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Screening and Potential Uses of Contaminated Spent Mushroom (*Pleurotus* spp.)

Mathipriya Shanmugavelu and Ganesan Sevugaperumal

Abstract

The commercial production of mushrooms generates a co-product, a virtually inexhaustible supply of spent mushroom substrate (SMS). It represents an ideal growth medium for plants and plant disease suppressive quality. Here we discussed about the contaminated microbial flora of SMS, potential antifungal and plant growth promoting activities, the results of these findings were also discussed in relation to the usage of SMS as a potential product for organic farming. SMS contained moisture content 72%, EC 1.75 mmho.cm⁻¹ and had pH of 6.1. The cellulose and hemicellulose content of paddy straw substrate were 30.25%, 23.18% and 15.31% dry weight respectively. Growth in terms of root and shoot weight of the seedlings of green gram, black gram, tomato and chili were significantly higher when grown in 60% SMS amended soil. Spent mushroom compost from *Pleurotus eous* used in this study harbored bacterial population including, *Bacillus* sp., *Clostridium* sp., *Pseudomonas* sp. and *E. coli*. Bacterial isolate B1 was identified as *Bacillus* sp., isolate B2 was identified as *Clostridium* sp., isolate B3 as *Pseudomonas* sp. and B4 as *Escherichia coli*. These bacterial strains showed significant antagonistic activity against soil borne pathogenic fungi viz., *Fusarium* sp., *Alternaria* sp., *Phytophthora* sp. and *Aspergillus* sp.

Keywords: SMS, *Pleurotus*, antifungal activity, contaminants

1. Introduction

Mushrooms have been recognized as the alternate source of good quality protein. They are capable of producing the highest quantity of protein per unit area and time from agro-wastes which are available to the tune of more than 300 million tons per annum in India. Many species of mushrooms are cultivated world-wide. Seventy percent of the global mushroom production is derived from three mushroom groups, *Agaricus bisporus*, *Pleurotus* spp., and *Lentinula edodes*. The remaining mushroom volume is generated by at least a dozen species [1].

Edible mushrooms commonly have insignificant lipid level with higher proportion of polyunsaturated fatty acids. All these result in low calorific yield from mushroom foods. Mushrooms do not have cholesterol. Instead, they have ergosterol that acts as a precursor for Vitamin D synthesis in human body. Similarly, ergosterol in button mushroom is converted in to vitamin D2 when exposed to UV radiation

or sunlight. The protein content of edible mushrooms is usually high, but varies greatly. The crude protein content of mushrooms varied from 12 to 35% dry weight depending upon the species. The free amino acids composition differs widely but in general they are rich in threonine and valine but deficient in sulfur containing amino acids (Methionine and cysteine) [2].

Antibiotic resistance has become a global concern [3]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens [4–6]. Already, a number of antibacterial compounds have been isolated from Basidiomycetes fungi, including Collybia and Frustulosin [7–9].

Pleurotus spp. are mushrooms which belong to the class basidiomycetes. They are generally understood to be called white rot fungi, because of their ability to degrade lignocellulosic materials. The oyster mushroom consists of a number of several edible *Pleurotus* species. This species represented 14% of the world production in 1997 [1]. *Pleurotus* mushrooms are edible with excellent flavor and taste. They have nutritional as well as medicinal properties [10]. They are low in calories, fats, sodium, carbohydrates and cholesterol, while being rich in proteins, minerals, vitamins and fibers [11]. *Pleurotus* spp. is promising as medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulation activities [12].

1.1 Spent mushroom substrate

After the cultivated mushroom have exhausted the nutrients within the substrates, and there were no more fruitbodies harvest, the so called remains, regarded as “the useless material” is known as spent mushroom substrate (SMS) [13].

Several agro industrial wastes could be used to prepare mushroom composts. These growing substrates may be composed from different wastes materials such as sawdust, rice straw, bedded horse manure, cotton wastes, paper wastes, cocoa shells, wheat straw, maize husks and various other wastes [14]. Additives such as rice bran, calcium carbonate or wheat bran may be added to enhance mushroom fructification [15].

