EVALUATION OF SPENT MUSHROOM SUBSTRATE FOR THE MANAGEMENT OF RHIZOME ROT OF GINGER

Thesis

by

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CERTIFICATE - I

This is to certify that the thesis titled, **“Evaluation of spent mushroom substrate for the management of rhizome rot of ginger”** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** in the discipline of **PLANT PROTECTION** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP)-173230 India is a bonafide research work carried out by **Mr. Aman Thakur (NH-2019-37-M)** son of Mr. Amar Nath my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation has been fully acknowledged.

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CERTIFICATE - II

This is to certify that the thesis entitled **"Evaluation of spent mushroom substrate for the management of rhizome rot of ginger"** submitted by **Mr. Aman Thakur (NH-2019-37-M)** son of Mr. Amar Nath to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (H.P.)- 173230 in the partial fulfilment for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** in the discipline of **PLANT PROTECTION** has been approved by the Advisory Committee after an oral examination of the same in collaboration with an external examiner.

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This is to certify that all the mistakes and errors as pointed out by the external examiner have been incorporated in the thesis entitled, **"Evaluation of spent mushroom substrate for the management of rhizome rot of ginger"** submitted by **Mr. Aman Thakur (NH-2019-37-M)** son of Mr. Amar Nath to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (H.P.)- 173230 India, in the partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** in the discipline of **PLANT PROTECTION**.

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Place: Neri, Hamirpur **(Aman Thakur)**

Dated:

CONTENTS

ABBREVIATIONS

ºC = degree Celsius
et al. = Co-workers
CRD = Completely Randomized design
g = Gram
Ha = Hectare
i.e. = that is
viz. = Namely
e.g. = Example
Mg = Milligram
Ml = Millilitre
PDA = Potato Dextrose Agar
A = Alfa
B = Beta
MT = Metric tone
% = Per cent
f. sp. = Forma specialis
spp. = Species
sp. = Specie
SMS = Spent mushroom substrate
SMC = Spent mushroom compost
Mm = Mili meter
Ml = Mili liter

Rpm = Revolution per minute
Min = Minute
Kg = Kilo gram
@ = At the rate
COC = Copper oxychloride
Q = Quintal
m^2 = Square meter
T = Tonne
w/w = Weight/weight
MSWC = Municipal solid waste compost
MIC = Minimal inhibitory concentration
Cm = Centi meter
DNA = Deoxy ribonucleic acid
EDTA = Ethylene diamine tetra acetic acid
CTAB = Cetryltrimethyl ammonium bromide
ITS = Internal Transcribed Spacer
PCR = Polymerase chain reaction
BLAST = Basic Local alignment Search Tool
No. = Number
w.r.t. = With respect to
CD = Critical difference
DF = Degree of freedom
P = Level of significance
ANOVA = Analysis of variance
$SE_{(d)}$ = Standard error of deviation

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Chapter -1

INTRODUTION

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a popular spice used globally especially in most of the Asian countries (Gao and Zhang, 2010). It is mentioned in ancient Chinese, Indian and Middle Eastern periodicals and has long been valued for its aromatic, culinary and medicinal properties (Langner *et al*., 1998). Ginger rhizome is used to attenuate and treat several common diseases, such as headaches, colds, nausea and emesis. Chemically, it contains over 400 different compounds (Prasad and Tyagi, 2015) and many bioactive compounds in ginger have been identified as phenolic and terpene compounds (Zhao *et al*., 2019). The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes and phenolic compounds (Grzanna *et al*., 2005). Terpene components of ginger include zingiberene, *β*-bisabolene, *α*-farnesene, *β*-sesquiphellandrene and *α*-curcumene, while, phenolic compounds include gingerol, paradols and shogaol out of which, gingerols (23–25%) and shogaol (18–25%) are found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A) and minerals are also present (Langner *et al*., 1998 and Shukla and Singh, 2007). Today, ginger is used around the world as a dietary supplement and food ingredient. Ginger is primarily used to treat nausea, but it is also used as an anti-inflammatory, a pain remedy, a warming remedy and a cholesterol-lowering herb (Potnuru and Saibabu, 2018).

It is the 3rd most important spice after chilly and garlic. India ranks at the top in ginger production worldwide. India produced around 996.041 Thousand MT of ginger during 2019 (Anonymous, 2020). As per data available from National Horticulture Board (2018), Assam is the leading producer of ginger in India with area and production of 18.70 thousand ha and 167.39 thousand MT followed by Maharashtra and West Bengal. In Himachal Pradesh, area and production under this crop is 2.51 thousand ha and 15.95 thousand MT respectively while, productivity of ginger in India is 3.58 tons/ha (Anonymous, 2018). In Himachal Pradesh, more than 3/4th of the area and production is restricted to district Sirmour. The other ginger growing districts are Solan, Mandi, Shimla, Kangra, Bilaspur, Hamirpur and Chamba (Shukla and Gupta, 2015).

Like any other crop, successful cultivation of ginger is also hampered by many fungal, bacterial and viral diseases. Out of these, rhizome rot is one of the most devastating disease causing huge losses to the crop. The term rhizome rot is generally used for all the diseases affecting the rhizome which result in the partial or complete rotting of the rhizomes irrespective of the pathogens involved (Shukla and Gupta, 2015). Mostly, the pathogens associated with rhizome rot are *Pythium* spp., *Fusarium oxysporum* f.sp. *zingiberi, F. solani* and *Ralstonia solanacearum,* rhizome rot caused by *Fusarium solani* being the most common (Kumar,1977). The rotten part sometimes attract dipteran flies which further hasten the rotting process. This disease is a major constraint for growing a healthy and clean ginger crop in view of the severe losses (40%) caused to the crop (Shukla and Gupta, 2015). It has become a major threat in all ginger growing areas of the state. The disease reduces potential yield of ginger to a greater extent in field, storage and market leading to losses of even more than fifty per cent (Joshi and Sharma, 1980).

The two diseases, soft rot and yellows are generally found together in same field affecting the same plant part and their symptoms often get mixed up. Soft rot is caused mostly by *Pythium apanidermatum* but other species, *P. deliense, P. myriotylum, P. pleroticum P. vexans* and *P. ultimum* were also reported by many workers from different states (Sharma *et al*., 1994). Yellows disease is mostly caused by *Fusarium oxysporum* f.sp. *zingiberi*. Other reported species causing ginger yellows are *F. solani and F. equiseti* (Mathur 2000). However, *Fusarium* species have also been reported to cause rotting in ginger rhizomes (Shanmugam *et al*., 2013 and Li *et al*., 2014).

This disease is both seed and soil borne. High soil moisture and soil temperature are the most important factors for development of rhizome rot. Irrigation water from the diseased field also helps in spread of disease (Dohroo *et al*., 2012).

Management of rhizome rot of ginger is difficult because *Pythium* spp. and *Fusarium* spp. can persist in soil for many years once introduced and single approach does not work effectively to suppress the pathogen under field conditions.

Biological method of managing plant diseases, including the use of composts and biological control agents, has received attention as a better alternative to the intensive use of chemically synthesized products. Biological method of approach is generally safer and has a minimal environmental impact (Brimmer and Boland, 2003). Rate of development of any disease can be reduced by an adequate and balanced mineral nutrition in crop. Nutrient management in crops is one of the disease management strategies gaining momentum these days in the direction of chemical free management of plant diseases. Compost is biodegradable and less expensive to develop, compared to fungicides. Use of spent mushroom substrate is one of the strategies in this direction.

Spent mushroom compost is the substrate left after a full mushroom crop harvest is a bye-product of mushroom production. It is rich in diverse microorganisms, such as disease antagonistic bacteria and fungi. It is biodegradable, safe to apply and less expensive to develop. It naturally suppresses pathogens in the soil that cause plant disease and decline in yields (Adedeji and Aduramigba, 2016). Effectiveness of composts including spent mushroom compost against plant diseases caused by a broad range of pathogens, such as bacteria, fungi and nematode species has been demonstrated in various studies (Litterick *et al*., 2004; Noble and Conventy, 2005 and Bonanomi *et al*., 2010). With the increasing mushroom production worldwide, if about five kilograms of fresh compost are needed to produce one kilogram of mushrooms, then about 15 tons of spent mushroom compost (SMC) are produced each year (Uzun, 2004). This spent mushroom compost / substrate cannot be used as such immediately after the mushroom crop is over, as it contains high concentrations of salts in it. However, after weathering for six months to one year, the extra salts are leached out and it becomes a suitable compost to be applied to crops (Uzun, 2004).

Keeping in the view the importance of disease and usefulness of spent mushroom compost in disease management, the present investigations were proposed with the following objectives:

Objectives

- To evaluate the different formulations/ extracts of spent mushroom substrate under *in vitro* conditions against associated pathogen(s) causing rhizome rot of ginger.
- To study the effect of spent mushroom substrate manuring on the development of rhizome rot of ginger under pot culture conditions.

Chapter - 2

REVIEW OF LITERATURE

Rhizome rot of ginger is mainly responsible for reducing potential yield of ginger to a greater extent in field and storage. Various pathogens have been reported to be associated with rhizome rot of ginger. The disease is both seed and soil borne and literature indicates that it can be managed by adopting organic amendments, fungicides and using antagonists. The available literature on pathogens associated and management of rhizome rot of ginger is being reviewed here in this chapter under the following heads:

- **PATHOGENS ASSOCIATED**
- **PATHOGENICITY**
- **MANAGEMENT**

2.1 PATHOGENS ASSOCIATED

The term rhizome rot is used to denote all the diseases affecting rhizome of ginger leading to complete rotting and many pathogens have been reported to cause such rotting in ginger rhizomes. The disease was reported from India by Haware and Joshi (1973) who identified *Fusarium oxysporum* f.sp. *zingiberi* to be the causal agent of the disease. According to Kumar (1977), *Pythium* spp. *F. oxysporum* f.sp. *zingiberi, F. solani* and *Ralstonia solanacearum* are the important pathogen associated with rhizome rot of ginger, *F. solani* being the most common. Species of *Pythium* and *Fusarium* have been reported to be associated with this disease out of which *P. zingiberium* and *F. oxysporum* f.sp. *zingiberi* are the main species (Yang *et al.,*1988). Bhardwaj and Gupta (1987) reported *P. aphanidermatum, F. equiseti, F. solani* and *Mucor* sp. to be associated with rhizome rot.

Other than fungi, *Ralstonia* (*Pseudomonas*) *solanacearum* has also been reported to be associated with the disease (Ramachandran *et al.*, 1989; Savita *et al.*, 2009). Among *Pythium* species, *P. aphanidermatum, P. myriotylum* and *P. zingiberium* have been reported to be predominant one (Dohroo *et al.,*1987; Yang *et al.,* 1988; Lanjewar and Shukla,1989; Kim *et al.,*1997; Rathaiah *et al.*, 2006; Savita *et al.,* 2009; Aliyu *et al.,* 2012 and Parveen and Sharma, 2013). Out of different *Fusarium* species, *F. oxysporum* f.sp. *zingiberi, F.solani, F. equiseti* and *F. monilliformae* have been found to be associated by various workers (Sharma and Jain, 1977; Dohroo *et al.,*1987; Bhardwaj and Gupta 1987; Yang *et al.,* 1988; Ramachandran *et al.*, 1989; Siddiqui and Kaushal,2000; Agarwal *et al.,* 2008; Savita *et al.,* 2009; Ramtake and Kamble, 2011 and Parveen and Sharma, 2013). Other than these, *Sclerotium rolfsii* (Dohroo *et al.,* 1987), *Colletotrichum dematum* (Sharma and Jain, 1977) have also been reported to be associated with rhizome rot of ginger.

2.2 PATHOGENECITY

For an isolated fungal culture to be pathogenic, it must have pathogenic genes in it and pathogenicity of any isolated culture is essential to prove the Koch's postulates. Yang *et al,* (1988) reported symptoms like yellowing, wilt and blighting of stem after 20 days of inoculation of ginger plant with *F. oxysporum* f.sp. *zingiberi.* Prachi *et al.* (2000) used the culture of *F. oxysporum* f.sp. *zingiberi* for the inoculation of ginger seedling and recorded the symptoms of ginger yellows with that, indicating culture of the pathogen to be pathogenic. Gupta *et al,* (2014) while studying the morphological, cultural, pathological and molecular variability among *F. oxysporum* f.sp. *zingiberi* isolates, inoculated the pathogen isolates on sterilized ginger rhizomes by placing a culture bit in the centre of cut rhizome and observed the development of brown lesion. An incubation period of 11 to 19 days was recorded for the development of brown lesion on inoculated rhizomes. The lesion size ranged from 8.5 to 18 mm in diameter after 10 days of its appearance in different pathogen isolates.

Chawla *et al*, (2021) inoculated the conidial suspension of *F. oxysporum* on the sterilized healthy rhizome of ginger by injecting the 3ml conidial suspension (10^8 spores/ml) into the rhizome and recorded the development of the yellowing and wilting from the inoculated and germinated rhizome which ultimately collapsed and no such symptoms were observed in un-inoculated control rhizomes. They re-isolated the pathogen from diseased inoculated plants. Tsegaye and Tesfaye (2020) while studying morphological and molecular density of *Fusarium* species causing wilt in ginger reported a pathogenicity index of 11.0 to 45.0 % in different inoculated samples.

2.3 MANAGEMENT

Rhizome rot is considered as a complex disease problem. Various available methods should be combined to obtain satisfactory control of this devastating disease. This disease is both seed and soil borne. High soil moisture and high soil temperature are the most important factors for development of rhizome rot. Irrigation water from the diseased field also help in spread of disease (Dohroo *et al*., 2012). Management of rhizome rot of ginger is difficult because *Pythium* spp. and *Fusarium* spp. can persist in soil for many years, once introduced and single approach does not work effectively to suppress the pathogen under field conditions.

2.3.1Biocontrol Agents

The use of composts and biological control agents a better option to the intensive use of chemically manufactured products for managing plant diseases. The biological approach is generally safer and has less influence on the environment (Brimmer and Boland, 2003). Applications of *Trichoderma* spp. are very effective biological mean for plant disease management especially for the soil borne pathogens (Sharma and Dohroo, 1991).

Thomas (1939) suggested biological control of *Pythium* sp. using *Trichoderma lignorum* as an antagonist. He observed that the increased acidity of the medium resulting by the antibiosis effect of *T. lignorum* might be responsible for reduced growth of *Pythium.* Dohroo and Sharma (1983) evaluated the suppression of ginger rhizome rot in storage. They used *Trichoderma viride* to treat the rhizomes and concluded that the rhizome rot disease was controlled to an extent of 80 per cent. Antagonism studies of *Trichoderma* sp. to *P. aphanidermatum*, *F. equiseti, F. solani, Cladosporium cladosporioides,* and *Mucor hiemalis* also have been studied *in vitro* (Bhardwaj and Gupta, 1987). *T. harzianum* and *Gliocladium virens* are also known to inhibit the growth of *F. oxysporum* f.sp. *zingiberi* when it caused rhizome rot in ginger (Sharma and Dohroo, 1991). To suppress the rhizome rot of ginger in storage, Bharadwaj *et al*. (1988) used *Trichoderma* spp. as biocontrol agents. *T. viride* and *T. hamatum* were applied to the rhizome by dipping it in spore suspension or smearing with antagonists. *P. aphanidermatum* and *F. equiseti*, which cause storage rot in ginger, were successfully controlled. Growth of *F. oxysporum* f.sp. *zingiberi* was inhibited effectively by *T. harzianum and Gliocladium virens* (Sharma and Dohroo, 1991), *T. viride* (Khatso and Tiameren, 2013 and Amreen and Kumar, 2013) and *T. harzianum* (Khatso and Tiameren, 2013). Rathore *et al*. (1992) evaluated the activity of *T. viride*'s volatile and non-volatile compounds. *P. myriotylum* and *F. solani*, which cause ginger rhizome rot, were inhibited by the non-volatile compounds by 70 per cent and 10 per cent, respectively. *T. viride*'s volatile compounds entirely stopped *P. myriotylum* from growing, but only lowered the colony diameter of *F. solani* by 3.4 per cent.

Dohroo (1995) suggested an integrated approach to combat the yellows disease of ginger which included treatment of seed rhizomes with mancozeb and carbendazim and use of biocontrol agents *T. harzianum, T. hamatum and G. virens* as seed treatment and soil application. Sharma (1998) studied the antifungal activities of biocontrol compounds derived from plant extracts against the fungi that cause yellow and ginger rhizome rot. It was observed that the biocontrol agents *viz*, *T. harzianum* and *T. viride* were particularly efficient in suppressing the mycelial growth of both pathogens, *F. oxysporum* f.sp. *zingiberi* and *P. aphanidermatum*, which cause yellow and rhizome rot of ginger, respectively.

Ram *et al*. (1999) tested *Psuedomonas* sp. alone and in combination with *T. harzianum*, as well as with fungicidal rhizome treatment against rhizome rot of ginger. Soil application of *T. harzianum* and rhizome treatment with *Pseudomonas* spp. and fungicides were found to be effective treatments. Shanmugam *et al*. (2000) used antagonistic microorganisms to control ginger rhizome rot. They used a dual culture approach to fight *P. aphanidermatum*, the cause of ginger rhizome rot, with *T. viride, T. harzianum* and *Bacillus* sp*., T. harzianum* and *T. viride* were found to be potential antagonists against ginger rhizome rot. Rajan *et al*. (2012) investigated ginger diseases and their management with *T. harzianum* and found that *T. harzianum* was extremely successful in controlling ginger diseases.

When *T. harzianum* was used in the field to treat turmeric (*F. solani*) rhizome rot, it reduced disease incidence and improved yield, (Reddy *et al*., 2003). Khare *et al*. (2010) studied the effect of *T. viride* 1433 mutant and wild type strains on *P. aphanidermatum*. In comparison to wild type strains, *T. viride* 1433 mutant strain controlled the pathogen in sterilized and natural soil up to 85 per cent by creating volatile and non-volatile metabolites. *In vitro* antagonism of *Trichoderma* species against *P. aphanidermatum* was investigated by Mishra (2010). He used a dual culture approach to screen ten *Trichoderma* species against *P. aphanidermatum.* He observed that *T. viride* 1433 is the most effective against *P. aphanidermatum* of the strains evaluated. According to Stirling *et al*. (2009), ginger rhizome rot caused by *P. myriotylum* can be reduced by microbial populations found in ginger-growing soils through antagonism, antibiosis or parasitism. Nasreen and Ghaffar (2010) studied the effects of several antagonists such as *T. harzianum*. In bitter gourd, bottle gourd, and cucumber, *T. viride, B. subtilis,* and *G. virens* reduced *F. solani* seedling and root infection

Bundyopadhayay and Bhattacharya (2012) evaluated the physical, chemical and biological methods of disease management in the field for three ginger growing seasons (2001-2003) to manage rhizome rot disease of ginger. Seed rhizomes were treated with hot water (51°C), with a fungicide mancozeb solution, a biocontrol agent *T. harzianum*, and soil application of neem cake individually and in combinations. Out of six treatments tested, rhizome treatment with hot water at 51°C for 10 min + soil application of inoculum mixed with 1 kg neem cake at the time of planting resulted in the lowest disease incidence (27.14%) and highest rhizome yield (6.46 kg plot).

In a pot culture study, Dohroo *et al*. (2012) found that soil treatment of *T. harzianum* bioformulation and seed application with onion and garlic were effective in suppressing soft rot of ginger and enhancing production and yield characteristics. Soft rot disease was also suppressed and improved the production by soil application of strobilurin compound (cabriotop) in a pot culture trial. Lokesh *et al*. (2012) examined rhizomes that had been treated with bioagents such as *T. harzianum* and bacterial consortia. The results showed that rhizome rot pathogen *Pythium* of ginger was reduced in both solarized and non-solarized treated rhizomes with *T. harzianum* and bacterial consortia. Dohroo and Gupta (2014) evaluated the efficacy of different fungal and bacterial biocontrol agents against rhizome diseases of ginger as well as nematode population under field conditions. Combined applications of bioagents were more effective in reducing the disease incidence than the individual treatments. *T. harzianum* + *P. fluorescens* + *B. subtilis* gave minimum disease incidence on rhizomes (8.64 %) as well as on tillers (12.50 %). Minimum population of root knot nematodes was recovered from a combination treatment of *T. harzianum* + *P. fluorescens* + *B. subtilis* (21.66/200 CC of soil). Gopi *et al.* (2016) was conducted the experiment during 2011-12 and 2012-13 to study the effect of various organic treatments on the incidence of soft rot, germination and yield of ginger. In the *in vitro* study, garlic @ 10 per cent concentration was found to be the most effective in reducing the growth of the soft rot pathogen with 62.3 per cent inhibition. Among the 10 selected *Trichoderma* isolates, *T. harzianum* South Sikkim was the most effective in reducing the *P. aphanidermatum* colony growth (72.0%). Among these best treatments evaluated against the soft rot, The hot water treatment at 47°C for 30 min

+ *T. harzianum* + COC (0.3 %) recorded maximum germination (91.3 and 90.3%) and highest yield (158 and 126 q/ha)

2.3.2 Organic amendments

Perennation and spread of soft rot are primarily due to infected rhizomes. Using disease-free rhizomes for planting is the most effective way to control the disease (Shahare and Asthana, 1962; Dohroo, 1993). Nutrient management in crops is one of the disease management strategies gaining momentum these days in the direction of chemical free management of plant diseases. Compost is biodegradable and less expensive to develop, compared to fungicides. Use of spent mushroom substrate is one of the strategies in this direction.

Das (1999) showed that the plots mulched with maha neem (*Melia azadirachta*) leaves (2.5 kg/m^2) were completely free from rhizome rot (*P. aphanidermatum*). The main mode of disease spread is through contaminated rhizomes. Therefore, the selection of healthy seed rhizomes has been found an effective control measure for the disease (Rana, 1991; Dohroo, 1993). Smith and Abbas (2011) found greatest (74.2 t/ha) rhizome yield and minimal (7.0%) losses to pathogens in the pasture lay that had been cultivated prior to ginger planting. Stirling *et al*. (2012) reported that organic inputs, tillage and rotation practices did not influence yellows disease.

Much literature is not available on the use of spent mushroom substrate/compost for the management of rhizome rot of ginger. But, Yohalem *et al*. (1996) suggested the control of apple scab (*Venturia inaequalis*) with aqueous extracts from spent mushroom substrate (SMS). Inhibitory activity of extracts, assessed as *in vitro* inhibition of *Venturia* conidia germination, was monitored over time for extracts prepared from SMS stored under different conditions. Viji *et al*. (2003) suggested that the bacteria isolated from spent mushroom substrate (SMS) were effective in suppressing the growth of of *Pyricularia grisea*, the causal agent of gray leaf spot of perennial ryegrass (*Lolium perenne*) turf as well as *Rhizoctonia solani, Sclerotinia homoeocarpa* and *Fusarium culmorum*. Ahlawat *et al*. (2006) reported that recomposted spent mushroom substrate significantly enhanced gross and net yield and quality attributes of cauliflower along with reduced incidence of black rot disease and caterpillar attack. Ntougias *et al*. (2008) used the compost derived from wastes and bye products of the olive oil, wine and *Agaricus* mushroom agro-industries, mixed with peat at 1:3 w/w ratios and comparatively evaluated in pot experiments to assess suppressiveness against soil-borne and foliar pathogens of tomato. All compost amendments demonstrated high levels of suppressiveness against *Phytophthora nicotianae* in tomato, when they were applied directly after curing indicating the occurrence of a "general suppression phenomenon" (81–100% decrease in plant disease incidence). Suppressiveness against *F. oxysporum* f.sp. *radicis-lycopersici* was relatively lower and varied widely among composts. The composts conferred induced systemic resistance against the foliar pathogen *Septoria lycopersici*. Sagar *et al*. (2009) reported the yield enhancement and lower incidence of disease in agricultural and horticultural crops along with changes in soil physical conditions by using spent mushroom substrate (SMS) as manure.

Ahlawat *et al*. (2011) evaluated the effect of button mushroom spent substrate (SMS) on yield, quality and disease incidence of different diseases of pea (*Pisum sativum*). Incidence of Fusarium wilts and powdery mildew was recorded lowest in 18 months old anaerobically composted SMS treatment, while, untreated control recorded maximum disease occurrence. Goonani *et al*. (2011) evaluated that leached spent mushroom compost (SMC), municipal solid waste compost (MSWC) and their extracts, to suppress *Phytophthora drechsleri* in cucumber plants. The results of the experiments showed that all applications rate of non-sterile SMC were significantly effective in controlling of the pathogen. The treatments amended with MSWC (15%) and SMC (25%) showed the most suppressive effect in controlling the pathogen. The extract of leached-SMC could inhibit *P. drechselri* in petri dish. Marin *et al*. (2014) prepared aerated compost tea and non-aerated compost tea from spent mushroom compost, grape marc compost, crop residues compost and vermicompost. *In vitro* inhibition of mycelial growth of the two tested pathogens was assessed, and *in vivo* effects of compost teas on disease severity, caused by *Phytophthora capsici* and *P. parasitica* were evaluated on pepper plants, in greenhouse experiments. The study demonstrated the clear effect of compost tea on disease suppression and plant growth promotion.

