* and *Trichoderma* sp. were also carried out by Dhanya (2007).

From the *in vitro* study, it is clear that FA-1 was the most effective fungal antagonist against *P. aphanidermatum*. Among the other fungal antagonists tested, reference culture *T. viride* recorded faster growth rate and was also compatible with FA-1, by intermingling of hyphae. Thus FA-1 + reference culture, *T. viride* was selected as fungal consortium against *P. aphanidermatum*. In the case of *R. solanacearum*, BA-5

was the only effective antagonistic bacterium isolated from SMS. And it was compatible with reference culture *P. fluorescens*. Thus in the preparation of bacterial consortium, BA-5 + *P. fluorescens* was selected and it was applied against *R. solanacearum*. For the fungal-bacterial consortium, FA-1 + BA-5 was selected against both the pathogens. Even though BA-4 was the most effective bacterial antagonist against *P. aphanidermatum*, it was not compatible with FA-1. Hence the next effective bacterium BA-5, was selected for the preparation of fungal-bacterial consortium with FA-1. Jain *et al.* (2012) selected *Pseudomonas aeruginosa* PJHU15, *Trichoderma harzianum* TNHU27 and *Bacillus subtilis* BHHU100 from rhizospheric soils based on compatibility, antagonistic and plant growth promotion activities. The compatible microbial consortia triggered defence responses in an enhanced level in pea than the microbes alone and provided better protection against Sclerotinia rot.

The selected fungal antagonist FA-1 was identified as *Trichoderma viride* by studying cultural and morphological characters. The characters of the isolate were in conformity with the C. M. I. Descriptions of pathogenic fungi and bacteria. The identity of this isolate was further got confirmed from 'National Centre of Fungal Taxonomy (NCFT)' New Delhi as per the accession number 4605.11.

The selected antagonist, *T. viride* from SMS vary considerably from the reference culture *T. viride* of KAU. The newly isolated *T. viride* culture produced dark brown metabolite from the fourth day onwards, whereas in the reference culture *T. viride*, the metabolite was absent. Difference in the time taken for sporulation was also noticed, it was on fourth day in the case of isolated culture and second day for reference culture. There was also difference in colony colour, it was pale green in the case of isolated culture and was dark green on reference culture. The days taken by the two cultures for initiating the over growth on the pathogen were also different. On third day after incubation, *T. viride* from SMS started over growing *P. aphanidermatum*. But reference culture *T. viride*, started over growing the pathogen on fourth day. In the case of *R. solanacearum*, both antagonists inhibited the pathogen on second day onwards. But on 2nd day, growth of *T. viride* from SMS against *R. solanacearum* was 46.3mm, whereas the growth of reference culture was 43.7mm. The selected antagonist,

Trichoderma was faster than the reference culture in inhibiting the pathogens. These differences may be because of the fact that *Trichoderma* culture varies from isolate to isolate in chemical and biological activity, and hence the two cultures can be considered as different strains.

Among the five bacterial isolates, only two were found to be effective against the pathogens. Cultural and morphological characters of the two selected bacterial antagonists were studied and they were identified by 16S rDNA sequence analysis. The isolate BA-4 was identified as *Pseudomonas aeruginosa* and BA-5 was identified as *Klebsiella pneumoniae.* The identification got confirmed by biochemical tests.

From the *in vitro* study, it was found that *Pseudomonas aeruginosa* was most effective against *P. aphanidermatum* and *Klebsiella pneumonia,* against *R. solanacearum.* Viji *et al.* (2003) obtained a total of 849 bacterial isolates from two spent mushroom substrate samples and tested for antagonism against the gray leaf spot pathogen. The six isolates were identified by fatty acid profile analysis as *Pseudomonas aeruginosa* (Schroeter) Migula. *P. aeruginosa* is one of the most predominant bacteria in fresh SMS tested. Previous studies indicated that *P. aeruginosa* produces different types of antibiotic such as phenazine-1-carboxylic acid and phenazine-1-carboximide and rhamnolipid-biosurfactants that play a major role in *Pythium* suppression (Tambong and Hofte, 2001; Perneel *et al.*, 2008). In addition, control of pathogens using *Pseudomonas* species has been reported to be due to competition for iron, antibiosis and induced systemic resistance in the host (Pieterse *et al.*, 2001; Berg *et al.*, 2002). However, further studies will be required to investigate factors involved in suppression of *P. aphanidermatum*-induced damping-off using *P. aeruginosa.*

