Studies on management of spent mushroom substrate

By

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CERTIFICATE – I

This is to certify that this thesis entitled: "**Studies on management of spent mushroom substrate"** submitted for the degree of Master of Science in the subject of **Plant Pathology** to **Chaudhary Charan Singh Haryana Agricultural University, Hisar** is a bonafide research work carried out by **Mr. Suresh Verma**, **Admn. No. 2012A77M** under my supervision and no part of the thesis has been submitted by him for any other degree.

The assistance and help received during the course of investigation have been duly acknowledged.

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CERTIFICATE – II

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CHAPTER-I

INTRODUCTION

The ever-increasing human demand for protein-rich food and the inefficiency of conventional methods have resulted in the need to explore alternatives for low cost production of unconventional protein-rich food (Mukherjee and Nandi, 2004). One of the options is edible fungi or mushrooms belonging to the Basidiomycetes. To date, the mushroom industry is an industry with world production greater than 25 mi tones. The current largest mushroom producer of mushrooms, China has attained more than 20 mi tones and accounted for over 80 per cent of the world's mushroom production (Li, 2012).

Spent Mushroom Substrate (SMS) or Spent Mushroom Compost (SMC) is a by-product of mushroom production. It results from the production growing media that is removed from the mushroom farm after the completion of harvest. In India, approximately 1,64,000 mi tones of SMS is produced (NHB, 2013). In Ireland, approximately 254,000 tones of SMS is generated each year (Barry *et al*., 2012) and in Netherlands, more than 800,000 tones of SMS is produced per year (Oei and Albert, 2012). In some countries, waste management of SMS is a major problem faced by farmers. Apparently, the obvious solution is to increase the demand for SMS through exploration of new applications for utilization. It would be more economical and favourable if SMS is to be recycled and reused. Often SMS is regarded as an agricultural waste product with little inherent value; yet there is still value in the substrate as it is rich in nutrients and organic matter and can provide benefits to other agricultural or non-agricultural sectors. SMS released after mushroom crop harvesting may cause various environmental problems because the large dumped piles of this substrate become anaerobic and give off offensive odour causing ground water contamination and nuisance (Beyer, 1996).

Mushroom industry needs to dispose off more than 50 mi tones of used mushroom compost each year (Fox and Chorover, 1999). The SMS has been found to be a good nutrient source for agriculture because of its rich nutrient status and slow mineralization rate which retain its quality as an organic matter (Dann, 1996). SMS possesses the quality of good organic manure for raising healthy crops of cereals, fruits, vegetables and ornamental plants. In addition to its ability of reclaiming the contaminated soil, SMS is utilized for nutritionally poor soil, neutralizing acidic soil and in other cases for improving polluted sites (Pannier, 1993; Ahlawat and Rai, 2002; Ahlawat *et al*., 2005). Although FYM is mostly being used for production of organic food but its poor availability has restricted the production of organic crops at large scale.

The spent mushroom substrate (SMS) released after button mushroom cultivation contains all the essential nutrients needed for raising a healthy field crop in addition to harbor fungal biomass and large population of heterotrophic microbes (Pill *et al*., 1993). The application of SMS plays vital role in disease management in crops, top dressing and soil amendments promotes a population of antagonistic microorganisms (crop friendly microorganisms), which interfere with the activities of pathogenic fungi. Aged compost, on recolonization with mesophilic bacteria, heterotrophic fungi or actinomycetes, mitigates plant diseases as well. This also stimulates a natural disease defense system in plants (Yohalem *et al.*, 1994).

The thousand tons of waste obtained from mushroom cultivation known as SMS, is being dumped on the road-side and awareness about its proper utilization is not much available. In Haryana, being the leading state in cultivation of button mushroom, SMS in excess of 50,000 tones is generated per year which is not properly utilized. So, keeping this in view, the present work was undertaken for following objectives:

1. Quantification of physico-chemical composition of SMS.
2. Antagonistic effect of beneficial mycoflora present in SMS against *Rhizoctonia solani* causing damping-off and root rot in tomato.

CHAPTER-II

REVIEW AND PATENT SEARCH

Spent mushroom substrate (SMS) is produced in very large quantity from the mushroom farms. Although it is known as 'spent' but it can be reused as an organic manure in agricultural fields. Being rich in macro and micro nutrients, it can act as a supplier of nutrients to the plants. SMS has been used for disease management in a number of crops, mainly against the root-borne and soil-borne plant pathogens. The subject of studies on management of spent mushroom substrate has been reviewed under the following headings:

2.1 SPENT MUSHROOM SUBSTRATE (SMS)

Growing of mushrooms on large scale have been providing employment for several people in the developed and developing countries of the world (Aina *et al.*, 2012a). It has been widely reported that commercial mushroom cultivation use substrates like wheat straw, poultry manure, gypsum, horse manure etc. (Beyer, 1996; Vijay, 2005; Jonathan and Adeoyo, 2011; Omarini, 2010; Jonathan *et al.*, 2012b). Correspondingly, for every kilogram of mushroom cultivated, 3-5 kg of SMS is generated (Semple *et al.*, 2001; Lau *et al.*, 2003). Singh *et al.* (2011) stated that average farm discards about 24 tones of SMS per month. Uncontrolled disposal of SMS may pose a problem to the environment, therefore, there is a need to convert this waste into harmless substances (waste to wealth) which will be useful for agricultural use or to products that will be environmentally friendly.

In India, the annual production of mushroom is 41000 mi tones from which approximately 1,64,000 mi tones of SMS is produced. In Haryana alone, more than 50000 tones of SMS is generated from the mushroom farms (NHB, 2013). Despite numerous benefit of mushroom cultivation, disposal of spent mushroom substrate have been known of constituting nuisance pollutants to our environment. These problems may include foul odour arising from the piling up of SMS after various flushes of mushrooms have been harvested. Dumping of SMS indiscriminately may also lead to disease outbreak or unwarranted health

risk especially when these were disposed at a close range to where people live. It has been reported that unwanted SMS disposal may serve as contamination to ground water sources in villages and rural area (Beyer, 1996). There are environmental problems associated with disposal of organic wastes, such as high levels of nutrients from organic wastes polluting waterways (Hoitink and Fahy, 1986). Further, regulations regarding disposal of organic materials into landfills have increased, making composting an attractive alternative (Stoffella and Kahn, 2001).

2.1.1 SMS AS ORGANIC MANURE

Conversion of SMS into an organic-mineral fertilizer is an alternate way of using spent mushroom compost for soil amelioration and to make it a balanced source of nutrition for plant growth. Thousand tones of SMS as a by-product obtained from mushroom cultivation recomposed through various methods such as natural weathering, aerobic recomposting and anaerobic recomposting, and used as organic manure in different agricultural and horticultural crops *viz.* wheat, tomato, ginger, cauliflower, cucurbits, brinjal and onion. SMC have been reported of containing nutrients which could be used for the growth of useful photosynthetic plants (Fasidi *et al.,* 2008 and Gbolagade *et al.,* 2006).

It serves as supplier of both, organic matter that improves soil structure and as source of macro and micro elements for plant nutrition (Bayer, 2006; Jordan *et al.,* 2008). It can supply nutrients and increase the water-holding capacity of the soil (Polat *et al.,* 2004). The demand for organic residues and compost has also increased several folds considering the ill effects of synthetic pesticides and fertilizers. Therefore, there is a need to look for an alternative source of organic fertilizers that will boost the growth and production of vegetables by the local farmers. Organic farms need to be supplied with nitrogen through sources such as SMC (Chefetz *et al.,* 2000). Also SMC has many attributes aiding its exploitation in place of inorganic farm yard manure (FYM) in raising organic field crops and environment management (Ahlawat *et al.,* 2007).

Spent mushroom substrate has the potential to improve soil structure and provide nutrients when tilled into the soil as an amendment to a planting bed or when used as top-dressing mulch during landscape establishment (American, 1995; Guo and Chorover, 2004). Further, composts such as SMS can increase soil organic matter which improves soil quality by improving soil structure, water infiltration and retention, nutrient content and buffering capacity (Steffen *et al.,* 1994). Stewart *et al.* (1998) reported that applications of SMS increased soil pH and provided plant-available nutrients. However, they also reported that SMS did not provide sufficient plant-available nitrogen and may need to be supplemented with inorganic nitrogen. Steffen *et al.* (1994) found that systems amended with well-rotted cattle manure and SMS had higher yields of different vegetable crops continuing through three growing seasons compared to inorganic fertilizer. Further, the higher costs of the

organic amendments would be compensated with greater yields and benefits continuing over several growing seasons. As SMS contains a considerable amount of nutrients and is generally not phytotoxic, it has been used as a soil amendment (Wang, 1977; Wang *et al.*, 1984; Kaddous and Morgan, 1986; Maynard, 1994). The phytonutritive capacity of compost has often been demonstrated to be analogous to that of manure; the same level of productivity, both quantitatively and qualitatively, can be maintained by replacing manure with compost (Beyca *et. al*., 1993). Weathered or outdoor-aged mushroom compost has been used as an organic fertilizer and soil amendment for plant production in agriculture and horticulture (Chong *et al.*, 1991b; Lohr *et al.*, 1984a; Maher, 1994).

2.1.2 PHYSICO-CHEMICAL PROPERTIES

The quantification of doses of SMS application in various crops has been worked out on the basis of total NPK requirements of the respective crops and the nutrient status of SMS. The recomposted SMS should be used singly as basal application or in combination with inorganic fertilizer (Maher, 1994). SMS being rich in nutrients adds nutrition to the soil, helps in neutralizing acidic soils, facilitates plant growth in barren areas and in some cases, it improve water quality along with bioremediation of contaminated industrial sites. In order to improve the physico-chemical characteristics of SMS for its use as manure recomposting method can be employed (Buswell, 1994). The SMS obtained from different sources usually has conductance in the range of 1.9 to 8.3 mmhos cm^{-1}. There are contradictory reports regarding the pH of fresh and weathered SMS. With the increasing recomposting period, pH decreases in SMS of all mushrooms, while conductivity increase in oyster and paddy straw mushroom spent substrates but not in button mushroom substrate. Lohr *et al.* (1984) observed that EC of fresh SMS was 1 mmhos cm^{-1} while that of aged SMS was 1.22 mmhos cm^{-1}. The pH of fresh SMS was found to be slightly alkaline in fresh SMS, which gradually decreased to 6.7 after aging. Fidanza *et al.* (2010) found that pH of SMS fall in range of 6.7 to 7.8 and EC was very high ranging from 10.5 to 14.9 mmhos cm^{-1}. According to Wuest and Fahy (1991), SMS has an initial pH of around 7.28 which increases during weathering while Devonald (1987) reported pH of the fresh SMS in the range of 7.01 to 8.04. The values of pH and EC in SMS are 6.6 to 9.0 and 21 to 66 mmhos cm^{-1}, respectively (Guo *et al.*, 2001) while Gupta *et al.* (2004) stated that the EC and pH of 6 months old SMS was 7.3 and 6.2 mmhos/cm, respectively.

Weathering causes a slow decrease in the organic matter contents (volatile solids) (Beyer, 1999). Guo *et al.* (2001) found that the leachate from 24 months old naturally weathered piles of 3’ and 5’ SMS depth contains 0.8-11.0 per cent of dissolved organic carbon. The organic carbon in 6 months old SMS was found to be 2.51 per cent by Gupta *et al.*, (2004).

SMS normally contains 1.9:0.4:2.4 per cent, N-P-K before weathering and 1.9:0.6:1.0, N-P-K after weathering for 8-16 months. Nitrogen and phosphorus do not leach out during weathering but potassium being more leachable is lost in significant amount during weathering (Gupta *et al.*, 2004). They reported that 6 months old SMS contain 2.73 per cent nitrogen and 0.31 per cent phosphorus while the potassium was 0.32 per cent. SMS is nutrient rich and contains about 80 per cent of the total nitrogen in bound form with high molecular weight fractions of lignin and humic substances. The absorption capacity of stable "humus" available in SMS helps in retention of nitrogen in the top soil (Grabbe, 1978). Fidanza *et al.* (2010) found that the percentage of nitrogen, phosphorus and potassium in SMS was 1.1, 0.29 and 1.04 respectively. Similarly Lohr *et al.* (1984) observed that N, P, K content of 6 months aged spent mushroom compost was 1580, 180 and 305 mmol kg^{-1}. The organic mineral fertilizers prepared with three different formulae, having 2, 7 and 10 per cent nitrogen and each supplemented with 2 per cent phosphate (P_2O_5) and 2 per cent of potassium (K_2O) has the potential of a balanced organic fertilizer (Sochtig and Grabbe, 1995).

The micronutrients present in SMS are in very trace amounts. According to Hy-Tech Mushroom Farm of PA, the percentage of iron (Fe), Manganese (Mn), Zinc (Zn) and Cooper (Cu) was 0.3, 0.03, 0.01 and 0.02 per cent, respectively. The micronutrients Fe, Mn, Cu and Zn were detected in mushroom compost at a very low average range of 0.02 to 0.43 per cent (Fidanza *et al.*, 2010). Similarly, Lohr *et al.* (1984) observed that the Fe, Mn and Zn content of fresh SMS was 67, 7.5 and 1.7 mmol kg^{-1} while that of aged SMS was 71, 7.1, and 2.1 mmol kg^{-1} respectively.

2.1.3 BIO-AGENTS ASSOCIATED WITH SMS

The spent mushroom substrate (SMS) released after button mushroom cultivation contains all the essential nutrients needed for harboring fungal biomass and large population of heterotrophic microbes (Pill *et al.*, 1993).

Weathering causes a slow decrease in the organic matter contents (volatile solids) and leads to different characteristics of weathered SMS because of on-going microbial activity (Beyer, 1999). The actinomycetes, bacteria and fungi inhabiting the compost, not only play role in its further decomposition but also exert antagonism to the normal pathogens surviving and multiplying in the soil ecosystem.

The biological analysis of SMS extract by Yohalem *et. al.* (1994) showed that it contains *Pseudomonas*, *Trichoderma* and *Bacillus.* SMS harbors different mycoflora and shows differences in its effect on inhibition of conidial germination and disease suppression. Spent substrate from *Pleurotus florida* (oyster mushroom) harbors 5 to 23 fold higher fungal population than other spent substrates. Among different fungi, *Trichoderma* spp. followed by *Aspergillus* spp. and *Mucor* spp. dominate in different spent substrates. *Trichoderma*

dominates in all spent substrates, while *Mucor* in paddy straw mushroom and *Aspergillus* in both paddy straw mushroom and oyster mushroom spent substrates.

Huang *et al.,* (1995; 1996) has also been reported that SMS stimulates the microbial population of *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp. and maintains it for 28 days.

Deacon (1997) stated that spent mushroom substrate, being a cellulosic agro-waste favoured growth of a well-known cellulose degrader, *T. harzianum*. Similarly, Butt *et. al.* (2001) and Lucas (1998) observed that organic manure like SMS are known to enhance the early growth and establishment of the bioagents including *Trichoderma* spp. Romaine and Holcomb (2001) found that organic manure including spent mushroom compost encouraged a population of antagonistic microorganisms such as *Trichoderma* spp., *Bacillus* spp. that interfere with the activity of plant pathogens.

2.2 THE FUNGUS *TRICHODERMA*

The fungus *Trichoderma* was described as early as 1794 by the mycologist Persoon. The potential for using *Trichoderma* as a biocontrol agent was suggested by Weindling (1932), who was the first to demonstrate the parasitic activity of the members of this fungus to soil borne plant pathogenic fungi e.g. *Rhizoctonia solani*. However, with the increasing interest in biological control, owing to environmental and economic concerns and with the rapid development of biotechnology, several *Trichoderma* species were formulated in a commercial production for the protection and growth enhancement of a number of crops in several countries (Mcspadden and Fravel, 2002).

The genus *Trichoderma* belongs to the class Deuteromycetes. It was, for the most parts, classified as an imperfect fungus, due to no known sexual stage (Gams and Bisset, 1998). Rifai (1969) distinguished nine species differentiated primarily by conidiophore branching patterns and conidium morphology based on microscopic characters; *Trichoderma aureoviride, T. hamatum, T. harzianum, T. koningii, T. longibrachiatum, T. piluliferum, T. polysporum, T. pseudokoningii* and *T. viride.* In the section *Trichoderma,* Persoon (1794) characterized species by narrow and flexuous conidiophores with branches and phialides uncrowded, frequently paired and seldom in verticals of more than three. In the section *Longibrachiatum,* Bissett (1984) indicated that conidiophores are sparingly and irregularly branched, with irregularly disposed and not usually in whorls or verticals and species in this section produce distinctive greenish yellow pigments in reverse of cultures. In the section *Saturnisporum,* conidiophores have a branching system with branches and phialides uncrowded and frequently paired and compact conidiogenous pustules as in section *Pachybasium.* However, it was differentiated by the wing-like conidial ornamentation. The section *Pachybasium,* have species with highly ramified, broad conidiophores usually arranged in compact pustules or fascicles, with branches and phialides broad or inflated, relatively short and disposed in crowded verticals. Some species are characterized by the

production of sterile conidiophores. The section *Hypocreanum*, characterized by effuse, usually spars conidiation, sparingly branched conidiophores, and cylindrical to subulate phialides frequently borne in *Verticillium*-like divergent verticals (Bissett, 1991a).