Compost is considered “spent substrate” when one full crop of mushroom has been taken and further extension becomes unremunerative [16–18]. Mushroom industry needs to dispose off more than 50 million tons of used mushroom compost each year called Spent Mushroom Substrate (SMS) [19]. Recently, the term spent compost or spent mushroom substrate has been replaced by a more appropriate term, “post mushroom substrate” because it is not “spent” and is ready to be further attacked by a new set of microorganisms. The large dumped piles of spent mushroom substrate become anaerobic and give off offensive odor. The run-off from such piles contaminates nearby water sources and pollutes them [20]. Under normal circumstances, the spent mushroom substrate is discarded as waste without considering environmental repercussion.

The disease suppressive properties of composting materials are known for many decades and much scientific evidence have revealed favorable properties of composts for the management of plant diseases [21, 22]. Due to the unique chemical constitution and the microflora present in SMS, its application can be more diversified than what is normally predicted.

1.2 The Management of Spent Mushroom Substrate (SMS)

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.

2. Review of literature

Mushroom is a macrofungus with a distinctive fruiting body, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand [23]. The number of mushroom species on the earth is estimated to be 1,40,000 suggesting that only 10% are known. Assuming that the proportion of useful mushrooms among the undiscovered and unexamined mushrooms will be 5%, which implies 7000 yet undiscovered species will be of possible benefit to mankind [24].

2.1 *Pleurotus spp.*

Mushrooms are considered as a functional food, which can provide health benefits beyond the traditional nutrients they contain [25, 26]. Nowadays, several species of *Pleurotus* are cultivated commercially because of their rich mineral contents and medicinal properties, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes and low demand on resources and technology.

2.2 Anti-microbial activity of *Pleurotus spp.*

Water and alcoholic extracts from *P. ostreatus* mycelium have been used in studies on antimicrobial activities against numerous types of microbes. The highest potency was shown by water extract, especially towards fungi, *Candida albicans*, *Cryptococcus humicola*, *Trichosporon cutaneum* and bacteria *Staphylococcus aureus* and *Escherichia coli* [27, 28].

The antimicrobial properties of mushroom extracts and highlighted some of the active compounds identified, including low- and high-molecular weight compounds which showed antagonistic activity against gram positive bacteria. LMW compounds are mainly secondary metabolites, such as sesquiterpenes and other terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolines, but also primary metabolites such as oxalic acid. HMW compounds are mainly peptides and proteins.

2.3 Spent mushroom substrate (SMS)

After mushroom cultivation, the partially degraded paddy or wheat straw and other agricultural waste, which form as valuable by-products of edible mushroom cultivation, have been termed as Spent Mushroom Substrate (SMS). Antibacterial activity of *H. erinaceus* SMS against phyto-pathogenic bacteria and evaluated the role of this extract in improving plant defense and growth [29, 30].

2.4 Composition of SMS

The macro and micronutrients of the raw material and the initial and spent substrates of *Pleurotus ostreatus* [31–33]. The mineral composition of the fruiting body varied with the substrates, which made possible the production of a fruiting body rich in K, P, Mg and Fe. Potassium was the mineral with the highest content in the fruiting body in all substrates tested. There was an increase in protein and mineral content in the spent substrate in relation to the initial one.

The pH of the compost was found to be 7.58 and the electrical conductivity of the compost was found to be 0.71 dms^{-1} . Chemical analysis of the compost showed varying organic matter and nutrient content. The carbon to nitrogen (C: N) ratio of a product was 13:1 and the bioavailability of total potassium (2.64%), magnesium

(2.26%) and calcium (5.16%) were comparatively higher to the availability of total phosphorous (0.48%) and sodium (0.29%).

2.5 Anti-microbial activity of SMS

The uncontrolled use of antibiotics has caused serious problems in human and animal health, causing that pathogens develop resistance to them, so World Health Organization considered the infections caused by pathogens resistant to drugs as a public health problem; therefore, it is necessary to find new pharmacological strategies, among which we can find natural products such as plants and fungi.