The next aspect of investigation was to find out the mechanism of antagonism of fungal and bacterial antagonists against both the pathogens. In the present study volatile metabolites from *T. viride* isolated from SMS produced no inhibition against *P. aphanidermatum.* In contrast to this, Rathore *et al.* (1990) reported that volatile substances produced by *T. viride* seemed to be effective against *P. myriotylum* as the

growth of this pathogen was completely inhibited by these substances. Howell (2003) reported that the enzyme chitinase or glucanase produced by the biocontrol agent are responsible for suppression of pathogen by breaking down the polysaccharides, chitin and β- glucans that are responsible for the rigidity of fungal cell wall, there by destroying the cell wall integrity. It was also observed that the type of mycoparasitism between *T. viride* from SMS and *P. aphanidermatum* is coiling. The mycelia of FA-1 coiled around the mycelia of *P. aphanidermatum* and might be producing the enzymes for the degradation of cell wall materials. Tong and LiangHao (2002) observed that chitinolytic enzymes of *Trichoderma* play an important role in mycoparasitism, which is considered as one of the mechanisms involved in the antagonism of *Trichoderma* sp. against plant fungal pathogens.

The effect of culture filtrates of fungal and bacterial antagonists against both the pathogens were also tested. Culture filtrates of bacterial antagonists did not show any effect on the pathogens. This may be because of the fact that culture filtrates of bacterial antagonists might be heat sensitive and inhibitory action might have lost during sterilization. But culture filtrate of fungal antagonist, *T. viride* showed 100 per cent inhibition against *P. aphanidermatum*. But it was not effective against *R. solanacearum*. The effectiveness of culture filtrate of *T. viride* against *P. aphanidermatum* might be due to its enzymatic properties. According to Muthukumar *et al.* (2011), the culture filtrate of *Trichoderma* isolate TVC_3 recorded complete inhibition on mycelial growth of *P. aphanidermatum* at 15% concentration.

Siderophore production was confirmed in the case of BA-4. It may be the mechanism of antagonism involved in the antagonistic efficiency of BA-4. Borowitz *et al.* (1992) reported that fluorescent pseudomonads suppress the pathogen by various modes of action like antibiosis, lytic enzymes, siderophore production and degradation of toxins. According to Heungens *et al.* (1992) siderophore production is involved in the antagonistic effect of *Pseudomonas aeruginosa* strain 7NSK2 against *Pythium* sp., particularly in control of post-emergence damping off of tomato seedlings.

The *in vitro* effect of selected fungal antagonist, *T. viride* (FA-1) and bacterial antagonists, *Pseudomonas aeruginosa* (BA-4) and *Klebsiella pneumoniae* (BA-5) against the mushroom species, *Pleurotus florida* and *P. sajor-caju* was tested by dual culture method. The results revealed that the bacterial antagonists were not inhibiting the mushroom fungi. During initial three days, there was slight reduction in growth of both *Pleurotus* sp. in dual culture compared to monoculture. The mushroom fungal growth touched the bacterial antagonist in dual culture on 4th day, and after that the rate of mushroom fungal growth was high in dual culture than in the monoculture. Complete growth in Petri dishes was noticed on 7th day in both mono and dual culture. The positive response of these organisms towards mushroom growth can be exploited in oyster mushroom cultivation. Similar reports have been made in the case of button mushroom cultivation by Ahlawat and Vijay (2003) and in oyster mushroom cultivation by Ahlawat (2000) in the preparation of bacterial fortified spawn. The isolated fungal antagonist *T. viride* was also tested against the mushroom species *in vitro*. It was found that *T. viride* from SMS gave 100 per cent inhibition against both the mushroom species. It started overgrowing the mushroom species on 4th day and the complete over growth was observed on 6th day. But in the present study, *T. viride* was isolated after the complete cycle of growth of mushroom. So it did not affect the growth and yield of mushroom fungus.

The inhibitory effects of aqueous extracts of SMS on both the pathogens were tested under *in vitro* condition. When filter sterilized aqueous extracts of paddy straw, saw dust and neopeat SMS of *P. florida* and *P. sajor-caju* were evaluated against *R. solanacearum*, only aqueous extract of paddy straw SMS of *P. sajor-caju* was found to be effective. Apart from other SMS, paddy straw SMS of *P. sajor-caju* may be having some unique properties to inhibit *R. solanacearum*, since *P. sajor-caju* is an efficient ligno-cellulolytic degrader compared to other *Pleurotus* sp., as reported by Owseph *et al.* (2001). When these aqueous extracts were evaluated against *P. aphanidermatum* by poisoned food technique, none of the treatments were found to be effective. The pathogen attained full growth on all the treatments. But the fluffiness of the pathogen was less in aqueous extract treatments compared to the control. In all reports with regard to the aqueous extract of SMS, they had used anaerobically fermented aqueous extract of SMS after *Agaricus* sp. mushroom cultivation. But in the present study, filter

sterilized fresh aqueous extracts were used. That may be the reason for absence of inhibition against the pathogen. Yohalem *et al*. (1994) reported that anaerobically fermented aqueous extract of spent mushroom substrate was most effective for the inhibition of germination of conidia of *Venturia inequalis,* causal agent of apple scab.