The genus *Trichoderma* is characterized by rapidly growing colonies bearing tufted or postulate, repeatedly branched conidiophore with lageniform phialides and hyaline or green conidia born in slimy heads (Bissett, 1984). The primary branches of conidiophore produce smaller secondary branches that also may produce tertiary branch and so on. The final branches are very simply constructed with a majority of singly phialides (Rifai, 1969). Conidiophore may end in sterile appendages with the phialides born on lateral branches in some species. Conidia are hyaline or more usually green, smooth walled or roughened. Hyaline chlamydospores are usually present in the mycelium of older cultures (Domsch *et al*, 1980). Phialides are ampulliform to lageniform, usually constricted at the base, more or less swollen near the middle and abruptly near the apex into short subcylindric neck. They are disposed in verticals terminally on branches of the conidiophore or less frequently singly or in whorls directly beneath septa along the conidiophore and its branches (Bissett, 1991c).

Trichoderma species are ubiquitous in the environment, especially in soils. They have been used or encountered in many human activities including commercial applications in production of enzymes and biological control of plant disease (Samuels, 1996). *Trichoderma* species are widely distributed all over the world (Domsch *et al.*, 1980) and found in all soils including forest humus layer (Wardle *et al.*, 1993) as well as in agricultural orchard soils (Roiger *et al.*, 1991) and natural habitats, especially in those containing or consisting of organic matter (Papavizas, 1985). Papavizas *et al.* (1982) found that the conidia of *T. harzianum* added to soil without nutrient supplying amendments survived between 110-130 days depending on the isolate. Lewis and Papavizas (1985) demonstrated the potential of various *Trichoderma* species aggregates to form chlamydospores readily and in great numbers in natural soil or in fragments of organic matter after the introduction of the fungus to the soil as conidia.

2.3 THE PATHOGEN *RHIZOCTONIA SOLANI*

The soil borne plant pathogen *Rhizoctonia solani* (Kuhn), [*Thanatephorus cucumeris* (Frank) Donk (anamorph)] is a basidiomycete that occurs worldwide and causes economically important diseases to a large variety of vegetable, field crops, turfgrasses, ornamentals and fruit and forest trees; inflicting yield losses averaging up to 20 per cent yearly in over 200 crops worldwide (Anderson, 1982; Adams, 1988; Sneh, 1996). *R. solani* is a complex and collective fungal species, which consists of strains that differ in host range, pathogenicity, cultural characteristics and the way they respond to the environment (Jones *et al.*, 1997; Dorrance *et al.*, 2001). It occurs in all parts of the world and is probably indigenous to uncultivated areas. History of taxonomy and nomenclature of the perfect stage, *T. cucumeris*

(Frank) Donk of *R. solani* Kuhn has been illustrated by Tarbot (1970). The anamorph, *R. solani* belongs to phylum *Ascomycota* from class *Deuteromycetes* and Order *Agnomycetales* (Alexopolus *et al.*, 1996). *R. solani* Kuhn is the cosmopolitan and destructive soil borne plant pathogen with a wide host range (Adams, 1988). It is seed as well as soil borne pathogen and its propagules are randomly distributed in the soil (Almeida *et al.*, 1980). It produces sclerotia which are the principle structures adapted for surviving under adverse conditions. The sclerotia of *R. solani* are dark brown and irregular in shape and size. The thermal death point of *R. solani* is 60 ^{0}C making it possible to survive even during hot summer of north India. The soil moisture of 20 per cent and temperature of 50 ^{0}C have been reported as most suitable for infection (Monga and Sheoraj, 1994).

Genus *Rhizoctonia* was first described by A. P. De Candolle in 1815 for the non-sporulating violet root rot pathogen and named *Rhizoctonia cruocorum* D. C. *Rhizoctonia solani* was first reported by Julius Kuhn on diseased potato in 1958 (Parmeter, 1970). Since then this fungus has gained the reputation of being a ubiquitous wide host range, destructive and versatile plant pathogen. Das Gupta (1992) described the mycelium of *R. solani* in culture as silvery in the beginning, becoming yellow and brown with maturity, 8-12 µm broad and infrequently straight. Saikia (1976) and Saikia and Roy (1976) reported that sclerotia of the fungus are white in the beginning but later on their colour change to chestnut brown. Sclerotia are globose to sub-globose, oval or cushion shaped, 2-3.5 mm in size, but some of those, aggregate together to form masses upto 1.2 cm in diameter and formed superficially. The sclerotia do not differentiate into rind and medulla. Burpee *et al.*, (1980) stated that sclerotia of *R. solani* were cartriage buff to mummy brown in colour and 0.3-10.0 mm in diameter.

R. solani is distributed in various agro-climatic regions because of its adaptability to diverse edaphic conditions due to high competitive and saprophytic ability. In a specific soil type, total population of the pathogen might be composed of different strains with varying degree of virulence and saprophytic ability (Papavizas *et al.*, 1975). In India *R. solani* occurs on different plants of economics value including maize, sorghum, fingermillet, pearlmillet, sugarcane, spinach, lettuce, greengram, blackgram, soyabean, pigeonpea, crucifers, turmeric, groundnut, pea, tomato, potato, rice etc. and several weeds namely *Cyperus rotundus, Digitaria longiflora, Penicurepens, Cynodon dactylon* (Singh and Saxena 1980; Baruah and Lal, 1981).

2.3 BIOLOGICAL CONTROL

Biological control is the reduction of amount of inoculum or disease reducing activity of a pathogen accomplished by or through one or more living organisms other than man (Cooc and Baker, 1983). It comprises use of living agents to control plant pathogens. The basis of biological control is exploitation of the antagonistic potential of biocontrol agents known as antagonists. An antagonist is a micro-organism that adversely affects another organism (target pathogen) growing in association with it (Baker and Cooc, 1974). Biological control of plant pathogens is an alternative to chemical control or as a part of integrated pest management system for disease control has been established since long (Baker, 1978). Before 1970, much of work on biocontrol involved the indirect enhancement of indigenous *Trichoderma* or *Gliocladium* by manipulating the environment. However, now it has increased greatly in recent years by introduced antagonists (Papavizas and Lumsden, 1980; Lewis and Lumsden, 1995). Most of the fungi used in biological control of soil borne pathogens are Hyphomycetes and among them genera *Trichoderma* and *Gliocladium* have received most attention (Harman *et al.*, 1980, 1981). The antagonistic ability of *Trichoderma* and *Gliocladium* spp. against plant pathogens were initially observed by Windling (1934; 1937). Since then many workers have reported that these two antagonists are highly effective against a wide range of soil borne plant pathogens (Cooc and Baker, 1983; Papavizas, 1985; Mukherjee *et al.*, 1989; Dubey, 1998; Harman, 2000; Laha and Venkatraman, 2001; Harman *et al*., 2004; Jash and Pan, 2004; Gaur *et al.*, 2005; Kapil and Kapoor, 2005; Tewari and Singh, 2005; Shabir *et al.*, 2012, 2013; Jan *et al.*, 2013).

There are several mechanisms of action suggested for *Trichoderma* spp. *viz.* mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solubilization and sequestration of inorganic nutrients and inactivation of the pathogen enzymes (Samuels, 1996). The initial interaction shows that the hypha of the mycoparasites grows directly towards its host (Chet *et al.*, 1981). When the mycoparasite reaches the host, its hypha coils it or attaches to it by forming a hook-like structure. Following these interactions hypha sometimes penetrates the host mycelium by partially degrading its cell wall (Elad *et al.*, 1983). The control of *Rhizoctonia solani* and *Pythium ultimum* by *Trichoderma* species including *T. harzianum* may be affected through direct penetration of host hyphae (Dennis & Webster, 1972; Benhamou & Chet, 1993).

Trichoderma spp. produces 43 substances that have antibiotic activity which do not include enzymes (Sivasithamparam and Ghisalbetri, 1998). Of these, alkyl pyrones, isonitriles, polyketides, peptaibols, dikeyopiperazines, sesquiterpenes and steroids have been associated with biocontrol activity of some species and strains of *Trichoderma* (Howell, 1998).

Competition for space or nutrients has also been considered as one of the classical mechanisms of biocontrol by *Trichoderma* spp. (Elad *et al.*, 1999). The competition for nutrients, primarily carbon, nitrogen and iron is one of the methods of the biological control of soil borne plant pathogens (Scher *et al*., 1984). *Trichoderma* species are generally considered to be aggressive competitors and the ability of *Trichoderma* to compete is species-dependent (Wardle *et al.*, 1993). It is an important mechanism because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere. Induction of resistance in host plant by treatment with the biocontrol agent *Trichoderma* species is another mechanism in biological control (Howell, 2003). Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which finally leads to induced systemic resistance (ISR) in the entire plant (De Meyer *et al.*, 1998).

Howell *et al.* (1999) reported that *Trichoderma* mutants that lacked both mycoparasitic ability and the capacity to produce antibiotics were more effective than the parental strains in biocontrol of *Rhizoctonia solani*.

Biocontrol potential of *Trichoderma* spp. against soil borne diseases has been reported by many workers. However, the first report that the pathogenicity of *Rhizoctonia* and soil causing damping-off of citrus seedlings could be reduced by *T. lignorum* was given by Weindling (1932) which was correlated with the production of a toxin.

Cherif and Benhamou (1990) and Monaco *et al.* (1991) mentioned that the antagonistic effect of *T. harzianum, T. koningii* and *T. viride* inhibited the growth of *F. oxysporum* f.sp. *lycopersici, Sclerotium rolfsii* and suppressed the germination of *R. solani in vitro*. The antagonistic effect of *Trichoderma* spp. might be attributed to producing cell wall degrading enzymes glucanase and chitinase which lyse the cell wall of the pathogenic fungi, inhibition of the host mycelium and hyphal penetration by *Trichoderma.*

Ghonim (1999) found that the application of *T. harzianum* as seed dressing and soil drenching gave the best control for tomato damping-off caused by *R. solani*, whereas soaking of tomato seedling roots in *T. harzianum* spores suspension has the lowest effect.

Sabaratnam and Traquair (2002) found that tomato seed treatment with *T. harzianum, T. viride, G. virens, B. subtilis,* and *Streptomyces* recorded the maximum protection against pre and post-emergence damping-off caused by *F. oxysporum* f.sp. *lycopersici, R. solani* and *S. rolfsii* and reduced the disease incidence .

Karthikeyan *et al.* (2001) reported that seed treatment by *T. viride* @ 0.4 per cent, *T. harzianum* @ 0.4 per cent significantly reduced damping-off incidence in vegetable crops. Vasudeva and Sikka (1941) reported that hyphae of *T. lignorum* and *A. niger* showed a dissolving effect on the hyphae of *R. bataticola* and *R. solani*. Hader *et al.* (1989) reported the control of damping-off of cotton caused by *R. solani* through *T. harzianum*. Lewis and

Papavizas (1985) reported that several species of *Trichoderma* (*T. viride, T. harzianum, T. hamatum* and *G. virens*) reduced the population of *R. solani* and consequently checked the damping-off of radish and sugarbeet. Biological control of root rot disease through mass introduction of *Trichoderma* spp. has also been reported by various workers (Mukhopadhyay, 1987; Lartey *et al.*, 1994; and Howell *et al*., 2003). The antagonistic activity of *T. harzianum* against several fungi including *R. solani* has been reported by several workers (Dennis and Webster, 1972; Hader *et al.*, 1989; Hermosa *et al.*, 2000 and Sanz *et al.*, 2004).

2.4 SMS FOR DISEASE MANAGEMENT

Majority of farmers noticed decrease in incidence of insects, pests and diseases in the crops manured with SMS (Ahlawat *et al.*, 2007). SMS in disease management can be more diversified due to its unique chemical constituents and the microflora.

The feasibility of using organic amendments such as compost, animal manures and organic industrial by-products in order to suppress soil borne plant pathogens has been well documented (Hoitink and Boehm, 1999; Ryckeboer, 2001; Cheuk *et al*., 2005 and Noble and Coventry, 2005). Composts prepared from agricultural waste and used in container media or as soil amendments may have highly suppressive effects against diseases caused by a variety of soil borne plant pathogens *viz. Pythium* spp. (Mandelbaum and Hadar, 1990; Pascual *et al*., 2000), *Phytophthora* spp. (Hoitink and Boehm, 1999; Widmer *et al*, 1999), *Rhizoctonia* spp. (Tuitert *et al.*, 1998**;** Rivera *et al*., 2004). The effectiveness of manure amendments against disease depends on the type of manure, type of soil and other factors. Mushroom compost and manure decreased damping-off of flax caused by *R. solani* with the compost being more efficient than the manure (Alabouvette *et al.*, 2004).

Noble and Coventry (2005) reviewed the various combinations of biological control agents (including *T. harzianum*) and organic amendment that were reported to control soil borne plant pathogens. They stated that such combinations could significantly reduce the disease caused by *R. solani*. Many authors have found that organic amendment or compost reduces disease caused by *R. solani* in a variety of crops (Tuitert *et al.*, 1998; Baily and Lazarovit; 2003; Alabouvette *et al.*, 2004; Rivera *et al*., 2004; Noble and Coventry, 2005).

Under *in vitro* conditions, the anaerobically fermented aqueous extract of SMS inhibit the conidial germination of *Venturia inaequalis.* The inhibitory property of SMS remain unaffected even after autoclaving and filter sterilization (Yohalem *et al.,* 1994). The aqueous extract obtained after 5-9 days of incubation in the ratio of 2:1 to 4:1 (water: SMS) maintain its efficacy for about 4 months on storage at -20 ^{0}C, 4 ^{0}C and room temperature. Due to the unique chemical constitution and the microflora present in SMS, its application can be more diversified than what is normally predicted. The extract also inhibits the conidial germination of *Cochliobolus carborum* and *Sphaeropsis sapinea* causing diseases on maize and red pine (*Pinus resinosa*), respectively.

Kaul and Chhabra (1993) used different organic amendments such as leaves of cabbage, mustard, radish, carrot, dried algal catch and spent mushroom compost and found them effective against many root diseases and nematodes. Hoitink *et al.* (1993; 1997a) has been worked on the suppression of plant pathogens by using different composts. They reported that the soil borne plant pathogens including *Rhizoctonia* are inhibited and restricted by a number of microorganisms e.g. *Trichoderma* spp. and *Gliocladium* spp. commonly found in lingo-cellulosic matter.

Harender *et al.* (1997) also observed that treatments with both natural weathered and anaerobic SMS resulted in lower disease incidence in different vegetable crops and also the caterpillar attack was minimum in these treatments.

Chiu and Huang (1997) reported that spent mushroom compost (SMC) inhibited the occurrence of *Fusarium* wilt of watermelon, club root of cabbage, root-knot disease of watermelon, tomato and pepper and root rot of tomato and watermelon.

Romaine and Holcomb (2001) reported that the percentage of compost in the growing medium affected the relationship between tomato (*Lycopersicon esculentum*) seedling survival and damping-off disease. They reported a general trend for increasing seedling survival as the proportion of compost increased up to 100 per cent and that compost at a level of 50 per cent or greater provided highly effective disease control.

SMS has also been shown to suppress various soil fungi (Davis *et al.*, 2005) and soil borne plant diseases (Segarra, 2007) as well as to increase microbial densities in soils (Perez-Piqueres *et. al.*, 2006). The presence and antagonism of different fungal microorganisms has been worked out by many researchers in which they have reported that the organic substrates such as spent mushroom compost can suppress a variety of plant pathogenic fungi including soil borne pathogens like *Rhizoctonia* spp. and *Pythium* spp. (Craft and Nelson, 1996; Grebus *et al.*, 1994; Hoitink *et al.*, 1997, Hoitink and Fahy, 1986; Nelson and Craft, 1992; Phae *et al.*, 1990; Viji *et al.*, 2003; Zhang *et al.*, 1998; Philippoussis *et al.*, 2004).

CHAPTER-III

MATERIALS AND METHODS

The present experiments were carried out at laboratories of Department of Soil Sciences and Department of Plant Pathology and at screen house and field of Department of Plant Pathology, CCS Haryana Agricultural University, Hisar during 2013-2014. The details of materials and methods used in present investigation are as follows:

3.1 QUANTIFICATION OF PHYSICO-CHEMICAL PROPERTIES OF SMS

Spent Mushroom Substrate was taken from Mushroom Production Unit of CCS Haryana Agricultural University and analysed for different properties i.e. pH, electrical conductivity, organic carbon, available nitrogen, available phosphorus, available potassium and available iron, manganese, zinc and copper.