The results showed that in the case of *Escherichia coli*, the greatest inhibition zone was of 12.66 mm at a concentration of 6 mg ml⁻¹, with treatment of *Lentinula edodes*/cedar; *Salmonella typhimurium* showed a greatest inhibition zone of 31.10 mm to a concentration of 5.12 mg mL⁻¹, with treatment of *Pleurotus ostreatus*/Barley straw.

2.6 Microbial flora of SMS

The bacterial diversity in SMC by using molecular techniques in order to reveal the origin of SMC microflora and its potential effect on soil microbial communities after incorporation into agricultural soils [30, 34, 35]. Fifty bacterial isolates were classified into 14 operational taxonomic units (OTUs) following ARDRA-PCR of the 16S rDNA gene. Sequencing of the 16S rDNA amplicon assigned 12 of the 14 OTUs to Gram-positive bacteria, associated with the genera *Bacillus*, *Paenibacillus*, *Exiguobacterium*, *Staphylococcus*, *Desemzia*, *Carnobacterium*, *Brevibacterium*, *Arthrobacter* and *Microbacterium* of the bacterial divisions *Firmicutes* and *Actinobacteria*.

Objectives of the study.

With the background research and the literature review on spent mushroom substrate (SMS), an attempt was made to isolate, identify and characterize the microbial flora of spent mushroom substrate of *Pleurotus eous*. The research involved the followings objectives:

- Analysis of the composition and physicochemical properties of spent mushroom substrate (SMS) of *Pleurotus eous*.
- Isolation of bacteria from SMS.
- Antimicrobial activity of the bacterial isolates against selected soil pathogenic fungi.

3. Materials and methods

Fresh Spent Mushroom Substrate (SMS) of *Pleurotus eous* was used for the present study. SMS was obtained by growing *Pleurotus eous* mushroom using paddy straw in the mushroom house at Post Graduate and Research Department of Botany, Thiagarajar College, Madurai. The spent paddy straw substrate after three yields of the mushroom *P. eous* was used fresh for characterization studies, dried in the sun and powdered for use in further analyses.

Laboratory tests measured the following properties: P^H, EC, soluble salts, moisture, organic matter, carbon, nitrogen and phosphorus, potassium, Carbon:Nitrogen (C:N) ratio, cellulose and hemicelluloses.

3.1 Growth studies

For pot culture studies, the powdered SMS was mixed with garden soil in different ratio as 20%,40%,60%80%100% and control.

Twenty seeds of green gram and black gram, fifty seeds of tomato and chili were sown in each pot containing garden soil with SMS in various concentrations. Pot contained only the garden soil was maintained as control for each treatment. The treatments were laid out in a completely randomized pattern with three replicates per treatment. Germination was performed under ambient conditions in the net house, and pots were irrigated daily. Growth was monitored up to 30 days after seeding.

3.2 Isolation of microbial flora from SMS

Sample of SMS of *Pleurotus eous* was mechanically stirred with distilled water. The SMS suspension was clarified by centrifugation, serially diluted (10^{-2} to 10^{-7}) with sterile water and inoculated on potato dextrose agar medium (PDA) and nutrient agar (NA) medium. All the strains observed growing on plates were arbitrarily selected, transferred and maintained in new PDA or NA plates for further use.

A representative sample colony of each visually differentiable bacterium was selected using a sterile inoculating loop. Each colony was transferred by streaking an inoculating loop in parallel lines over four quadrants of NA plate. The plates were incubated at 37°C for twenty-four hour. The isolated colonies were used for initial observations about the shape, color, size and other visual properties of each isolate were recorded. The bacterial strains isolated from the SMS of *Pleurotus eous* were characterized and identified by using the standard procedures.

3.3 Antifungal activity of bacteria isolated from SMS in dual culture plate assay

The fungal pathogens viz., *Fusarium sp.*, *Alternaria sp.*, *Phytophthora sp.*, and *Aspergillus sp.*, were obtained from Biotechnology lab at Post Graduate and Research Department of Botany, Thiagarajar College, Madurai.