Milovanovic *et al.* (2014) evaluated antifungal, antioxidant and anticancer potentials of *P. eryngii, P. ostreatus* and *P. pulmonarius* mycelial extracts and the influence of mycelium enrichment with selenium on these activities. Both Se-amended and non-amended extracts showed the same or similar minimal inhibitory concentration for 14 studied micromycetes, while a fungicidal effect was not noted, contrary to ketoconazole, which had inhibitory and fungicidal effects at very low concentrations. Se-non-amended extracts exhibited antioxidant activity, especially at higher concentrations. Remya and Paul (2014) evaluated spent mushroom substrate (SMS) from *P. florida* and *P. sajor-caju* on growth parameters, yield and soft rot incidence by *P. aphanidermatum* in ginger (*Zingiber officinale). P. sajor-caju* as mulch was significantly superior to all other treatments and the incidence of soft rot was not observed in the treatment, whereas control plants recorded 100 per cent disease incidence. Martínez *et al*. (2015) studied the antibacterial activity of spent substrate of *P. ostraetus* combined with medicinal plants and suggested the possible use of the spent substrate of *P. ostreatus* (barley straw) as source of extracts with antibacterial activity, being the best option in combination with *Mentha piperita* L.

Adedeji and Aduramigba (2016) evaluated that aqueous extracts of spent mushroom compost (SMC) *in vitro* for antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* (Fol). Autoclaved SMC extracts at 15 per cent concentration significantly reduced the mycelial growth of test pathogen. However, 1, 5, 10 and 15 per cent concentration of the non-autoclaved extracts significantly inhibited the growth of the pathogen by 69.7, 88.1, 80.3 and 85.5 per cent, respectively. The SMC of oyster mushroom contained diverse microorganisms including fluorescent *Pseudomonas* spp., *Trichoderma viridae, Baccilus* spp., *Penicillum* spp., and *Aspergillus terrus.*

Verma *et al.* (2017) isolated and cultured the fungal isolates predominantly present in spent mushroom substrate (SMS) and then identified them as *Trichoderma harzianum* and *T. viride.* Antifungal activities of these isolated bio-agents were determined by testing their effect against *Rhizoctonia solani* with dual culture method. They reported that spent mushroom substrate (SMS) not only supplies nutrition to the soil but also helps in management of soil-borne plant pathogens. Yusidah and Istifadah (2018) were investigated the potential and application method of spent substrate of oyster mushroom (*Pleurotus ostreatus*), straw mushroom (*Volvariella volvaceae*) and shiitake (*Lentinula edodes*) to control basal rot disease caused by *F. oxysporum* f.sp. *cepae* in shallot. The results showed that the SMS of oyster mushroom, straw mushroom and shiitake reduced the intensity of basal rot disease in shallot by 44-76.80 per cent. The treatment that showed highest disease reduction and shallot growth was the application of *V. volvaceae* SMS in the planting site and drenching its water extract every two weeks.

Wang *et al.* (2020) evaluated the suppressive capacity of different spent mushroom substrate (SMS) amendments against Fusarium wilt and analysed their effects on soil microbiological properties. The *Flammulina velutipes* substrate, spent *Lentinus edodes* substrate and spent *Pleurotus ostreatus* substrate treatments significantly reduced disease incidence by 53.3, 25.7 and 37.9 per cent, respectively. Singh *et al.* (2021) reported that *T. asperellum* grown in supplemented spent paddy strw substrate of *P. ostreatus* resulted in 66.26 per cent inhibition of *F. oxysporum* f.sp. *lycopersici.*

2.3.3 Antimicrobial properties of mushroom or spent mushroom substrate extracts

Successful determination of biologically active compound from plant material is mainly depending on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include less toxic, ease of evaporation at low heat, increase the physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate. The solvent should be non-toxic because the ultimate product will contain traces of residual solvent and should not interfere with the bioassay. The decision will also be affected by the target compounds to be extracted. Although, water is normally used solvent for extract plant products with antimicrobial activity, but plant extracts from organic solvents have been reported more consistent antimicrobial activity compared to aqueous extract (Sharma and Gupta, 2003; Parekh *et al*., 2005; Gurjar *et al.,* 2012). Additionally, water-soluble flavenoids (mostly anthocyanins) have less significance antimicrobial and water-soluble phenolics are antioxidant compounds, according to a study, and extraction of other phenolics and tannins was better in aqueous acetone than in aqueous methanol. (Parekh *et al*., 2005). The most commonly used organic solvents include ethanol, methanol, acetone and ethyl acetate (Peschel *et al.,* 2006).

Bawadekji *et al.* (2017) investigated antibacterial and antifungal activity of cold crude extract of basidiocarps of *P. ostreatus.* The fruit bodies were dried, reduced to powder and then extracted by cold water. Antibacterial effect against *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 254992) and *Staphylococcus aureus* (ATCC 254996) and antifungal activity toward *Candida albicans* (ATCC 10231) were investigated. All strains were tested by well diffusion technique. Crude extract of *P. ostreatus* fruit-bodies showed an important zone of inhibition only toward *C. albicans*, *P. aeruginosa* and *S. aureus*. Owaid *et al.* (2017) evaluated the antifungal activity of four fruiting bodies of oyster mushroom harvested from three agro-substrates *in vitro*. At three concentrations (2, 4 and 8 mg/disc), extracts discs of *P. ostreatus* (grey), *P. ostreatus* var. *florida*, *P. cornucopiae* var. *citrinopileatus* and *P. salmoneostramineus* were tested against three fungal pathogens *viz., T. harzianum* (after 2 days), *Verticillium* sp. and *Pythium* sp. (after 5 days) via the disc diffusion method. The highest overall activity was by the extract disc *P.*

cornucopiae grown on substrate; 70 per cent wheat straw, 20 per cent hardwood sawdust and 10 per cent date palm fibers and the lowest by *P. cornucopiae* grown on wheat straw. The best inhibition zone was toward *T. harzianum* by extract disc *P. ostreatus* var. *florida*, compared with Nystatin disc, followed *P. salmoneostramineus* grown on 50 per cent wheat straw, 30 per cent hardwood sawdust and 20 per cent date palm fibers extract disc against *Pythium* sp. and against *Verticillium* sp..

Ishihara *et al*. (2019) evaluated the water extracts from the spent mushroom substrates (SMSs) of *L. edodes* and *Hypsizygus marmoreus* on the resistance of rice leaves to *Pyricularia oryzae* infection. The spraying of the SMS extracts clearly suppressed the development of lesions caused by *P. oryzae* infection. The accumulation of phytoalexins momilactones A and B, oryzalexin A, and sakuranetin was markedly induced by the spraying of extracts. The enhanced expression of defense related genes PR1b and PBZ was also found in leaves sprayed with the extracts. Treatments with the extracts also affected phytohormone levels. Gutef *et al*. (2020) studied the antimicrobial properties of different types (chloroform, methanol and aqueous) of extracts of *P. ostreatus* against *Candida albicans* and found that methanol extracts of *P. ostreatus* were most effective in inhibiting the growth of *C. albicans in vitro.*

Mossebo *et al*. (2020) evaluated antifungal activity of crude extracts of three tropical mushrooms including *P. sajor-caju, P. tuber-regium* and *Lentinus squarrosulus* on three species of fungal pathogens. For the pathogenic fungi, the minimal inhibitory concentration (MIC) of carpophores extracts ranged from 0.39 mg/ml to 6.25 mg/ml for *Candida albicans,* 0.78 mg/ml to 6.25 mg/ml for *Aspergillus fumigetus* and 1.56 mg/ml to 6.25 mg/ml for *A. ochraceus.* Sultan (2020) studied antifungal activity of aqueous and ethanol extracts of *Agaricus bisporus in vitro* against *Aspergillus flavus* following Poison food technique using different concentrations. The maximum effect of ethanolic extract against *A. flavus* growth was achieved at concentration 10 mg/ml, when the growth rate reached 1.25 cm while the maximum effect of ethanolic extract was achieved at concentration 16 mg/ml, when the growth rate reached 2.5 cm.

Chapter - 3

MATERIALS AND METHODS

The present investigations entitled **"Evaluation of spent mushroom substrate for the management of rhizome rot of ginger"** were conducted during 2019-2021 in the Plant Pathology Laboratory, Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur. The details of materials used and methods followed during the experimentation are presented in this chapter.

3.1 MATERIALS USED

The following materials were used during the present investigations.

3.1.1 Diseased and healthy rhizomes of ginger

To conduct the various experiments during the present studies on rhizome rot of ginger, diseased as well as healthy rhizomes of ginger were procured from local Farmer at Bilaspur, Himachal Pradesh.

3.1.2 Glassware

The standard brand Borosil glassware *viz*., Petri plates, test tubes, conical flasks, measuring cylinder, slides, cover slip, pipette and spirit jar were used for laboratory work.

3.1.3 Equipments

Common laboratory equipments used for laboratory studies included autoclave, laminar air flow cabinet, BOD incubator, refrigerator, microscope, electronic balance and hot air oven.

3.1.4 Culture media

The common laboratory potato dextrose agar (PDA) medium was used for all *in vitro* studies including isolation and maintenance of the pure cultures of causal fungi for management studies.

3.1.5 Procurement of spent mushroom substrate

Spent mushroom compost of *Agaricus bisporus* and substrate of *Pleurotus* spp. viz. *P. sajor caju, P. florida, P. ostreatus* was procured from the mushroom production unit, Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur (H.P.).

3.1.6 Miscellaneous material

Inoculating needle, forceps, spirit lamp, non absorbent cotton, polythene sheets, tape, blade, scissors, cork borer, marker, mixer, sterilized distilled water, scale, plastic trays, glass marking pencils, test tube stand, parafilms etc.

3.2 METHODS

The methodology adopted for investigation is described under the following heads:

3.2.1 Isolation and Purification of the pathogen

3.2.2 Pathogenicity of associated pathogen

3.2.3 Identification of the pathogen

3.2.4 Colonization of spent substrate with *Trichoderma viride*

3.2.5 Leaching of spent substrate

3.2.6 Management studies

3.2.7 Statistical analysis

3.2.1 Isolation and Purification of the pathogen

The ginger rhizomes showing the typical symptoms of rotting of rhizome were collected from ginger growing areas of Bilaspur district and were brought to laboratory for isolation of the pathogen. Isolation was done by cutting small pieces of infected portion of rhizome along with the healthy portion and immersed in sodium hypochloride solution (0.1 %) for 30 seconds. Then, these diseased rhizome portions were kept thrice in Petri plates having sterile distilled water and finally placed aseptically on the sterilized Petri plates containing sterilized potato dextrose agar (PDA) medium. These plates were incubated at 25°C temperature for growth of the pathogen. The Petri plates were observed periodically for the growth of the pathogen. The culture thus obtained was purified by sub culturing mycelium on to the PDA slants (Dhingra and Sinclair, 1985).

3.2.2 Pathogenicity of associated pathogen

The pathogenicity was tested by following Koch's postulates. Healthy rhizomes of ginger were selected from the ginger seed/rhizome or procured from the local market, washed thoroughly with tap water, swabbed with sodium hypochlorite (1 part sodium hypochlorite and 3 parts distilled water) for 30 seconds and washed with sterile distilled water to remove the traces of sodium hypochlorite.

The surface inoculation on sterilized rhizome was done with the help of blade injury. Sterilized blade was used to make wound on sterilized fruit under aseptic conditions and the culture bit of the particular pathogen was placed on it and sealed with a tape. The inoculated rhizomes were placed in Petri plate and covered with beaker and internally sprayed with sterilized distilled water so as to maintain the appropriate humidity. These were then incubated at room temperature and data were recorded in terms of incubation period (days) and symptoms appearance.

3.2.3 Identification of the pathogen

The isolated pathogen was identified preliminarily on the basis of cultural and microscopic characters. Temporary mounts of the culture where prepared on glass slide and observed under microscope. Various characters like hyphal color, conidial morphology and septation etc. were recorded. Finally, the pathogen was identified on the basis of molecular characterized. For molecular characterization, genomic DNA of test pathogen was isolated and puried by CTAB (Cetryltrimethyl ammonium bromide method) given by Doyle and Doyle (1987). The DNA was extracted from 5-7 days old fungal culture grown in culture plate. The mycelium along with spores was scraped out with the help of sterilized slide and crushed into a fine powder in liquid nitrogen in pre-chilled pestle and mortar. The fine powder was placed in 2 ml microcentrifuge tubes and 1 ml DNA extraction buffer (100 mM Tris-HCL, 20m M EDTA, 1.4M NaCl, 2% CTAB powder, 0.2% β-merceptoethanol) was added. The centrifuge tubes were kept in hot water bath at 65°C for one hour for lysis of fungal cells. Equal volume of phenol: chloroform (24:1) was added to the solution and gently mixed. The homogenate was centrifuged at 12000 rpm for 10 min and supernatant was recovered and transferred to a fresh 1.5 ml microcentrifuge tubes. 1/3rd volume of isopropanol was added to supernatant obtained for precaution of DNA. The content of microcentrifuge tube was gently mixed. The DNA was precipitated by centrifugation at 12000 rpm for 10 min at 4°C. DNA pellet was raised with 70 per cent ethanol. The DNA was than dissolved in TE buffer. Isolated DNA was stored in refrigerator at 4°C for further use. The quality and quantity of DNA was checked on 0.8 per cent agrose gel in horizontal gel electrophoresis. Amplification of internal transcribed spacer (ITS) region of associated fungal pathogen was carried out using universal primer pair ITS1 and ITS4 (Sambrooke *et al.*, 1989). The amplified PCR product was outsourced for sequencing. The obtained sequences were analysed *in silico* using bioinformatics tools *viz.,* NCBI BLAST, MEGA X.

3.**2.4 Colonization of spent substrate with *Trichoderma viride***

One part of the procured substrate was colonized with the culture of *T. viride.* For this, pure culture of *T. viride* procured from Department of Plant Pathology, COHF, Neri, grown on maize grains was mixed with the spent substrate and left for 10-15 days so as to get the compost and substrate fully colonized with *T. viride.* This was used for further experiments.

3.2.5 Leaching of spent substrate

Some part of both uncolonized and *T. viride* colonized substrate was leached for 10 days. To leach these materials, both uncolonized and *T. viride* colonized substrate were filled in bag of jute sack each and sacks were hung with the top of sacks half open. Water was added to the sacks with help of a mug from the open top till the water drops started coming out from the bottom of the sack, so as to leach out excessive nutrients and other chemical from these substrates. Same process was repeated twice a day up to 10 days. On the completion of tenth day, the sacks were taken down and leached materials were stored safely in the laboratory for use in further experiments. Leachate of all substrate was also collected separately in different containers and stored for use in further experiments.

3.2.6 Management studies

Aqueous and ethanol extracts of spent mushroom substrate of four mushroom species *viz*., *Agaricus bisporus* and *Pleurotus* spp. *viz*. *P. sajor caju, P. florida* and *P. ostreatus* were used. These spent mushroom substrates were first evaluated *in- vitro* against isolated pathogen by using their water and ethanolic extracts as well as their leachate. Effective treatments were further evaluated for the management of pre and post emergence rot in ginger under pot culture conditions.

3.2.6.1 Preparation of aqueous extract of the spent mushroom substrate

Aqueous extracts of each spent substrate under study were evaluated against the isolated pathogen under *in vitro* conditions. To make the aqueous extract, 100g of the spent substrate was boiled in 300ml of water in a covered vessel so as to reduce the volume to 1/3rd of the original. Finally, 100ml of the extract was extracted after filtration from sterilized muslin cloth under aseptic conditions. This extract served as 100 per cent concentration and was stored in refrigerator for further use.

3.2.6.2 *In vitro* evaluation of aqueous extract of spent mushroom substrate

All the aqueous extracts were evaluated *in vitro* against all test pathogens at 5, 10, 15 and 20 per cent concentration by using poisoned food technique (Falck, 1907). A parallel control devoid of aqueous extract was also maintained. Required concentration of aqueous extract was poured into Petri plates (90 mm diameter) along with PDA and allowed to solidify. A culture disc (5 mm diameter) of test pathogen was placed in the centre of solidified poured Petri dishes and sealed with parafilms. These plates were incubated at 25±2°C. Data were recorded in terms of diametric mycelial growth (mm) until the growth in the control plate was completed (90 mm). Inhibition (%) in diametric growth was further calculated as follows by the formula given by Vincent (1947):

Per cent Inhibition $=\frac{C-T}{C} \times 100$

Where,

C = Diametric growth (mm) of the test fungus in control

T = Diametric growth (mm) of the test fungus in treatment

3.2.6.3 Preparation of ethanol extract of the spent mushroom substrate

Ethanol was used as solvent for preparing crude extracts of all substrates under evaluation. Fifty gram powder of each substrate was soaked in 200 ml of solvent in 500 ml capacity conical flask which was plugged tightly with cotton and wrapped with paper. It was kept on the rotary shaker at 150 rpm at room temperature for three days and then allowed to stand so as to settle the substrate debris. Supernatant from each flask was filtered separately through Whatman No. 1 filter paper and evaporated at room temperature or 60°C in water bath for 30 min to completely remove the solvent. Residual substrate was re-extracted thrice with 10 ml ethanol in totality to harvest maximum metabolites. Concentrated extracts then were considered as 100 per cent concentrate and transferred into small vials and kept in the refrigerator at 5 ºC for further use. The resultant crude extracts were evaluated at four different concentrations by adding to sterilized PDA before solidification to obtain the proposed concentrations (Birari *et al.,* 2018.)

3.2.6.4 *In vitro* evaluation of ethanol extract of spent mushroom substrate

All the ethanol extracts were evaluated at 1, 2, 3 and 4 per cent concentrations *in vitro* against the test pathogen by poisoned food technique. Observations were recorded in terms of diametric growth (mm) and per cent inhibition with respect to control was further calculated.

3.2.6.5 Evaluation of the spent mushroom substrate under pot culture conditions

2.6.5.1 Effect of spent mushroom substrate on pre and post emergence rot in naturally infected ginger

An experiment was conducted under pot culture conditions in which infected seed of ginger was sown in different pots. The soil in each pot was amended with different spent substrate treatment. Untreated healthy and diseased seed in pots served as positive and negative control respectively. Each treatment was replicated thrice and observations were recorded in terms of following parameters

1. Pre-emergence rot incidence (%)
2. Post emergence rot disease severity (%)

For the calculation of the disease severity, data was recorded on 0-4 scale and disease severity was further calculated on the basis of the formula given by Mc Kinney(1923) as follow:-

$$\text{Disease severity (\%)} = \frac{\text{Sum of all diseased rating}}{\text{Total number of seedling} \times \text{maximum disease grade}} \times 100$$

Disease severity index

Severity Grade	**Severity Scale (%)**	**Description**
0	0	No symptoms
1	0-25	Less than 25 per cent leaf area covered by disease
2	25-50	25-50 per cent leaf area covered by disease
3	50-75	50-75 per cent leaf area covered by disease
4	75-100	75-100 per cent leaf area covered by disease

3.2.6.5.2 Effect of spent mushroom substrates on pre and post emergence rot artificially inoculated ginger

Healthy seed of ginger was sown in pot soil amended with spent substrate treatments. After that the soil was artificially inoculated with the associated pathogen. Data were recorded in terms of pre and post emergence disease incidence (%) and disease severity (%) respectively. Uninoculated and un-amended pots having healthy seedlings served as positive control while, inoculated un-amended pots served as negative control.

2.7. STATISTICAL ANALYSIS

All the laboratory as well as field experiments were conducted with 3 replications each while, results were statistically analysed by using completely randomized design (CRD) for lab and pot experiments as per Panse and Sukhatme (2000). Statistical analysis was also performed by two-way ANOVA using OPSTAT software (Sheoran *et al.,* 1998).

Chapter- 4

RESULTS AND DISCUSSION

The results of present studies entitled **"Evaluation of spent mushroom substrate for the management of rhizome rot of ginger"** are being presented in this chapter and discussed here in the light of available literature.

4.1 Isolation, maintenance and identification of pathogen

4.1.1 Isolation and purification of the pathogen

Pathogen was isolated from ginger rhizomes showing typical symptoms of the disease. Standard tissue isolation technique was followed after surface disinfection as described in "Materials and Methods". The pure culture of the fungus was obtained by hyphal tip isolation method after 15 days of inoculation which showed white mycelial growth of fungus. The pure culture thus obtained was again sub cultured in potato dextrose agar (PDA) slants once in 15 days and kept in the refrigerator at 5°C for further studies.

4.1.2 Pathogenicity test of test fungus

Pathogenicity test of test fungus were done in control conditions in laboratory on healthy rhizomes of ginger. The rhizomes were inoculated the pathogen isolates on sterilized ginger rhizomes by placing a culture bit in the centre of cut rhizome and observed the development of brown lesion. An incubation period of 12 days was recorded for the development of brown lesion on inoculated rhizomes. Reisolation was made from artificially inoculated rhizome showing typical symptoms of rotting and compared with original culture. It was observed that reisolated culture was morphologically similar to that of original culture (Plate 4.1).

4.1.3 Identification of the isolated organism

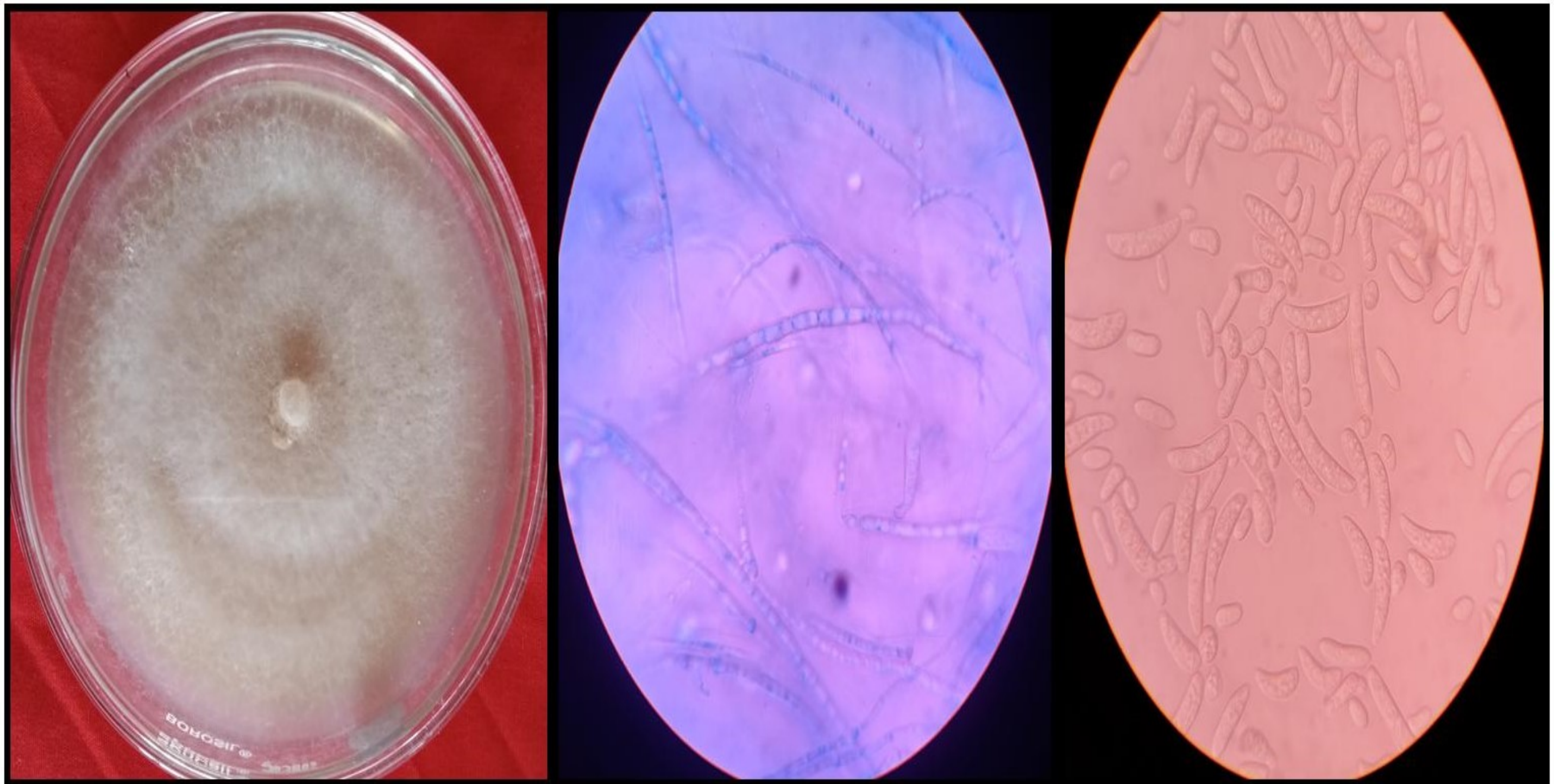
The pathogen was identified by observing growth on media, cultural characteristics and microscopic observations made from the microscopic mounts on glass slides prepared directly from active cultures. The color of the mycelium was white initially which turn to light pink with the passage of time. When observed under microscope, the mycelium was observed to be septate producing micro and macro conidia. Macro-conidia were two to five septate (Plate 4.2). The pathogen was preliminarily identified as *Fusarium* sp.. The ITS region of the fungus was amplified and sequenced and sequence analysis through NCBI, BLAST and MEGA X revealed the identity of pathogen to be 100 per cent similar to *Fusarium solani* (Plate 4.3 and 4.4).