3.1.1 SMS SAMPLING

After the harvesting of white button mushroom (*Agaricus bisporus*) SMS was transferred to SMS pit of 5 meter quadrate and left for 6 months (April to September) for natural weathering (Fig. 1). After 6 months, SMS samples were taken from different depths of SMS pit i.e. 0-30, 30-60 and 60-90 cm using hand auger. These samples were then transported in sealed aluminum foil to the laboratory of Department of Soil Sciences, where stones and other materials were removed and the SMS is homogenized through a 2 mm sieve. The SMS samples were stored in the dark bottles till further analysis of its physico-chemical properties.

3.1.2 ELECTRICAL CONDUCTIVITY (EC) AND PH

Five gm of SMS was taken from each composite sample and mixed with 50 ml of distilled water in a 100 ml beaker to make a suspension of 1:5. The aliquots were allowed to rest for 2 hours for settling down the sediments. EC and pH were determined by using Elico EC meter and Elico pH analyser respectively.

3.1.3 ORGANIC CARBON

Dry combustion method (Nelson and Sommer, 1982) was used in the present study for calculating organic carbon in SMS samples. The dry combustion method is based on oxidation of carbon and thermal decomposition of carbonate materials in a medium-temperature resistance furnace.

3.1.4 AVAILABLE NITROGEN

In the present study, Colorimetric (Nessler's reagent) method (Lindner, 1944) was used to determine available nitrogen in SMS samples. The samples were digested in diacid mixture of H_2SO_4 and $HClO_4$ in the ratio of 9:1 and then Nessler's reagent, potassium

tetraiodomercuriate (I_4HgK_2) was added, from which an orange color complex of oxidimercurammonium iodide, $HgOHg(NH_2)I$ was obtained. Intensity of coloured complex was measured on a spectrophotometer at 440 nm wavelength by using blue filter and nitrogen content was calculated using the standard curve against the measured transmittance.

3.1.5 AVAILABLE PHOSPHORUS

Vanadomolybdophosphoric (VMP) yellow color method (Koenig and Johnson, 1942) was used to determine available phosphorus in SMS samples. The SMS samples were digested in diacid mixture of HNO_3 and $HClO_4$ in a ratio of 4:1 and then Vanadate-molybdate reagent was used to develop vanadomolybdo-phosphoric acid yellow color. The intensity of yellow color was measured on spectrophotometer by using blue filter at 440 nm wavelength and phosphorus content was calculated using the standard curve against the measured transmittance.

3.1.6 AVAILABLE POTASSIUM

Potassium in the SMS samples was determined by using flame photometer. It is based on the principle that atoms of some specific element absorb energy from flame and get excited to the higher orbit. Such atoms release energy of a characteristic wavelength, which is specific for that element and is proportional to the concentration of atoms of that element in a sample. The samples were digested in diacid mixture of HNO_3 and $HClO_4$ in a ratio of 4:1 and the intensity of filterate was measured with a spectrophotometer at 660 nm wavelength by using a red filter. Potassium content in samples was then calculated using the standard curve against the measured transmittance.

3.1.7 ZINC, IRON, MANGANESE AND COPPER

Atomic Absorption Spectrophotometer (AAS) was used in the present study to determine content of micronutrients in SMS samples. The AAS is based on the principle that atoms of metallic elements (Zn, Mn, Fe, Cu), which normally remain in ground state, under flame conditions, absorb energy when subjected to radiation of specific wavelength. The absorption of radiation is proportional to the concentration of atoms of that element. The SMS samples were digested in diacid mixture of HNO_3 and $HClO_4$ in a ratio of 4:1 and the reading of Zn/Mn/Fe/Cu in sample was measured from AAS and concentration was calculated from standard curve against the sample readings.

3.2 ISOLATION OF MYCOFLORA FROM SMS

Serial dilution and plating method was followed for isolation of fungal microorganisms from SMS. One gram SMS was mixed into 9 ml of sterile distilled water, then 1 ml of SMS suspension was taken into another tube containing 9 ml of sterile distilled water. This serial dilution technique was continued up to 1: 10,000. From the final dilutions, 1:1,000 and 1: 10,000, 1 ml suspension was transferred to each of the Petri dishes containing 20 ml of melted PDA and mixed by giving a gentle whirling motion to the plate and allowed

them to incubate at 26±1 ºC. The fungal colonies which were abundant and predominantly present, were aseptically transferred to PDA slants separately and kept at 26±1 ºC for further growth. The culture was further purified by single hyphal tip method. The pure culture thus obtained was maintained by subculturing it at monthly interval on PDA and stored in a refrigerator for further studies.

3.3 COLLECTION, ISOLATION, PURIFICATION AND MULTIPLICATION OF *R. SOLANI*

Tomato plants showing damping-off and root rot symptoms were collected from Vegetable Research area of CCSHAU farm. The isolation of fungus was done following standard tissue isolation technique. Those parts of root showing typical symptoms of disease were washed in running tap water and cut into small bits. These bits were surface sterilized with 0.01 per cent mercuric chloride solution for 30 seconds. The bits were washed thoroughly in sterile distilled water for three times to remove traces of mercuric chloride and then aseptically transferred to sterilized potato dextrose agar (PDA) plates and were incubated at 27±1ºC for three days for mycelium growth of fungus. Later, the mycelial bit of fungal growth was transferred to PDA slants. The culture was purified by single hyphal tip method. The pure culture thus obtained was maintained by subculturing it at monthly interval on PDA and stored in a refrigerator at 4 ^{0}C for further use.

3.4 PATHOGENICITY TEST

The pathogenicity of *R. solani* was tested on tomato cultivar Hisar Arun by the following method. *R. solani* was raised on Potato Dextrose Broth (PDB) for 7 days at 27±1 °C. The mycelia mats were harvested, washed and blended at the rate of 10.0 g fresh mycelia per litre sterilised water. The mycelia suspension was added in earthern pots of 15 cm diameter at the rate of 100 ml suspension per kg soil. The fungus was allowed to stabilize for 3 days. 15 tomato seeds were sown after 3 days after inoculation under screenhouse. Uninoculated pots were also kept as control. Observations were taken on pre and post emergence mortality and the appearance of symptoms on seedlings up to 30 days of sowing. These symptoms were compared with the early described symptoms of the disease. The pathogen was reisolated from the inoculated plants examined under microscope and compared to the original fungus used for inoculation of the pots to prove Koch's postulates.

3.5 EVALUATION OF ISOLATED FUNGAL BIO-AGENTS AGAINST *R. SOLANI (IN VITRO)*

Fungal bio-agents isolated from SMS were tested for their antagonism against *R. solani* under *in vitro* conditions using dual culture method (Morton and Strouble, 1955) on PDA in Petriplates. Discs of 5 mm size cut from the margins of actively growing cultures of the pathogen and antagonist were placed at opposite points in Petriplates 4 cm apart from each other. Side by side control plates (*R. solani* only) were also maintained for each isolate. For each treatment six replications were maintained. The plates were incubated at 27 ± 1°C.

Observations on the colony diameter and radial growth of pathogen were taken daily till pathogen occupied the full Petri-plate in the control. The colony diameter of the fungus was recorded in metric scale (mm) by taking measurement horizontally and another vertically of the size of the fungal colony and mean of these two was the colony diameter. Half of the measurement of the colony diameter gave radial growth of the pathogen. Per cent growth inhibition of the pathogen was calculated by the formula given by Vincent (1947):

$$I = C - T / C \times 100$$

Where,

I = per cent growth inhibition

C = radial growth of *R. solani* mycelium in control (mm)

T = radial growth of *R. solani* mycelium in treatment (mm)

3.6 FORMULATION OF ANTAGONISTS

The fungal bio-agents were grown in Petri plates on PDA medium for 15 days for abundant conidial production. The conidia was harvested from well sporulated agar culture plates with the help of a cotton swab into a clean beaker. One ml of spore suspension of containing 10^5 spores was prepared with the help of Haemocytometer. Tomato seeds were added to 1 ml of spore suspension for seed treatment.

3.7 PREPARATION OF *RHIZOCTONIA SOLANI* INOCULUM

Wheat grains were used for preparing inoculum of *R. solani*. Wheat grains were boiled in water in Erlenmayer flasks and then autoclaved at 15psi at 121.6 ^{0}C for 2 consecutive days. After 2 days the grains were inoculated with mycelial bits of *R. solani* and incubated at 27±1 °C. The flasks were shaked every 3-4 day for uniform growth of the pathogen.

3.8 EVALUATION OF BIO-AGENTS AND ADDITION OF SMS AGAINST *R. SOLANI*

Isolated fungal bio-agents were evaluated for their efficacy by seed treatments along with addition of SMS in managing *R. solani* in tomato cultivar 'Hisar Arun' in screen house. Evaluation of addition of SMS was also studied in nursery conditions on same cultivar.

3.8.1 EVALUATION OF FUNGAL BIO-AGENTS AS SEED TREATMENT ALONG WITH ADDITION OF SMS IN SCREENHOUSE

The experiment was laid down in pots in completely randomized design to determine the combined effects of seed treatment with antagonists and addition of SMS for the management of *R. solani* in tomato.

Field soil filled in 12" diameter earthern pots was inoculated with *R. solani* inoculum prepared by above said method @ 5 gm/kg soil. The soil was kept moist for three days for the stabilization of the pathogen. After three days, SMS was added in the pots at three different rates i.e. 50, 100 and 150 gm per kg of soil i.e. 5, 10 and 15 per cent respectively. After 3 days of addition of SMS in pots, 15 seeds treated with bio-agents were sown in each pot. Untreated seeds sown in inoculated soil served as control treatments. Each treatment has three

replications. Observations on disease data were recorded upto 30 days. Per cent seed germination was calculated after two weeks and per cent seedling mortality and per cent disease control was calculated after four weeks of sowing by following formulas:

$$\% \text{ Seed germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

$$\% \text{ Seedling mortality} = \frac{\text{Seedling stand after two weeks of sowing} - \text{Seedling stand after four weeks of sowing}}{\text{Seedling stand after two weeks of sowing}} \times 100$$

$$\% \text{ Disease control} = \frac{\text{Seedling mortality in control} - \text{Seedling mortality in treatment}}{\text{Seedling mortality in control}} \times 100$$

3.8.2 Evaluation of addition of SMS in nursery beds

This study was conducted on Plant Pathology field using sick plot technique. The experiments were laid down in 1 m^2 nursery beds in randomized block design to determine the effect of addition of SMS for management of *R. solani* in tomato.

Flat nursery beds each of size 1 m^2 and 6 cm height were prepared and fine soil tilth was made. Each nursery bed was inoculated with *R. solani* inoculum @ 150 gm/m^2. The soil was kept moist for three days for the stabilization of the pathogen. After three days, SMS was added at different rates i.e. 1.5 , 2 and 2.5 kg per bed and the beds was left as such for 5 days. Untreated seeds of tomato were sown in each bed at the spacing of 20x10 cm. Seeds sown in unadded inoculated nursery beds served as control treatments. Each treatment has three replications. Observations on disease data were recorded upto 30 days. Per cent seed germination was calculated after two weeks; per cent seedling mortality and per cent disease control were calculated after four weeks of sowing.

3.9 STATISTICAL ANALYSIS

The experimental data were statistically analyzed by “Analysis of Variance” and the significance of treatment was judged with the help of F-test and t-test.

CHAPTER-IV

RESULTS

4.1 QUANTIFICATION OF PHYSICO-CHEMICAL PROPERTIES OF SMS

The results presented in Table 1 show that except EC all the parameters decreased with the increase in depth of SMS pit (Fig. 2). The range of all the parameters is as under:

4.1.1 ELECTRICAL CONDUCTIVITY (EC) AND pH

Electrical conductivity of SMS increased with the increase in depth of SMS pit. It was observed that maximum EC was recorded in the sample taken from 60-90 cm depth (1.74 mmhos cm^{-1}) followed by sample taken from 30-60 cm depth (1.07 mmhos cm^{-1}) with minimum in the sample taken from 0-30 cm depth (1.01 mmhos cm^{-1}). The overall EC of SMS was found to be 1.27 mmhos cm^{-1}.

SMS samples taken from 0-30 cm depth have the maximum pH (7.66) followed by sample taken from 30-60 cm depth (7.51) with minimum in the sample taken from 60-90 cm depth (7.40). The overall pH of SMS was 7.52.

4.1.2 ORGANIC CARBON (OC)

Mean OC content of the samples taken from different depths was 2.19 per cent. The OC content of different depths i.e. 0-30 cm, 30-60 cm and 60-90 cm was found to be 2.56, 2.13 and 1.89 per cent respectively.

4.1.3 AVAILABLE NITROGEN

The results showed that the nitrogen content of SMS decreased with the increase in depth of the SMS pit. The maximum nitrogen content was present in the sample taken from 0-30 cm depth (2.42%) followed by the sample taken from 30-60 cm depth (1.91%) and sample from 60-90 cm depth (1.60%). The overall nitrogen content in SMS sample was 1.98 per cent.

4.1.4 AVAILABLE PHOSPHORUS

The maximum phosphorus content was found to be in the sample taken from 0-30 cm depth (1.16%) followed by the samples taken from 30-60 cm depth (1.15%) and 60-90 cm depth (1.08%) with overall percentage of 1.13 per cent.

4.1.5 AVAILABLE POTASSIUM

Potassium content of SMS samples from different depths decreased with the increase in pit depth. The maximum P content was in the sample taken from 0-30 cm depth (1.08%) followed by depth of 30-60 cm (0.96%) and 60-90 cm (0.93%). The overall content of potassium in SMS samples was 0.99 per cent.

Table 1: Properties of naturally weathered spent mushroom substrate*

SMS pit depth	EC (mmhos cm^{-1})	pH	OC (%)	N (%)	P (%)	K (%)	Fe (%)	Mn %)	Zn (%)	Cu (%)
0-30 cm	1.01	7.66	2.56	2.42	1.16	1.08	0.34	0.02	0.01	0.01
30-60 cm	1.07	7.51	2.13	1.91	1.15	0.96	0.28	0.01	< 0.01	< 0.01
60-90 cm	1.74	7.40	1.89	1.60	1.08	0.93	0.18	0.01	< 0.01	< 0.01
CD (p=0.05)	0.11	0.09	0.34	N/A	N/A	N/A	0.1	N/A	N/A	N/A
Overall mean	1.27	7.52	2.19	1.98	1.13	0.99	0.26	0.01	< 0.01	< 0.01

*after 6 months

4.1.6 ZINC, IRON, MANGANESE AND COPPER

Table 1 showed that all the micronutrients are present in very trace amount. Iron content was in the range of 0.18 per cent (0-30 cm depth) to 0.34 per cent (60-90 cm) with a value of 0.28 per cent in the sample taken from 30-60 cm depth. The overall iron content in SMS samples was 0.26 per cent. The maximum amount of manganese, zinc and copper was found in samples taken from 0-30 cm depth having percentage of 0.02, 0.01 and 0.01 per cent respectively. The samples taken from 30-60 cm and 60-90 cm depth have very low content of manganese (0.01%), zinc (<0.01%) and copper (0.01%).