The effect of spent mushroom substrate for the management of rhizome rot complex disease of ginger was assessed under pot culture conditions. Among the various treatments for the management of both the pathogens associated with rhizome rot complex disease, *T. viride* from SMS and paddy straw SMS of *P. sajor-caju* as mulch were found to be the best giving cent per cent disease control. In control treatment, cent per cent disease incidence was noticed. Shibuya and Minami (2001) reported that carbohydrate and protein elicitors that induce defence mechanism in plants are released from the mycelia of fungal pathogens. Therefore, the mycelia of mushrooms that are prevalent in SMS are abundant sources of elicitors and thus application of SMS to plants may be useful for the control of plant diseases. The fragments of glucan and chitin from the mycelial cell walls of mushrooms in SMS may serve as elicitors for inducing systemic acquired resistance (SAR) in ginger plants. And in the case of *T. viride*, the glucanolytic enzymes play an important role in its mycoparasitism, which is considered as the major mechanism of antagonism against *Pythium*. According to Zembek *et al.* (2011) antagonism of *Trichoderma* spp. against phytopathogenic fungi is widely exploited for biocontrol of plant diseases. A crucial role in the biocontrol mechanism is attributed to cell-wall-degrading enzymes secreted by *Trichoderma* spp. More over SMS is rich in microorganisms, such as disease fighting bacteria and fungus. It naturally suppresses pathogens in the soil that cause plant damage. Craft and Nelson (1996) reported that spent mushroom compost stimulates the natural defence system in plants. The disease suppressiveness of SMS against damping off disease (*Pythium ultimum)* was evaluated and addition of SMS significantly provided effective control of damping off disease in tomato. The lowest wilt disease incidence and the highest yield of aubergine has been recorded by Yohalem *et al.* (1996) from the plots applied with SMS+paddy straw (1 ton/ ha + 2 tons/ ha) along with the recommended doze of NPK as compared to FYM (10 tons/ ha) and poultry manure (1.4 tons/ ha). Sanam and Gokulapalan (2010) also reported the effect of SMS on disease suppression (damping off caused by *Pythium aphanidermatum*) and

plant growth promotion in tomato. In the case of both the experiments for the management of *P. aphanidermatum* and *R. solanacearum*, biological treatments were having less disease incidence compared to chemical treatments.

In the experiment for the management of *P. aphanidermatum*, hundred per cent germination was noticed in the treatment with *T. viride* from SMS + reference culture *T. viride*, which was followed by the treatment *T. viride* from SMS and then the treatment with *P. aeruginosa*. As a whole, the study revealed that bioagents treated pots gave better percentage of germination especially with *T. viride* from SMS. It may be due to the growth enhancement activity of *T. viride*. According to Windham *et al*. (1986) *Trichoderma* sp. produced growth regulatory factors that increased the rate of emergence of tomato and tobacco seedling. Among the treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* showed better germination percentage. But in the experiment for the management of *R. solanacearum*, the treatment applied with *Klebsiella pneumoniae* showed 100 per cent germination followed by the treatment applied with *T. viride* from SMS. This may be because of the higher degradation capacity of *P. sajor-caju* that may facilitate better colonization by plant growth promoting organisms like *P. aeruginosa* and *K. pneumoniae*. Eudoxie and Alexander (2011) evaluated the quality and effectiveness of spent mushroom substrate (SMS) as a complete substitute for promix, in the germination, growth and development of tomato seedling. Germination percentage was >95% for both SMS and promix treatments. Yadav *et al*. (2010) reported that plant growth promoting rhizobacteria like *Pseudomonas* sp. and *Klebsiella* sp. have better effect on better seed germination and plant growth in chick pea under *in vitro* conditions.

The growth parameters like number of tillers, number of leaves per tiller and height of tillers were highest in the treatment with *T. viride* from SMS, at two, three, four and five months after planting, in the experiment for the management of *P. aphanidermatum*. Vijayaraghavan (2003) reported that *T. viride* incorporated treatments produced maximum leaf number in pepper cuttings. Among the treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* was the best. The results of the experiments indicated that the extent of degradation of the spent mushroom compost might have a positive effect on improving the biological activity of the soil and which has got a direct

impact on the sustainability of soil health. Thus the application of SMS enhanced the growth parameters of the plant. Ahmed and Khair (2000) reported the significant increase in plant height of maize using spent mushroom compost. Chang *et al.* (1986) and Mukhopadhyay (1988) demonstrated increased growth response of several crop plants in the presence of biological control agents, which may be caused by a direct effect on the plant as a biofertilizer or by control of plant pathogens. Dhanya (2007) evaluated *Trichoderma* sp. for its efficacy in the production of growth promoting substances like IAA under *in vitro* condition. She observed IAA production for all the *Trichoderma* isolates tested, which ranging from 0.031-0.064 mg/ml.