Antifungal activity of bacteria isolated from SMS of *Pleurotus eous* mushroom was evaluated in a dual plate assay against four fungal pathogens of plants viz., *Fusarium sp.*, *Alternaria sp.*, *Phytophthora sp.* and *Aspergillus sp.* The bacteria and fungi were cultured in opposing fashion on PDA plates. Bacterial isolate broth (100 µl) of approximately 108 cells/ml was inoculated on PDA plates using spread plate technique. Mycelial agar plugs of one day old culture of the test fungi and the bacterial isolate were inoculated opposite to each other. All the plates were maintained at room temperature and observed for the appearance of zone of inhibition surrounding the bacterial colony where the fungal mycelium failed to grow.

4. Observations and results

4.1 Composition of spent mushroom substrate (SMS)

The empirical data depicted in **Tables 1** and **2** show the composition of Spent Mushroom Substrate (SMS) of *Pleurotus spp.*, compared with the substrate, paddy straw. The cellulose and hemicellulose content of paddy straw substrate were 30.25% and 23.18% and dry weight respectively. Cultivation of *Pleurotus eous* on the substrate had lowered the cellulose content by 24.89%. The hemicellulose

Component	Untreated substrate (paddy straw)	Spent mushroom substrate (paddy straw)	Degradation %
Cellulose (% dry wt.)	30.25 ± 0.73	23.97 ±0.65	24.89
Hemicellulose (% dry wt.)	23.18 ±0.43	22.23 ±0.64	3.35

Table 1.
Biochemical properties of fresh SMS.

pH	6.1
EC	1.75 mmho.cm ⁻¹
Moisture content	72%
N	0.87%
P	0.26%
K	0.19%
Organic carbon	15%
C:N	17.24:1

Table 2.
Physico chemical properties of fresh SMS.

utilization was less comparatively, showing a degradation rate of 3.35% from the initial content. The physico-chemical properties of SMS of Fresh SMS had an acidic pH 6.1 and its conductivity was 1.75 mmho.cm⁻¹. It had 72% moisture content. The fresh SMS had 0.87% nitrogen, 0.26% phosphorus, 0.19% potassium and 15% organic carbon. The C/N ratio of fresh SMS was 17.24:1.

4.2 Growth studies

4.2.1 Green gram

The effect of different concentrations of SMS extract on seedling fresh weight and dry weight of green gram revealed that it is also concentration dependent, as in case of seed germination. Green gram seedlings grown in 60% w/v aqueous SMS extract showed the highest shoot weight (2.112 g). The lowest fresh weight of shoot was observed in 100% of SMS extract (0.621 g). The highest root fresh weight (0.498 g) was found in the green gram seedlings treated with 60% of SMS extract and the lowest root fresh weight (0.157 g) in 100% SMS extract treatment. In case of whole plant, fresh weight of the whole plant was also the highest in 60% of SMS treated seedlings and the lowest in 100% concentration of SMS extract. Control seedlings had 2.074 g as whole plant fresh weight.

The observations regarding the dry weight of shoot, root and whole plant of green gram showed the same response shown for fresh weight. i.e. the seedlings treated with 60% SMS extract produced the highest dry weight values of 0.234 g, 0.056 g and 0.291 g for shoot, root and whole plant respectively. The lowest values of 0.069 g, 0.019 g and 0.069 g for shoot, root and whole plant respectively, were observed on 100% SMS extract treated seedlings. The control seedlings showed 0.187 g dry weight for shoot, 0.043 g for root and 0.230 g for whole plant (Table 3).