4.2 Effect of the spent mushroom substrate leachate on the mycelial growth of *Fusarium solani*

In order to study the effect of leachate of different spent mushroom substrates *viz. Agaricus bisporus, Pleurotus sajor-caju, Pleurotus florida* and *Pleurotus ostreatus,* leachate was collected from both uncolonized and *Trichoderma viride* colonized spent substrate after leaching the substrate for 10 days. This leachate was evaluated *in vitro* against *F. solani* at 5, 10, 15 and 20 per cent concentration by poisoned food technique and data were recorded in terms of diametric growth (mm). Normal nutrient medium devoid of any leachate served as control. Per cent inhibition in relation to growth in untreated control was further calculated and has been presented in Table 4.1 and figure 4.1.

Data presented in the Table 4.1 clearly reveal that all the leachates of different spent substrates were effective against the pathogen to certain extent. Irrespective of the concentration used, significantly minimum mean diametric mycelial growth (48.33 mm) was recorded in leachate of *P. sajor-caju* spent substrate colonized with *T. viride* (PSCTVLe) leading to 46.29 per cent growth inhibition of pathogen (Plate 4.5) followed by that in leachate of *P. florida* spent substrate colonized with *T. viride* (PFTVLe, 52.08 mm) leading to 42.12 per cent growth inhibition of pathogen and that in leachate of *P. ostreatus* spent (53.58 mm) substrate colonized with *T. viride* (POTVLe) leading to 40.27 per cent growth inhibition of pathogen. However, significantly maximum growth (66.67 mm) next to control (90.00 mm)was recorded in leachate *P. ostreatus* (POLe) leading to only 25.92 per cent growth inhibition of

Plate 4.1 Pathogenicity test (a) Diseased ginger (b) Pure culture of isolated pathogen (c) Artificially inoculated rhizome shows symptoms (d) Untreated control.



Pure culture

Mycelium

Micro and macro conidia

Plate 4.2 Cultural and microscopic identification of *Fuasrium solani*

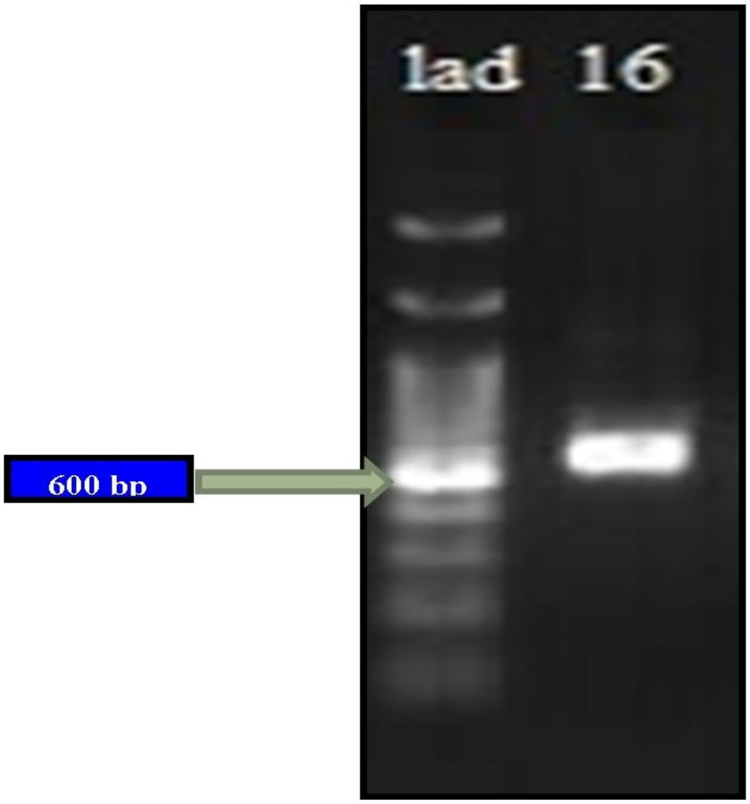


Plate 4.3 Amplified product of DNA isolated from test pathogen

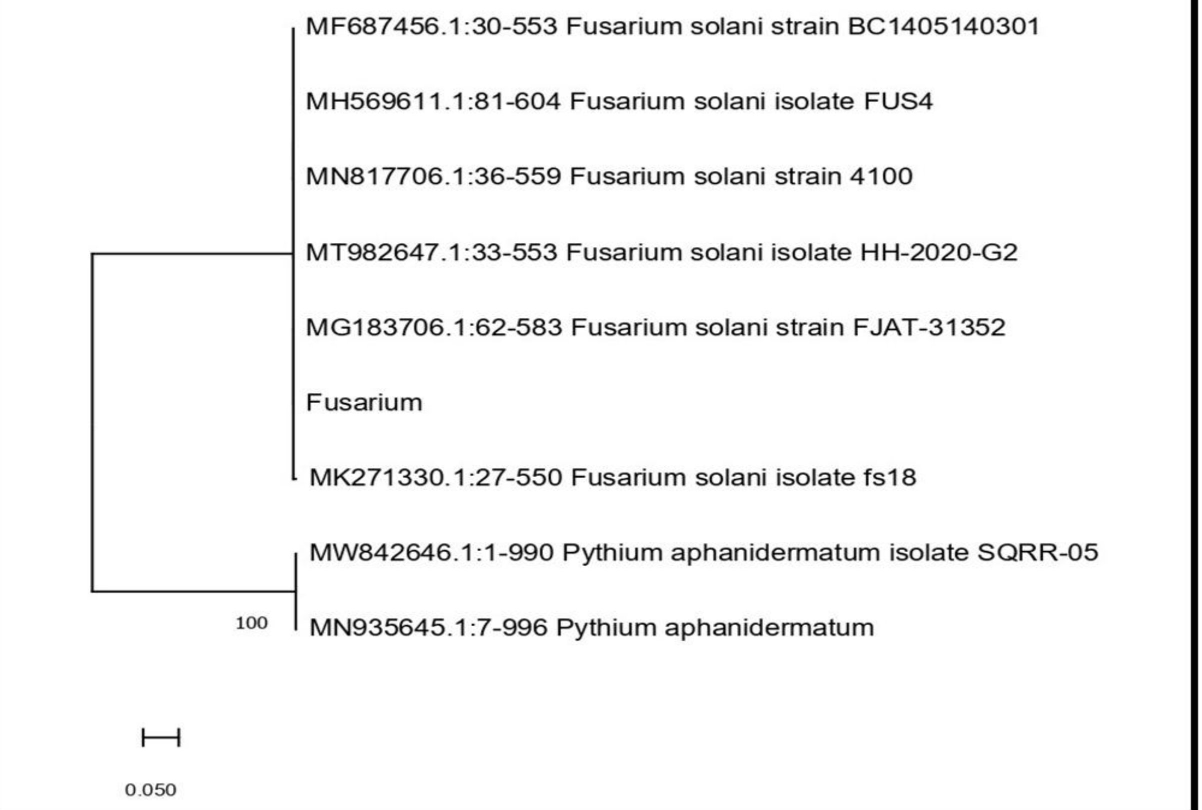


Plate 4.4 Phylogenetic analysis of ITS sequence of isolated fungus causing rhizome rot of ginger.

pathogen. Among the different concentrations evaluated, the mean diametric mycelial growth of the pathogen decreased significantly with each 5 per cent increase in concentration being significantly maximum at 5 per cent concentration (72.15 mm) leading to 22.29 per cent growth inhibition of pathogen and minimum at 20 per cent (51.89 mm) concentration leading to 47.54 per cent growth inhibition of pathogen irrespective of the leachate used.

Table 4.1 Effect of the spent mushroom substrate leachate on the mycelial growth of *Fusarium solani*

Treatment	**Mycelial growth (mm) of the pathogen at concentration (%)**				**Overall Mean**	**Growth inhibition (%) at concentration (%)**				**Overall Mean**
	5	**10**	**15**	**20**		**5**	**10**	**15**	**20**	
ABLe	75.00	65.00	58.67	50.33	62.25	16.49	27.77	34.81	44.07	30.79
ABTVLe	64.67	58.67	52.00	45.33	55.17	28.14	34.81	42.22	49.63	38.70
PSCLe	73.33	63.00	55.67	51.00	60.75	18.51	30.00	38.14	43.33	32.49
PSCTVLe	59.33	51.00	45.33	37.67	48.33	34.07	43.33	49.63	58.14	46.29
PFLe	80.33	72.00	59.00	52.67	66.00	10.74	20.00	34.44	41.48	26.66
PFTVLe	64.00	55.33	48.33	40.67	52.08	28.88	38.51	46.29	54.81	42.12
POLe	78.00	70.67	63.33	54.67	66.67	13.33	21.48	29.63	39.25	25.92
POTVLe	64.67	55.67	49.33	44.67	53.58	28.14	38.14	45.18	49.63	40.27
Contol	90.00	90.00	90.00	90.00	90.00					
Overall mean	72.15	64.59	57.96	51.89		22.29	31.75	40.04	47.54	

Factors	**CD$_{P\geq0.05}$**	**SE$_{(d)}$**
Treatment	0.57	0.28
Concentration	0.38	0.19
Treatment X Concentration	1.13	0.57

Where: ABLe = Leachate of *A. bisporus* spent mushroom compost, ABTVLe= Leachate of *A. bisporus* spent mushroom compost colonized with *T. viride*, PSCLe= Leachate of *P. sajor-caju* spent mushroom substrate, PSCTVLe= Leachate of *P. sajor-caju* spent mushroom compost colonized with *T. viride*, PFLe= Leachate of *P. florida* spent mushroom substrate, PFTVLe= Leachate of *P. florida* spent mushroom compost colonized with *T. viride*, POLe= Leachate of *P. ostreatus* spent mushroom substrate, PSCTVLe= Leachate of *P. ostreatus* spent mushroom compost colonized with *T. viride*,

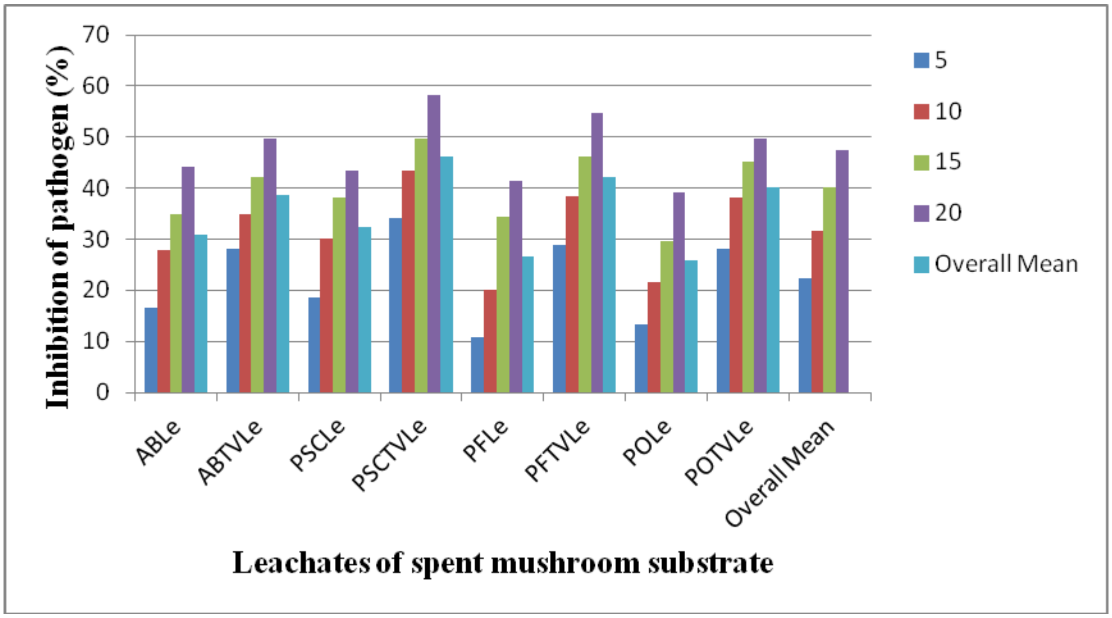


Fig. 4.1 Effect of spent mushroom substrate leachates on per cent inhibition of mycelial growth of *Fusarium solani*

The body of the table reveals that significantly minimum diametric mycelial growth of the pathogen was recorded in PSCTVLe when used at 20 per cent concentration (37.67 mm) leading to 58.14 per cent growth inhibition of pathogen followed by PFTVLe (40.67 mm) leading to 54.81 per cent growth inhibition of the pathogen and that with POTVLe (44.67 mm) at same concentration leading to 49.63 per cent growth inhibition of the pathogen. However, significantly maximum mycelial growth of the pathogen next to control (90.00 mm) was recorded in PFLe at 5 per cent concentration (80.33 mm) leading to 10.74 per cent growth inhibition of pathogen followed by leachate of POLe (78.00 mm) at same concentration leading to 13.33 per cent growth inhibition of pathogen.

During present studies, leachate of different spent mushroom substrates (SMS) resulted in inhibiting the *F. solani* to certain extent. Generally, the leaching of the SMS is advised to get rid of heavy metals from the substrate. Certain organic and inorganic salts are also leached from the SMS. However, no such studies have yet been conducted to see the effects of leachate of any kind of SMS on the fungal growth, so these results cannot be compared with. But, the growth inhibition of the pathogen by leachate of different types of SMS can be attributed to the leaching of

Plate 4.5 Mycelial growth of *Fusarium solani* as affected by leachate of *Pleurotus sajor-caju* spent substrate colonized with *Trichoderma viride* at different concentrations: (a) 20% (b) 15% (c) 10% (d) 5% and (e) Control

antimicrobial substances along with the organic and inorganic salts. When the spent substrates were colonized with *Trichoderma viride* and leached, the inhibitory potential of the leachate was further increased. It can be attributed to the fact that the antimicrobial compounds synthesized by *T. viride* might have additionally inhibited the pathogen growth.

Among the different leachates, leachate of *P. sajor-caju* proved best at 20 per cent concentration followed by that of *P. florida.* Spent substrate of these two mushrooms have earlier been reported to manage rhizome rot in ginger caused by *Pythium aphanidermatum* (Remya and Paul, 2014) which indicate the presence of some antimicrobial compounds in these substrates. So these studies support our finding to certain extent. Further, Verma *et al*. (2017) also reported *Trichoderma* spp. present in SMS to be effective against *Rhizoctonia solani* causing damping-off and root rot of tomato indicating that *Trichoderma* spp. increases the antimicrobial capacity of SMS.

4.3 Effect of aqueous extracts of spent mushroom substrates on the mycelial growth of *Fusarium solani*

In order to study the effect of aqueous extract of spent substrates of *Agaricus bisporus, Pleurotus sajor-caju, Pleurotus florida* and *Pleurotus ostreatus* on the mycelial growth of *F. solani*, crude water extracts were prepared from the *A. bisporus* spent substrate (AB), *A. bisporus* spent substrate-leached (ABL) for 10 days, *A. bisporus* spent substrate colonized with *T. viride* (ABTV), *A. bisporus* spent substrate colonized with *T. viride*-leached (ABTVL) for 10 days, *P. sajor-caju* spent substrate (PSC), *P. sajor-caju* spent substrate-leached (PSCL) for 10 days, *P. sajor-caju* spent substrate colonized with *T. viride* (PSCTV), *P. sajor-caju* spent substrate colonized with *T. viride*-leached (PSCTVL) for 10 days, *P. florida* spent substrate (PF), *P. florida* spent substrate-leached (PFL) for 10 days, *P. florida* spent substrate colonized with *T. viride* (PFTV), *P. florida* spent substrate colonized with *T. viride*-leached (PFTVL) for 10 days, *P. ostreatus* spent substrate (PO), *P. ostreatus* spent substrate-leached (POL) for 10 days, *P. ostreatus* spent substrate colonized with *T. viride* (POTV) and *P. ostreatus* spent substrate colonized with *T. viride*-leached (POTVL) for 10 days. These extracts were evaluated *in vitro* against the test pathogen at 5, 10, 15 and 20 per cent concentration by poisoned food technique and data were recorded in terms of mycelial growth (mm). Per cent inhibition in relation to control was further calculated and data recorded have been presented in Table(s) and figures 4.2, 4.3, 4.4 and 4.5

Data presented in the Table 4.2 clearly reveal that all the aqueous extracts of the *A. bisporus* spent substrate were effective against the pathogen to certain extent. Irrespective of the concentration used, significantly minimum mean diametric mycelial growth (44.08 mm) was recorded in aqueous extract of ABTVL spent substrate (Plate 4.6) followed by extract of ABTV spent substrate (46.58 mm) and ABL spent substrate (50.08 mm) leading to 51.02, 48.24 and 44.35 per cent inhibition, respectively. However, significantly maximum growth next to control (90.00 mm) was recorded in AB spent substrate (52.83 mm) leading to 41.39 per cent inhibition. Among the different concentrations evaluated, the mean diametric mycelial growth of the pathogen decreased significantly with each 5 per cent increase in concentration being significantly maximum at 5 per cent (64.20 mm) and minimum at 20 per cent (49.67 mm) concentration leading to 35.83 and 56.11per cent inhibition, respectively irrespective of the extract used.

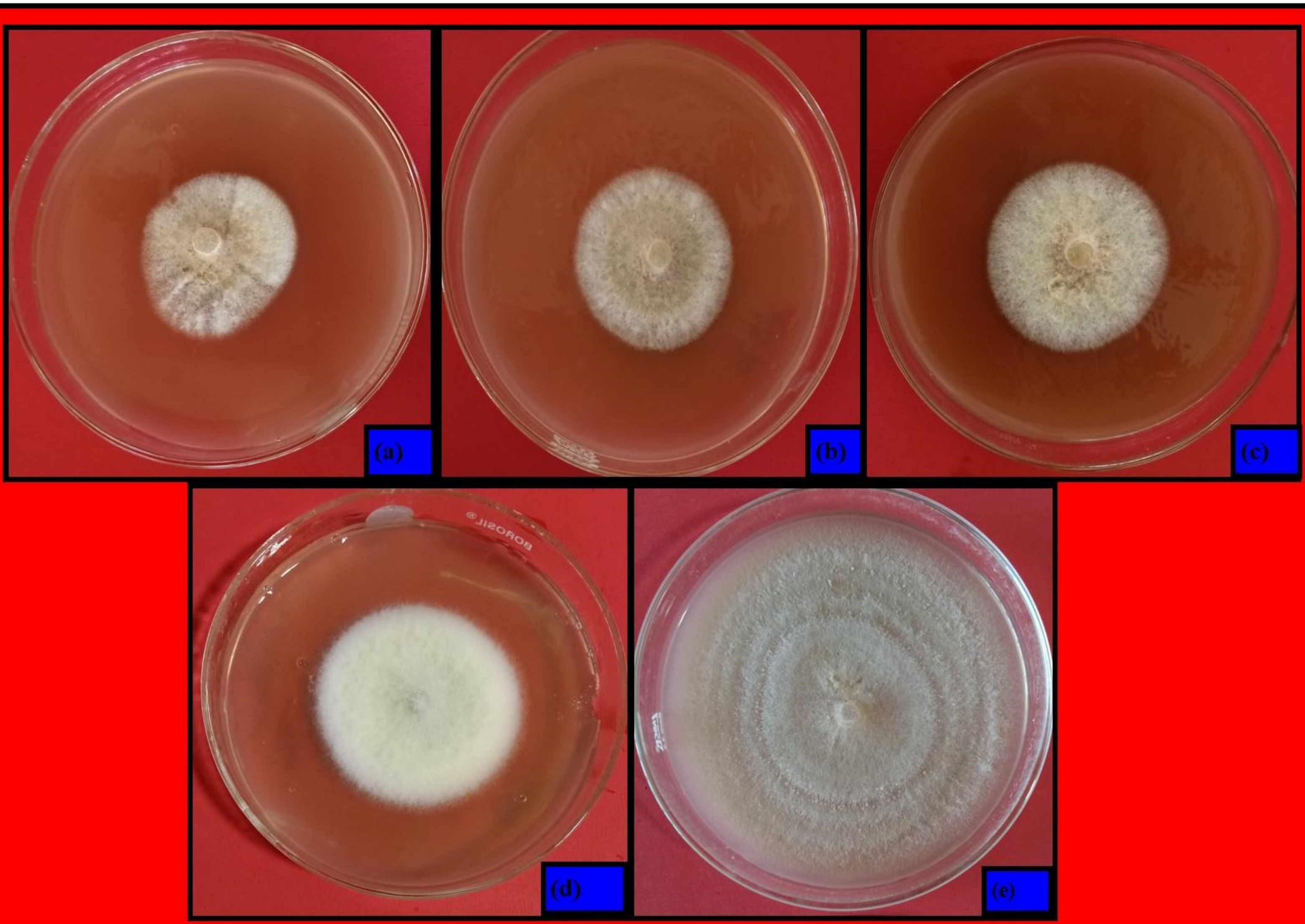


Plate 4.6 Mycelial growth of *Fusarium solani* as affected by aqueous extracts of *Agaricus bisporus* spent substrate colonized with *Trichoderma viride*-leached at different concentrations: (a) 20% (b) 15% (c) 10% (d) 5% and (e) Control

Table 4.2 Effect of the aqueous extracts of *Agaricus bisporus* substrate on the mycelial growth of *Fusarium solani*

Aqueous Extract	Mycelial growth (mm) of the pathogen at concentration (%)				Overall Mean	Growth inhibition (%) at concentration (%)				Overall Mean
	5	10	15	20		5	10	15	20	
AB	60.33	55.33	50.00	45.67	52.83	32.96	38.52	44.44	49.63	41.39
ABL	59.00	52.67	47.67	41.00	50.08	34.43	41.48	47.04	54.44	44.35
ABTV	56.00	49.67	44.33	36.33	46.58	37.78	44.81	50.74	59.63	48.24
ABTVL	55.67	45.67	39.67	35.33	44.08	38.15	49.26	55.93	60.74	51.02
Control	90.00	90.00	90.00	90.00	90.00					
Overall Mean	64.20	58.67	54.33	49.67		35.83	43.52	49.54	56.11	

Factors	**$CD_{P\geq0.05}$**	**$SE_{(d)}$**
Treatment	0.56	0.27
Concentration	0.50	0.25
Treatment X Concentration	1.11	0.55

Where: AB= *A. bisporus* spent substrate, ABL= *A. bisporus* spent substrate-leached, ABTV= *A. bisporus* spent substrate colonized with *T. viride*, ABTVL= *A. bisporus* spent substrate colonized with *T. viride*-leached.

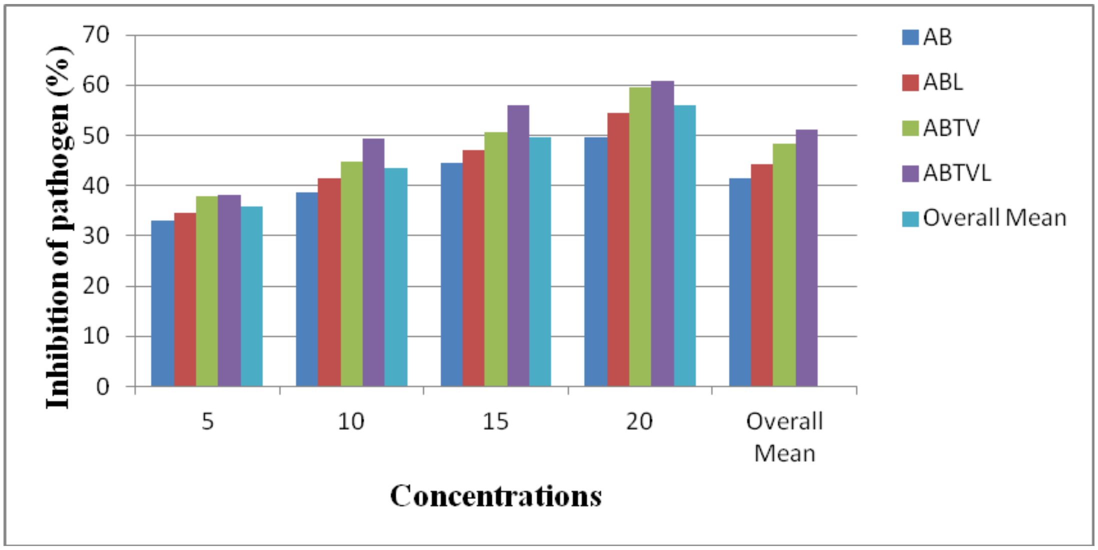


Fig. 4.2 Effect of the aqueous extracts of *Agaricus bisporus* spent substrate on per cent inhibition of mycelial growth of *Fusarium solani*

The body of table reveals that significantly minimum diametric mycelial growth of the pathogen was recorded in aqueous extract of ABTVL spent substrate when used at 20 per cent concentration (35.33 mm) followed by aqueous extract of ABTV spent substrate (36.33 mm) and aqueous extract of ABL spent substrate (41.00 mm) substrate at same concentration leading to 60.74, 59.63 and 54.44 per cent inhibition respectively. However , significantly maximum mycelial growth of the pathogen next to control (90.00 mm) was recorded in aqueous extract of AB spent substrate at 5 per cent concentration (60.33 mm) followed by extract of ABL spent substrate (59.00 mm) at same concentration leading to 32.96 and 34.43 per cent inhibition, respectively. An intermediate level of growth and inhibition was recorded with test of extracts at various concentrations under study.