4.2 ISOLATION OF MYCOFLORA FROM SMS

Two fungal isolates (antagonists) that were predominantly present in SMS were isolated by serial dilution and plating technique. These isolates were cultured on potato dextrose agar (PDA) medium and were identified based on colony and morphological characters mentioned below. The first isolate produced light green colony on PDA media with formation of concentric circles in Petriplate with colony growth rate of 8-9 cm in 3-4 days. In early stages of growth, the colour of colony was light green; it gradually became yellowish dark green. The culture gave the smell of 'Malt'. The conidiation was in ring-like zones with highly branched, regular conidiophore branching with size of 2-3 µm. Based on these characteristics the first fungal isolate was identified as *Trichoderma harzianum* (Fig.3). The second isolate produced yellowish to dark green colony on PDA with colony growth rate of 8-9 cm in 5 days. The reverse colony colour was deep yellow with wavy edges and the culture smell lie coconut. The conidiophores were irregularly and moderately branched with size of 4-5µm. Based on these observations, the second fungal isolate was identified as *Trichoderma viride.*

4.3 COLLECTION, ISOLATION, PURIFICATION AND MULTIPLICATION OF *R. SOLANI*

The fungus isolated from diseased tomato seedlings was characterized as having light brown coloured vegetative hyphae with 5-13 µm width. The branching was almost at right angles to the hyphal cell showing a septation at the origin of the branch. The fungus also

Fig 1. Weather parameters during natural weathering of SMS for 6 months (April to September, 2013)

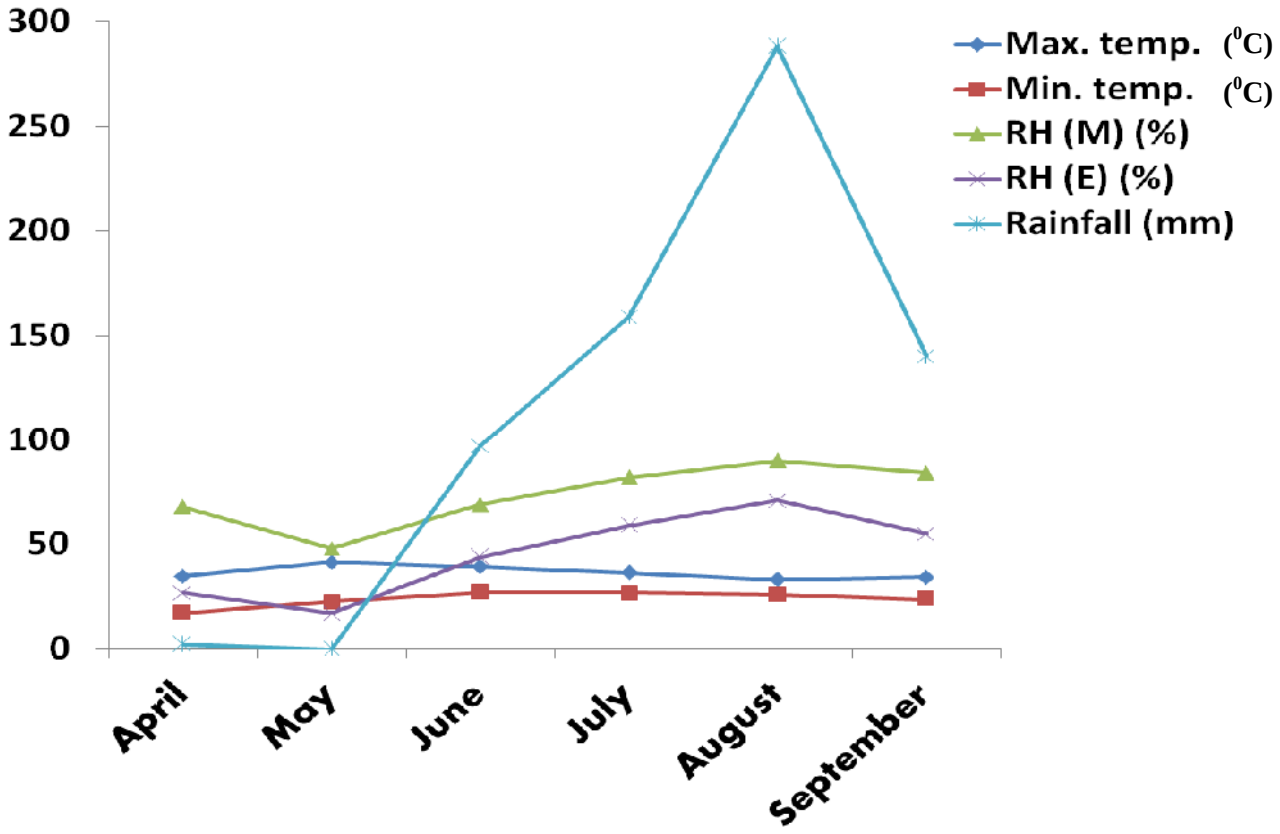


Fig 2. Properties of 6 months old naturally weathered spent mushroom substrate

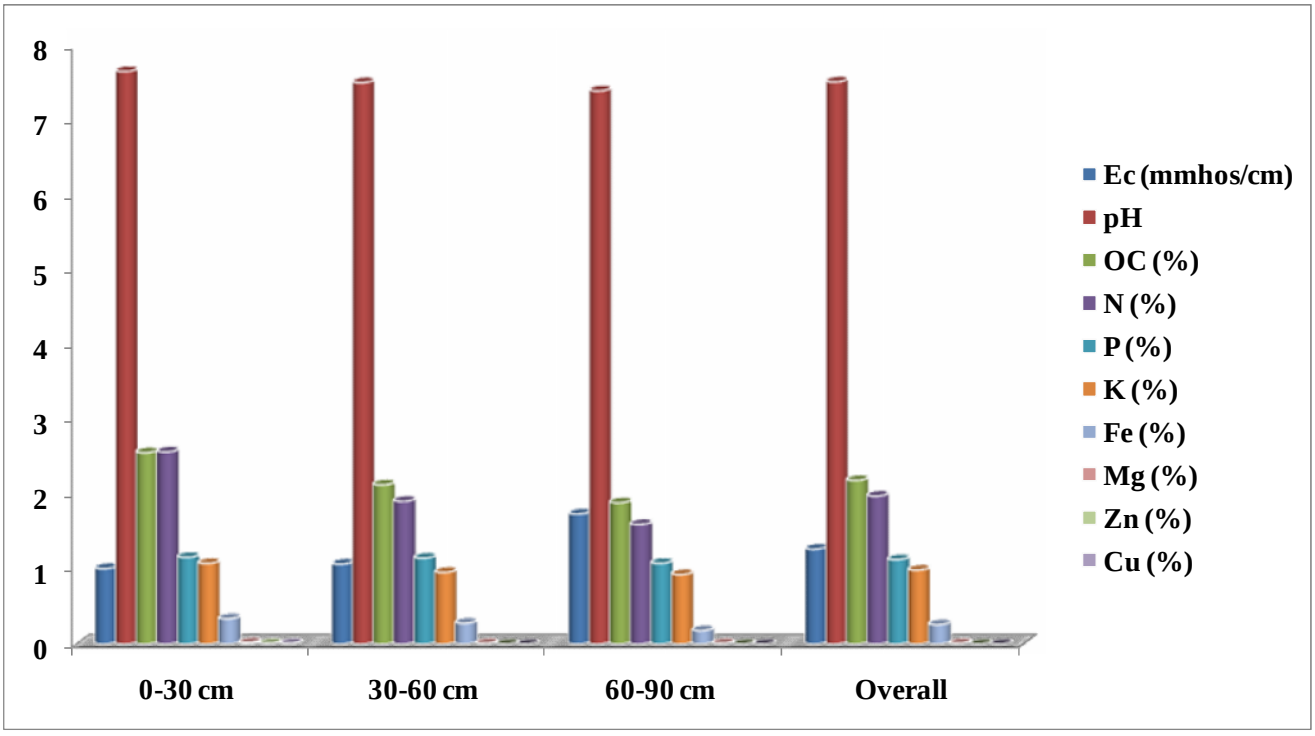


Fig 3. ***Trichoderma*** **spp. isolated from spent mushroom substrate (a)** ***T. harzianum*** **(b)** ***T. viride***

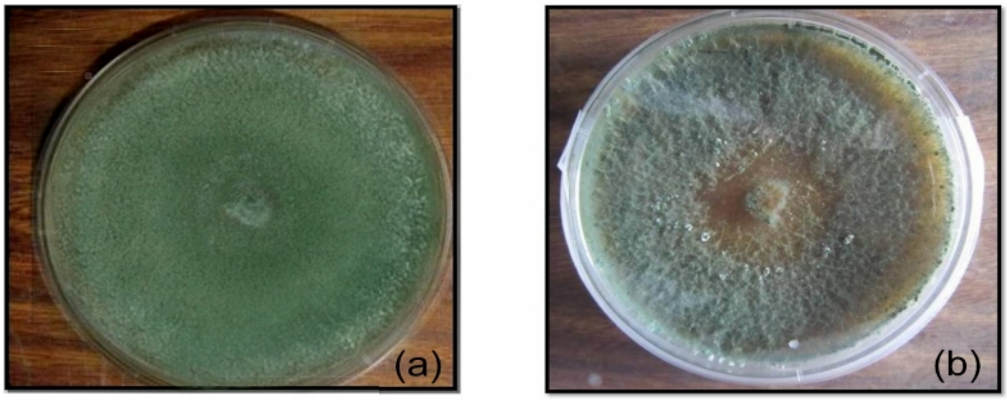


Fig 4. Culture of ***Rhizoctonia solani*** **on PDA (a) light brown mycelial growth (b) dark brown sclerotia produced in concentric circles (c) PDA turned dark as sclerotia mature**

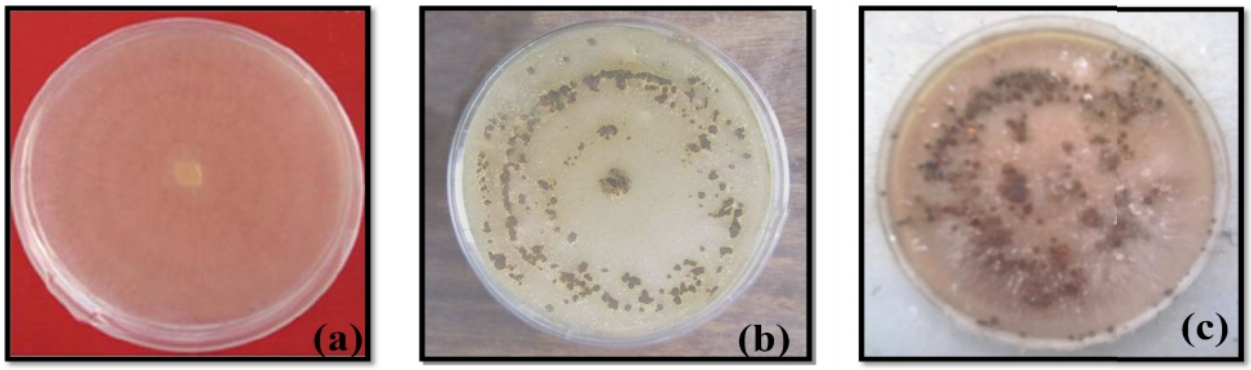


Fig 5. Growth of ***Rhizoctonia solani*** **in dual culture against (a)** ***T. harzianum*** **(b)** ***T. viride***

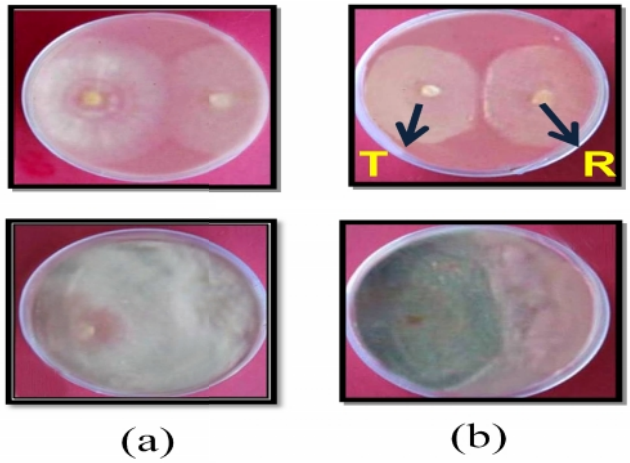
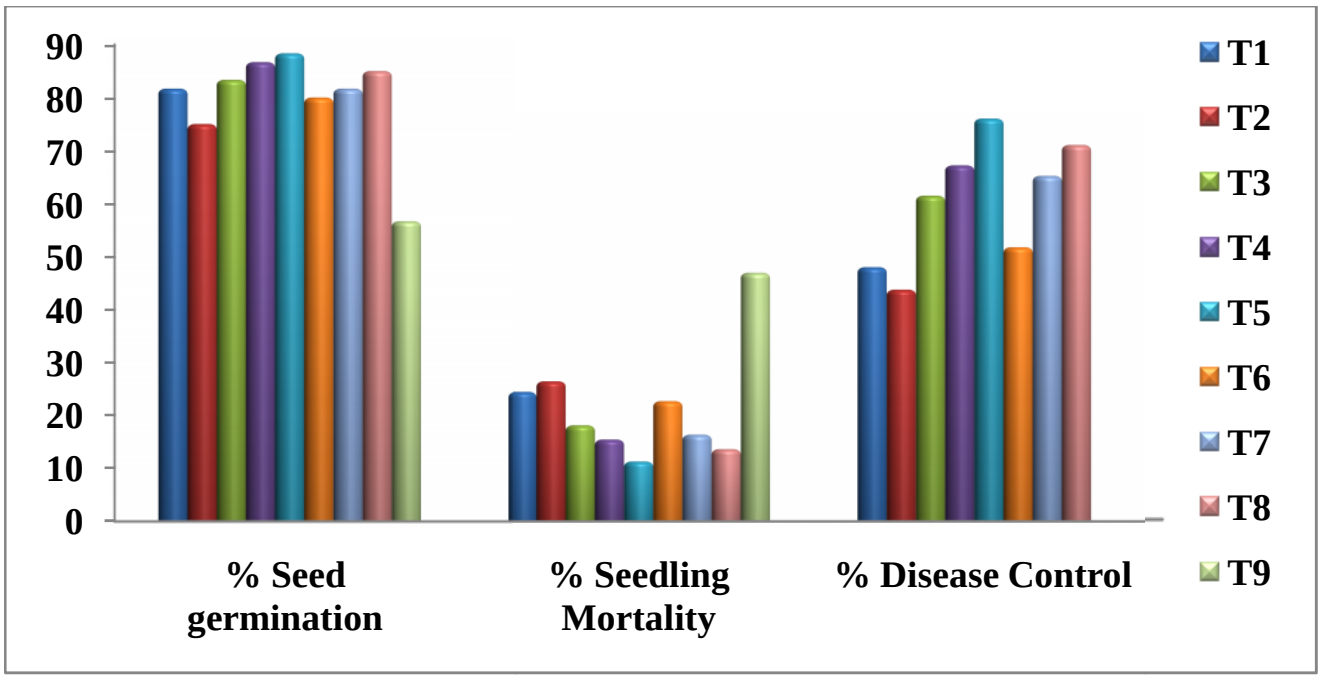
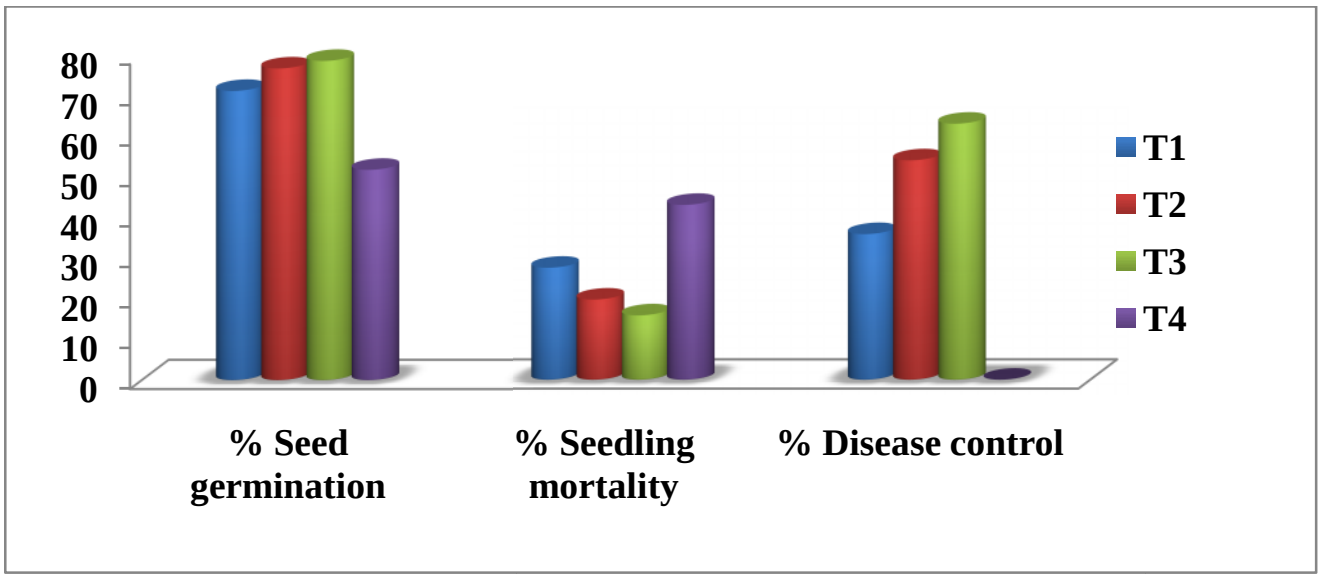


Fig 6. Effect of antagonists as seed treatment with addition of SMS on tomato against *R. solani* under screen house conditions



T1 = ST with *T. harzianum;* T2 = ST with *T. viride;* T3 = ST with *T. harzianum* + 5% SMS; T4= ST with *T. harzianum* + 10% SMS; T5 = ST with *T. harzianum* + 15% SMS; T6 = ST with *T. viride* + 5% SMS; T7 = ST with *T. viride* + 10% SMS; T8 = ST with *T. viride* + 15% SMS; T9 = Control

Fig 7. Effect of addition of spent mushroom substrate on tomato in nursery beds inoculated with *Rhizoctonia solani*



T1= 1.5 kg SMS/m^2; T2= 2.0 kg SMS/m^2; T3= 2.5 kg SMS/m^2; T4= control

produced barrel shaped comparatively smaller cells in groups, which were more than twice as wide as the vegetative cells ranging from 10-20 μm in length. They may be loosely aggregated in the form of round compact structures often called sclerotia. The sclerotia were dark brown at young stage while it became darker producing a brown pigment on PDA turning its colour to dark brown. On the basis of these morphological and cultural characteristics, fungus was identified as *Rhizoctonia solani* (Fig. 4).