But in the experiment for the management of *R. solanacearum*, the treatment with paddy straw SMS of *P. sajor-caju* as mulch showed highest number of tillers. Number of leaves per tiller was highest in the treatment applied with *K. pneumoniae* + reference culture *P. fluorescens,* in the first three months and the treatment with paddy straw SMS of *P. sajor-caju* as mulch for the next two months. Height of tillers was observed maximum in the treatments applied with *T. viride* from SMS. According to Oei *et al.* (2008), SMS from *Agaricus* mushroom can have a number of beneficial effects when added to soils. Because it contains significant amount of essential plant nutrients. The phosphorus and potassium requirements of the crop plants can be fulfilled by incorporating 5% of SMS by volume, while nitrogen requirement can be fully met by 25% of SMS by volume. The actinomycetes, bacteria and fungi inhabiting the compost not only play role in its further decomposition but also exert some antagonism to the normal pathogens surviving and multiplying in the soil eco-system. Because SMS has high organic matter content, it can have important benefits in improving the physical structure of the soil. Ahlawat *et al.* (2009) evaluated spent mushroom substrate (SMS) as manure for nutritionally poor soil and its effects were studied on vegetative growth of tomato.

The same trend was noticed in the case of yield also. In the experiment for the management of *P. aphanidermatum*, highest yield was observed in the treatment with *T. viride* from SMS alone, which was followed by the treatment with *T. viride* from SMS

+ reference culture *T. viride*. But in the experiment for the management of *R. solanacearum*, the treatment with paddy straw SMS of *P. sajor-caju* as mulch recorded highest yield followed by the treatment with *T. viride* from SMS. All the treatments were having more than cent per cent increase in yield over control. So as a whole we can say that better degradation of spent mushroom substrate, along with growth enhancement and antagonistic property of bioagents must have contributed to higher yield. Among the isolated microorganisms, *T. viride* was found to be the best in increasing the crop vigour and yield. Mechanism involved in the phenomenon might be the elimination of pathogens in the rhizosphere and production of growth promoting substances. The above results are in agreement with the findings of Krishnamoorthy (1987), and Neelamegam and Govindarajan (2002) who reported increased seedling vigour in tomato with *T. viride.* According to Kaviyarasan and Siva (2007) isolation and identification of effective local isolate of *T. harzianum* and formulation based on the *Pleurotus* spent compost produced more chitinase, indicate that compost is a suitable carrier, not only to supply nutrients but a good substrate to support the survival time in the soil. Polat *et al.* (2009) reported that all of the spent mushroom compost treatments resulted in higher yield than control treatment in cucumber grown in green houses. Rhodas and Olson (1995) postulated that increased yield was due to the nitrogen availability which was resulted with SMS treatments. According to Siddiqui and Akhtar (2007), *P. aeruginosa* is having the phosphate solubilising capacity and Dong *et al.* (2003) reported *K. pneumoniae* as a diazotrophic endophytic bacterium that can provide fixed N_2 to plants. In our treatments, these factors might have contributed to better yield in the mulch treatments.

After five months of planting, rhizome maggot infestation was observed in the plants in both the experiments. Effect of each treatment against the pest incidence was also determined. In the experiment for the management of *P. aphanidermatum*, the treatment applied with *T. viride* from SMS showed maximum tolerance against the pest attack, followed by the treatment with *P. aeruginosa*, *T. viride* from SMS + reference culture *T. viride* and the treatment with paddy straw SMS of *P. sajor-caju* as mulch. In the experiment for the management of *R. solanacearum*, the treatment applied with *T. viride* from SMS and paddy straw SMS of *P. sajor-caju* as mulch showed maximum

tolerance. The application of bioagents and SMS might have provided a stimulus for activating the defence mechanism in ginger plants against pest attack.

The spent mushroom substrate obtained by the degradation of paddy straw and saw dust by using *P. florida* and *P. sajor-caju* can be used as mulch for ginger crops. Among these, paddy straw SMS from *P. sajor-caju* was found to be the best giving maximum enhancement of growth parameters and also reduces the incidence of rhizome rot complex disease of ginger. Among the different fungal antagonists, *T. viride* isolated after the harvest of mushroom was the best in inhibiting the growth of P. *aphanidermatum* and *R. solanacearum*. The consortium treatment, *T. viride* from SMS + reference *culture T. viride* also gave better result. So from the present study, it can be concluded that the use of paddy straw SMS of *P. sajor-caju* as mulch, along with the application of *T. viride* from SMS can be considered as an effective management practice against the rhizome rot complex disease of ginger.