Data presented in the Table 4.3 reveal that aqueous extracts of spent substrate of *P. sajor caju* were effective to certain extent against the test pathogen. Irrespective of the concentration used, significantly minimum mean diametric mycelial growth was recorded in aqueous extract of PSCTVL spent substrate (43.50 mm) leading to 51.66 per cent growth inhibition of pathogen (Plate 4.7) followed by aqueous extract of PSCTV spent substrate (44.25 mm) leading to 50.83 per cent growth inhibition and PSCL spent substrate (46.58 mm) leading to 48.24 per cent growth inhibition of the pathogen. However, significantly maximum growth next to control (90.00 mm) was recorded in PSC spent substrate (51.67 mm) leading to 42.59 per cent growth inhibition of the pathogen. Among the different concentrations evaluated, there was a significant decrease in the mean diametric mycelial growth of pathogen with each 5 per cent increase in concentration used being significantly maximum at 5 per cent (61.80 mm) concentration leading to 39.16 per cent growth inhibition of pathogen and minimum at 20 per cent (48.47 mm) concentration leading to 57.68 per cent growth inhibition of pathogen.

Table 4.3 Effect of the aqueous extracts of *Pleurotus sajor-caju* spent substrate on the mycelial growth of *Fusarium solani*

Aqueous extract	**Mycelial growth (mm) of the pathogen at concentration (%)**				**Overall Mean**	**Growth inhibition (%) at concentration (%)**				**Overall Mean**
	5	**10**	**15**	**20**		**5**	**10**	**15**	**20**	
PSC	59.00	53.33	50.33	44.00	51.67	34.43	40.74	44.07	51.11	42.59
PSCL	53.67	48.33	44.67	39.67	46.58	40.37	46.30	50.37	55.93	48.24
PSCTV	54.00	45.67	41.00	36.33	44.25	40.00	49.30	54.44	59.63	50.83
PSCTVL	52.33	48.33	41.00	32.33	43.50	41.85	46.29	54.44	64.07	51.66
Control	90.00	90.00	90.00	90.00	90.00					
Overall Mean	61.80	57.13	53.40	48.47		39.16	45.65	50.83	57.68	

Factors	**$CD_{P\geq0.05}$**	**$SE_{(d)}$**
Treatment	0.55	0.27
Concentration	0.49	0.24
Treatment X Concentration	1.10	0.54

Where: PSC = *P. sajor-caju* spent substrate, PSCL= *P. sajor-caju* spent substrate-leached , PSCTV= *P. sajor-caju* spent substrate colonized with *T. viride*, PSCTVL= *P. sajor-caju* spent substrate colonized with *T. viride*-leached.

The body of the table reveals that significantly minimum diametric mycelial growth of the pathogen was recorded in aqueous extract of PSCTVL spent substrate when used at 20 per cent concentration (32.33 mm) leading to 64.07 per cent growth inhibition of the pathogen followed by aqueous extract of PSCTV spent substrate (36.33 mm) leading to 59.63 per cent growth inhibition of the pathogen and aqueous extract of PSCL (39.67 mm) both at 20 per cent concentration leading to 55.93 per cent growth inhibition of the pathogen. However, maximum diametric mycelial growth next to the control (90.00 mm) was recorded in aqueous extract of PSC spent substrate at 5 per cent concentration (59.00 mm) leading to 34.43 per cent growth inhibition of the pathogen followed by aqueous extract of PSCTV spent substrate (54.00 mm) leading to 40.00 per cent growth inhibition of pathogen.

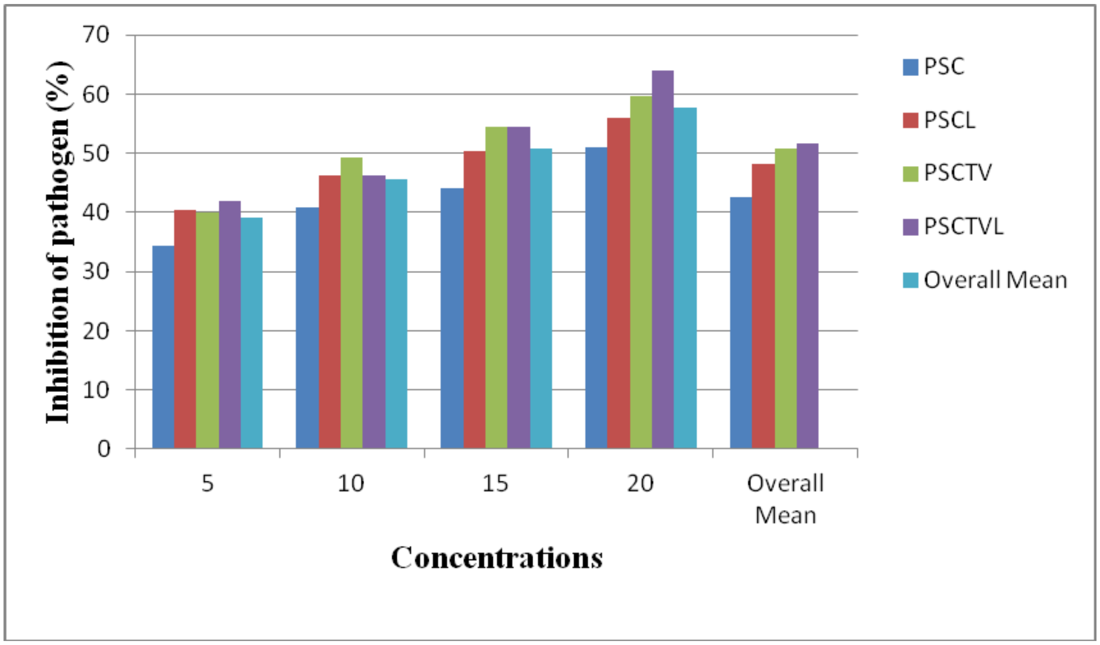


Fig. 4.3 Effect of the aqueous extracts of *Pleurotus sajor-caju* spent substrate on per cent inhibition of mycelial growth of *Fusarium solani*

It is clear from the data presented in Table 4.4 that in general, all the aqueous extracts of the *P. florida* spent substrate were effective against the pathogen to certain extent. Irrespective of the concentrations used, significantly minimum mean diametric mycelial growth was recorded in aqueous extract of PFTVL (46.08 mm)

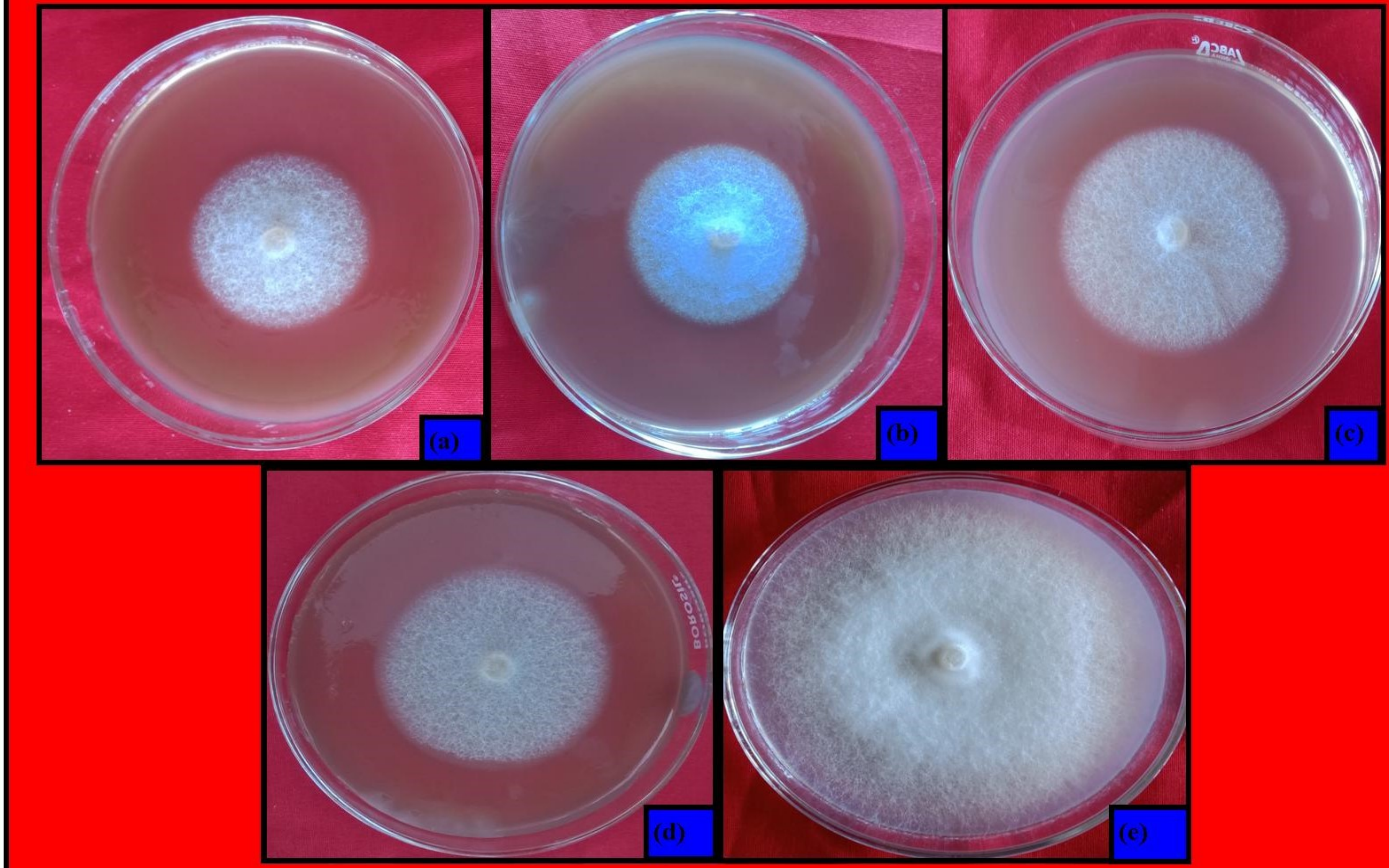


Plate 4.7 Mycelial growth of *Fusarium solani* as affected by aqueous extracts of *Pleurotus sajor-caju* spent substrate colonized with *Trichoderma viride*-leached at different concentrations: (a) 20% (b) 15% (c) 10% (d) 5% and (e) Control

leading to 48.80 per cent growth inhibition of pathogen (Plate 4.8) followed by aqueous extract of PFTV (47.17 mm) leading to 47.59 per cent growth inhibition of pathogen which was statistically at par with that recorded in PFL (47.58 mm) leading to 47.13 per cent growth inhibition. However significantly maximum growth next to control (90.00 mm) was recorded in PF substrate (47.92 mm) leading to 46.76 per cent growth inhibition of the pathogen. Among the different concentrations evaluated, the mean diametric mycelial growth of pathogen decreased significantly with each 5 per cent increase in the concentration, being significantly maximum at 5 per cent concentration (61.47 mm) leading to 39.63 per cent growth inhibition of pathogen and minimum at 20 per cent concentration (49.73 mm) leading to 55.92 per cent growth inhibition of pathogen.

Table 4.4 Effect of the aqueous extracts of *Pleurotus florida* spent substrate on the mycelial growth of *Fusarium solani*

Aqueous extract	**Mycelial growth (mm) of the pathogen at concentration (%)**				**Overall Mean**	**Growth inhibition (%) at concentration (%)**				**Overall Mean**
	5	**10**	**15**	**20**		**5**	**10**	**15**	**20**	
PF	54.67	49.67	46.33	41.00	47.92	39.26	44.81	48.52	54.44	46.76
PFL	54.33	50.33	44.67	41.00	47.58	39.63	44.07	50.37	54.44	47.13
PFTV	53.67	49.67	45.00	40.33	47.17	40.37	44.81	50.00	55.18	47.59
PFTVL	54.67	49.33	44.00	36.33	46.08	39.26	45.18	51.11	59.63	48.80
Control	90.00	90.00	90.00	90.00	90.00					
Overall Mean	61.47	57.80	54.00	49.73		39.63	44.72	50.00	55.92	

Factors	**$CD_{P\geq0.05}$**	**$SE_{(d)}$**
Treatment	0.52	0.26
Concentration	0.47	0.23
Treatment X Concentration	1.05	0.52

Where: PF= *P. florida* spent substrate, PFL= *P. florida* spent substrate-leached, PFTV = *P. florida* spent substrate colonized with *T. viride*, PFTVL = *P. florida* spent substrate colonized with *T. viride*-leached.

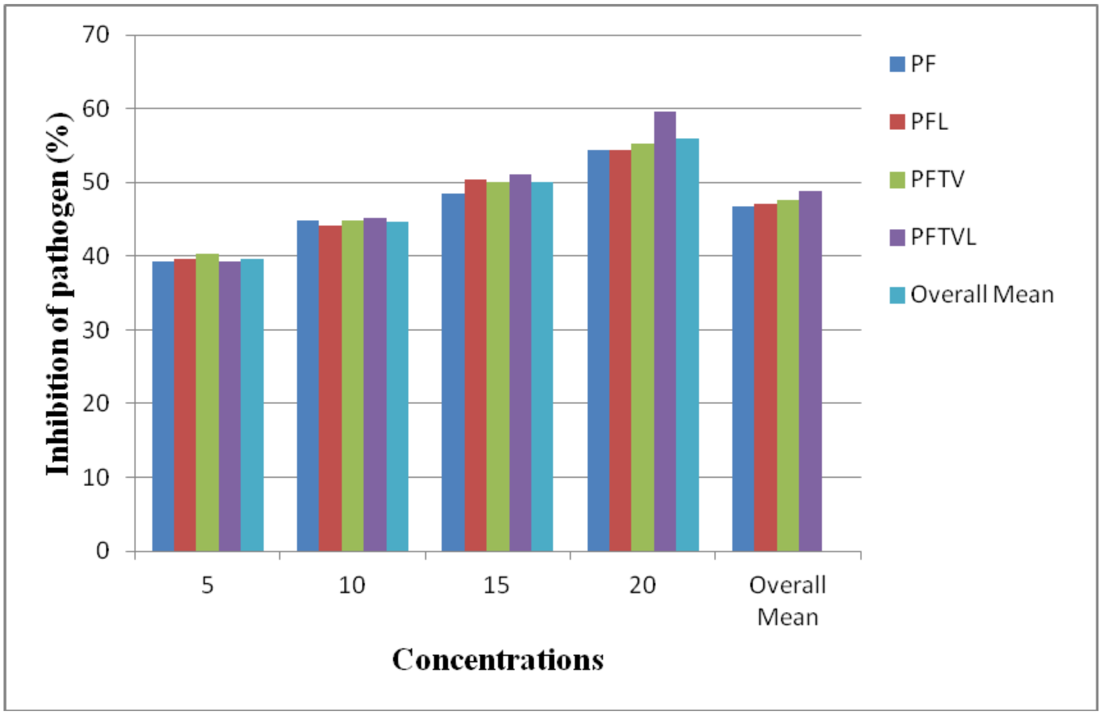


Fig. 4.4 Effect of the aqueous extracts of *Pleurotus florida* spent substrate on per cent inhibition of mycelial growth of *Fusarium solani*

The body of the table reveals that significantly minimum diametric growth of pathogen was recorded in aqueous extract of PFTVL spent substrate when used at 20 per cent concentration (36.33 mm) leading to 59.63 per cent growth inhibition of pathogen followed by aqueous extract of PFTV spent substrate (40.33 mm) used at same concentration, leading to 55.18 per cent growth inhibition of pathogen. However, significantly maximum mycelial growth of the pathogen next to control (90.00 mm) was recorded in aqueous extract of PF spent substrate at 5 per cent concentration (54.67 mm) leading to 39.26 per cent growth inhibition of pathogen which was statistically at par with that recorded in PFL spent substrate (54.33 mm) leading to 39.63 per cent growth inhibition of pathogen. An intermediate level of growth inhibition was recorded in rest of the extracts at different concentrations under study.

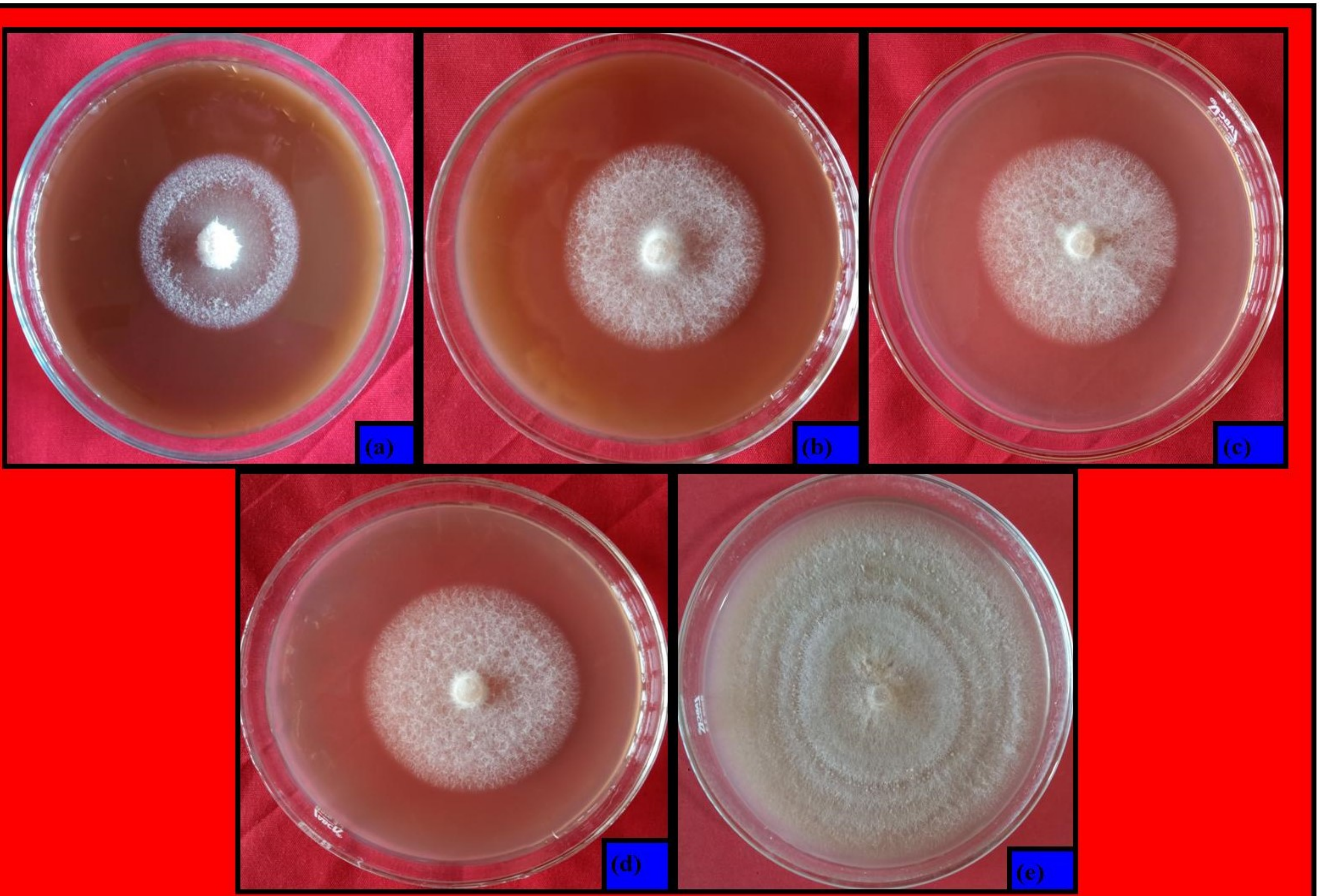


Plate 4.8 Mycelial growth of *Fusarium solani* as affected by aqueous extracts of *Pleurotus florida* spent substrate colonized with *Trichoderma viride*-leached at different concentrations: (a) 20% (b) 15% (c) 10% (d) 5% and (e) Control

A perusal of the data presented in Table 4.5 shows that irrespective of the concentrations used, significantly minimum mean diametric mycelial growth was recorded in aqueous extract of POTVL spent substrate (23.58 mm, Plate 4.9) followed by aqueous extract of POTV spent substrate (32.67 mm) and POL spent substrate (34.42 mm) leading to 73.80, 63.70 and 61.76 per cent growth inhibition of pathogen, respectively. However significantly maximum growth next to control (90.00 mm) was recorded in PO spent substrate (40.67 mm) leading to 54.82 per cent growth inhibition of the pathogen. However, among the different concentrations evaluated, the mean diametric mycelial growth of pathogen decreased significantly with each 5 per cent increase in the concentration, being significantly maximum at 5 per cent concentration (56.87 mm) leading to 46.02 per cent growth inhibition of pathogen and minimum at 20 per cent concentration (33.47 mm) leading to 78.52 per cent growth inhibition of pathogen.

Table 4.5 Effect of the aqueous extract of *Pleurotus ostreatus* spent substrate on the mycelial growth of *Fusarium solani*

Aqueous extract	Mycelial growth (mm) of the pathogen at concentration (%)				Overall Mean	Growth inhibition (%) at concentration (%)				Overall Mean
	5	10	15	20		5	10	15	20	
PO	57.00	46.67	35.00	24.00	40.67	36.67	48.15	61.11	73.33	54.82
POL	51.00	36.67	28.67	21.33	34.42	43.33	59.26	68.15	76.30	61.76
POTV	52.33	34.33	26.67	17.33	32.67	41.85	61.85	70.37	80.74	63.70
POTVL	34.00	26.00	19.67	14.67	23.58	62.22	71.11	78.15	83.70	73.80
Control	90.00	90.00	90.00	90.00	90.00					
Overall Mean	56.87	46.73	40.00	33.47		46.02	60.09	69.45	78.52	

Factors	**$CD_{P \geq 0.05}$**	**$SE_{(d)}$**
Treatment	0.57	0.28
Concentration	0.51	0.25
Treatment X Concentration	1.13	0.56

Where: PO= *P. ostreatus* spent substrate, POL= *P. ostreatus* spent substrate-leached, POTV= *P. ostreatus* spent substrate colonized with *T. viride* and POTVL= *P. ostreatus* spent substrate colonized with *T. viride*-leached.

Interaction of extracts and concentrations reveals that significantly minimum diametric growth of pathogen was recorded in aqueous extract of POTVL spent substrate when used at 20 per cent concentration (14.67 mm) followed by aqueous extract of POTV spent substrate (17.33 mm) and aqueous extract of POL spent substrate (21.33 mm) at same concentration leading to 83.70, 80.74 and 76.30 per cent growth inhibition of pathogen, respectively. However, maximum diametric mycelial growth next to the control (90.00 mm) was recorded in aqueous extract of PO spent substrate at 5 per cent concentration (57.00 mm) followed significantly by POL spent substrate (51.00 mm) leading to 36.67 and 43.33 per cent growth inhibition of pathogen, respectively. Rest of the extracts at remaining concentrations resulted in intermediate diametric growth of the pathogen leading to respective levels of growth inhibition.