4.4 PATHOGENICITY TEST

R. solani was isolated from diseased tomato plant, multiplied on Potato Dextrose Broth and inoculated in earthern pots containing field soil. Seeds of tomato cultivar Hisar Arun were sown in these pots. In inoculated pots few seedlings emerged from the soil as compared to uninoculated pots. Also the emerged seedlings started showing decay at the point where the stem touch the soil. Water soaked sunken lesions developed on young seedlings at soil level. At the emergence of cotyledonary leaves the lesions became brown, girdled the periphery and moved towards root. The affected portion becomes constricted and affected parts became soft, watery and ultimately resulted in seedling collapse. Plants remained weaker and ultimately dried after 15-20 days. For pathogenecity test, seedlings showing distinguished symptoms were uprooted. Fungus was isolated from the diseased seedlings and compared with the fungus used for the inoculation. Reisolated fungus resembled the original isolate in all its morphological characters. Hence pathogenicity was proved.

4.5 EVALUATION OF ISOLATED FUNGAL BIO-AGENTS AGAINST *R. SOLANI (IN VITRO)*

The antifungal activity of bio-agents was determined by testing their effect on radial growth of the pathogen with dual culture method (Fig. 5). Isolated bio-agents namely *T. harzianum* and *T. viride* were tested against *R. solani*. The results are presented in Table 2 which show that *T. harzianum* showed maximum antifungal activity as compared to *T. viride.* The radial growth of pathogen was more in presence of *T. viride* at 3DAI (27.83 mm) and 4DAI (16.16 mm) while it is less in presence of *T. harzianum* (23.5 mm and 12.35 mm respectively). *T. harzianum*

Table 2: Effect of *Trichoderma* spp. on the growth of *Rhizoctonia solani* in dual culture

Growth of *R. solani* against	Radial growth* (mm)		% Growth inhibition	
	3 DAI	4 DAI	3 DAI	4 DAI
T. harzianum	23.5	12.33	33.48	72.29
T. viride	27.83	16.16	21.22	63.68
Control (*R. solani)*	35.33	44.50	-	-
t-value			11.25	6.41
Sig. (p=0.05)			0.43	0.03

*average of six replications

showed maximum inhibition of mycelial growth, 33.48 per cent and 72.29 per cent, followed by *T. viride* with 21.22 per cent and 63.68 per cent inhibition over control at 3DAI and 4DAI, respectively.

4.6 EVALUATION OF FUNGAL BIO-AGENTS AS SEED TREATMENT ALONG WITH ADDITION OF SMS IN SCREEN HOUSE

An evident from Table 3 that, the seed germination after 15 DAS was significantly increased when seeds were treated with bio-agents, however, *T. harzianum* found to be superior over *T. viride* as these showed significant difference in seed germination. When the seeds were treated with bio-agents without addition of SMS, the highest per cent seed germination was observed in the pots treated with *T. harzianum* (81.66%) followed by *T. viride* (74.99%). Addition of SMS significantly increased the seed germination of tomato. The results revels that the maximum per cent seed germination was observed when the SMS was added at the rate of 15 per cent alongwith seed treatment with bio-agents. *T. harzianum* showed highest per cent seed germination (88.33%) followed by *T. viride* (84.99%). At 5 per cent and 10 per cent addition of SMS, the per cent seed germination in *T. harzianum* and *T. viride* treatments were 83.33 per cent and 79.99 per cent; and 86.66 per cent and 81.66 per cent while that of control treatment was 56.66 per cent.

4.7 EVALUATION OF ADDITION OF SMS IN NURSERY BEDS

SMS was added to the flat nursery beds of 1x1 m^2 size which were inoculated with *R. solani* infested wheat grains. Tomato seeds were sown and per cent seed germination and per cent seedling mortality were calculated.

The results presented in table 5 and table 6 show that addition of SMS @ 2.5 kg/m^2 showed maximum per cent seed germination (78.70%) and minimum per cent seedling mortality (15.88%). The minimum per cent seed germination (71.29%) and maximum per cent seedling mortality (27.64%) was observed when the rate of SMS added was 1.5 kg/m^2 while 76.84 per cent seed germination and 19.78 per cent seedling mortality was obtained

from the beds where SMS was added @ 2 kg/m^2 while the control beds showed 51.84 per cent seed germination and 43.15 per cent seedling mortality where no SMS was added.

Table 3: Efficacy of *Trichoderma* spp. as seed treatment with addition of SMS on seed germination of tomato under screen house conditions

Treatment[#]	**% Seed Germination***	**% increase over control**
ST with *T. harzianum*	81.66 (64.84)	30.61
ST with *T. viride*	74.99 (60.10)	24.44
ST with *T. harzianum* + 5% SMS	83.33 (65.98)	32.00
ST with *T. harzianum* + 10% SMS	86.66 (68.87)	34.61
ST with *T. harzianum* + 15% SMS	88.33 (70.49)	35.25
ST with *T. viride* + 5% SMS	79.99 (63.56)	29.16
ST with *T. viride* + 10% SMS	81.66 (64.69)	30.61
ST with *T. viride* + 15% SMS	84.99 (67.26)	33.33
Control (Untreated inoculated)	56.66 (48.81)	-
C.D. (p=0.05)	5.66	

[#] ST = seed treatment
* Figures in parentheses are angular transformed values

Table 4: Efficacy of *Trichoderma* spp. as seed treatment with addition of SMS on seedling mortality of tomato under screen house conditions

Treatment[#]	**% Seedling Mortality***	**% Disease Control**
ST with *T. harzianum*	24.41 (29.43)	47.91
ST with *T. viride*	26.40 (30.81)	43.67
ST with *T. harzianum* + 5% SMS	18.10 (25.05)	61.38
ST with *T. harzianum* + 10% SMS	15.42 (23.10)	67.10
ST with *T. harzianum* + 15% SMS	11.28 (19.38)	75.93
ST with *T. viride* + 5% SMS	22.65 (28.24)	51.67
ST with *T. viride* + 10% SMS	16.34 (23.83)	65.13
ST with *T. viride* + 15% SMS	13.61 (21.15)	70.96
Control (Untreated inoculated)	46.87 (43.17)	-
C.D. (p=0.05)	5.62	

[#] ST = seed treatment
* Figures in parentheses are angular transformed values

Table 5: Effect of addition of SMS on seed germination of tomato in nursery beds inoculated with *Rhizoctonia solani*

SMS added (kg/m^2)#	% Seed germination*	% increase over control
1.5 kg	71.29 (57.65)	27.28
2.0 kg	76.84 (61.27)	32.53
2.5 kg	78.70 (62.56)	34.12
Control (Untreated inoculated)	51.84 (46.04)	-
C.D. (p=0.05)	5.65	

size of bed = 1x1 m
* Figures in parentheses are angular transformed values

Table 6: Effect of addition of SMS on seedling mortality of tomato in nursery beds inoculated with *Rhizoctonia solani*

SMS added (kg/m^2)#	% Seedling Mortality*	% Disease control
1.5 kg	27.64 (31.67)	35.94
2.0 kg	19.78 (26.39)	54.15
2.5 kg	15.88 (23.34)	63.19
Control (Untreated inoculated)	43.15 (41.00)	-
C.D. (p=0.05)	7.58	

size of bed = 1x1 m
* Figures in parentheses are angular transformed values

CHAPTER-V

DISCUSSION

Spent mushroom substrate (SMS) is the material which is removed from the mushroom house after the completion of the mushroom crop. The farmers usually dump it out in the fields and along the road side, but it has many advantages over chemical fertilizers and also over some other manures which are used for providing nutrients to the soil and plants. Damping-off and root rot of tomato is a serious disease caused by *Rhizoctonia solani*. The seed germination and the mortality of seedlings are greatly affected by this pathogen, especially under nursery conditions. It can be controlled biologically by application of organic manures which are good source of bio-agents. The presence of essential nutrients and antagonists in SMS enhance its ability to be used as organic manure and as a biological control for plant diseases. To date scanty information is available on above mentioned aspects of SMS. Hence, results obtained are discussed here:

In the present study, the analysis of 6 months old SMS from three different depths of the SMS pit showed that up to 60 cm depth, EC was 1.07 mmhos cm^{-1} while it increased after 60 cm to 1.74 mmhos cm^{-1}. All the three samples collected from three different depths have alkaline pH, which differed significantly. The uppermost layer (0-30 cm) has the maximum pH of 7.66 which is significantly higher than other two samples (30-60 cm and 60-90 cm). SMS samples taken from different depths have average electrical conductivity of 1.27 mmhos cm^{-1} and pH of 7.52. Gupta *et al.* (2004) calculated the physico-chemical properties of SMS and found that the EC of 6 and 9 months old SMS was 6.22 mmhos cm^{-1} and 0.95 mmhos cm^{-1} while the pH was 7.3 and 6.7, respectively. Wuest and Fahy (1991) reported that pH of fresh SMS was 7.28 which increased to 8.05 on weathering of 8-16 months.

Organic carbon content was found to be maximum, 2.56 per cent, in upper layer of 0-30 cm which gradually decreased as depth increased to 90 cm. The overall content of OC in SMS samples was recorded 2.19 per cent. OC content decreased gradually upon aging as reported by Gupta *et al.* (2004). They found that OC of 6 and 9 months old SMS was 2.51 and 1.46 per cent respectively. The macronutrients i.e. nitrogen, phosphorus and potassium in SMS are present in appreciable amount. The NPK content was 1.98, 1.13 and 0.98 per cent giving ratio of approximately 2:1:1. Although the maximum NPK was recorded in the samples of 0-30 cm but there was no significant difference among all the three samples taken from three different depths. The percentage of micro-nutrients was in traces and was very low in all the SMS samples. The analysis showed that in the upper layer of 0-30 cm depth, iron has a maximum percentage of 0.34 which was followed by manganese (0.02%), zinc (0.01%)

and copper (0.01%). The mean values of different parameters such as EC, pH, OC, N, P, K, Fe, Mn, Zn and Cu was recorded 1.27 mmhos cm^{-1}, 7.52, 2.19 per cent, 1.98 per cent, 1.13 per cent, 0.99 per cent, 0.26 per cent, 0.01 per cent, <0.01 per cent and <0.01 per cent respectively. According to Wuest and Fahy (1991) the NPK content of fresh and old SMS was 1.93, 0.36, 2.35 per cent and 1.92, 0.55, 1.03 per cent respectively and ratio of NPK of 8-16 months old SMS was 1.9:0.6:1.0. The micronutrients were present in very little amounts (Fe-0.44%, Cu-46.26 ppm, Zn-103.88ppm). Similarly, Gupta *et al.* (2004) found that the content of nitrogen, phosphorus and potassium in 6 and 9 months old SMS was 2.73, 0.31, 0.32 per cent and 1.96, 0.20, 0 per cent respectively.

Ahlawat *et al*. (2010) stated that the indigenous fungi associated to the SMS of *A. bisporus* included *Trichoderma* spp. In the present study, two isolates of genus *Trichoderma*, *T. harzianum* and *T. viride* were found to be present abundantly in SMS. Being a cellulose degrader it grows well on many organic manures like SMS and predominate all other competitive fungi. The pre-dominant presence of *Trichoderma* spp. in SMS has been studied by many research workers (Yohalem *et al.*, 1994; Deacon, 1997, Huang *et al*., 1995; 1996; Romaine and Holcomb, 2001). The bio-agents were isolated using dilution plate technique at 25 ^{0}C. This is proved that the optimum temperature for growing of *T. harzianum* is in the range of 15-35 ºC (Domsch *et al*., 1980). These isolates were identified on the basis of cultural and morphological characteristics. Seaby (1996) was also of the same opinion where he reported that the different morphological traits are subjected to environmental influence and can vary substantially from culture to culture. Supported by Park *et al*. (2005) that based on cultural and morphological characteristics, the *Trichoderma* isolates isolated from oyster mushroom substrates could be divided separately into seven groups using growth of the isolates on PDA.

Fungus isolated from diseased tomato plants on PDA had light brown coloured, septate mycelium having branches almost at right angles to the hyphal cell. The fungus also produced barrel shaped small sized cells in groups which later on aggregate to form round compact structures called sclerotia. The sclerotia were dark brown. These sclerotia later on coalesce to form bigger sclerotia and lose their individual identity. The sclerotia were initially white in colour but later on they changed to chestnut brown (Saikia, 1976; Saikia and Roy, 1976). On the basis of these characteristics the fungus was identified as *Rhizoctonia solani* Kuhn as described by Parmeter and Whiteny (1970).

Biological control, especially using fungal antagonists against fungal plant pathogens has gained considerable attention and appears to be promising as a viable supplement or alternative to chemical control (Papavizas, 1985). *Rhizoctonia solani* is capable of attacking a tremendous range of host plants causing seed decay, damping-off, stem cankers, root rot, fruit decay, and foliage disease (Elad *et al*., 1980). In the present investigation, two *Trichoderma*

isolates were tested for mycelial inhibition of *R. solani in vitro. T. harzianum* showed maximum antifungal activity with 72.29 per cent inhibition of mycelial growth of the pathogen followed by *T. viride* (63.68%). The radial growth of pathogen at 3DAI and 4DAI was found to be greatly influenced by these bio-agents. At 3rd day, no significant difference was observed in inhibition but at 4th day, *T. harzianum* overlapped the mycelia of pathogen inhibiting its further growth more than *T. viride*. Gagwar *et al.* (2004) also reported the comparative antagonistic performance of *T. harzianum* and *T. viride* which showed that *T. harzianum* exhibited maximum (75.55%) mycelial growth inhibition of *R. solani* followed by *T. viride* which showed 65.93 per cent growth inhibition of the pathogen. Bunker and Mathur (2001) reported that *Trichoderma* spp. suppress the growth of *R. solani in vitro*. Other workers have also reported the effectiveness of *T. viride* and *T. harzianum in vitro* against *R. solani* (Roy, 1977; Elad *et al*., 1980; Papavizas, 1985; Dubey, 1998). Prasad and Gupta (2002) evaluated the bio-efficacy of *T. harzianum* in controlling stem rot of potato caused by *R. solani*. They found that *T. harzianum* significantly inhibited the mycelial growth and sclerotial production of *R. solani*.

The potentiality of *Trichoderma* spp. as biocontrol agents of phytopathogenic fungi in several crops is well known especially to *Fusarium* spp. and *Rhizoctonia* spp. (Poddar *et al.*, 2004 and Rojo *et al.*, 2007). The antagonistic activity of *Trichoderma* spp. could be related to their ability to act as biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Cook, 2000 and Chakraborty *et al.*, 1994). The seed treatment with *Trichoderma* spp. in the present study and the addition of SMS along with effectively controlled the disease in tomato caused by *R. solani*. In screen house conditions, seed treatment with *T. harzianum* has maximum influence on the pathogen. Between these two bio-agents, seed treatment with *T. harzianum* without any addition of SMS showed 81.66 per cent seed germination with 30.61 per cent increase over control whereas seed treatment with *T. viride* showed 24.44 per cent increase over control with 74.99 per cent seed germination. These figures increased significantly with the addition of SMS and increase in SMS rate.

Among different treatments seed treatment with *T. harzianum* with SMS @ 15% gave maximum seed germination (88.33%) which decreased with the decrease in rate of SMS i.e. 86.66 per cent @ 10% and 83.33 per cent @ 5%. In case of seed treatment with *T. viride*, seed germination was maximum with addition of 15 per cent SMS (88.99%) followed by 10 per cent (81.66%) and 5 per cent (79.99%). After 4 weeks the recorded seedling mortality in SMS unadded treatments is highest as compared to SMS added treatments. The maximum disease control, 75.93 per cent, was found to be in seed treatment of *T. harzianum* added with 15 per cent SMS. Per cent seedling mortality decreased as the rate of SMS was increased. Both, *T.*

harzianum and *T. viride* has the significant effect in managing the pathogen when added with SMS @ 10 and 15%. *T. harzianum* showed 18.10, 15.42 and 11.28 per cent seedling moratilty and *T. viride* showed 22.65, 16.34 and 13.61 per cent seedling mortality when the rate of SMS added was 5, 10 and 15 per cent, respectively. The ability of *Trichoderma* to reduce diseases caused by soil borne pathogens is well known and it is related to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogenic fungi and/or competition for limiting factors in the rhizosphere mainly iron and carbon (Sivan & Chet, 1986). Another mechanism has been suggested by Kleifeld and Chet (1992) related to *Trichoderma*-induced resistance in host plants to fungal attack. *T. viride* and *T. harzianum* were reported by several workers as the best antagonists against several soil and seed borne plant pathogens (Poddar *et al.*, 2004).