The effectiveness of paddy straw SMS of *P. sajor-caju* in the management of rhizome rot diseases of ginger can be attributed to its unique chemical composition including the nutrient status which has to be further explored. The SMS is rich in microflora with antagonistic activity against pathogenic microorganisms associated with ginger plants. High cellulolytic capacity of *P. sajor-caju* favour the maximum degradation of the substrate, thereby provides a niche for the multiplication of favourable microorganisms with antagonistic effect. These microorganisms along with the available macro and micro elements in SMS stimulate the defence mechanism in plants which in turn fight against the soil borne pathogens like *P. aphanidermatum* and *R. solanacearum*. In addition, the beneficial microorganisms may be having plant growth promoting activity which contributes to increased yield in various treatments.

Summary

6. SUMMARY

The present study on "Exploitation of spent mushroom substrate as mulch for the management of rhizome rot complex disease of ginger" was carried out to study the effectiveness of spent mushroom substrate as mulch for ginger and to evaluate its efficacy in the management of rhizome rot complex disease.

Ginger plants with typical soft rot and bacterial wilt symptoms were collected from the farmer's field and fungal and bacterial pathogens were isolated. Based on cultural and morphological characters, fungal pathogen was identified as *Pythium aphanidermatum* and bacterial pathogen was identified as *Ralstonia solanacearum*. After purification, pure cultures of both the pathogens were maintained.

For the production of spent mushroom substrate, *Pleurotus florida* and *P. sajor-caju* were the mushroom species selected and they were grown in paddy straw, saw dust and neopeat. The performance of mushrooms were estimated by recording the nature of mycelial growth, time taken for spawn run, time taken for mushroom production and weight of sporocarps. In paddy straw and saw dust, *P. florida* and *P. sajor-caju* showed very good mycelial growth. But in neopeat growth was thickened at the point of spawn inoculation and mycelial spreading was very sparse. In the case of both the species, the time taken for spawn run and first harvest were minimum in paddy straw compared to saw dust and neopeat. The mushroom species, *P. sajor-caju* took minimum days for spawn run and first harvest compared to *P. florida.* Among the different substrates significantly higher yield was obtained in saw dust, followed by paddy straw, for both the species. Average yield of *P. sajor-caju* was less compared to *P. florida.*

The microbial population associated with different substrates at the time of bed preparation, spawn running period (15^{th} day), during harvest (30^{th} day) and after harvest (50^{th} day) were evaluated by serial dilution technique. In all the treatments, the number of fungal and bacterial colonies varies from stage to stage. In the case of *P. florida* and

P. sajor-caju, the fungal population in all the treatments were less at the time of spawn run compared to that at the time of bed preparation. But during harvest, the population again starts increasing and it was maximum after harvest. The fungi associated with the substrates were tentatively identified as *Aspergillus* sp. (FA-2 and FA-3) and *Trichoderma* sp. (FA-1). In the case of bacterial population, maximum population was obtained at the time of spawn run, in both *P. florida* and *P. sajor-caju*. But during harvest, it started decreasing and was minimum after harvest. From four different stages of mushroom growth, five different types of bacteria *viz*., BA-1, BA-2, BA-3, BA-4 and BA-5 were obtained.

An i*n vitro* evaluation study was conducted to find out the efficiency of these isolated organisms against the pathogens *P. aphanidermatum* and *R. solanacearum*. Among the bacterial isolates tested against *P. aphanidermatum,* BA-4 and BA-5 were effective, whereas against *R. solanacearum,* only BA-5 was found effective. All the three fungal isolates, FA-1, FA-2 and FA-3 gave 100 per cent inhibition against both the pathogens. But FA-1 was the most effective one. So these five antagonists were selected and the compatibility between these antagonistic organisms was studied by dual culture technique. Compatibility was tested among fungal antagonists, bacterial antagonists and between fungal and bacterial antagonists. Intermingling was the type of reaction noticed in the case of all compatible pairs.

The selected fungal antagonist FA-1 was identified as *Trichoderma viride* by studying cultural and morphological characters. The characters of the isolate were in conformity with the C. M. I. Descriptions of pathogenic fungi and bacteria. The identity of this isolate was further got confirmed from 'National Centre of Fungal Taxonomy (NCFT)' New Delhi as per the accession number 4605.11.

The selected antagonist, *T. viride* from SMS was different from our reference culture *T. viride.* It was found that the isolated culture was having thick dark brown metabolite production from the fourth day onwards, whereas in the reference culture *T. viride*, it was absent. Difference in the time taken for sporulation was also noticed, it

was on fourth day in the case of isolated culture and second day for reference culture. There was also difference in colony colour due to sporulation, it was pale green in the case of isolated culture and dark green in the case of reference culture. The days taken by the two cultures for over growth on the pathogen were also different. The selected antagonist, *Trichoderma* was faster than the reference culture in inhibiting the pathogens.