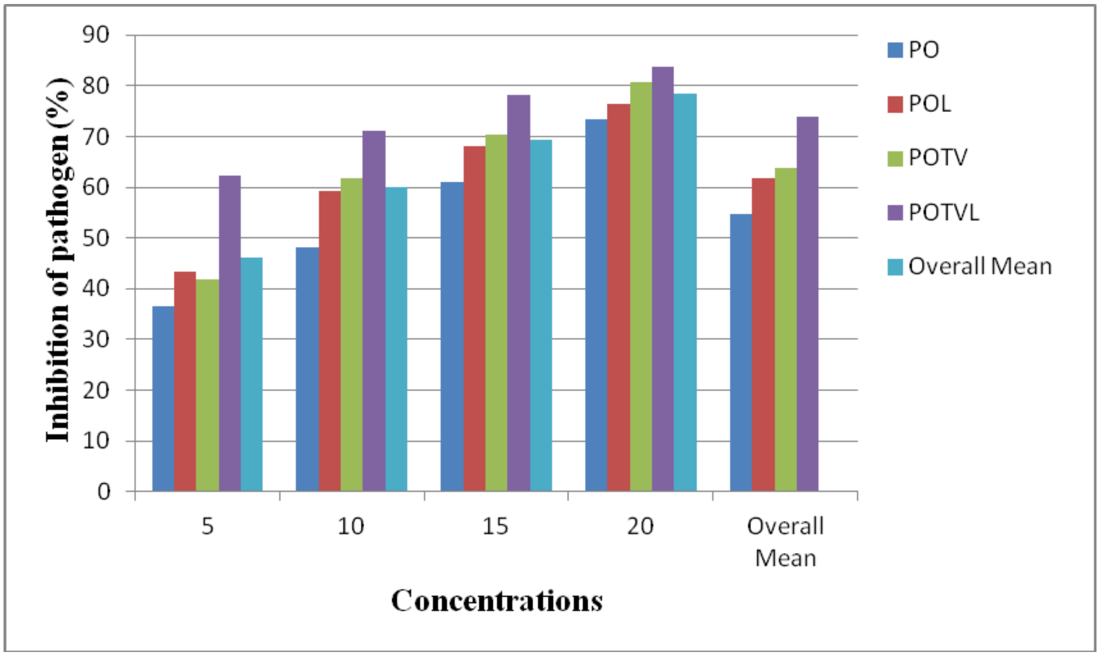


Fig. 4.5 Effect of the aqueous extracts of *Pleurotus ostreatus* spent substrate on per cent inhibition of the mycelial growth of *Fusarium solani*

However, much reports are not available in literature pertaining to the efficacy of aqueous extracts of *A. bisporus* spent compost against any of the pathogen, but,

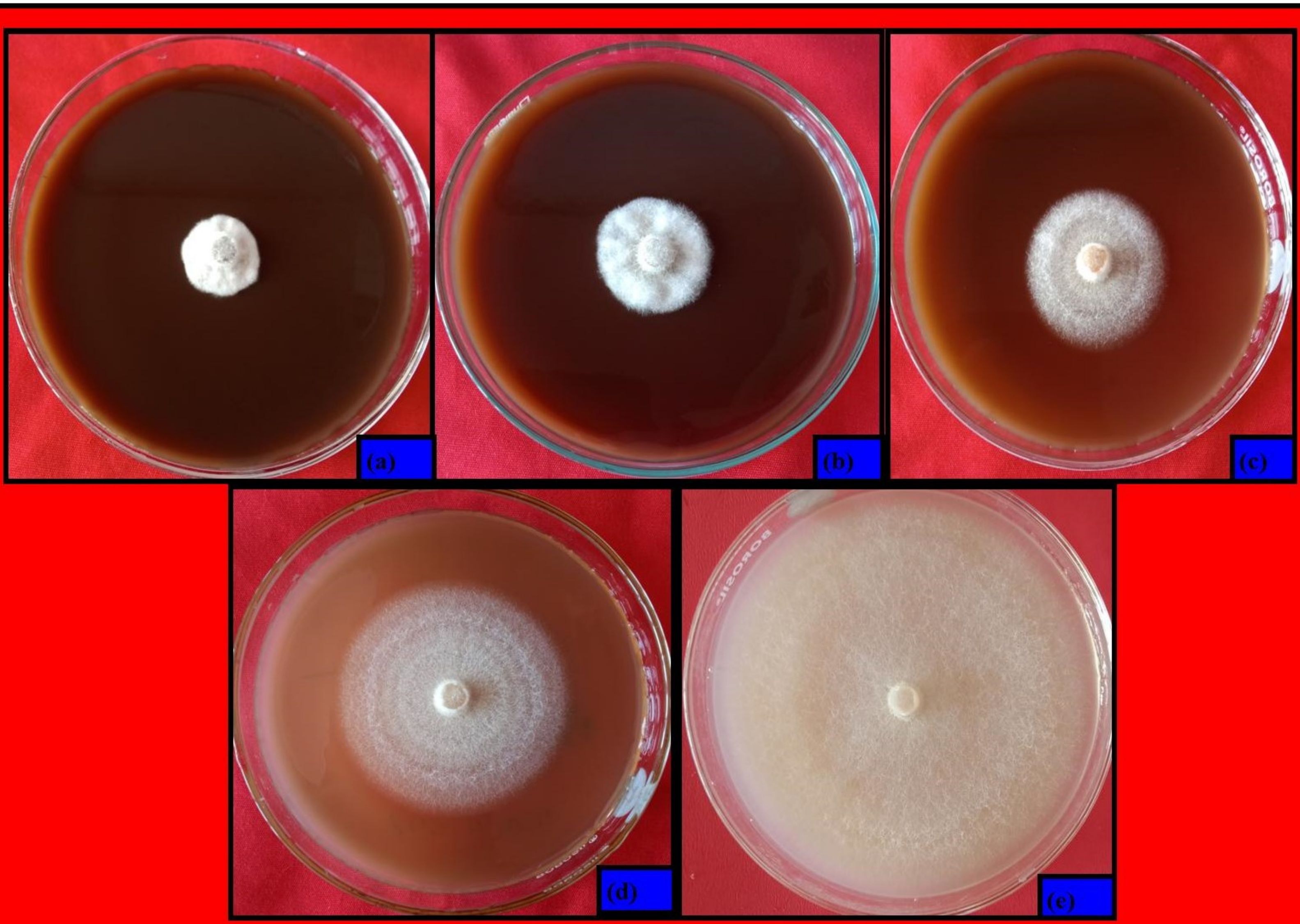


Plate 4.9 Mycelial growth of *Fusarium solani* as affected by aqueous extracts of *Pleurotus ostreatus* spent substrate colonized with *Trichoderma viride*-leached at different concentrations: (a) 20% (b) 15% (c) 10% (d) 5% and (e) Control

Ntougias *et al*. (2008) reported the effective management of *Phytophthora nicotianae, F. oxysporum* f.sp. *radicis-lycopersici* and *Septoria lycopersici* by use of SMS of *A. bisporus*. Further Yohalem *et al*. (1996) have also reported aqueous extract of SMS to control apple scab disease caused by *Venturia inaequalis.* Sultan *et al.* (2020) however, reported the aqueous extracts of *A. bisporus* fruiting bodies to be effective against *Aspergillus flavus* under *in vitro* assays. The extracts of SMS of different species of oyster mushroom were more effective against the test pathogen than that of button mushroom. These results are in conformity with Adedeji and Aduramigbo (2016) who also reported the water extracts of SMS of the oyster mushroom to be effective against *F. oxysporum* f. sp. *lycopersici.* These findings are also supported by Remya and Paul (2014) who studied the SMS of *P. sajor* caju to be effective against *P. aphanidermatum* causing soft rot of ginger. Antifungal properties of crude water extracts of *P. sajor-caju* have earlier been reported by Mossebo *et al.* (2020) which further strengthen our findings. Antifungal properties of *P. florida* have been reported against *Pythium* spp. by Owaid *et al.* (2017) which also support our findings. These findings are also in agreement with Martínez *et al*. (2015) who studied the antimicrobial activity of spent substrate of *P. ostraetus* combined with medicinal plants and suggested the possible use of the spent substrate of *P. ostreatus* (barley straw) as source of extracts with antimicrobial activity, being the best option in combination with *Mentha piperita* L. Bawadekji *et al.* (2017) have also reported the antimicrobial potential of crude water extracts of *P. ostreatus* which further strengthen our results.

T. viride when colonized into SMS, might have released some antibiotic compounds in the SMS and those might have been extracted in aqueous extract to increase the efficacy of the extract. Individually however, *T. harzianum* has been reported to be antagonistic to *F. saloni* by Nasreen and Ghaffar (2010).

4.4 Effect of the ethanol extract of spent mushroom substrate on the mycelial growth of *Fusarium solani*

In order to study the effect of ethanol extract of spent mushroom substrate on the mycelial growth of *F. solani*, crude ethanol extracts were prepared from the *A. bisporus* spent substrate (AB), *A. bisporus* spent substrate-leached (ABL) for 10 days, *A. bisporus* spent substrate colonized with *T. viride* (ABTV), *A. bisporus* spent substrate colonized with *T. viride*-leached (ABTVL) for 10 days, *P. sajor-caju* spent substrate (PSC), *P. sajor-caju* spent substrate-leached (PSCL) for 10 days, *P. sajor-caju* spent substrate colonized with *T. viride* (PSCTV), *P. sajor-caju* spent substrate colonized with *T. viride*-leached (PSCTVL) for 10 days, *P. florida* spent substrate (PF), *P. florida* spent substrate-leached (PFL) for 10 days, *P. florida* spent substrate colonized with *T. viride* (PFTV), *P. florida* spent substrate colonized with *T. viride* -leached (PFTVL) for 10 days, *P. ostreatus* spent substrate (PO), *P. ostreatus* spent substrate-leached (POL) for 10 days, *P. ostreatus* spent substrate colonized with *T. viride* (POTV) and *P. ostreatus* spent substrate colonized with *T. viride*-leached (POTVL) for 10 days. These extracts were evaluated *in vitro* against the test pathogen at 1, 2, 3 and 4 per cent concentration by poisoned food technique and data were recorded in terms of mycelial growth (mm). Normal nutrient medium devoid of any extract served as control. Per cent inhibition in relation to control was further calculated and data recorded have been presented in Table(s) and figures 4.6, 4.7, 4.8 and 4.9

Data presented in the Table 4.6 clearly reveal that is respective of concentrations used, significantly minimum mean diametric mycelial growth was recorded in ethanol extract of ABTVL spent substrate (37.33 mm) leading to 58.51 per cent growth inhibition of test pathogen w.r.t. control (Plate 4.10) followed by ABTV spent substrate (40.17 mm) leading to 55.37 per cent growth inhibition and ABL spent substrate (43.00 mm) leading to 52.22 per cent growth inhibition of pathogen. Among the different concentrations evaluated, there was a significant decrease in the mean diametric mycelial growth of pathogen with each 1 per cent increase in concentration, being significantly maximum at 1 per cent (59.07 mm) followed by 2 (52.80 mm), 3 (48.20 mm) and 4 per cent (44.93 mm) concentration leading to 42.96, 51.66, 58.05 and 62.59 per cent growth inhibition of pathogen, respectively.

Table 4.6 Effect of the ethanol extracts of *A. bisporus* spent substrate on the mycelial growth of *Fusarium solani*

Ethanol Extract	Mycelial growth (mm) of the pathogen at concentration (%)				Overall Mean	Growth inhibition (%) at concentration (%)				Overall Mean
	1	2	3	4		1	2	3	4	
AB	54.67	47.33	43.00	38.00	45.75	39.25	47.40	52.22	57.77	49.16
ABL	52.00	44.67	39.67	35.67	43.00	42.22	50.37	55.92	60.37	52.22
ABTV	50.33	42.33	36.00	32.00	40.17	44.07	52.96	60.00	64.43	55.37
ABTVL	48.33	39.67	32.33	29.00	37.33	46.29	55.92	64.07	67.77	58.51
Control	90.00	90.00	90.00	90.00	90.00					
Overall Mean	59.07	52.80	48.20	44.93		42.96	51.66	58.05	62.59	

Factors	**$CD_{P\geq0.05}$**	**$SE_{(d)}$**
Treatment	0.47	0.23
Concentration	0.42	0.21
Treatment X Concentration	0.93	0.46

AB= *A. bisporus* spent substrate, ABL= *A. bisporus* spent substrate-leached, ABTV= *A. bisporus* spent substrate colonized with *T. viride*, ABTVL= *A. bisporus* spent substrate colonized with *T. viride*-leached.

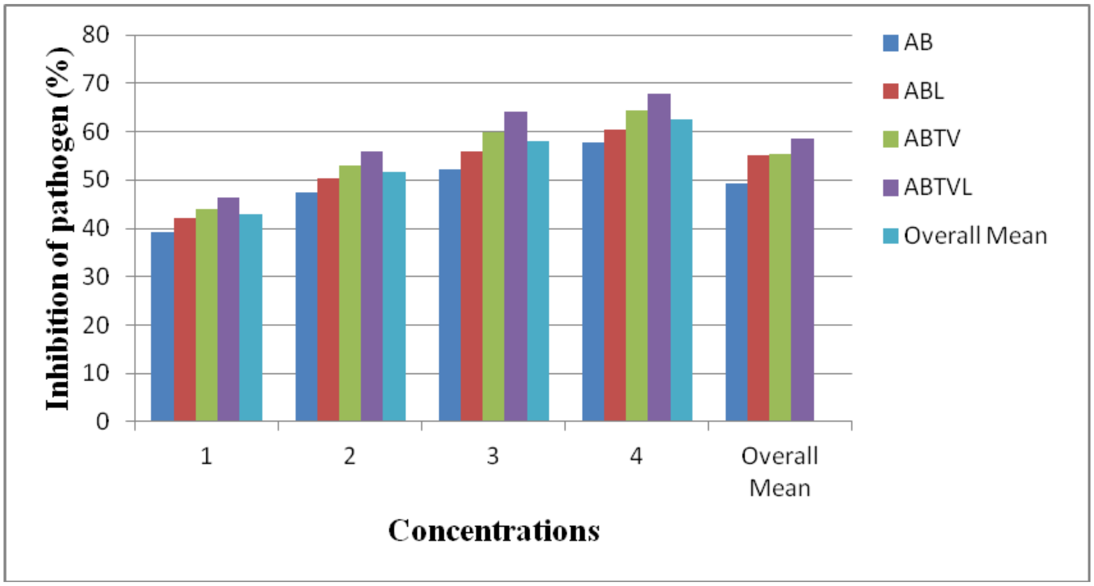


Fig. 4.6 Effect of the ethanol extracts of *Agaricus bisporus* spent substrate on per cent inhibition of the mycelial growth of *Fusarium solani*

The body of table reveals that significantly minimum diametric mycelial growth of the pathogen was recorded in ethanol extract of ABTVL spent substrate when used at 4 per cent concentration (29.00 mm) leading to 67.77 per cent growth inhibition of pathogen followed by that in ethanol extract of ABTV spent substrate at same concentration (32.00 mm) leading to 64.43 per cent growth inhibition of pathogen and ABL spent substrate (35.67 mm) at 4 per cent same concentration leading to 60.37 per cent growth inhibition of pathogen. However, maximum diametric mycelial growth next to the control (90.00 mm) was recorded in ethanol extract of AB spent substrate used at 1 per cent concentration (54.67 mm) leading to only 39.25 per cent growth inhibition of pathogen followed by ethanol extract of ABL spent substrate (52.00 mm) leading to 42.22 per cent growth inhibition of pathogen. An intermediate level of growth inhibition was recorded with remaining extracts at different concentrations evaluated.

A perusal of the data presented in Table 4.7 clearly reveals that all the ethanol extracts of *P. sajor-caju* spent substrate were effective against the pathogen to certain extent. Irrespective of the concentrations used, significantly minimum mean diametric mycelial growth was recorded in ethanol extract of PSCTVL spent substrate (12.33 mm, Plate 4.11) followed by that in ethanol extract of PSCTV spent substrate (15.67 mm) and PSCL spent substrate (22.42 mm) leading to 86.29, 82.58 and 75.09 per cent growth inhibition, respectively. However, significantly maximum growth next to control (90.00 mm) was recorded in ethanol extract of PSC spent substrate (25.75 mm) leading to 71.39 per cent growth inhibition. Among the different concentrations evaluated, the mean diametric mycelial growth of the pathogen decreased significantly with each 1 per cent increase in concentration, being significantly maximum at 1 per cent concentration (44.13 mm) and minimum at 4 per cent concentration (24.20 mm) leading to 63.70 and 91.39 per cent growth inhibition, respectively, irrespective of the extract used.

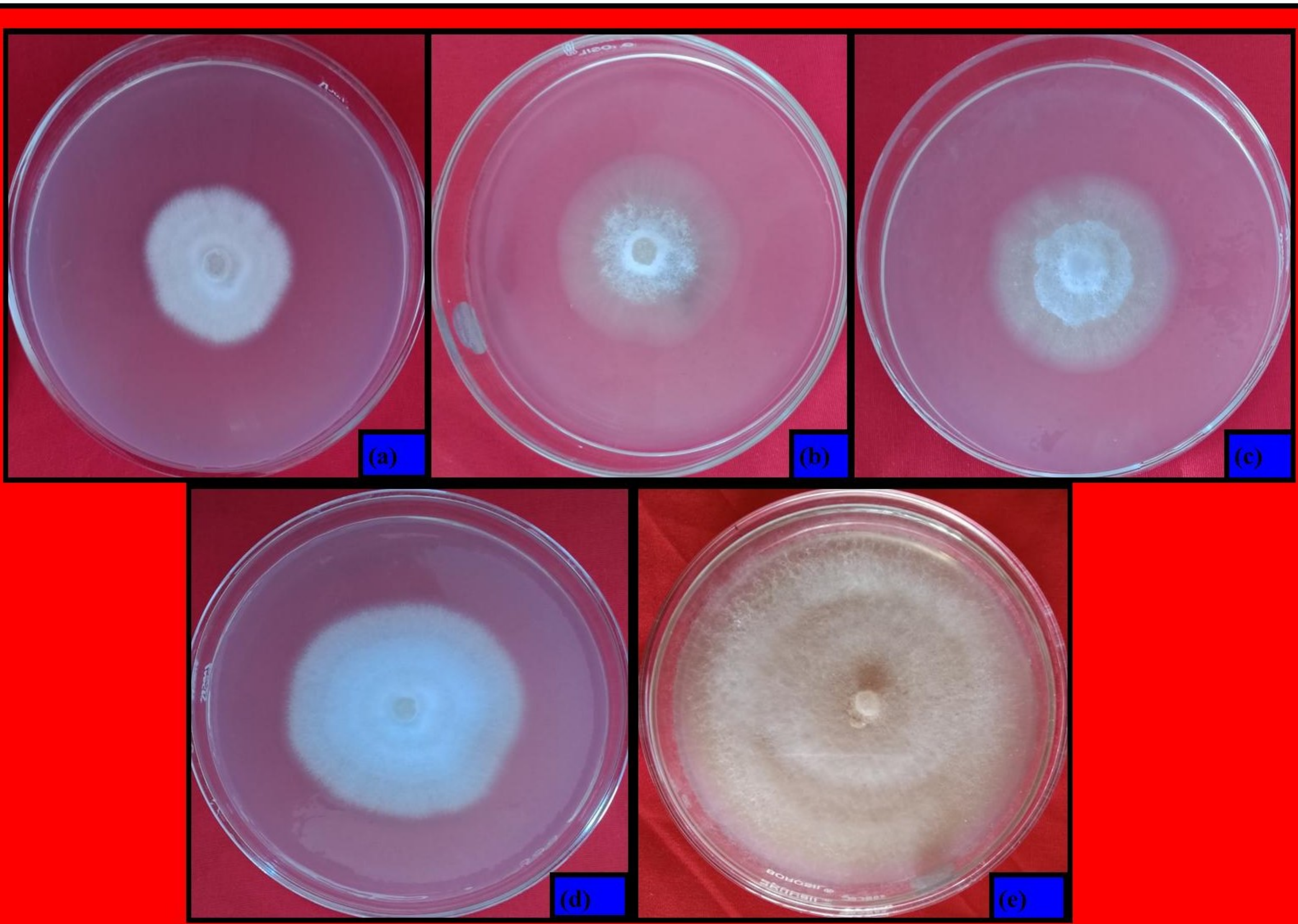


Plate 4.10 Mycelial growth of *Fusarium solani* as affected by ethanol extracts of *Agaricus bisporus* spent substrate colonized with *Trichoderma viride*-leached at different concentrations: (a) 4% (b) 3% (c) 2% (d) 1% and (e) Control

Table 4.7 Effect of the ethanol extracts of *Pleurotus sajor-caju* spent substrate on the mycelial growth of *Fusarium solani*

Ethanol Extract	Mycelial growth (mm) of the pathogen at concentration (%)				Overall Mean	Growth inhibition (%) at concentration (%)				Overall Mean
	1	2	3	4		1	2	3	4	
PSC	43.00 (40.96)	27.00 (31.29)	19.00 (25.83)	14.00 (21.96)	25.75 (30.01)	52.22	70.00	78.88	84.44	71.39
PSCL	35.67 (36.66)	24.67 (29.77)	17.33 (24.59)	12.00 (20.25)	22.42 (27.82)	60.37	72.59	80.74	86.66	75.09
PSCTV	28.00 (31.93)	20.00 (26.55)	9.67 (18.10)	5.00 (12.92)	15.67 (22.38)	68.86	77.77	89.25	94.44	82.58
PSCTVL	24.00 (29.32)	18.00 (25.09)	7.33 (15.70)	0.00 (4.05)	12.33 (18.54)	73.33	80.00	91.86	100.00	86.29
Control	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)					
Overall mean	44.13 (41.99)	35.93 (36.75)	28.67 (31.06)	24.20 (26.05)		63.70	75.10	85.18	91.39	

Factors	**$CD_{P\geq0.05}$**	**$SE_{(d)}$**
Treatment	0.45	0.22
Concentration	0.40	0.20
Treatment X Concentration	0.90	0.44

Figures in parentheses indicate angular transformed values

Where: PSC = *P. sajor-caju* spent substrate, PSCL= *P. sajor-caju* spent substrate-leached , PSCTV= *P. sajor-caju* spent substrate colonized with *T. viride*, PSCTVL= *P. sajor-caju* spent substrate colonized with *T. viride*-leached

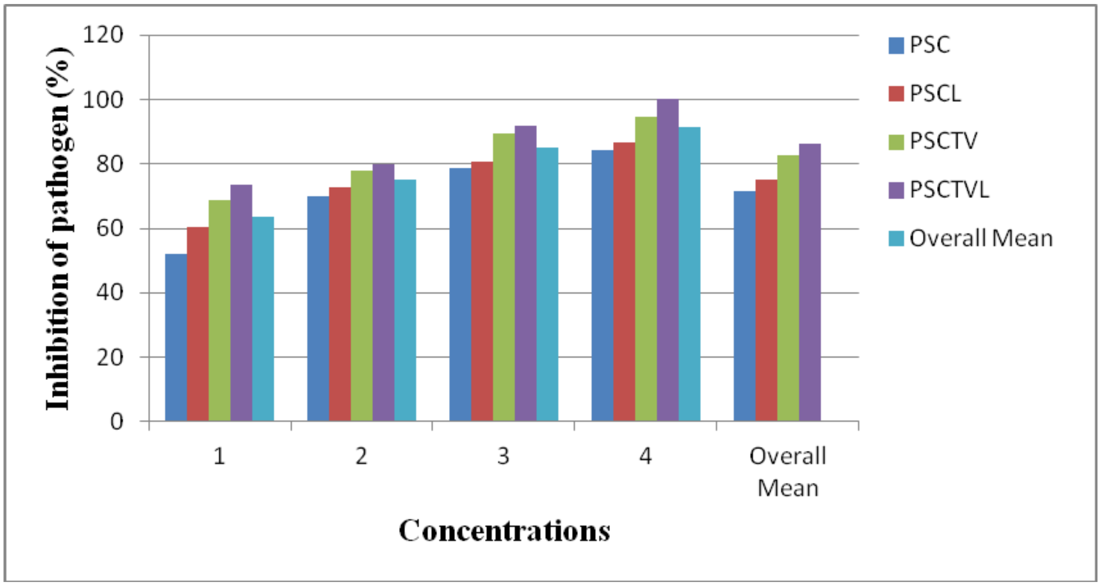


Fig. 4.7 Effect of the ethanol extracts of *Pleurotus sajor-caju* spent substrate on per cent inhibition of the mycelial growth of *Fusarium solani*

The body of table reveals that no mycelial growth of the pathogen was recorded in ethanol extract of PSCTVL spent substrate when used at 4 per cent concentration (0.00 mm) followed significantly by ethanol extracts of PSCTV spent substrate (5.00 mm), PSCL spent substrate (12.00 mm) and PSC spent substrate (14.00 mm) leading to 100.00, 94.44, 86.66 and 84.44 per cent growth inhibition, respectively. However, significantly maximum diametric mycelial growth next to the control (90.00 mm) was recorded in ethanol extract of PSC spent substrate at 1 per cent concentration (43.00 mm) followed by ethanol extract of PSCL spent substrate (35.67 mm) at same concentration leading to 52.22 and 60.37 per cent growth inhibition, respectively.