Mukhopadhyay (1995) reported that biological seed treatment in tomato, potato, chickpea, lentil and peanut with *T. harzianum* resulted in excellent against a wide range of pathogens including *R. solani* and the treatment were consistently as effective as or better than fungicidal seed treatment. Noble and Coventry (2005) reviewed the various combinations of biological control agents (including *T. harzianum*) and organic amendment that were reported to control soil borne plant pathogens. They stated that such combinations could significantly reduce the disease caused by *R. solani*.

Barakat *et al*. (2007) found that the addition of an organic amendment, however, the *Trichoderma* population was enabled to increase and to reach a critical concentration that enabled it to bring about a reduction in damping off. The organic amendment may have acted as a nutrient source for *Trichoderma*, and thereby increased its population.

Roy *et al*. (1998) found that seed treatments of *T. viride, T. harzianum and T. koningii* reduced damping off of cabbage caused by *Rhizoctonia solani* in both sterilized and unsterilised soil. The antagonistic ability of *Trichoderma* isolates is highly variable (Chet *et al*., 1979), as was shown in this study in which only 11.54 per cent and 5.77 per cent of the *Trichoderma* isolates tested were effective in controlling *R. solani* and *S. rolfsii*, respectively.

Jayaraj *et al.* (2006) reported that the seed treatment with *T. harzianum* formulations reduced the incidence of damping-off disease of tomato by up to 74 per cent and enhanced plant biomass under greenhouse and field (Tamil Nadu, India) conditions.

In nursery conditions, SMS showed a significant effect on the seed germination and seedling mortality when added @ 2.0 and 2.5 kg/m^2. The maximum seed germination (78.70%) was resulted when 2.5 kg was added in 1 m^2 area. The increase in seed germination over the un-added control was 27.28 per cent @ 1.5 kg which increased to 32.53 per cent @ 2.0 kg and to 34.12 per cent @ 2.5 kg. Similar trend was followed at 30 DAS where the per cent seedling mortality was found to be decreased from 43.15 to 27.64 per cent, 19.78 per cent and 15.88 per cent with the increase in rate of SMS from 0 kg to 1.5 kg, 2.0 kg and 2.5

kg respectively. The maximum disease control (63.19%) was in the beds added with 2.5 kg SMS which was significantly higher than the beds aided with 1.5 kg (35.94%).

Organic soil amendment is another important option and eco-friendly approach for controlling damping off disease by developing suppressive nature of soil (Hilocks and Waller, 1997; Dey, 2005; Islam *et al.*, 2007). In plant-fungal interactions, carbohydrate and protein elicitors that induce defense mechanism in plants are released from the mycelia of fungal pathogens (Shibuya and Minami, 2001). Once the plants recognize elicitors, many plants develop an enhanced resistance to further pathogen attack also in the uninoculated organs. This type of induced resistance is called systemic acquired resistance (SAR) (Durrant and Dong, 2004; Vallad and Goodman, 2004; Da Rocha and Hammerschmidt, 2005; Walters *et al*., 2005; Conrath, 2006). Therefore, the mycelia of mushrooms that are prevalent in SMS are abundant sources of elicitors, and thus application of SMS to plants may be useful for the control of plant diseases. However, the study of the potential role of SMS in disease control has not received adequate attention. In the few studies that have addressed this topic, the emphasis has been on exploiting the antibiotic-producing microorganisms in SMS by applying SMS as compost (Yohalem *et al*., 1994; Cronin *et al*., 1996; Viji *et al*., 2003; Choi *et al*., 2007). Alabouvette *et al*. (2004) also reported that mushroom compost and manure decreased damping off of flax caused by *R. solani*, with the compost being more efficient than the manure.Tuitert *et al*. (1998) found that the control of damping off caused by *R. solani* on cucumber in compost-amended pot media was greatly affected by the stage of maturation of the compost fresh compost actually stimulated pathogen growth and infection, but long-matured compost consistently suppressed it.The severity of bean root rot caused by *R. solani* was reduced by compost (Ferrara *et al*., 1996). Several authors studying a variety of crops have found that organic amendment or compost reduces disease caused by *R. solani* (Tuitert *et al*., 1998; Baily and Lazarovit; 2003; Alabouvette *et al*., 2004; Rivera *et al*., 2004; Noble and Coventry, 2005).

CHAPTER-VI

SUMMARY AND CONCLUSION

The cultivation of mushroom end up with the harvesting of mushroom which results in production of spent mushroom substrate (SMS). SMS contains many macro- and micro-nutrients which can give nutrition to soil and plants. The presence of fungal antagonists in SMS made it suitable for controlling plant diseases biologically. It is often considered as a waste but it has many advantages including its ability to add nutrients to the soil and in controlling the plant diseases. Hence, detailed studies including quantitative analysis of physico-chemical properties of SMS, isolation and identification of fungal bio-agents present in SMS, isolation and identification of *Rhizoctonia solani*, effect of isolated bio-agents on this pathogen *in vitro*, testing the efficacy of bio-agents along with addition of SMS under screen house conditions and evaluation of SMS in controlling the pathogen in nursery conditions had been carried out.

The physico-chemical analysis of SMS showed that the average EC and pH of SMS samples collected from three different depths was 1.27 mmhos cm^{-1} and 7.52 respectively. The organic carbon content of SMS collected from three different depths of SMS pit i.e. 0-30 cm, 30-60 cm and 60-90 cm was 2.56, 2.13 and 1.89 per cent respectively. The amount of available nitrogen, phosphorus and potassium had not differed significantly with different depths. The ratio of NPK in the SMS sample was 2:1.2:1 with maximum amount in uppermost layer (2.42% N, 1.16% P and 1.08% K). The presence of micro-nutrients in SMS samples was in traces. Among different micro-nutrients the average iron (Fe) content was found to be 0.26 per cent followed by manganese (Mn) (0.01%) while the zinc (Zn) and copper (Cu) content was less than 0.01 per cent.

Isolation of two *Trichoderma* spp. i.e. *T. harzianum* and *T. viride* from SMS was done by using serial dilution and plating method. These bio-agents were identified by their different cultural and morphological characteristics.

R. solani was isolated from the tomato plants showing typical symptoms of disease, later it was identified based on light brown mycelium, its branching and production of brown sclerotia on PDA culture. Its pathogenicity to tomato was proved.

Efficacy of isolated bio-agents was tested *in vitro* under laboratory conditions for the per cent mycelial growth inhibition of *R. solani*. *T. harzianum* showed maximum antifungal activity restricting its growth to 12.33 mm with 72.29 per cent inhibition of mycelial growth followed by *T. viride* (16.16 mm) which showed 63.68 per cent growth inhibition.

The seed treatment of *Trichoderma* spp. along with addition of SMS had significantly reduced the effect of fungus *R. solani*. SMS added and treated seeds had higher seed germination in comparison to control treatments. For instance, addition of 10 per cent and 15 per cent of SMS had 86.66 per cent and 88.33 per cent germination for seeds treated with *T. harzianum,* 81.66 per cent and 84.99 per cent germination for seeds treated with *T. viride*, respectively. Without addition of SMS *T. harzianum* treated seeds had 81.66 per cent germination while *T. viride* treated seeds had only 74.99 per cent germination. Similarly, seedling mortality was significantly reduced by addition of SMS along with seed treatment with bio-agents. The disease control was enhanced by 67.10 per cent and 75.93 per cent with seed treatment of *T. harzianum* added with 10 per cent and 15 per cent SMS respectively. For seeds treated with *T. viride* and added with 10 per cent and 15 per cent SMS showed 67.10 per cent and 75.93 per cent disease control.

On the other hand, under nursery conditions, the amendment of SMS had a significant increase in seed germination in comparison to un-added beds. The maximum seed germination was in the beds amended with 2.5 kg SMS (78.70%). The beds aided with 1.5 kg and 2.0 kg of SMS showed 71.29 per cent and 76.84 per cent seed germination respectively while control unadded beds had seed germination of 51.84 per cent. Similarly after four weeks of sowing, the disease control was maximum (63.19%) in the beds aided with 2.5 kg SMS followed by the beds aided with 2 kg (54.15%) and 1.5 kg (35.94%).

BIBLIOGRAPHY

Adams, G.C. (1988). *Thanatephorus cucumeris* (*Rhizoctonia solani*), a species complex of wide host range. *In*: Inggram D.S. and P.H. Williams (Eds). *Advances in Plant Pathology: Genetics of Plant Pathogenic Fungi*. Academic Press, London. **6**: 535-552.

Ahlawat, O.P. and Rai, R.D. (2002). Recycling of spent mushroom substrate. *In*: *Recent Advances in the Cultivation Technology of Edible Mushrooms*, R.N. Verma and B. Vijay (eds.), pp. 261-282, National Research Centre for Mushroom, Solan, India.

Ahlawat, O.P., Gupta, P., Kumar, S., Sharma, D.K. and Ahlawat, K. (2010). Bioremediation of fungicides by spent mushroom substrate and its associated microflora. *Indian Journal of Microbiology.* **50**: 390-395.

Ahlawat, O.P., Indu Rani, C. and Sagar, M.P. (2005). Spent mushroom substrate- properties and recycling for beneficial purposes. *In: Frontiers in Mushroom Biotechnology*, R.D. Rai, R.C. Upadhyay and S.R. Sharma (eds.), pp. 314-334, National Research Centre for Mushroom, Solan, India.

Ahlawat, O.P., Sagar, M.P., Raj, D., Indu Rani, C., Gupta, P. and Vijay, B. (2007). Effect of spent mushroom substrate on yield and quality of capsicum (*Capsicum annuum*). *Indian Journal of Horticulture.* **64**(4): 430-434.

Aina, D.A., Oloke, J.K., Jonathan, S.G. and Olawuyi O. J. (2012a). Comparative assessment of mycelial biomass and exo-polysaccharide production of wild type and mutant strains of *Schizophyllum commune* grown in submerged liquid medium. *Nature and Science.* **10**(10): 82-89.

Alabouvette C., Backhouse, D., Steinberg, C., Donovan, N.J., Edel-Hermann V. and Burgess, L.W. (2004). Microbial diversity in soil-effect on crop health. *In*: Managing Soil Quality: Challenges in Modern Agriculture (P. Schjonning, S. Elmholt, B.T. Christensen, ed.). CAB International, Wallingford, UK, 121-138.

Alexopoulus, C.J., Mims, C.W. and Blackwell, M. (1996). *Introductory Mycology*. Wiley Eastern, New Delhi. pp. 869.

Almeidia , O.C., Robbs, C.F., Akiba, F. and Kimura (1980). A new pepper disease caused by *Rhizoctonia solani* Kuhn in Brazil. *Fitopat.* (Brazil). **5**: 7-10.

American Mushroom Institute. (1995). Environmental, agricultural, and industrial uses for spent mushroom substrate from mushroom farms: proceedings from Spent Mushroom Substrate Symposium. Emmaus, PA: JG Press.

Anderson, N.A. (1982). The genetics and pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology.* **20**: 329-347.

Baily K.L. and Lazarovits, G. (2003). Suppressing soil-borne diseases with residue management and organic amendments. *Soil and Tillage Research.* **72**: 169-180.

Baker, K.F. and Cook, R.J. (1974). Biological control of Plant Pathogens. W.H. Freeman, San Francisco. pp. 433

Baker, R. (1978). Inoculum potential. *In*: Plant Disease. An Advanced Treatise. Vol. 2. Academic Press, New York. pp. 436.

Barakat, R., Al-Mahareeq, F., Ali-Shtayeh, M. and Al-Masri, M. (2007). Biological control of *Rhizoctonia solani* by using indigenous *Trichoderma* spp. isolates from Palestine. *Hebron University Research Journal.* **3**: 1–15.

Barry, J., Doyle, O., Grant, J., Grogan, H. (2012). Supplementary of spent mushroom substrate (SMS) to improve the structure and productivity as a casing material. *In*: 18th Congress of the International Society for Mushroom Science. Beijing, China. pp. 735–742.

Bayer, D. (2006). Spent mushroom substrate (SMS) research in the US. *Mushrooms International.* **103**: 19-21.

Benhamou, N. and Chet, I. (1993). Hyphal interaction between *Trichoderma harzianum* and *Rhizoctonia solani*: Ultrastructure and gold chemistry of the mycoparasitic process. *Phytopathology*. **83**(10): 1062-1071.

Beyca, J., De Chant, L., Conditt, M. and Jones, B., (1993). Wet bag composting trial yields promising results. *Biocycle*. **34**(4): 72-75.

Beyer, D.M. (1999). Spent mushroom substrate fact sheet. http:/mushroom spawn.cas.psu.edu/ spent.htm.

Beyer, M. (1996). The impact of the mushroom industry on the environment. *Mushroom news.* **44**(11): 6-13.

Bissett, J. (1984). A revision of the genus *Trichoderma*. I. Section *Longibrachiatum*. *Canadian Journal of Botany.* **62**: 924-931.

Bissett, J. (1991c). A revision of the genus *Trichoderma*. IV. Additional notes on section *Longibrachiatum*. *Canadian Journal of Botany.* **69**: 2418-2420.

Bunker, R. N. and Mathur, K. (2001). Antagonism of local biocontrol agents to *Rhizoctonia solani* inciting dry root-rot of chilli. *Journal of Mycology Plant Pathology*, **31**(1): 50-53.

Burpee, L.L., Sanders, P.L., Cole, H. Jr., Shrewood, R.T. (1980). Anastomosis groups among isolates of *cerato-basidium cornigerum. Mycologia.* **72**: 689-701.

Buruah, P. and Lal, S. (1981). Host range of *Rhizoctonia solani* sasakii, the incitant of banded sclerotial disease of maize. *Indian Phytopathology.* **34**: 494-496.

Buswell, J.A. (1994). Potential of spent mushroom substrate for bioremediation purposes. *Mushroom news*. **43**(5): 28–34.

Butt, T.M., Jackson, C.W. and Magan, N. (2001). Fungi as biocontrol agent: Progress, problems and potential. *CABI, Press,* Oxon, U.K., pp. 390.

Chakraborty, B.N., Das, S.K. and Chakraborty, U. (1994). Phyllosphere microphlora of tea (*Camellia sinensis* (L.) O. Kuntze) and their interaction with *Glomerella cingulata* the causal agent of brown light disease. *Tea.* **15**(1): 27-34.

Chefetz, B.J., van Heemst, D.H., Chen, Y., Romaine, C.P., Chorover, J., Rosario, R., Guo, M. and Hatcher, P.G. (2000). Organic matter transformations during the weathering process of spent mushroom substrate. *Journal of Environmental Quality* **29**(2): 592-602.

Cherif, M. and Benhamou, N. (1990): Cytochemical aspects of chitin break down during the parasitic action of *Trichoderma* sp. on *Fusarium oxysporum* f.sp *radicis lycopersici. Phytopathology*. **80**: 1406-1414.

Chet, I., Hadar, Y., Elad, Y., Katan, J. and Hennis, Y. (1979). Biological control of soil-borne plant pathogens by *Trichoderma harzianum*. *In*: Soil- borne plant pathogens. Eds., B. Schippers and W. Gams. Academic Press, London. pp. 585-591.

Chet, I., Harman, G.E. and Baker, R. (1981). *Trichoderma hamatum*, its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microb. Ecol*. **7**: 29-38.

Cheuk, W., Victor, L.K., Copeman, R., Joliffe, P. and Fraser, B. (2005). Disease suppression on greenhouse tomatoes using plant waste compost. *Journal of Environmental Science and Health.* **40**: 449–461.

Chiu, A.L. and Huang, J.W. (1997). Effect of composted agricultural and industrial wastes on the growth of vegetable seedlings and suppression of their root diseases. *Plant Pathology Bulletin.* **6**: 67-75.

Choi, H.W., Chung, I.M., Sin, M.H., Kim, Y.S., Sim, J.B., Kim, J.W., Kim, K.D. and Chun, S.C. (2007). The effect of spent mushroom sawdust compost mixes, calcium cyanamide and solarization on basal stem rot of the cactus *Hylocereus trigonus* caused by *Fusarium oxysporum*. *Crop. Protection.* **26**: 162-168.

Chong, C., Cline, R.A. and Rinker, D.L. (1991). Rapid leaching of salts - key to successful plant growth in spent mushroom compost potting mixes. *Mushroom news.* **39**(10): 12-13.