Among the five bacterial isolates, only two were found effective against the pathogens. Cultural and morphological characters of the two selected bacterial antagonists were studied and they were identified by 16S rDNA sequence analysis. The isolate BA-4 was identified as *Pseudomonas aeruginosa* and BA-5 was identified as *Klebsiella pneumoniae.* The identification got confirmed by biochemical tests.

The mechanisms involved in the antagonism of fungal and bacterial isolates were also studied. Non volatile metabolites of *T. viride* isolated from SMS produced 100 per cent inhibition against *P. aphanidermatum*. But it was not effective against *R. solanacearum*. Non volatile metabolites of bacterial antagonists do not have any effect on both the pathogens. It was also observed that the type of mycoparasitism between *T. viride* from SMS and *P. aphanidermatum* is coiling. The mycelia of *T. viride* coiled around the mycelia of *P. aphanidermatum.* Siderophore production was confirmed in the case of *P. aeruginosa* by CAS assay.

The *in vitro* effect of selected fungal antagonist, *T. viride* (FA-1) and bacterial antagonists, *Pseudomonas aeruginosa* (BA-4) and *Klebsiella pneumoniae* (BA-5) against the mushroom species, *Pleurotus florida* and *P. sajor-caju* was tested by dual culture method. The results showed that the bacterial antagonists were not inhibiting the mushroom fungi. During initial three days, there was slight reduction in growth of both *Pleurotus* sp. in dual culture compared to monoculture, but after that it started slightly increasing. The isolated fungal antagonist *T. viride* was also tested against the mushroom species *in vitro*. It was found that *T. viride* from SMS gave cent per cent inhibition against both the mushroom species. But in the present study, *T. viride* was

isolated after the complete cycle of growth of mushroom. So it did not affect the growth and yield of mushroom fungus.

The inhibitory effects of aqueous extracts of SMS on both the pathogens were tested under *in vitro* condition. When filter sterilized aqueous extracts of paddy straw, saw dust and neopeat SMS of *P. florida* and *P. sajor-caju* were evaluated against *R. solanacearum*, only aqueous extract of paddy straw SMS of *P. sajor-caju* was found to be effective. When these aqueous extracts were evaluated against *P. aphanidermatum* by poisoned food technique, none of the treatments were found to be effective. The pathogen attained full growth on all the treatments. But the fluffiness of the pathogen was less in aqueous extract compared to the control.

The effect of spent mushroom substrate for the management of rhizome rot complex disease of ginger was assessed under pot culture conditions. Two separate experiments were conducted for the management of *P. aphanidermatum* and *R. solanacearum*. All the treatments were applied at three times- at the time of planting, 60 DAP and 120 DAP. Among the various treatments for the management of both the pathogens associated with rhizome rot complex disease, *T. viride* from SMS and paddy straw SMS of *P. sajor-caju* as mulch were found to be the best giving cent per cent disease control. The other SMS as mulch treatments and microbial treatments also showed better result. In control treatment, cent per cent disease incidence was noticed.

Cent per cent germination was noticed in the treatment, *T. viride* from SMS + reference culture *T. viride* in the experiment for the management of *P. aphanidermatum.* Among the treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* showed better germination percentage. But in the experiment for the management of *R. solanacearum,* the treatment *Klebsiella pneumoniae* showed 100 per cent germination followed by the treatment *T. viride* from SMS.

In the experiment for the management of *P. aphanidermatum*, the growth parameters like number of tillers, number of leaves per tiller and height of tillers were highest in the treatment *T. viride* from SMS, after two, three, four and five months of planting. But in the experiment for the management of *R. solanacearum*, the treatment paddy straw SMS of *P. sajor-caju* as mulch showed highest number of tillers. Number of leaves per tiller was highest in the treatment *K. pneumoniae* + reference culture *P. fluorescens,* in the first three months and the treatment with paddy straw SMS of *P. sajor-caju* as mulch for the next two months. Height of tillers was maximum in the treatments applied with *T. viride* from SMS. The same trend was noticed in the case of yield also. In the experiment for the management of *P. aphanidermatum*, highest yield was observed in the treatment *T. viride* from SMS. But in the experiment for the management of *R. solanacearum*, the treatment paddy straw SMS of *P. sajor-caju* as mulch produced highest yield.

The SMS is rich in microflora with antagonistic effect against pathogenic microorganisms associated with ginger plants. High cellulolytic capacity of *P. sajor-caju* favour the maximum degradation of the substrate, thereby provides a niche for the multiplication of favourable microorganisms with antagonistic effect. These microorganisms may stimulate the defence mechanism in plants which allow the plants to fight against the soil borne pathogens like *P. aphanidermatum* and *R. solanacearum*. So as a whole we can say that better degradation of spent mushroom substrate, along with growth enhancement and antagonistic property of bioagents must have contributed to better yield by reducing the disease incidence.