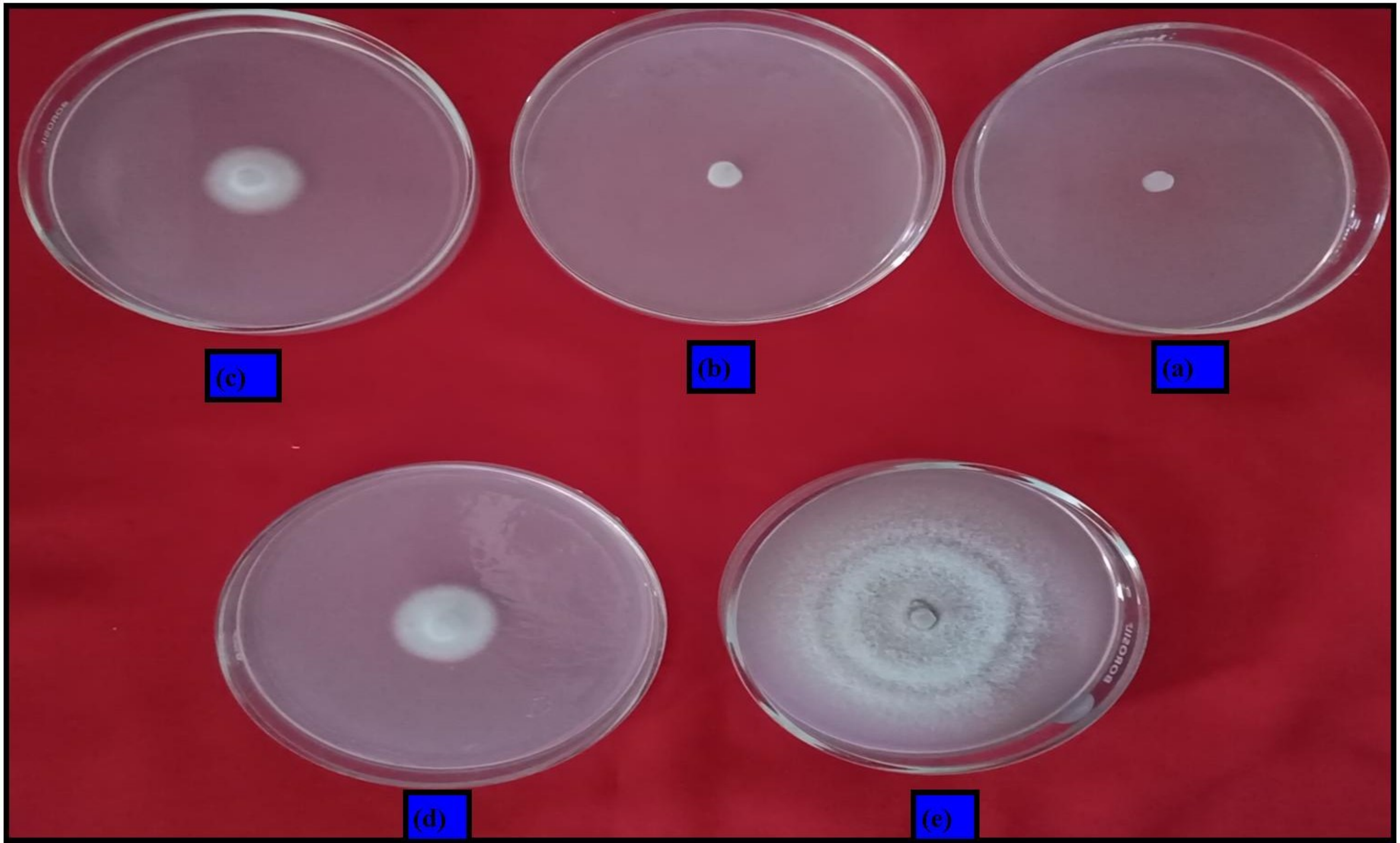


Plate 4.11 Mycelial growth of *Fusarium solani* as affected by ethanol extracts of *Pleurotus sajor-caju* spent substrate colonized with *Trichoderma viride*-leached at different concentrations: (a) 4% (b) 3% (c) 2% (d) 1% and (e) Control

Table 4.8 Effect of the ethanol extracts of *Pleurotus florida* substrate on the mycelial growth of *Fusarium solani*

Ethanol Extract	Mycelial growth (mm) of the pathogen at concentration (%)				Overall Mean	Growth inhibition (%) at concentration (%)				Overall Mean
	1	2	3	4		1	2	3	4	
PF	20.00 (26.55)	12.67 (20.84)	9.67 (18.10)	5.33 (13.34)	11.92 (19.70)	77.78	85.93	89.26	94.07	86.76
PFL	18.00 (25.09)	11.00 (19.35)	7.67 (16.06)	5.00 (12.92)	10.42 (18.35)	80.00	87.78	91.48	94.44	88.43
PFTV	19.67 (26.31)	10.33 (18.74)	7.33 (15.70)	0.00 (4.05)	9.33 (16.20)	78.15	88.52	91.85	100.00	89.63
PFTVL	11.33 (19.66)	7.67 (16.06)	5.33 (13.34)	0.00 (4.05)	6.08 (13.28)	87.41	90.74	94.07	100.00	93.06
Control	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)					
Overall Mean	31.80 (33.74)	26.33 (29.21)	24.00 (26.85)	20.07 (21.08)		80.84	88.24	91.67	`97.13	

Factors	$\text{CD}_{P\geq 0.05}$	$\text{SE}_{(d)}$
Treatment	0.43	0.21
Concentration	0.39	0.19
Treatment X Concentration	0.86	0.43

Figures in parentheses indicate angular transformed values

Where: PF= *P. florida* spent substrate, PFL= *P. florida* spent substrate-leached, PFTV = *P. florida* spent substrate colonized with *T. viride*, PFTVL = *P. florida* spent substrate colonized with *T. viride*-leached.

It is clear from the data presented in the Table 4.8 that all the ethanol extracts of *P. florida* spent substrate were quite effective against the pathogen. Irrespective of the concentrations used, significantly minimum mean diametric mycelial growth was recorded in ethanol extract of PFTVL spent substrate (6.08 mm) leading to 93.06 per cent growth inhibition (Plate 4.12) followed by ethanol extract of PFTV spent substrate (9.33 mm) leading to 89.63 per cent growth inhibition of the pathogen and PFL spent substrate (10.42 mm) leading to 88.43 per cent growth inhibition of the pathogen. However, significantly maximum growth next to control (90.00 mm) was recorded in PF spent substrate (11.92 mm) leading to 86.76 per cent growth inhibition of the pathogen. Among the different concentrations evaluated, the mean diametric mycelial growth of the pathogen decreased significantly with each 1 per cent increase in concentration evaluated, being significantly maximum at 1 per cent concentration (31.80 mm) and minimum at 4 per cent concentration (20.07 mm) leading to 80.84 and 97.13 per cent growth inhibition of the pathogen, respectively, irrespective of the extract used.

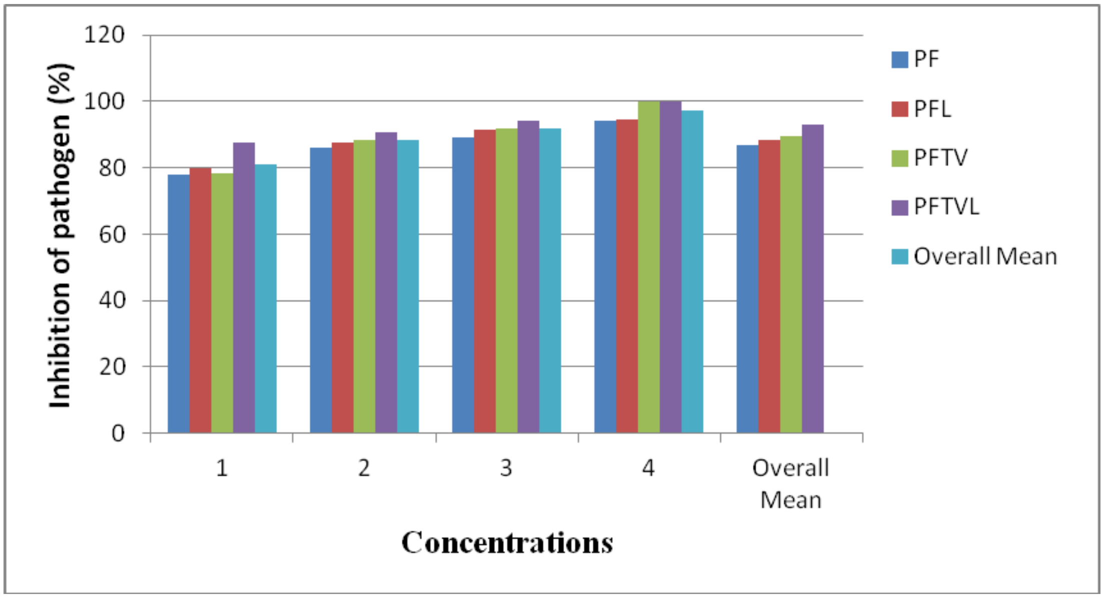


Fig. 4.8 Effect of the ethanol extracts of *Pleurotus florida* spent substrate on per cent inhibition of the mycelial growth of *Fusarium solani*

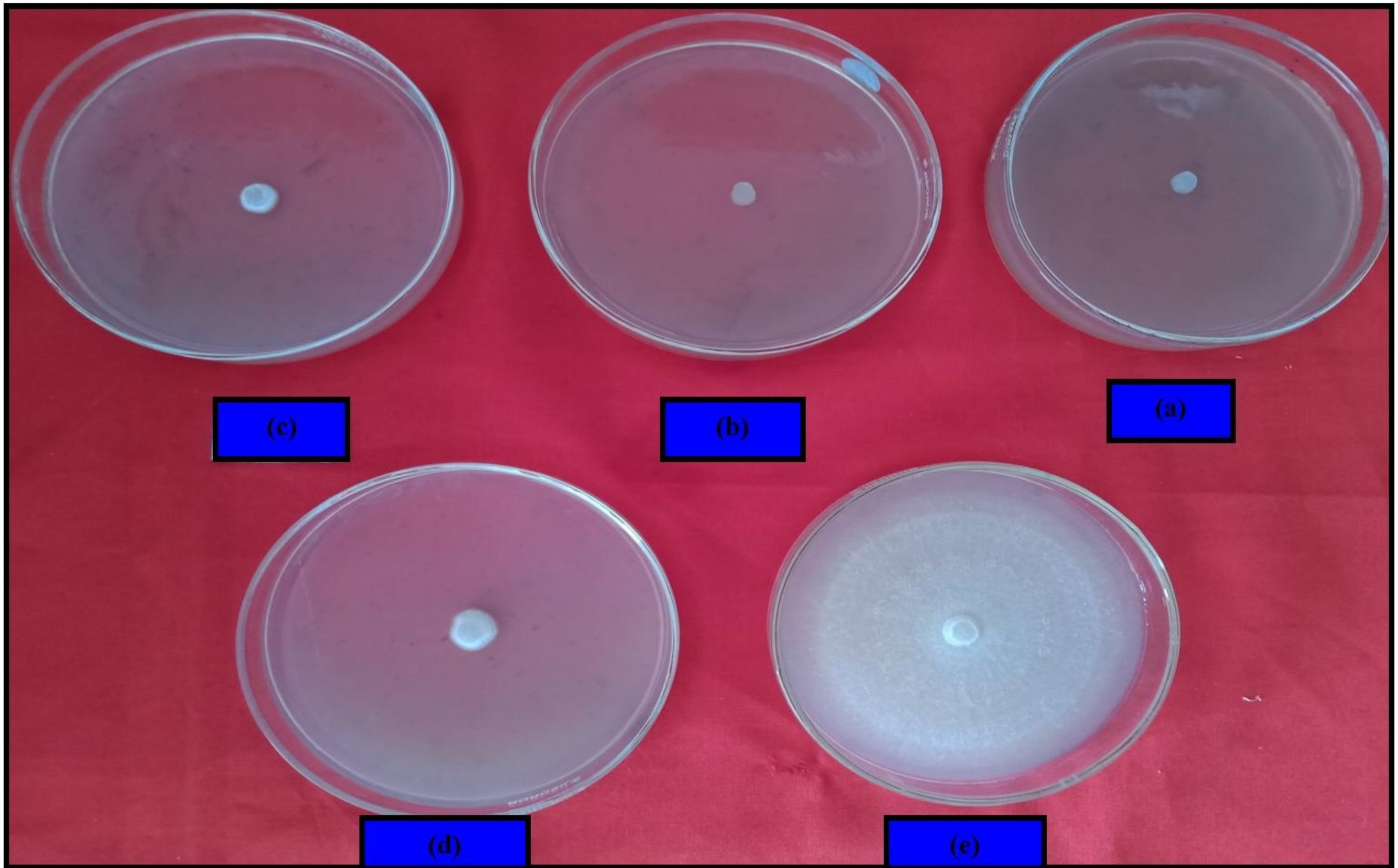


Plate 4.12 Mycelial growth of *Fusarium solani* as affected by ethanol extracts of *Pleurotus florida* spent substrate colonized with *Trichoderma viride*-leached at different concentrations: (a) 4% (b) 3% (c) 2% (d) 1% and (e) Control

The body of table reveals that pathogen completely failed to grow in ethanol extract of PFTVL spent substrate and PFTV spent substrate when used at 4 per cent concentration (0.00 mm) leading to 100.00 per cent growth inhibition of the pathogen. However, significantly minimum diametric growth was recorded in ethanol extract of PFL spent substrate at 4 per cent concentration (5.00 mm) leading to 94.44 per cent growth inhibition which was statistically at par with the growth recorded in ethanol extract of PF spent substrate (5.33 mm) at same concentration leading to 94.07 per cent growth inhibition of the pathogen. However, maximum mycelial growth of the pathogen was recorded in ethanol extract of PF spent substrate at 1 per cent concentration (20.00 mm) leading to 77.78 per cent growth inhibition of the pathogen which was statistically at par with the growth recorded in ethanol extract of PFTV spent substrate used at 1 per cent concentration (19.67) leading to 78.51 per cent growth inhibition of pathogen.

Data presented in the Table 4.9 clearly reveal that all the ethanol extracts of *P. ostreatus* spent substrate were effective against the pathogen to certain extent. Irrespective of the concentrations used, significantly minimum mean diametric mycelial growth was recorded in ethanol extract of POTVL spent substrate (18.50 mm, Plate 4.13) which was followed by POTV spent substrate(18.67 mm) and POL spent substrate (21.42 mm) leading to 79.45, 79.26 and 76.20 per cent growth inhibition, respectively. However, significantly maximum growth next to control (90.00 mm) was recorded in PO spent substrate (25.92 mm) leading to 69.54 per cent growth inhibition. Among the different concentrations evaluated, the mean diametric mycelial growth of the pathogen was recorded to be significantly maximum at 1 per cent concentration (47.13 mm) and significantly minimum at 4 per cent concentration (23.07 mm) leading to 59.54 and 92.96 per cent growth inhibition, respectively, irrespective of the extract used.

Table 4.9 Effect of the ethanol extracts of *Pleurotus ostreatus* spent substrate on the mycelial growth of *Fusarium solani*

Ethanol Extract	Mycelial growth (mm) of the pathogen at concentration (%)				Overall Mean	Growth inhibition (%) at concentration (%)				Overall Mean
	1	2	3	4		1	2	3	4	
PO	40.33 (39.41)	29.00 (32.57)	24.00 (29.32)	10.33 (18.74)	25.92 (30.01)	55.19	67.78	66.66	88.52	69.54
POL	35.67 (36.66)	28.00 (31.93)	14.33 (22.24)	7.67 (16.06)	21.42 (26.72)	60.37	68.89	84.07	91.48	76.20
POTV	30.33 (33.41)	25.00 (29.99)	12.00 (20.25)	7.33 (15.70)	18.67 (24.84)	66.30	72.22	86.67	91.85	79.26
POTVL	39.33 (38.83)	25.67 (30.43)	9.00 (17.43)	0.00 (4.05)	18.50 (22.69)	56.30	71.48	90.00	100.00	79.45
Control	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)	90.00 (71.06)					
Overall Mean	47.13 (43.87)	39.53 (39.20)	29.87 (32.06)	23.07 (25.12)		59.54	70.09	81.85	92.96	

Factors	**$CD_{P\geq0.05}$**	**$SE_{(d)}$**
Treatment	0.43	0.21
Concentration	0.39	0.19
Treatment X Concentration	0.86	0.42

Figures in parentheses indicate angular transformed values

Where: PO= *P. ostreatus* spent substrate, POL= *P. ostreatus* spent substrate-leached, POTV= *P. ostreatus* spent substrate colonized with *T. viride* and POTVL= *P. ostreatus* spent substrate colonized with *T. viride*-leached.

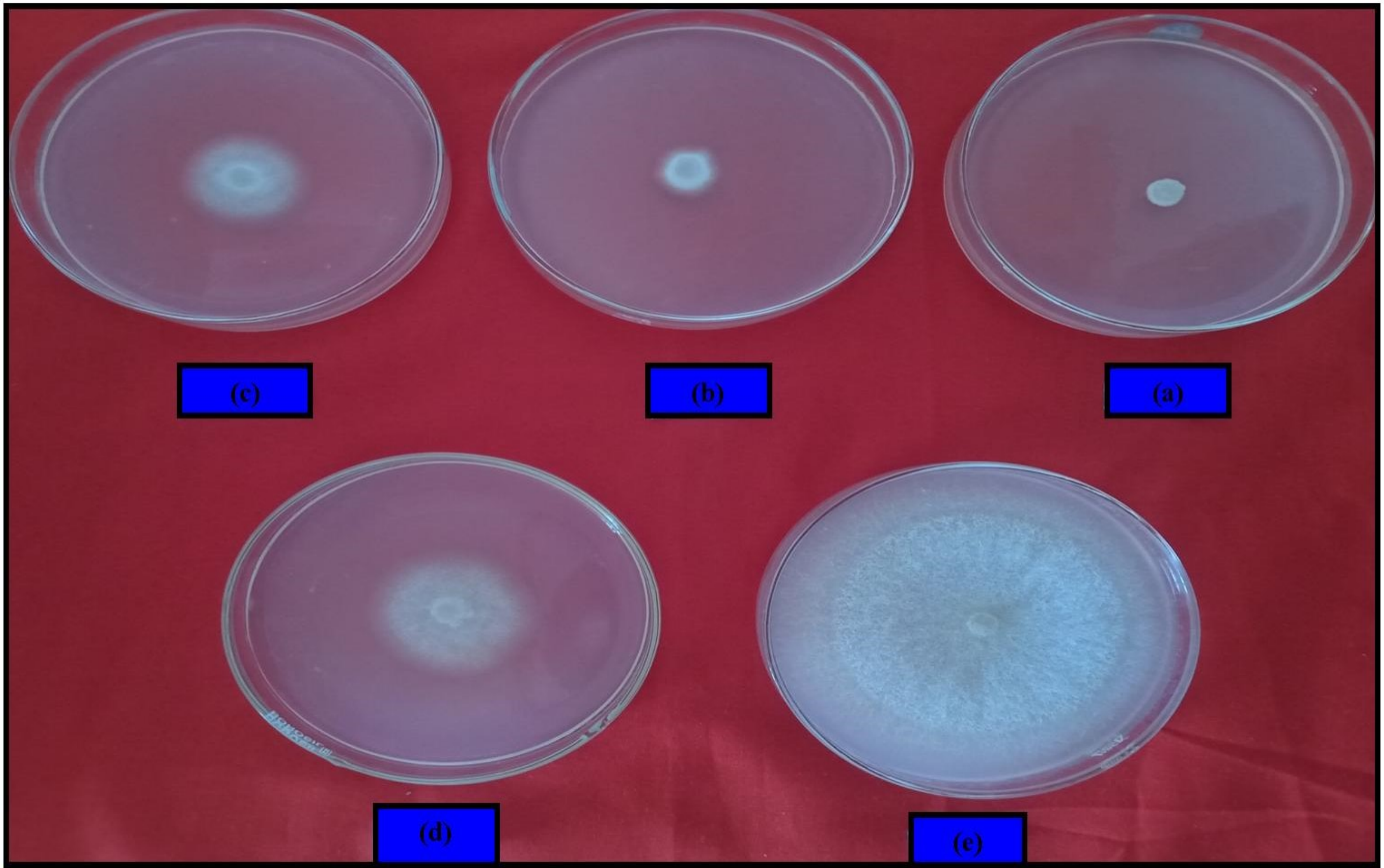


Plate 4.13 Mycelial growth of *Fusarium solani* as affected by ethanol extracts of *Pleurotus ostreatus* spent substrate colonized with *Trichoderma viride*-leached at different concentrations: (a) 4% (b) 3% (c) 2% (d) 1% and (e) Control

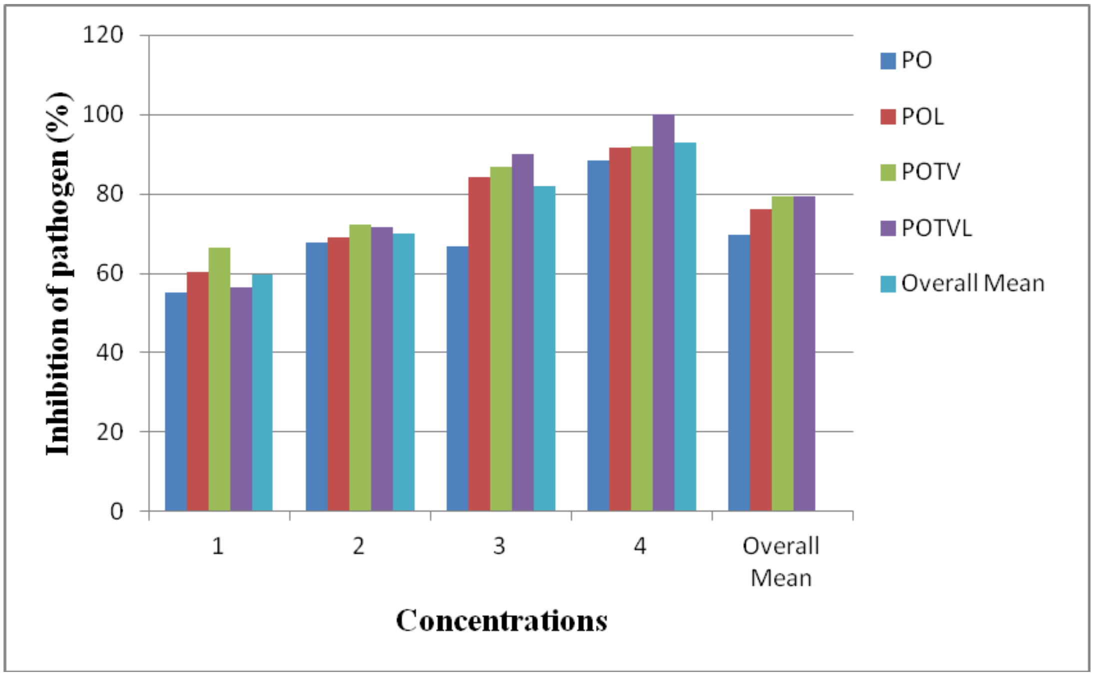


Fig. 4.9 Effect of the ethanol extracts of *Pleurotus ostreatus* spent substrate on per cent inhibition the mycelial growth of *Fusarium solani*

The body of table reveals that no growth of the pathogen at all was recorded in ethanol extract of POTVL spent substrate when used at 4 per cent concentration (0.00 mm) leading to cent per cent growth inhibition followed significantly by ethanol extract of POTV spent substrate (7.33 mm) which was statistically at par with ethanol extract of POL spent substrate (7.67 mm) leading to 91.85 and 91.48 per cent growth inhibition, respectively. However, maximum diametric mycelial growth next to the control (90.00 mm) was recorded in ethanol extract of PO spent substrate at 1 per cent concentration (40.33 mm) followed by ethanol extract of POTVL spent substrate (39.33 mm) at same concentration leading to 55.19 and 56.30 per cent growth inhibition, respectively.

It was evident from present studies that ethanol extracts of the spent mushroom substrates were more effective even at low concentrations than the crude water extracts of same substrates. This high efficiency of ethanol extracts may be attributed to the fact that organic antimicrobial compounds which might have been water insoluble, could have been extracted with organic solvent and exhibited their effect strongly. The results of present study are in agreement with Sultan (2020) who also reported ethanol extracts of *A.bisporus* fruit bodies to be effective against *Aspergillus flavus.*The present finding are also in accordance with the finding of Milovonovic *et al.* (2014) who reported the antifungal properties in ethanol extracts of *P. ostreatus, P. oryngii* and *P. pulmonarius* mycelia. The results are further strengthened by Gutef *et al.* (2020) who reported antifungal potential in the methanol extracts of *P. ostreatus* fruit bodies. As the SMS of each mushroom species remains impregnated with the respective mushroom mycelia, the antimicrobial compounds present in these mycelia must be extracted along with those present in substrate.

4.5. Effect of spent mushroom substrate on the rhizome rot of ginger under pot culture conditions

4.5.1 Naturally infected ginger

In order to study the effect of spent mushroom substrate on the pre emergence rot in naturally infected ginger, spent substrates of all four mushroom species were further evaluated under pot culture conditions because all the extracts gave certain level of pathogen inhibition in the previous experiments. For this, spent substrates were prepared as spent mushroom substrate (SMS), spent mushroom substrate-leached (SMSL), spent mushroom substrate colonized with *T. viride* (SMSTV), spent mushroom substrate colonized with *T. viride*-leached (SMSTVL), for each substrate. These all substrates were applied as soil dressing in the pots at the time of sowing of naturally infected ginger seeds. Two control treatments *viz.* untreated healthy and naturally infected/diseased seed in pots served as positive and negative control, respectively. The data were recorded in terms of pre-emergence rot (%) and post emergence rot (%) and disease control in relation to negative control were further calculated. Data recorded have been presented in Table(s) and figure 4.10 and 4.11.

A perusal of the data presented in table 4.10 clearly reveals that irrespective of the mushroom spent substrate, no pre-emergence rot at all was recorded in SMSTVL leading to cent per cent disease control in this treatment. However, significantly minimum mean (20.58 %) pre-emergence rot was recorded in SMSTV treatment leading to 79.33 per cent disease control. Significantly maximum mean pre-emergence rot (52.67 %) next to control (100 %) was recorded in SMS treatment followed by that in SMSL treatment (40.25 %) leading to 47.33 and 59.58 per cent disease control, respectively. However, no disease at all was recorded in healthy positive control treatment. Irrespective of treatments, significantly minimum mean pre emergence rot (33.44 %) was recorded in *A. bisporus* spent substrate treatment followed by that in *P. sajor-caju* (35.00 %), *P. florida* (36.39 %) and *P. ostreatus* (37.50 %) spent substrate treatments leading to 74.83, 72.42, 70.72 and 68.58 per cent disease control, respectively.