Conrath, U. (2006). Systemic acquired resistance. *Plant Signal Behaviour*. **1**(4): 179-184.

Cook, R. J. and Baker, K.F. (1983). The Nature and Practices of Biological Control of Plant Pathogens. *Annual Phytopathogical Society, St. Paul, Minn.* pp. 39.

Cook, R.G. (2000). Advances in plant health management in the 20th centry. *Annual Review of Phytopathology.* **38**: 95-116.

Craft, C.M. and Nelson, E.B. (1996). Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. *Applied Environmental Microbiology.* **62**: 1550–1557.

Cronin, M.J., Yohalem, D.S., Harris, R.F. and Andrews, J.H. (1996). Putative mechanism and dynamics of inhibition of the apple scab pathogen Venturia inequalis by compost extracts. *Soil Biology and Biochemistry.* **28**: 1241-1249.

Da Rocha, A.R. and Hammerschmidt, R. (2005). History and perspectives on the use of disease resistance inducers on horticultural crops. *Horticulture Technology* **15**: 518-529.

Dann, M.S. (1996). The many uses of spent mushroom substrate. *Mushroom news.* **44**(8): 24-27.

Dasgupta, M.K. (1992). Rice Sheath blight: The challenge continues. *In:* Plant disease if international importance: Disease of cereals and pulses. Eds., Singh, U.S., Mukhopadhyay, A.N., Kumar, J. and Chaube, H.S. Prentice Hall Englewood cliffs, New Jersey. pp. 130-150.

Davis, D.D., Kuhns, L.J. and Harpster, T.L. (2005). Use of mushroom compost to suppress artillery fungi. *Journal of Environmental Horticulture*. **23**(4): 212-215.

De Meyer, G., Bigirimana, J., Elad, Y. and Hofte, M. (1998). Induced systemic resistance in *Trichoderma harzianum* biocontrol of *Botrytis cinerea*. *European Journal of Plant Pathology.* **104**: 279-286.

Deacon, J.W. (1997b). *Modern mycology*. Blackwell Science Ltd. pp. 303.

Dennis, C. and Webster, J. (1972). Antagonism properties of species groups of *Trichoderma*. III. Hyphal interaction. *Transactionsof the British Mycological Society.* **57**: 363-369.

Devonald, V.G. (1987). Spent mushroom compost, a possible growing medium ingredient. *In*: Compost: Production, Quality and Use. Els., April Science. London. 785-791.

Dey, T. K. (2005). Effect of soil solarization in controlling damping-off disease of true potato seedlings. *Bangladesh Journal of Plant pathology.* **21**(1&2): 93.

Domsch, K.H., Gams, W. and Anderson, T.H. (1980). Compendium of soil fungi. Vol.1. New York: Academic Press.

Dorrance, A.E., Kleinhenz, M.D., Mc Clure, S.A. and Tuttle, N.T. (2001). Temperature, moisture, and seed treatment effects on *Rhizoctonia solani* root rot of Soyabean. *Plant Disease.* **87**: 533-538.

Dubey, S.C. (1998). Evaluation of fungal antagonists of *Thanatephorus cucumeris* causing web blight of horsegram. *Journal of Mycology and Plant Pathology.* **28**(1): 15-18

Durrant,W.E. and Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology*. **42:** 185-209.

Elad, Y., Chet, I. and Boyle, P. and Henis, Y. (1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii*-scanning electron microscopy and fluorescence microscopy. *Phytopathology*. **73**: 418-422.

Elad, Y., Chet, I. and Katan, J. (1980). *Trichoderma harzianum:* A biocontrol agent effective against *sclerotium rolfsii* and *Rhizoctonia solani. Phytopathology*. **70**: 119-121.

Elad, Y., David, D.R., Levi, T., Kapat, A., Kirshner, B., Guvrin, E. and Levine, A. (1999). *Trichoderma harzianum* T-39 mechanisms of biocontrol of foliar pathogens. *In:* H. Lyr, (Ed). *Modern Fungicides and Antifungal Compounds* II. Intercept Ltd., Andover, Hampshire, UK.

Fasidi, I.O., Kadiri, M., Jonathan, S.G., Adenipekun, C.O. and Kuforiji, O.O. (2008). Cultivation of tropical mushrooms. Ibadan: Ibadan University Press. pp. 81.

Ferrara, A. M., Avataneo, M. and Nappi, P. (1996). First experiments of compost suppressiveness to some phytopathogens. In The Science of Composting. *Ed:* de Bertoldi M., Sequi P., Lemmes B. and Papi T. pp. 1157-1160. London: Blackie Academic& Professional.

Fidanza, M. A., Sanford, D. L., Beyer, D. M., and Aurentz, D. J. (2010). Analysis of Fresh Mushroom Compost. *Horticulture Technology.* **20**(2): 449-453.

Fox, R. and Chorover, J. (1999). Seeking a cash crop in spent substrate. http:/aginfo.psu.edu/psa/sgg/mushrooms.html.

Gagwar, R.K., Prajapati, R.K. and Kumar, K. (2004). Evaluation of fungal antagonists against *Fusarium oxysporum* f.sp. *ciceris. Annals of Plant Protection. Sciences.* **12**: 444-445.

Gams, W. and Bissett, J. (1998). Morphology and identification of *Trichoderma*. *In*: G.E. Harman and C.P. Kubicek (Eds.), *Trichoderma* and *Gliocladium*. **1** (3-43).

Gaur, R.B., Sharma, P.N. and Singh, V. (2005). Manipulations in the mycoparasite application techniques against *Rhizoctonia* root rot of cotton. *Indian Phytopathogy*. **58**(4): 402-409.

Gbolagade, J.S., Ajayi, A.I., Oku, I. and Wankasi, D.O. (2006). Nutritive value of common wild edible mushrooms from southern Nigeria. *Global Journal of Biotechnology and Biochemistry* **1**(1): 16-21.

Ghonim, M.I. (1999). Induction of systemic resistance against *Fusarium* wilting in tomato by seed treatment with the biocontrol agent *Bacillus subtilis. Bulletin of Faculty of Agriculture Cairo University*, **50**: 313 - 328.

Grabbe, K. (1978). Verfahren zur Herstellung eines Dung emittels. BRD – patent NR. 2831583.

Grebus, M.E., Watson, M.E. and Hoitink, H.A.J. (1994). Biological, chemical, and physical properties of composted yard trimmings as indicators of maturity and plant disease suppression. *Compost Science and Utilisation*. **2**: 57–71.

Guo, M. and Chorover, J. (2004). Solute release from weathering of spent mushroom substrate under controlled conditions. *Compost Science* and *Utilization*. **12**(3): 225-234.

Guo, M., Chorover, J. and Fox, R.H. (2001). Effects of spent mushroom substrate weathering on the chemistry of underlying soils. *Journal of Environmental Quality*. **30**: 2127-2134.

Gupta, P., Indurani, C., Ahlawat, O.P., Vijay, B. and Mediratta, V. (2004). Physico-chemical properties of spent mushroom substrates of *Agaricus bisporus*. *Mushroom Research*. **13**(2): 84-94.

Hadar, Y., Chet, I. and Hennis, y. (1989). Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum. Phytopathology.* **69:** 64-68.

Harender, R.J., Kapoor and Raj, H. (1997). Possible management of *Fusarium* wilts of capsicum by soil amendments with composts. *Indian Phytopathology*. **50**: 387-394.

Harman, G.E. (2000). Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease.* **84**: 377-393.

Harman, G.E. Chet, I. and Baker , R. (1981). Factors affecting *Trichoderma hamatum* applied to seed as a biocontrol agent. *Phytopathology*. **71**: 569-572.

Harman, G.E. Chet, I. and Baker, R. (1980). *Trichoderma hamatum* on seeding diseases induced in radish and pea by *Pythium* spp. and *Rhizoctonia solani. Phytopathology* **70**: 1107-1172.

Harman, G.E., Howell, C.R. Viterbo, A., Chet, I. and Lorito, M. (2004). *Trichoderma* species opportunistic avirulent plant symbionts. *Nature Reviews, Microbiology.* **2:** 43-56.

Hermosa, M.R., Grondona, I., Iturriaga, E.A., Diaz-Minguaz, J.M., Castro, C., Monte, E. and Garcia-Acha, I. (2000). Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. *Applied Environmental Microbiology.* **66:** 1890-1898.

Hilocks, R. J. and Waller, J. M. (1997). Soil borne diseases of tropical crops, (ed.). CAB International, New York, USA. 77-397 pp.

Hoitink, H. and Fahy P.C. (1986). Basis for the control of soil borne plant pathogens with composts. *Annual Review of Phytopathology*. **24**: 93-114.

Hoitink, H.A.J. and Boehm, M.J. (1999). Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annual Review of Phytopathology*. **37**: 427–446.

Hoitink, H.A.J. and Fahy, P.C. (1986). Basis for the control of soilborne plant pathogens with composts. *Annual Review of Phytopathology*. **24**: 93–114.

Hoitink, H.A.J., Boehm, M.J., Hadar Y. (1993). Mechanisms of suppression of soil-borne plant pathogens in compost-amended substrates. Science and Engineering of composting: Design, Environmental, microbiological and Utilization aspects, Renaissance Publications, Worthington, Ohio. pp. 601-621.

Hoitink, H.A.J., Stone, A.G. and Han, D.Y. (1997). Suppression of plant diseases by composts. *Horticultural Science* **32**: 184–187.

Howell, C. R.(2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Disease.* **87**: 4-10.

Howell, C.R. (1998). The role of antibiosis in biocontrol. *In*: G.E. Harman and C.P. Kubicek (Eds.) *Trichoderma and Glioladium*. **2** (173-184).

Howell, C.R., Hanson, L.E., Stipanovic, R.D. and Puckhaber, L.S. (1999). Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Phytopathology*. **90**: 248-252.

Huang, J.W., Hu, C.K. and Shih, S.D. (1996). The role of soil microorganisms in alleviation of root injury of garden pea seedlings by alachlor with spent golden mushroom compost. *Plant Pathology Bulletin*. **5**(3): 137-145.

Huang, J.W., Hu, C.K., Tzeng, D.D.S. and Ng, K.H. (1995). Effect of soil amended with spent golden mushroom compost on alleviating phytotoxicity of alachlor to seedling of garden pea. *Plant Pathology Bulletin*. **4**(2): 76- 82.

Islam, M.T. and Faruq, A.N. (2007). Effect of selected soil amendments on seed germination, seedling growth and control of damping-off of chilli seedlings. *Journal of Sher-e-Bangla Agricultural University*. **2**(2): 12-16.

Jan, M.J., Nisar, A.D., Tariq, A.B., Arif, H.B. and Mudasir, A.B. (2013). Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *International Journal of Modern Plant Animal Science* **1**(2): 39-57.

Jash, S. and Pan, S. (2004). Stable mutants of *Trichoderma virens* for biological control of *Rhizoctonia solani* that causes seedling blight in green gram. *Journal of Mycology and Plant Pathology*. **34**(2): 476-79.

Jayaraj, J., Radhakrishnan, N. V. and Velazhahan, R. (2006). Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum*. *Phytopathology and Plant Protection.* **39**(1): 1-8.

Jonathan, S.G., and Adeoyo, O.R. (2011). Evaluation of ten wild Nigerian mushrooms for amylase and cellulase activities. *Mycobiology*. **39**: 103-8.

Jonathan, S.G., Okorie, A.N., Babayemi, O.J., Oyelakin, A.O. and Akinfemi, A. (2012b). Biodegradation of agricultural wastes (rice straw and sorghum stalk) into substrates of utilizable products using white rot fungus (*Pleurotus florida). Nature and Science*. **10**(9): 131-137.

Jones, J.B., Jones, J.P., Stall, R.E. and Zitter, T.A. (1997). Compedium of tomato Diseases. The *American Phytopathological Society*, United States of America. pp. 20-25.

Jordan, S.N., Mullen, G.J. and Murphy, M.C. (2008). Composition variability of spent mushroom compost in Ireland. *Bioresource Technology*. **99**(2): 411-418.

Kaddous, F.G.A. and Morgans, A.S. (1986). Spent mushroom compost and deep litter fowl manure as a soil ameliorant for vegetables. Surface Soil Management. *In*: 'Proceedings New Zealand Society of Soil Science Australian Society of Soil Science Joint Conference', pp. 138-47. Rotorua. (NZSSS/ASSSI: Rotorua.)

Kapil, R. and Kapoor A.S. (2005). Management of white rot of pea incited by *Sclerotinia sclerotiorum* using *Trichoderma* spp. and biopesticides. *Indian Phytopathogy*. **58**(1). 10-16.

Karthikeyan G., Sabitha Ooraiswamy and Shivkumar, C.V. (2001). Biological and chemical control of *Pythium aphanidermatum, Aleloidogyne incognita* disease complex in chilli and brinjal. *Madras Agricultural Journal.* **87** (1/3)*:* 105-108.

Kaul, V.K. and Chhabra, H.K. (1993). Control of *Meloidogyne incognita* by incorporation of organic wastes. *Plant Disease Research*. **8**: 35-41.

Kleifeld, O., & Chet, I. (1992). *Trichoderma harzianum* – interaction with plants and effect on growth response. *Plant and Soil.* **144**: 267-272.

Koenig, R.A.and Johnson, C.R. (1942). Colorimetric determination of P in biological materials. *Industrial and Engineering Chemical Analysis.* **14**: 155-56.

Laha, G. S. and Venkataramana, S. (2001). Sheath blight management in rice with biocontrol agents. *Indian Phytopathology.* **54**(4): 461-464.

Lartey, R.T., Curl, E.A. and Peterson, C.M. (1994). Interaction of Mycophagous collembola and biological control fungi in the suppression of *Rhizoctonia solani. Soil Biology and Biochemistry.* **26**: 81-88.

Lau, K.L., Tsang, Y.Y. and Chiu, S.W. (2003). Use of spent mushroom compost to bioremediate PAH-contaminated samples. *Chemosphere.* **52**: 1539–1546.

Lewis J.A. and Lumsden, R.D. (1995). Do pathogenic fungi have the potential to inhibit biocontrol fungi? *Journal of Phytopathology.* **143**: 585-588.

Lewis J.A. and Papavizas, G.C. (1985). Effect of mycelial preparations of *Trichoderma* and *Gliocladium* on population of *Rhizoctonia solani* and the incidence of damping-off. *Phytopathology.* **75**: 812-817.

Li, Y. (2012). Present development situation and tendency of edible mushroom industry in China. *In*: 18th Congress of the International Society for Mushroom Science. Beijing, China, pp. 3–9.

Lindner, R.C. (1944). Rapid analytical method for some of the more common inorganic constituents of plant tissues. *Plant Physiology.* **19**: 76-89.

Lohr, V.I., Wang, S.H. and Wolt, J.D. (1984). Physical and chemical characteristics of fresh and aged spent mushroom compost. *Horticultural Science.* **19**(5): 681-683.

Lucas, J.A. (1998). *Plant pathology and plant pathogens.* 3rd Edition. pp. 273.

Maher, M.J. (1994). Spent mushroom compost (SMC) as a nutrient source in peat based potting substrate. *Mushroom Science.* **13**(2): 645-650.

Mandelbaum, R. and Hadar, Y. (1990). Effects of available carbon source on microbial activity and suppression of *Pythium aphanidermatum* in compost and peat container media. *Phytopathology.* **80**: 794–804.

Maynard, A.A. (1994). Sustained vegetable production for three years using composted animal manures. *Compost Science and Utilization*. **2**: 88-96.

Mcspadden Gardner, B.B. and Fravel, D.R. (2002). Biological control of plant pathogens: Research, commercialization, and application in the USA. *Plant Health Progress*. Online, publication dio:10.1049/PHP- 2002-0510-01-RV.

Monaco, C., Perello, A., Alippi, H.E. and Pasquare, A. (1991). *Trichoderma spp.* a biocontrol agent of *Fusarium spp.* and *Sclerotium rolfsii* by seed treatment. *Advances in Horticultural Science*. **5**: 92-95.

Monga, D. and Sheoraj. (1994). Progress of root rot in American (*Gossypium hirsutum*) and desi (*G. arboretum*) cotton varieties in northern region. National symposium on "current trends in the management of plant disease" held at CCS HAU, Hisar in Nov. 10-11.

Morton, D.J and Strouble, U.H (1955).Antagonistic and stimulatory effect of soil microorganisms upon *Sclerotium rolfsii. Phytopathology,* **45**: 417-420.

Mukherjee, P.K., Upadhyay, J.P. and Mukhopadhyay, A.N. (1989). Biological control of *Pythium* damping-off of cauliflower by *Trichoderma harzianum*. *Jornal of Biological Control*. **8**: 105-110.