From the pot culture experiment it was clear that for the management of *R. solanacearum* and plant growth promotion, best treatment is Paddy straw SMS of *P. sajor-caju* as mulch. This may be because of the higher degradation capacity of *P. sajor-caju* that facilitate better colonization by plant growth promoting and antagonistic microorganisms. It also helps to improve the biological activity of the soil and got a direct impact on the sustainability of soil health. Whereas, best treatment for the management of *P. aphanidermatum* and plant growth enhancement is *T. viride* from SMS. It was found to be better than reference culture *T. viride*. The beneficial

microorganisms may be having plant growth promoting activity which contributes to increased yield in the various treatments. So from the present study, it can be concluded that the use of paddy straw SMS of *P. sajor-caju* as mulch, along with the application of *T. viride* from SMS can be considered as an effective management practice against the rhizome rot complex disease of ginger. Depending on the location and climatic condition in which the mushroom crop is raised, the microorganisms harbouring in SMS will vary and they can be used for disease management in various crops.

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Appendix

APPENDIX –I

MEDIA COMPOSITION

Potato Dextrose Agar (PDA)

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

Triphenyl Tetrazolium Chlorode (TZC) medium

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

Martin Rose Bengal Streptomycin Agar (MRBA)

KH_2PO_4	- 1 g
$MgSO_4.7H_2O$	- 0.5g
Peptone	- 5g

Dextrose	- 10 g
Rose Bengal	- 0.03 g
Streptomycin	- 30 g
Agar	- 20 g
Distilled water	- 1000 ml

Nutrient Agar medium (NA)

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

King's B medium

Peptone	- 20 g
Glycerol	- 10 ml
K_2HPO_4	- 10 g
$MgSO_4.7H_2O$	- 1.5 g
Agar	- 20 g
Distilled water	- 1000ml
pH	- 7.2 - 7.4

Kenknight's Agar medium (KAM)

Glucose	- 1 g
KH_2PO_4	- 0.1 g
$NaNO_3$	- 0.1 g
KCl	- 0.1 g
$MgSO_4.7H_2O$	- 0.1 g
Agar	- 20 g
Distilled water	- 1000 ml

Succinate medium

Succinic acid	- 4 g
K_2PO_4	- 3 g
NH_4SO_4	- 0.2 g
Agar	- 20 g
Distilled water	- 1000 ml
pH	- 7.0

Starch medium

Peptone	- 10 g
Beef extract	- 5 g
Starch solution	- 2 g
Agar	-15 g
pH	- 7.0

Peptone beef extract medium

Peptone	- 10 g
Beef extract	- 5 g
Sucrose	- 50 g
Agar	-20 g

Gelatin medium

Peptone	- 10 g
Beef extract	- 5 g
Gelatin	- 4 g
Agar	- 20 g
Distilled water	- 1000 ml

Christensen's Urea agar

$NH_4H_2PO_4$	- 0.5 g
K_2HPO_4	- 0.5 g
$MgSO_4.7H_2O$	- 0.2 g
NaCl	- 5 g
Yeast extract	- 1.0 g
Agar	- 20 g
Phenol red	- 0.012 g
Distilled water	- 1000 ml

Thornley's medium

Peptone	- 1.0 g
NaCl	- 5.0 g
K_2HPO_4	- 0.3 g
Agar	- 3.0 g
Phenol red	- 0.01 g
L arginine	- 1.0 g
Distilled water	- 1000ml
pH	-7.2

Peptone water medium

Peptone	- 10 g
NaCl	- 5 g
Distilled water	- 1000ml

Nitrate agar medium

KNO_3	- 1 g
Peptone	- 10 g
Beef extract	- 5 g
Agar	- 15g
pH	- 7.0

Hayward's semi solid medium

Peptone	- 1 g
$NH_4H_2PO_4$	-1 g
KCl	- 0.2 g
$MgSO_4$. $7H_2O$	- 0.2 g
Bromothymol blue	- 0.08 g
Agar	- 3 g
Distilled water	- 1000ml
pH	- 7.0

EXPLOITATION OF SPENT MUSHROOM SUBSTRATE AS MULCH FOR THE MANAGEMENT OF RHIZOME ROT COMPLEX DISEASE OF GINGER

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Spent mushroom substrate (SMS) is the composted organic material remained after the harvest of a crop of mushroom and it is rich in plant nutrients including minerals and was used as manure in different crops including ginger. Recent findings illustrate that substrate after one cycle cultivation of mushroom can be used for disease management. So far no attempt has been made on the use of spent mushroom substrate from oyster mushroom, against rhizome rot complex disease of ginger. Under these circumstances, a study was conducted to know the effectiveness of oyster mushroom SMS to control the rhizome rot complex disease of ginger.