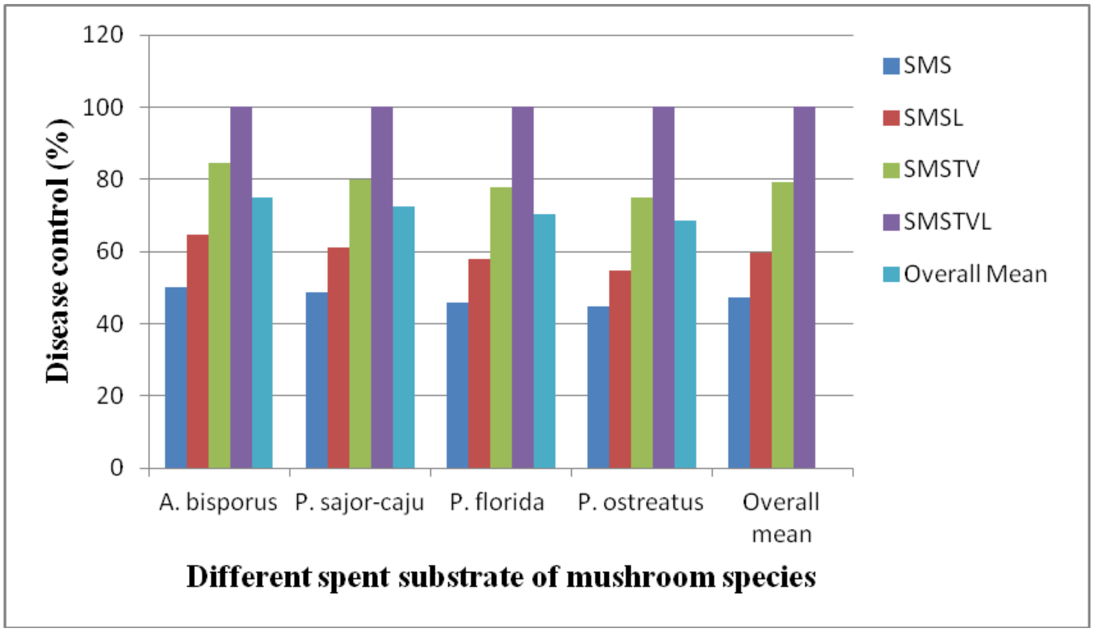


Fig. 4.10 Effect of spent mushroom substrate on per cent disease control of the pre emergence rot in naturally infected ginger under pot conditions

Table 4.10 Effect of spent mushroom substrate on the pre emergence rot in naturally infected ginger under pot conditions

Treatments	**Pre emergence rot (%)**				**Overall Mean**	**Disease Control (%)**				**Overall Mean**
	A. bisporus	***P. sajor caju***	***P. florida***	***P. ostreatus***		***A. bisporus***	***P. sajor caju***	***P. florida***	***P. ostreatus***	
SMS	50.00 (44.98)	51.33 (45.75)	54.00 (47.28)	55.33 (48.04)	52.67 (46.51)	50.00	48.67	46.00	44.67	47.33
SMSL	35.33 (36.46)	39.00 (38.63)	42.00 (40.38)	44.67 (41.92)	40.25 (39.35)	64.67	61.00	58.00	54.67	59.58
SMSTV	15.33 (23.04)	19.67 (26.31)	22.33 (28.19)	25.00 (29.99)	20.58 (26.88)	84.67	80.00	77.67	75.00	79.33
SMSTVL	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100.00	100.00	100.00	100.00	100.00
Control (+)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)					
Control (-)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)					
Overall Mean	33.44 (33.08)	35.00 (34.12)	36.39 (34.98)	37.50 (35.66)		74.83	72.42	70.42	68.58	

Factors	**$CD_{P\geq0.05}$**	**$SE_{(d)}$**
Treatment	0.24	0.12
Substrate	0.19	0.10
Treatment X Substrate	0.48	0.24

Figures in parentheses indicate angular transformed value

Where: SMS= Spent mushroom substrate, SMSL= Spent mushroom substrate leached, SMSTV= Spent mushroom substrate colonized *T. viride,* SMSTVL= Spent mushroom substrate colonized with *T. viride* leached

Body of the table reveals that no pre-emergence rot was recorded in the spent mushroom substrate colonized with *T. viride*-leached of any of the four mushroom species while, significantly minimum (15.33 %) pre-emergence rot was recorded in SMS of *A. bisporus* colonized with *T. viride*-leached treatment leading to 84.67 per cent disease control. Significantly maximum (55.33 %) pre-emergence rot was however recorded in the pots treated with SMS of *P. ostreatus* leading to only 44.67 per cent disease control. Rest of the treatment exhibited intermediate level of pre-emergence rot and disease control.

It is clear from that Table 4.11 that irrespective of mushroom substrate, significantly minimum (11.07 %) mean disease severity was recorded in SMSTVL treatment followed by that in SMSTV (18.54 %), SMSL (24.22 %) and SMS (29.07 %) treatments leading to 88.17, 81.46, 75.98 and 70.93 per cent disease control, respectively (Plate 4.14 and 4.15). Irrespectively of the treatments, significantly minimum mean disease severity (28.24 %) was recorded with *P. sajor-caju* SMS followed by that with *P. florida* (30.22 %) and *P. ostreatus* (31.62 %) spent substrate which was statistically at par with that recorded with *A. bisporus* (31.85 %) spent substrate leading to 82.63, 79.69, 77.57 and 77.41 per cent disease control, respectively. As pre-emergence rot in negative control was recorded to be 100 per cent, the disease severity was also considered to be 100 per cent in this treatment.

Body of the table reveals that significantly minimum disease severity (7.78 %) was recorded in pots treated with *P. sajor-caju* SMSTVL while, maximum disease (31.55 %) next to control (100.00 %) was recorded in pots treated with *A. bisporus* SMS. However, no disease appeared in untreated positive control. Rest all treatments exhibited intermediate level of disease control.

Table 4.11 Effect of spent mushroom substrates on the post emergence rot in naturally infected ginger under pot conditions

Treatments	Post emergence rot (%)				Overall Mean	Disease control (%)				Overall Mean
	A. bisporus	*P. sajor caju*	*P. florida*	*P. oystreatus*		*A. bisporus*	*P. sajor caju*	*P. florida*	*P. oystreatus*	
SMS	31.55 (34.16)	27.27 (31.47)	28.21 (32.07)	29.25 (32.72)	29.07 (32.60)	68.45	72.73	71.79	70.75	70.93
SMSL	25.76 (30.48)	21.25 (27.44)	24.86 (29.89)	25.00 (29.99)	24.22 (29.45)	75.00	78.75	75.19	75.00	75.98
SMSTV	20.97 (27.23)	13.17 (21.26)	18.79 (25.67)	21.25 (27.44)	18.54 (25.40)	79.03	86.83	81.21	78.75	81.46
SMSTVL	12.83 (20.98)	7.78 (16.18)	9.45 (17.89)	14.22 (22.14)	11.07 (19.30)	87.17	92.23	90.55	85.78	88.93
Control (+)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)					
Control (-)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)					
Overall Mean	31.85 (33.80)	28.24 (31.05)	30.22 (32.58)	31.62 (33.71)		77.41	82.63	79.69	77.57	

Factors	**CD$_{P\geq0.05}$**	**SE$_{(d)}$**
Treatment	0.43	0.21
Substrate	0.35	0.17
Treatment X Substrate	0.85	0.42

Figures in parentheses indicate angular transformed values

Where: SMS= Spent mushroom substrate, SMSL= Spent mushroom substrate leached, SMSTV= Spent mushroom substrate colonized *T. viride,* SMSTVL= Spent mushroom substrate colonized with *T. viride* leached

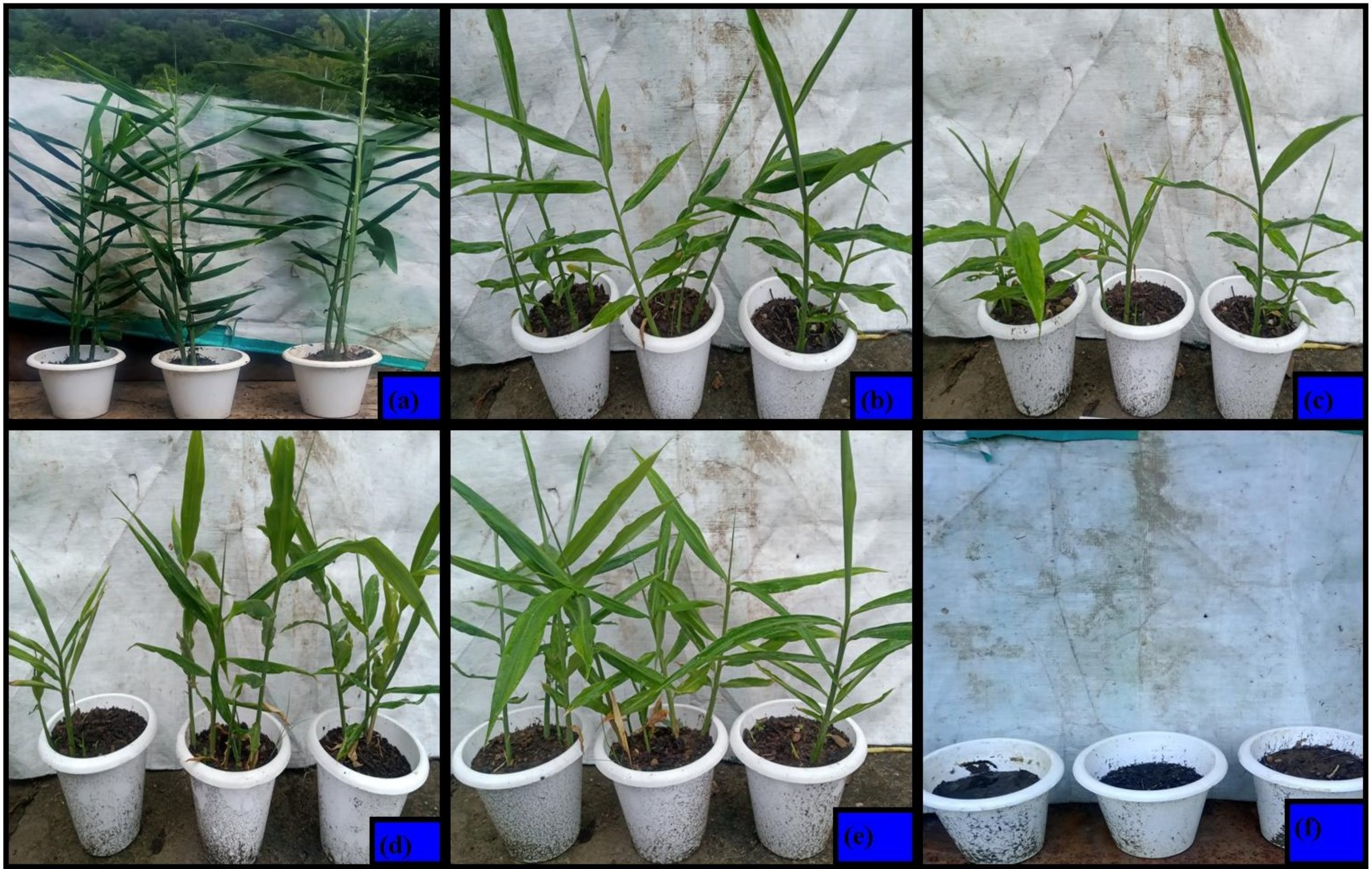


Plate 4.14 Effect of *P. sajor-caju* spent mushroom substrates on the post emergence rot in naturally infected ginger under pot culture conditions (a) Control (+) (b) Spent mushroom substrate colonized with *T. viride*-leached (c) Spent mushroom substrate colonized with *T. viride* (d) Spent mushroom substrate-leached (e) Spent mushroom substrate and (f) Control (-)

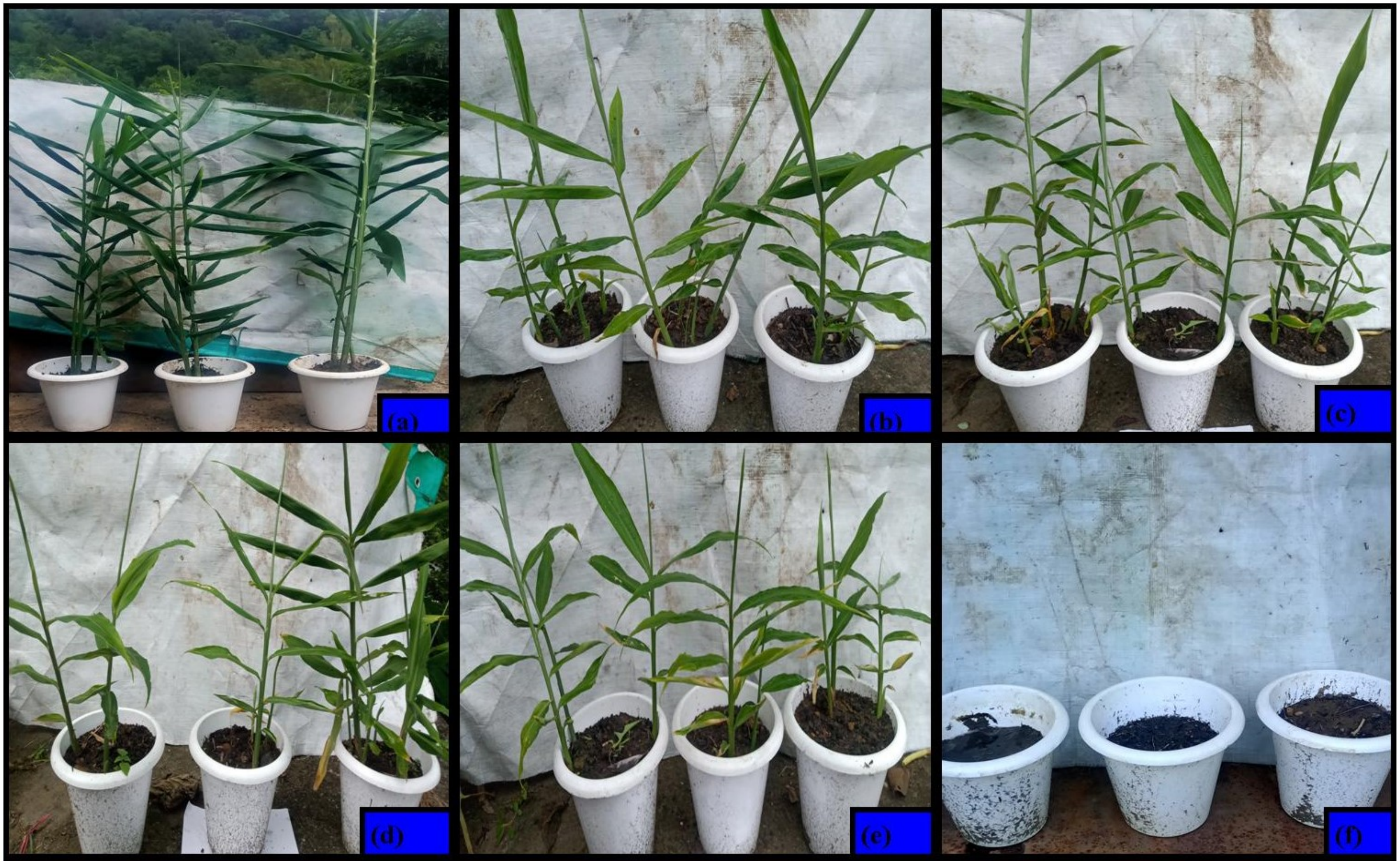


Plate 4.15 Effect of spent mushroom substrates colonized with *Trichoderma viride* on the post emergence rot in naturally infected ginger under pot culture conditions (a) control(+) (b) *Pleurotus sajor-caju* (c) *P. florida* (d) *Agaricus bisporus* (e) *P. ostreatus* and (f) Control(-)

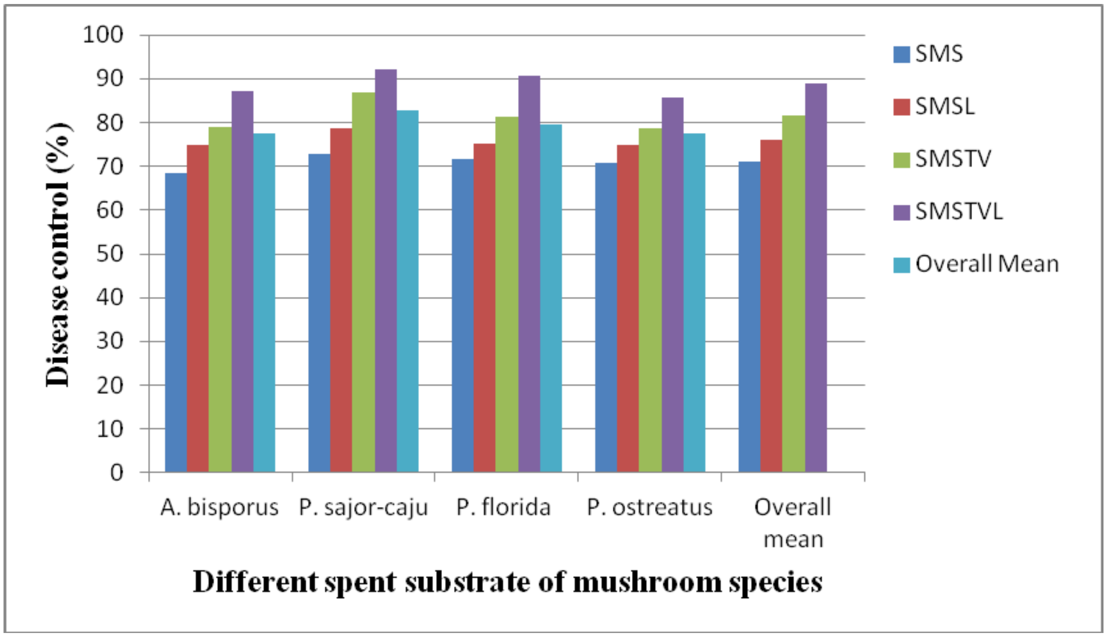


Fig. 4.11 Effect of spent mushroom substrate on per cent disease control of the post emergence rot in naturally infected ginger under pot conditions

4.5.2 Artificially inoculated ginger

In order to study the effect of spent mushroom substrate on the pre emergence rot in artificially inoculated ginger, spent substrates of all four mushroom species were further evaluated under pot culture. For this, spent substrates were prepared as spent mushroom substrate (SMS), spent mushroom substrate-Leached (SMSL), spent mushroom substrate colonized with *T. viride* (SMSTV), spent mushroom substrate colonized with *T. viride*-leached (SMSTVL) for each substrate. These all substrates were applied as soil dressing in the pots at the time of sowing of artificially inoculated ginger seeds. Two control treatments *viz*. un-inoculated un-amended healthy and inoculated un-amended seed in pots served as positive and negative control, respectively. The data were recorded in the terms of pre-emergence rot (%) and post emergence severity (%). Disease control in relation to negative control was further calculated. Data recorded have been presented in table(s) and figures 4.12 and 4.13.

Data presented in Table 4.12 reveals that in addition to positive healthy control, no disease development in any of the SMS Treatment leading to cent per cent disease control. However, irrespective of the specific mushroom substrate, significantly minimum mean pre emergence rot (21.25 %) was recorded in SMSTV treatment leading to 78.75 per cent disease control followed by that in SMSL (32.67 %) and SMS (49.83 %) treatments leading to 67.33 and 50.17 per cent disease control, respectively. Among the various mushroom spent substrates, significantly minimum mean pre-emergence (31.17 %) was recorded in *A. bisporus* spent substrate followed by that in *P. sajor-caju* (32.89 %), *P. florida* (34.94 %) and *P. ostreatus* (36.83 %) leading to 78.25, 75.67, 72.58 and 69.75 per cent disease control, respectively.

Body of the table reveals that SMSTVL treatment of all substrates controlled the disease completely. However, significantly minimum (15.33 %) disease severity was recorded in SMSTVL of *A. bisporus* while, significantly maximum (54.33 %) disease level was recorded in rest of the treatments.

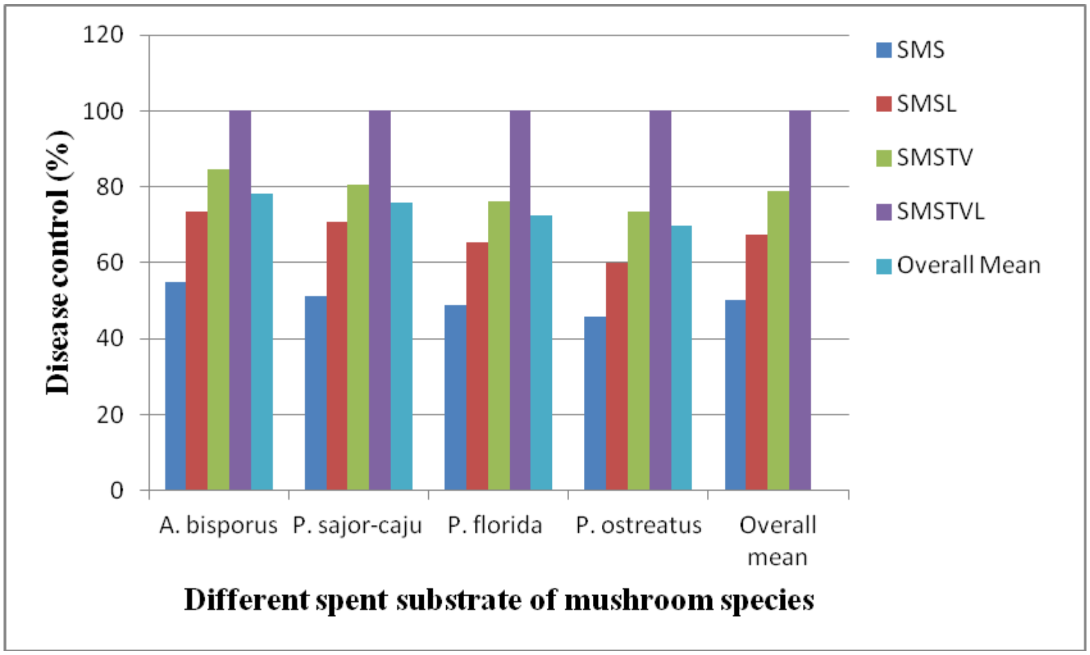


Fig. 4.12 Effect of spent mushroom substrate on per cent disease control of pre emergence rot in artificially inoculated ginger under pot conditions

Table 4.12 Evaluation of spent mushroom substrate on pre emergence rot in artificially inoculated ginger under pot conditions

Treatments	**Pre emergence rot (%)**				**Overall Mean**	**Disease control (%)**				**Overall Mean**
	A. bisporus	***P. sajor caju***	***P. florida***	***P. ostreatus***		***A. bisporus***	***P. sajor caju***	***P. florida***	***P. ostreatus***	
SMS	45.00 (42.11)	48.67 (44.22)	51.33 (45.75)	54.33 (47.47)	49.83 (44.89)	55.00	51.33	48.67	45.67	50.17
SMSL	26.67 (31.06)	29.33 (32.78)	34.67 (36.06)	40.00 (39.22)	32.67 (34.78)	73.33	70.67	65.33	60.00	67.33
SMSTV	15.33 (23.04)	19.33 (26.07)	23.67 (29.10)	26.67 (31.08)	21.25 (27.32)	84.67	80.67	76.33	73.33	78.75
SMSTVL	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100.00	100.00	100.00	100.00	100.00
Control (+)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)					
Control (-)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)					
Overall Mean	31.17 (31.71)	32.89 (32.85)	34.94 (34.15)	36.83 (35.30)		78.25	75.67	72.58	69.75	

Factors	**$CD_{P \geq 0.05}$**	**$SE_{(d)}$**
Treatment	0.42	0.21
Substrate	0.34	0.17
Treatment X Substrate	0.84	0.42

Figures in parentheses indicate angular transformed values

Where: SMS= Spent mushroom substrate, SMSL= Spent mushroom substrate leached, SMSTV= Spent mushroom substrate colonized *T. viride,* SMSTVL= Spent mushroom substrate colonized with *T. viride* leached

Disease severity of post emergence rot in artificially inoculated ginger has been presented in Table 4.13. it is clear from the table that irrespective of mushroom species spent substrate , significantly minimum (8.62 %) disease severity was recorded in SMSTVL followed by that in SMSTV (13.49 %) , SMSL (17.71 %) and SMS (23.26 %) leading to 91.68, 86.51, 82.29 and 76.74 per cent disease control, respectively (Plate 4.16 and 4.17). Irrespective of type of substrate, significantly minimum (25.61 %) disease severity was recorded in *A. bisporus* spent substrate leading to 86.59 per cent disease control while, significantly maximum (29.61 %) disease severity was recorded in *P. ostreatus* spent substrate leading to 80.75 per cent disease control, respectively. As pre-emergence rot in negative control was recorded to be 100 per cent, the disease severity was also considered to be 100 per cent in this treatment.

Body of the table reveals that minimum (7.08 %) disease severity was recorded in SMSTVL of *P. sajor-caju* which was statistically at par with that recorded in SMSTVL of *A. bisporus* (7.39 %) leading to 92.92 and 92.68 per cent disease control, respectively. However, significantly maximum (25.22 %) severity was recorded in SMS of *P. ostreatus* leading to 74.78 per cent disease control. Rest of the treatments resulted in intermediate level of disease control.