Mukherjee, R. and Nandi, B. (2004). Improvement of *in vitro* digestibility through biological treatment of water hyacinth biomass by two *Pleurotus* spp. *International Biodeterioration & Biodegradation*. **53**: 7–12.

Mukhopadhyay, A. N. (1995). Exploitation of *Trichoderma harzianum* and *Gliocladium virens* for biological seed treatment against seed borne disease. *Indian Journal of Mycology and Plant Pathology*. **2**(1&2): 124.

Mukhopadhyay, A.N. (1987). Biological control of soil borne plant pathogens by *Trichoderma* spp. *Indian Journal of Mycology and Plant Pathology*. **17**: 1-10.

National Horticultural Board. (2013).

Nelson, D.W. and Sommer, L.E. (1982). A rapid and accurate procedure for estimation of organic carbon in soil. *Proceedings|Indiana Academy of Science*. **84**: 456-62.

Nelson, E.B. and Craft, C.M. (1992). Suppression of dollar spot on creeping bentgrass and annual bluegrass turf with compost-amended topdressings. *Plant Disease.* **76**: 954–958.

Noble, R. and Coventry, E. (2005). Suppression of soilborne plant diseases with composts: a review. *Biocontrol Science and Technology.* **15**(1): 3–20.

Oei, P. and Albert, G. (2012). Recycling casing soil. *In*: 18th Congress of the International Society for Mushroom Science. Beijing, China, pp. 757–765.

Omarini, A., Nepote, V., Grosso, N.R., Zygadlo, J.A. and Alberto, E. (2010). Sensory analysis and fruiting bodies characterization of the edible mushrooms *Pleurotus ostreatus* and *Polyporus tenuiculus* obtained on leaf waste from the essential oil production industry. *International Journal of Food Science and Technology*. **45**: 466-474.

Pannier, W. (1993). Spent mushroom compost: A natural resource that provides solution to environmental problems. *Mushroom news.* **41**: 10-11.

Papavizas, G.C. (1985). *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. *Annual Review of Phytopathology*. **23**: 23-54.

Papavizas, G.C. and Lumsden, R.D. (1980). Biological control of soil-borne fungal propagulas. *Annual Review of Phytopathology*. **18**: 389-413.

Papavizas, G.C., Adams, P.B.,Lumsden, R.D., Lewis, J.A., Dow, R.L., Ayers, W.A., Kantzes, J.G. (1975). Ecology and epidemiology of *Rhizoctonia solani* in field soil. *Phytopathology*. **65:** 871-77.

Papavizas, G.C., Lewis, J.A. and Abd-El Moity, T.H. (1982). Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. *Phytopathology.* **72**: 126-132.

Park., M.S., Seo, G.S., Lee, K.H., Bae, K.S. and Yu, S.H. (2005). Morphological and cultural characteristics of *Trichoderma* spp. associated with green mould of oyster mushroom in Korea. *Journal of Plant Pathology.* **21**(3): 221-228.

Parmeter, J.R. (Jr.) (1970). *Rhizoctonia solani*, Biology and Pathology, Univ. of California Press. pp. 255.

Parmeter, J.R. (Jr.) and Whitney, H.S. (1970). Taxonomy and nomenclature of imperfect state. *In*: Parmeter, J.R., Jr. Ed. *R. solani*, Biology and Pathology, Univ. of California press, Berkeley. pp. 7-9.

Pascual J.A., Hernandez, T., Garcia, C., De Leij, F.A.A.M. and Lynch, J.M. (2000). Long-term suppression of *Pythium ultimum* in arid soils using fresh and composted municipal wastes. *Biology and Fertility of Soils.* **30**: 478-484.

Perez-Piqueres, A., Edel-Hermann, V., Alabouvette, C. and Steinberg, C. (2006). Response of soil microbial communities to compost amendments. *Soil Biology* and *Biochemistry*. **38**: 460-470.

Persoon, C.H. (1794). Dispositio methodica fungorum. Römer´s neues. *Magazin Botanische.* **1**: 81-128.

Phae, C.G., Sasaki, M., Shoda, M. and Kubota, H. (1990). Characteristics of *Bacillus subtilis* isolated from composts suppressing phytopathogenic microorganisms. *Soil Science and Plant Nutrition.* **36**: 575-586.

Philippoussis, A., Zervakis, G.I., Diamantpoulou, P., Papadopoulou, K. and Ehaliotis, C. (2004). Use of spent mushroom compost as a substrate for plant growth and against plant infections caused by *Phytophthora. Mushroom Science*. **16**: 579-584.

Pill, W.G., Evans, T.A. and Garrison, S.A. (1993). Forcing white button asparagus in various substrates under cool and warm regimes. *Horticultural Science.* **28**(10): 996-998.

Poddar, R.K., Singh, D.V. and Dubey, S.C. (2004). Integrated application of *Trichoderma harzianum* mutants and carbendazim to manage chickpea wilt (*Fusarium oxysporum* f.sp. *ciceri*). *Indian Journal of Agricultural Science.* **74**: 346–348.

Polat, E., Onus, A.N. and Demir, H. (2004). The effects of spent mushroom compost on yield and quality in lettuce growing. *Journal of Faculty of Agricultural Akdeniz University.* **17**(2): 149-154.

Prasad, C.S. and Gupta, V. (2002). Studies on bio-efficacy of *Trichoderma harzianum* in management of stem rot of potato (*Rhizoctonia solani*). *National Academy Science Letters.* **25**(11/12): 357-359..

Rifai, M.A. (1969). A revision of the genus *Trichoderma*. *Mycology*. pp. 116-156.

Rivera, M.C., Wright, E.R., Lopez, M.V., Garda, D. and Barrague, M.Y. (2004). Promotion of growth and control of damping off (*Rhizoctonia solani*) of greenhouse tomatoes amended with vermicompost. Phyton, *International Journal of Experimental Botany.* **53**: 229-235.

Roiger, D.J., Jeffer, S.N. and Caldwell, R.W. (1991). Occurrence of *Trichoderma* species in apple orchard and woodland soil. *Soil Biology and Biochemistry.* **23**: 353-359.

Rojo, F.G., Reynoso, M.M., Sofia, M.F., Chulze1, N. and Torres, A.M. (2007). Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. *Crop Protection*. **26**: 549-555.

Romaine, C.P. and Holcomb E.J. (2001). Spent mushroom substrate: a novel multifunctional constituent of potting medium for plants. *Mushroom news.* **49**: 4-15.

Roy, A.K. (1977). Parasitic activity o\f *Trichoderma viride* on sheath blight fungus of rice. *Jornal of Plant Disease Protection.* **84**: 675-683.

Roy, S. K., Das, B. C. and Bora, L. C. (1998). Non-pesticidal management of damping-off of cabbage and cauliflower caused by *Rhizoctonia solani* Kuhn. *Journal of Agricultural Sciences Society*. **11**(2): 127-130.

Ryckeboer, J. (2001). Biowaste and Yard Waste Compost: Microbiological and Hygienic Aspects-Suppressiveness to Plant Diseases. PhD Thesis, Katholieke Universiteit Leuven, Belgium.

Sabaratnam, S. and Traquair, J.A. (2002). Formulation of a *Streptomyces* as a biocontrol agent for the suppression of *Rhizoctonia* damping-off in tomato Transplants. *Biological Control*. **23**: 245-253.

Saikia, U.N. (1976). Blight of mung caused by *Corticium sasakii* a new disease recorded from Assam. *Indian Phytopathology*. **29:** 61-62.

Saikia, U.N. and Roy, A.K. (1976). Natural occurrence of *Corticium sasakii* on few plants in Jorhat. *Assam Science Cul*. **42:** 228-229.

Samuels, G.J. (1996). *Trichoderma*: a review of biology and systematic of the genus. *Mycology Research.* **100**: 923-935.

Sanz, L., Montera, M., Grondon, I., Vizcaino, J. A. and Monte, E. (2004). Cell well degrading isoenzyme profiles of *Trichoderma* biocontrol strain have correlation with rDNA taxonomical species. *Current Genetics.* **46:** 277-286.

Scher, F.M., Ziegle, J.S., and Klopper, J.W. (1984). A method for assessing the root colonization capacity of bacteria on maize. *Canadian Journal of Microbiology* **30**: 151-157.

Seaby, D. (1996). Differentiation of *Trichoderma* taxa associated with mushroom production. *Plant Patho*logy. **45**: 905-912.

Segarra, G., Casanova, E., Borrero, C., Aviles, M., and Trillas, I. (2007). The suppressive effects of composts used as growth media against *Botrytis cinerea* in cucumber plants. *European Journal of Plant Pathology*. **117**: 393-402.

Semple, K.T., Reid , B.J. and Fermor, T.R. (2001). Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environmental Pollution*. **112**: 269–283.

Shabir, R., Rubina, L, Ebenezer, J.K., Talat, M.A., Shabir, A.G., Waseem, A.D. and Javid, A.B. (2013). Eco-friendly management of root-rot of chilli caused by *Rhizoctonia solani* Kuhn. *Africal Journal of Agricultural Research.* **8**(21): 2563-2566.

Shabir, R., Rubina, L., Ebenezer, J.K. and Zaffar, A.B. (2012). Comparative efficacy of *Trichoderma viride, T. harzianum* and carbendazim against damping-off disease of cauliflower caused by *Rhizoctonia solani* Kuhn. *Journal of Biopesticide.* **5**(1): 23-27.

Shibuya, N. and Minami, E. (2001). Oligosaccharide signaling for defense responses in plant. *Physiological & Molecular Plant Pathology*. **59**: 223-233.

Singh, A.D., Vikineswary, S., Abdullah, N. and Sekaran, M. (2011). Enzymes from spent mushroom substrate of *Pleurotus sajor-caju* for the decolourisation and detoxification of textile dyes. *World Journal of Microbiology & Biotechnology*. **27**: 535-545.

Singh, S.B. and Saksena, H.K. (1980). A new sheath and leaf blight of Bajra. *Indian Phytopatholgy*. **33:** 127-129.

Sivan, A. and Chet, I. (1986). Degradation of fungal cell walls by lytic enzymes of *Trichoderma harzianum. Journal of Genetics & Microbiology*. **135**: 675-682.

Sivasithamparam, K. and Ghisalberti, E.L. (1998). Secondary metabolism in *Trichoderma* and *Glioladium. In*: C.P. Kubicek and G.E. Harman (Eds.). *Trichoderma* and *Glioladium.* **1**: 139-191.

Sneh, B. (1996). Nonpathogenic isolates of *Rhizoctonia* spp. (np-R) and their role in biological control. *In*: Biology, ecology, pathology and disease control. pp. 473-483.

Sochtig, H. and Grabbe, K. (1995). The production and utilization of organic-mineral fertilizer from spent mushroom compost. *Mushroom Science*. **14**(II): 907-915.

Steffen, K.L., Dann, M.S., Fager, K., Fleisher, S.J., and Harper, J.K. (1994). Short-term and long-term impact of an initial large scale SMS soil amendment on vegetable crop productivity and resource use efficiency. *Compost Science* and *Utilization*. **2**(4): 75-83.

Stewart, D. P. C., Cameron, K. C., Cornforth I. S. and Sedcole, J. R. (1998). Effects of spent mushroom substrate on soil physical conditions and plant growth in an intensive horticultural system. *Australian Journal of Soil Research.* **36**(6): 899-912.

Stoffella, P.J. and Kahn, B.A. (Eds.). (2001). Compost utilization in horticultural cropping systems. Boca Raton: Lewis Publishers.

Talbot, P.H.B. (1970). Taxonomy and nomenclature of the perfect state. *In*: J.R. Parameter (Jr.) *Rhizoctonia solani*, biology and pathology, University of California Press, Berkeley. pp. 20-31.

Tewari, L. and Singh, R. (2005). Biological control of sheath blight of rice by *Trichoderma harzianum* using different delivery systems. *Indian Phytopathogy*. **58** (1): 35-40.

Tuitert, G., Szczech, M. and Bollen, G.J. (1998). Suppression of *Rhizoctonia solani* in potting mixtures amended with compost made from organic household waste. *Phytopathology*. **88**: 764-773.

Vallad, G.E. and Goodman, R.M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Scence.* **44**:1920-1934.

Vasudeva, R.S and Sikka, M.R. (1941). Studies on the root rot disease of cotton in the Punjab. Effect of certain fungi on the growth of root fungi. *Indian Journal of Agricultural Science*. **11:** 422-431.

Vijay, B. (2005). Formulations for compost for white button mushroom. *In*: *Frontiers in Mushroom Biotechnology*, R.D. Rai, R.C. Upadhyay and S.*R.* Sharma (eds.), pp. 80-87, National Research Centre for Mushroom, Solan.

Viji, G., Uddin, W. and Romaine, C.P. (2003). Suppression of gray leaf spot (blast) of perennial ryegrass turf by *Pseudomonas aeruginosa* from spent mushroom substrate. *Biological Control.* **26**: 233-243.

Vincent, J.M. (1947). Distortion of fungal hyphae in presence of certain inhibitor. *Nature*, **150**:850.

Walters, D., Walsh, D., Newton, A. and Lyon, G. (2005). Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. *Phytopathology*. **95**: 1368-1373.

Wang, S. H., Lohr, V. I., and Coffey, D. L. (1984). Spent mushroom compost as a soil amendment for vegetables. *Journal of American Society of Horticultural Science*. **109**: 698-702.

Wang, S.T. (1977). Comparison of the effect of [NH.sub.4-] nitrohumate, garbage compost and waste mushroom compost on sweetcorn yield. *In*: 'Proceedings of the International Seminar on Soil Environment and Fertility Management in Intensive Agriculture', pp. 725-30. (The Society of the Science of Soil and Manure: Tokyo).

Wardle, D.A., Parkinson, D. and Waller, J. E. (1993). Interspecific competitive interaction between pairs of fungal species in natural substrates. *Ecologia*. **94**: 165-172.

Weindling, R. (1932). *Trichoderma lignorum* as a parasitic of other fungi. *Phytopathology.* **22**: 837-845.

Weindling, R. (1934). Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology.* **24:** 1153-1179.

Weindling, R. (1937). Isolation of toxic substances from culture filtrates of *Trichoderma* and *Gliocladium*. *Phytopathology*. **27:** 1175-1177.

Widmer, T.L., Graham, J.H. and Mitchell, D.J. (1999). Composted municipal solid wastes promote growth of young citrus trees infested with *Phytophthora nicotiancae*. *Compost Science and Utilization.* **7**: 6–16.

Wuest, P.J. and Fahy, H.K. (1991). Spent mushroom compost traits and uses. *Mushroom news.* **39**(12): 9- 15.

Yohalem, D.S., Harris, R.F. and Andrews, J.H. (1994). Aqueous extracts of spent mushroom substrate for foliar disease control. *Compost Science and Utilization.* **2**(4): 67-74.

Zhang, W., Han, D.Y., Dick, W.A., Davis, K.*R.* and Hoitink, H.A.J. (1998). Compost and compost water extract-induced systemic acquired resistance in cucumber and *Arabidopsis. Phytopathology.* **88**: 450–455.

ABSTRACT

Title of thesis	:	**Studies on management of spent mushroom substrate**
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Admission number	:	2012A77M
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Name and address of Major Advisor	:	**Dr. R. S. Rana** Department of Plant Pathology CCS Haryana Agricultural University Hisar, Haryana
Degree awarding University	:	Chaudhary Charan Singh Haryana Agricultural University, Hisar
Year of award of degree	:	2014
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Keywords: Spent mushroom substrate, physico-chemical properties, *Trichoderma, Rhizoctonia solani*, bio-control

Spent mushroom substrate (SMS) is the result of mushroom houses which remain after the harvest of mushroom crop. It is rich in nutrients and bio-agents which can not only supply nutrition to the soil but can also help in management of soil-borne plant pathogens. The physico-chemical analysis of SMS showed that electrical conductivity and pH of SMS was 1.27 mmhos cm^{-1} and 7.52 respectively. The NPK content was in the ratio 2:1.2:1 and the micronutrients viz. Fe, Zn, Cu, and Mg were present in traces. Two *Trichoderma* spp., *T. harzianum* and *T. viride* were isolated from SMS using serial dilution and plating method and efficacy of these bio-agents was tested *in vitro* for the per cent mycelial growth inhibition of *R. solani*. *T. harzianum* showed maximum antifungal activity restricting its mycelial growth to 12.33 mm with 72.29 per cent inhibition followed by *T. viride* (16.16 mm) which showed 63.68 per cent growth inhibition. In screen house, these bio-agents were evaluated against *R. solani* and it was found that seed treatment of tomato with *T. harzianum* along with addition of SMS @ 15% showed maximum seed germination and minimum seedling mortality. In nursery conditions, addition of SMS @ 2.5 kg/m^2 was found to be most effective which showed the maximum seed germination and maximum disease control.

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I, hereby, declare that all the information given in the resume is true to the best of my knowledge.

Dated:

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