Bacterial wilt and soft rot are the two common rhizome rot diseases of ginger. Ginger plants showing typical wilt symptoms and soft rot symptoms were collected from the farmer's field. The fungal pathogen *Pythium aphanidermatum* and the bacterial pathogen, *Ralstonia solanacearum* were isolated from the rhizomes showing typical symptoms, and pure cultures were maintained. Since *Pleurotus florida* and *P. sajor-caju* are the most suitable mushroom species in Kerala, they were selected for the production of spent mushroom substrate. The substrate used were agricultural waste materials like paddy straw, saw dust and neopeat. The enumeration of microorganisms at different stages of mushroom growth were made and the fungi commonly observed were *Aspergillus* sp. (FA-2 and FA-3) and *Trichoderma* sp. (FA-1). Maximum number of microbial population was noticed in paddy straw, whereas it was minimum in neopeat. From four different stages of mushroom growth, five different types of bacteria, BA-1, BA-2, BA-3, BA-4 and BA-5 were observed.

An *in vitro* evaluation study was conducted to find out the efficiency of isolated organisms against the pathogens *P. aphanidermatum* and *R. solanacearum*. Among the bacterial isolates tested against *P. aphanidermatum,* BA-4 and BA-5 were effective, whereas against *R. solanacearum,* only BA-5 was found effective. All the three fungal isolates, FA-1, FA-2 and FA-3 gave 100 per cent inhibition against both the pathogens. But FA-1 was the most effective one. These five antagonists were selected for the compatibility studies by dual culture technique. The selected fungal antagonist FA-1

was identified as *Trichoderma viride* by studying cultural and morphological characters. The bacterial isolates BA-4 and BA-5 were identified as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively.

The effect of spent mushroom substrate for the management of rhizome rot complex disease of ginger was assessed under pot culture conditions. Two separate experiments were conducted for the management of *P. aphanidermatum* and *R. solanacearum*. All the treatments were applied at three times- at the time of planting, 60 DAP and 120 DAP. Among the various treatments for the management of both the pathogens associated with rhizome rot complex disease, *T. viride* from SMS and paddy straw SMS of *P. sajor-caju* as mulch were found to be the best giving cent per cent disease control. In control treatment, cent per cent disease incidence was noticed.

In the experiment for the management of *P. aphanidermatum,* hundred per cent germination was noticed in the treatment, *T. viride* from SMS + Reference culture *T. viride*. Among the treatments with SMS as mulch, paddy straw SMS of *P. sajor-caju* showed better germination percentage. But in the experiment for the management of *R. solanacearum*, the treatment *Klebsiella pneumoniae* showed 100 per cent germination followed by the treatment *T. viride* from SMS.

The growth parameters like number of tillers, number of leaves per tiller and height of tillers were highest in the treatment *T. viride* from SMS, at two, three, four and five months of planting, in the experiment for the management of *P. aphanidermatum*. But in the experiment for the management of *R. solanacearum*, the treatment paddy straw SMS of *P. sajor-caju* as mulch showed highest number of tillers. Number of leaves per tiller was highest in the treatment *K. pneumoniae* + reference culture *P. fluorescens,* in the first three months and the treatment with paddy straw SMS of *P. sajor-caju* as mulch for the next two months. Height of tillers was observed maximum in the treatments applied with *T. viride* from SMS. The same trend was noticed in the case of yield also. In the experiment for the management of *P. aphanidermatum*, highest yield was observed in the treatment *T. viride* from SMS. But in the experiment for the

management of *R. solanacearum*, the treatment paddy straw SMS of *P. sajor-caju* as mulch produced highest yield.

SMS is rich in microflora with antagonistic effect against pathogenic microorganisms associated with ginger plants. From the pot culture experiment it was clear that for the management of *R. solanacearum* and plant growth promotion, best treatment is paddy straw SMS of *P. sajor-caju* as mulch. High cellulolytic capacity of *P. sajor-caju* favour the maximum degradation of the substrate, thereby provides a niche for the multiplication of favourable microorganisms with antagonistic effect. Whereas, best treatment for the management of *P. aphanidermatum* and plant growth enhancement is *T. viride* from SMS. It was found to be better than reference culture *T. viride*. In addition the beneficial microorganisms may be having plant growth promoting activity which contributes to increased yield in the various treatments. So from the present study, it can be concluded that the use of paddy straw SMS of *P. sajor-caju* as mulch, along with the application of *T. viride* from SMS can be considered as an effective management practice against the rhizome rot complex disease of ginger. Depending on the location and climatic condition in which the mushroom crop is raised, the microorganisms harbouring in SMS will vary and they can be used for disease management in various crops.