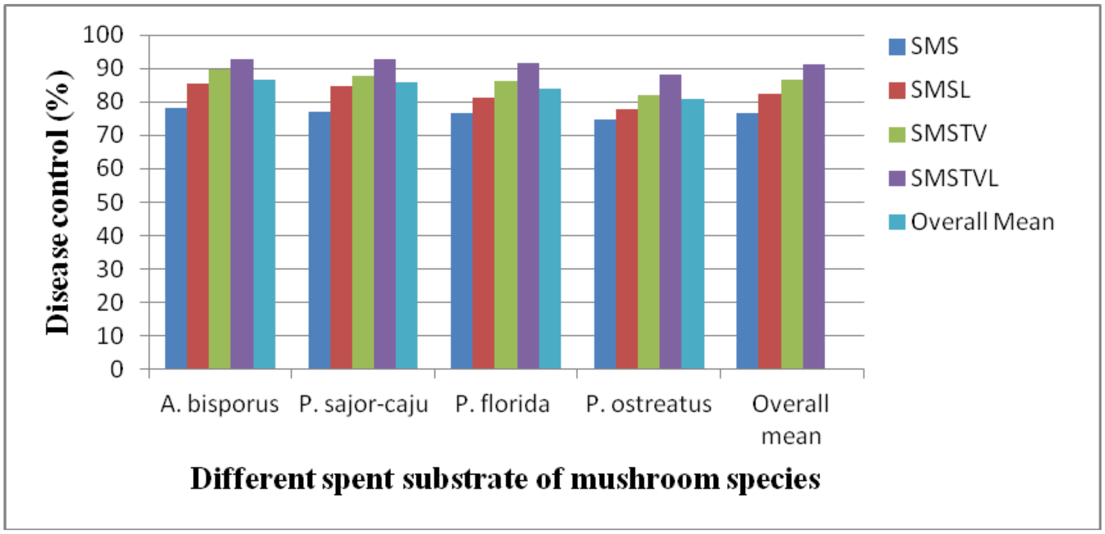


Fig. 4.13 Effect of spent mushroom substrate on per cent disease control of post emergence rot in artificially inoculated ginger under pot conditions

Table 4.13 Effect of spent mushroom substrates on the post emergence rot in artificially inoculated ginger under pot conditions

Treatments	Post emergence rot (%)				Overall Mean	Disease control (%)				Overall Mean
	A. bisporus	*P. sajor caju*	*P. florida*	*P. oystreatus*		*A. bisporus*	*P. sajor caju*	*P. florida*	*P. oystreatus*	
SMS	21.69 (27.74)	22.92 (28.59)	23.22 (28.80)	25.22 (30.13)	23.26 (28.82)	78.31	77.07	76.78	74.78	76.74
SMSL	14.49 (22.37)	15.25 (22.98)	18.94 (25.78)	22.16 (28.07)	17.71 (24.80)	85.51	84.75	81.06	77.84	82.29
SMSTV	10.14 (18.56)	12.12 (20.36)	13.83 (21.82)	17.89 (25.01)	13.49 (21.44)	89.86	87.88	86.17	82.11	86.51
SMSTVL	7.32 (15.69)	7.08 (15.41)	8.33 (16.77)	11.74 (20.03)	8.62 (16.97)	92.68	92.92	91.67	88.26	91.38
Control (+)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)					
Control (-)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)					
Overall Mean	25.61 (29.05)	26.23 (29.55)	27.39 (30.52)	29.50 (32.20)	25.61 (29.05)	86.59	85.66	83.92	80.75	

Factors	**CD$_{P\geq0.05}$**	**SE$_{(d)}$**
Treatment	0.33	0.16
Substrate	0.27	0.13
Treatment X Substrate	0.66	0.33

Figures in parentheses indicate angular transformed values

Where: SMS= Spent mushroom substrate, SMSL= Spent mushroom substrate leached, SMSTV= Spent mushroom substrate colonized *T. viride,* SMSTVL= Spent mushroom substrate colonized with *T. viride* leached

These findings are supported by Remya and Paul (2014) who reported that SMS of *P. sajor* caju as well as *P. florida* was an effective management practice against *P. aphanidermatum* causing soft rot of ginger. These findings are further supported by Ntougias *et al.* (2008) who reported the SMS of *A. bisporus* to be effective against soil-borne and foliar pathogens *viz., Phyophthora nicotianae*, *F. oxysporum* f.sp. *radicis-lycopersici* and *Septoria lycopersici* of tomato. Our results are also in agreement with Yusidah and Istifadah (2018) who reported that basal rot disease of shallot caused by *F. oxysporum* f.sp *cepae* was managed by use of organic matters including spent mushroom substrate (SMS) of oyster mushroom (*Pleurotus ostreatus*), straw mushroom (*Volvariella volvaceae*) and shiitake (*Lentinula edodes*). The results are also in conformity with Wang *et al.* (2020) who reported the suppressive capacity oft spent mushroom substrate (SMS) of *P. ostreatus* amendments against Fusarium wilt.

Colonization of spent mushroom substrates with *T. viride* increased the level of disease control in all the type of substrates and when this colonized substrate was leached for 10 days, it further improved its efficacy against the pathogen. *Trichoderma* spp. have already been reported to reduce the incidence of rhizome rot of ginger (Bundopadhyay and Bhattacharya, 2018; Khatso and Tiameren, 2013). Leaching of this colonized substrate for 10 days might have leached out excessive organic and inorganic salts along with some antibiotic compounds from the substrate, but might have intensified the antibiotic activity as well as better mycelial spread of *T. viride* in the substrate leading to better disease control in these treatments.

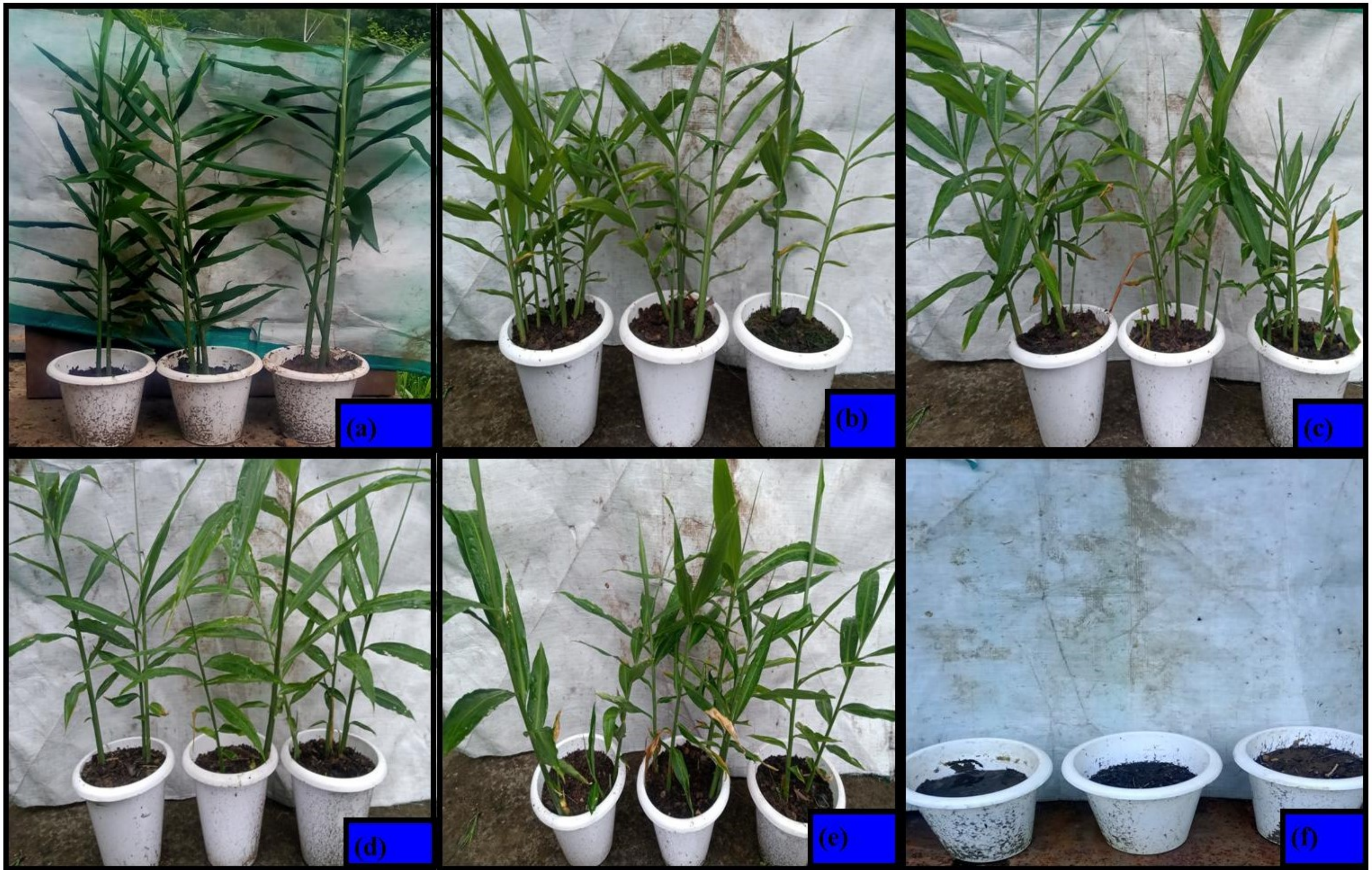


Plate 4.16 Effect of *Agaricus bisporus* spent mushroom substrates on the post emergence rot in artificially inoculated ginger under pot culture conditions (a) Control (+) (b) Spent mushroom substrate colonized with *T. viride*-leached (c) Spent mushroom substrate colonized with *T. viride* (d) Spent mushroom substrate-leached (e) Spent mushroom substrate and (f) Control (-)

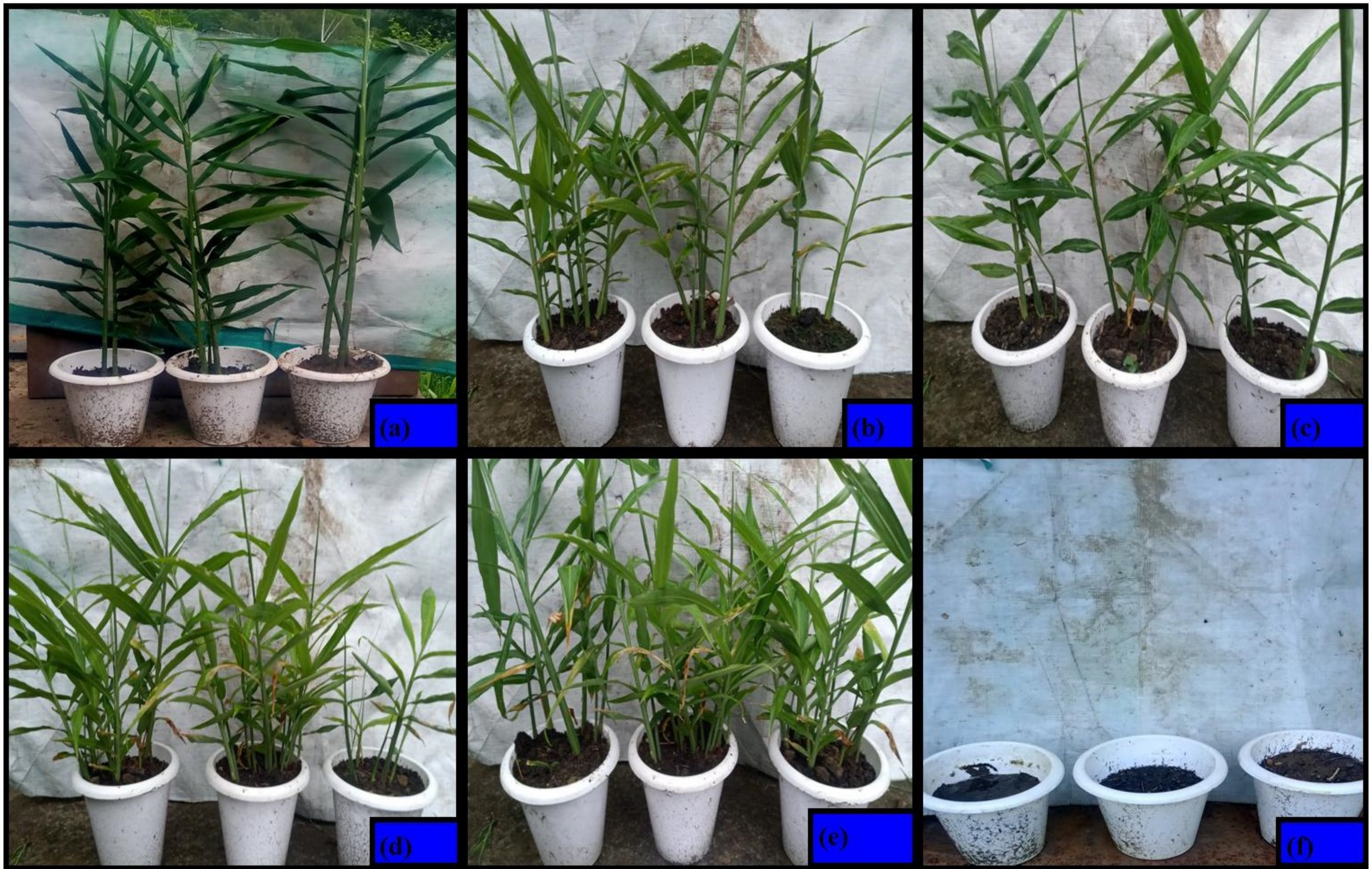


Plate 4.17 Effect of spent mushroom substrates colonized with *Trichoderma viride* on the post emergence rot in artificially inoculated ginger under pot culture conditions (a) control(+) (b) *Pleurotus sajor-caju* (c) *Agaricus bisporus* (d) *Pleurotus florida* (e) *Pleurotus ostreatus* and (f) Control (-)

Chapter-5

SUMMARY AND CONCLUSION

Present investigation entitled **"Evaluation of spent mushroom substrate for the management of rhizome rot of ginger"** were carried out during the year 2020-2021. The results obtained from present studies are briefly summarized as under:

5.1 *Fusarium solani* was found to be associated with rhizome rot of ginger.

5.2 Among the leachates of different spent substrates tested, all the leachates were found to be effective against the pathogen to certain extent. Maximum per cent inhibition of pathogen (46.29 %) was recorded in leachate of *P. sajor-caju* spent substrate colonized with *T. viride* (PSCTVLe) followed by that in leachate of *P. florida* spent substrate (42.12 %) colonized with *T. viride* (PFTVLe) and that in leachate of *P. ostreatus* (40.27%) spent substrate colonized with *T. viride* (POTVLe).

5.3 Effect of aqueous extracts of *A. bisporus* spent substrate was studied and it was observed that all the aqueous extracts of the *A. bisporus* spent substrate were effective against the pathogen to certain extent. Maximum per cent inhibition of pathogen (51.02 %) was recorded in aqueous extract of *A. bisporus* spent substrate colonized with *T. viride*-leached (ABTVL) followed by extract of *A. bisporus* spent substrate (48.24 %) colonized with *T. viride* (ABTV) and *A. bisporus* spent substrate-leached (ABL, 44.35 %).

5.4 Out of four different aqueous extracts of *P. sajor-caju* spent substrate evaluated *in vitro* against test pathogen, maximum per cent inhibition of pathogen (51.66 %) was recorded in aqueous extract of *P. sajor-caju* spent substrate colonized with *T. viride*-leached (PSCTVL) followed by extract of *P. sajor-caju* spent substrate (50.83 %) colonized with *T. viride* (PSCTV) and *P. sajor-caju* (48.24 %) spent substrate-leached (PSCL).

5.5 Among the aqueous extracts of *P. florida* spent substrate tested, maximum per cent inhibition of pathogen (48.80 %) was recorded in aqueous extract of *P. florida* spent substrate colonized with *T. viride*-leached (PFTVL) followed by extract of *P. florida* spent substrate (47.59 %) colonized with *T. viride* (PFTV).

5.6 Out of four different aqueous extracts of *P. ostreatus* spent substrate evaluated *in vitro* against test pathogen, maximum per cent inhibition of pathogen (73.80 %)

was recorded in aqueous extract of *P. ostreatus* spent substrate colonized with *T. viride*-leached (POTVL) followed by extract of *P. ostreatus* (63.70 %) spent substrate colonized with *T. viride* (POTV) and *P. ostreatus* (61.76 %) spent substrate-leached (POL).

5.7 Among the ethanol extracts of *A. bisporus* spent substrate tested, all the extracts were found to be effective against the pathogen. Maximum per cent inhibition of pathogen (58.51 %) was recorded in ethanol extract of ABTVL followed by that in extract of ABTVL (55.37 %) and that in extract of ABL spent substrate (52.22 %).

5.8 Effect of ethanol extracts of *P. sajor-caju* spent substrate was studied and it was observed that all the ethanolic extracts were effective against the pathogen to certain extent. In ethanol extract of PSCTVL at 20 per cent concentration, cent per cent inhibition were recorded next to that maximum per cent inhibition of pathogen (86.29 %) was recorded in ethanol extract of PSCTVL spent substrate followed by extract of PSCTV spent substrate (82.58 %) and PSCL spent substrate (75.09 %).

5.9 Among the ethanol extracts of *P. florida* spent substrate tested, all the extracts were found to be effective against the pathogen to certain extent. In ethanol extract of PFTVL and PFTV at 20 per cent concentration, cent per cent inhibition were recorded next to that maximum per cent inhibition of pathogen (93.06 %) was recorded in ethanol extract of PFTVL spent substrate followed by extract of PFTV spent substrate (89.63 %) and PFL spent substrate (88.43 %).

5.10 Out of four different ethanol extracts of *P. ostreatus* spent substrate was evaluated *in vitro* against test pathogen. In ethanol extract of POTVL at 20 per cent concentration resulted in cent per cent inhibition of the pathogen next to that maximum per cent inhibition of pathogen (79.45 %) was recorded in aqueous extract of POTVL spent substrate followed by extract of POTV spent substrate (79.26 %) and POL spent substrate (76.20 %).

5.11 Among the different spent mushroom substrates evaluated under pot culture condition, all the substrates were found to be effective against the pathogen to certain extent. In naturally infected ginger, spent mushroom substrate colonized with *T. viride* in all substrate and leached for 10 days resulted in cent per cent reduction of pre emergence rot in both naturally infected and artificially inoculated ginger followed by disease control recorded in *P. sajor-caju* substrate (88.11%). Same treatment proved best in managing the post emergence rot in both naturally infected (88.93%) and artificially inoculated ginger (91.38%).

From these studies it is conducted that *Fusarium solani* was associated with rhizome rot of ginger. The pathogen could be managed *in vitro* with crude aqueous and ethanol extracts of spent mushroom substrate of *A. bisporus, Pleurotus sajor-caju, P. florida* and *P. ostreatus* in different variants. However, ethanol extractswre found more effective than aqueous extracts. Under pot culture also the spent substrates of all these mushrooms colonized with *T. viride* and leached for 10 days were quite effective in managing the pre and post emergence rot of ginger.

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APPENDIX

Anova 4.1 Effect of the spent mushroom substrate leachate on the mycelial growth of *Fusarium solani*

Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**F-Calculated**	**Significance**
Treatment	8	14,699.296	1,837.412	3,816.163	0.00000
Concentration	3	6,149.074	2,049.691	4,257.051	0.00000
Treatment X Concentration	24	927.593	38.650	80.272	0.00000
Error	72	34.667	0.481		
Total	107	21,810.630			

Anova 4.2 Effect of the aqueous extracts of *A. bisporus* substrate on the mycelial growth of *Fusarium solani*

Source of Variation	**DF**	**Sum of Squares**	**Mean Squares**	**F-Calculated**	**Significance**
Treatment	4	17,149.767	4,287.442	9,527.648	0.00000
Concentration	3	1,727.783	575.928	1,279.840	0.00000
Treatment X Concentration	12	484.633	40.386	89.747	0.00000
Error	40	18.000	0.450		
Total	59	19,380.183			

Anova 4.3 Effect of the aqueous extracts of *Pleurotus sajor-caju* spent substrate on the mycelial growth of *Fusarium solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	4	18,654.767	4,663.692	10,762.365	0.00000
Concentration	3	1,438.133	479.378	1,106.256	0.00000
Treatment X Concentration	12	435.367	36.281	83.724	0.00000
Error	40	17.333	0.433		
Total	59	20,545.600			

Anova 4.4 Effect of the aqueous extracts of *Pleurotus florida* spent substrate on the mycelial growth of *Fusarium solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	4	17,618.833	4,404.708	11,011.771	0.00000
Concentration	3	1,142.183	380.728	951.819	-0.00000
Treatment X Concentration	12	320.233	26.686	66.715	-0.00000
Error	40	16.000	0.400		
Total	59	19,097.250			

Anova 4.5 Effect of the aqueous extract of *Pleurotus ostreatus* spent substrate on the mycelial growth of *Fusarium solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	4	33,166.567	8,291.642	17,767.804	0.00000
Concentration	3	4,495.333	1,498.444	3,210.952	0.00000
Treatment X Concentration	12	1,405.167	117.097	250.923	0.00000
Error	40	18.667	0.467		
Total	59	39,085.733			

Anova 4.6 Effect of the ethanol extracts of *Agaricus bisporus* spent substrate on the mycelial growth of *Fusarium solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	22,996.667	5,749.167	18,155.263	0.00000
Concentration	3	1,690.583	563.528	1,779.561	-0.00000
Treatment X Concentration	12	443.333	36.944	116.667	0.00000
Error	40	12.667	0.317		
Total	59	25,143.250			

Anova 4.7 Effect of the ethanol extracts of *Pleurotus sajor-caju* spent substrate on the mycelial growth of *Fusarium solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	4	21,625.016	5,406.254	18,519.552	0.00000
Concentration	3	2,148.757	716.252	2,453.579	0.00000
Treatment X Concentration	12	692.313	57.693	197.631	0.00000
Error	40	11.677	0.292		
Total	59	24,477.763			

Anova 4.8 Effect of the ethanol extracts of *Pleurotus florida* substrate on the mycelial growth of *Fusarium solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	4	28,463.314	7,115.829	26,125.291	0.00000
Concentration	3	1,247.896	415.965	1,527.190	0.00000
Treatment X Concentration	12	439.833	36.653	134.568	-0.00000
Error	40	10.895	0.272		
Total	59	30,161.939			

Anova 4.9 Effect of the ethanol extracts of *Pleurotus ostreatus* spent substrate on the mycelial growth of *Fusarium solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	4	19,788.814	4,947.203	18,338.718	0.00000
Concentration	3	3,037.383	1,012.461	3,753.078	0.00000
Treatment X Concentration	12	1,107.620	92.302	342.152	0.00000
Error	40	10.791	0.270		
Total	59	23,944.608			

Anova 4.10 Effect of spent mushroom substrate on the pre emergence rot in naturally infected ginger under pot house conditions

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	5	56,674.117	11,334.823	136,498.689	0.00000
Substrate	3	67.052	22.351	269.156	-0.00000
Treatment X Substrate	15	79.508	5.301	63.831	-0.00000
Error	48	3.986	0.083		
Total	71	56,824.663			

Anova 4.11 Effect of spent mushroom substrates on the severity of rhizome rot in naturally infected ginger under pot house conditions

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	5	46,745.002	9,349.000	34,989.628	0.00000
Substrate	3	88.765	29.588	110.738	0.00000
Treatment X Substrate	15	81.911	5.461	20.437	0.00000
Error	48	12.825	0.267		
Total	71	46,928.503			

Anova 4.12 Evaluation of spent mushroom substrate on pre emergence rot in artificially inoculated ginger

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	5	55,806.693	11,161.339	42,631.026	0.00000
Substrate	3	131.429	43.810	167.332	0.00000
Treatment X Substrate	15	144.148	9.610	36.705	-0.00000
Error	48	12.567	0.262		
Total	71	56,094.837			

Anova 4.13 Effect of spent mushroom substrates on the post emergence severity of rhizome rot in artificially inoculated ginger

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	5	48,841.102	9,768.220	60,973.574	-0.00000
Substrate	3	103.934	34.645	216.254	-0.00000
Treatment X Substrate	15	75.011	5.001	31.215	0.00000
Error	48	7.690	0.160		
Total	71	49,027.737			

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ABSTRACT

The present study entitled **"Evaluation of spent mushroom substrate for the management of rhizome rot of ginger"** was conducted in Department of Plant Pathology, COHF, Neri. During the study, it was found that *Fuasrium solani* was associated with rhizome rot of ginger. Among eight leachates of four different mushroom species, leachate of *Pleurotus sajor-caju* spent substrate colonized with *Trichoderma viride* (46.29 % inhibition) was found superior to all other leachates. Out of different aqueous extracts of four mushroom spent substrate under study, spent substrate colonized with *T. Viride*-leached of *Agaricus bisporus* (51.02 % inhibition,) *P. sajor-caju* (51.66 % inhibition), *P. florida* (48.80 % inhibition) and *P. ostreatus* (73.80 % inhibition) proved better than their respective counterpart. Among the four different ethanol extracts of *A. bisporus* spent substrate, ethanol extract of *A. bisporus* spent substrate colonized with *T. viride*-leached (58.51 % inhibition) was found best to inhibit the pathogen. Out of four different ethanol extracts of *P. sajor-caju* spent substrate, ethanol extract of *P. sajor-caju* spent substrate (86.29 % inhibition) colonized with *T. viride*-leached used at 4 per cent concentration was found best in which cent per cent inhibition of pathogen was recorded. Among the four different ethanol extracts of *P. florida* spent substrate, ethanol extract of *P. florida* spent substrate colonized with *T. viride*-leached (93.06 % mean inhibition) followed by *P. florida* spent substrate colonized with *T. viride* (89.63 % mean inhibition) both used at 4 per cent concentration were found to completely inhibition of pathogen (100% inhibition). Out of four different ethanol extracts of *P. ostreatus* spent substrate, ethanol extract of *P. ostreatus* spent substrate colonized with *T. viride*-leached (79.45 % inhibition) used at 4 per cent concentration was found to completely inhibit the pathogen. Among the different spent mushroom substrates evaluated under pot culture conditions, spent mushroom substrate colonized with *T. viride* and leached for 10 days of all substrates resulted in cent per cent reduction of pre emergence rot in both naturally infected and artificially inoculated ginger. Among the four species, spent substrate of *P. sajor-caju* substrate (88.11%) was found to be best in disease management. Spent mushroom substrate colonized with *T. viride* of all substrates and leached for 10 days treatment proved best in managing the post emergence rot in both naturally infected (88.93%) and artificially inoculated ginger (91.38%) and was found to be best in disease management.

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