Aqueous SMS conc. (%) (w/v)	Fresh weight (g)			Dry weight (g)		
	Shoot	Root	Whole plant	Shoot	Root	Whole plant
20	1.693 ± 0.028	0.430 ± 0.014	2.124 ± 0.038	0.188 ± 0.043	0.046 ± 0.003	0.234 ± 0.005
40	1.620 ± 0.173	0.445 ± 0.012	2.066 ± 0.168	0.180 ± 0.019	0.049 ± 0.001	0.229 ± 0.019
60	2.112 ± 0.053	0.498 ± 0.012	2.610 ± 0.058	0.234 ± 0.005	0.056 ± 0.003	0.291 ± 0.008
80	1.891 ± 0.067	0.453 ± 0.013	2.344 ± 0.079	0.210 ± 0.017	0.049 ± 0.002	0.259 ± 0.010
100	0.621 ± 0.081	0.157 ± 0.033	0.778 ± 0.114	0.069 ± 0.009	0.019 ± 0.003	0.069 ± 0.009
Control	1.683 ± 0.020	0.390 ± 0.007	2.074 ± 0.016	0.187 ± 0.002	0.043 ± 0.002	0.230 ± 0.001

Table 3.
Effect of SMS on seedling growth of green gram.

4.2.2 Black gram

The results on the effect of SMS extracts on biomass of black gram are given and shown in **Table 4**. In shoot, the fresh weight, 1.824 g was the highest in 60% of SMS treated seedlings and the lowest fresh weight, 1.4 g was observed in 100% concentration of SMS extract. Similarly 60% SMS extract treated seedlings produced the highest fresh root weight value (0.536 g) and 100% of SMS extract treatment showed the least value (0.353 g). The highest whole plant fresh weight value (2.361 g) was found in the seedlings treated with 60% of SMS extract and the lowest value of whole plant fresh weight (1.753 g) was observed in 100% SMS extract treatment compared with the untreated control experiments (1.917 g).

In case of the dry weight of black gram seedlings, the seedlings treated with 40% SMS extract produced the highest dry weight values of 0.212 g and 0.265 g for shoot and whole plant respectively. In root, 0.059 g was found as the highest dry weight in seedlings treated with 60% of SMS extract, followed by 0.053 g in 40% SMS extract treatment. The lowest values of 0.155 g, 0.039 g and 0.194 g for shoot, root and whole plant respectively, were observed on 100% SMS extract treated seedlings. The control seedlings showed 0.171 g dry weight value for shoot, 0.041 g value for root and 0.213 g for whole plant (**Table 4**).

4.2.3 Tomato

Table 5 presents the results of the effect of aqueous extract of SMS in different concentrations on biomass of tomato. The highest shoot fresh weight value (4.446 g) was found in the seedlings treated with 60% of SMS extract, followed by 4.048 g in 80% SMS extract treated seedlings. Control seedlings had the lowest value of fresh weight (1.259 g). 1.108 g of root fresh weight was found as the highest in 60% of SMS treated seedlings and 0.301 g as the lowest root fresh weight in control. The 60% SMS treated seedlings produced the highest whole plant fresh weight value (5.554 g) followed by 80% treated seedlings (5.061 g) compared to the control seedlings (1.560 g).

Tomato seedlings, the seedlings treated with 60% of SMS extract produced the highest dry weight values of 0.264 g, 0.065 g and 0.330 g for shoot, root and whole plant respectively. The lowest dry weight values of 0.129 g in shoot of 100% SMS extract treated seedling, 0.039 g and 0.173 g for root and whole plant seedlings respectively, were observed in the seedlings treated as control. (**Table 5**).

4.2.4 Chili

Table 6 show the results on the effect of aqueous extract of SMS in different concentrations on biomass of chili. The highest shoot fresh weight value (8.887 g) was found in the seedlings treated with 60% of SMS extract, followed by 5.560 g in 40% treated seedlings and the lowest value of shoot fresh weight (0.779 g) in control. 1.488 g of root fresh weight was found as the highest in 60% of SMS treated seedlings and 0.253 g as the lowest fresh weight of root in control. The 60% SMS extract treated seedlings produced the highest whole plant fresh weight value (10.375 g) followed by 40% treated seedlings (6.848 g). The lowest fresh weight of shoot was observed in control seedlings (1.033 g).

The seedlings treated with 60% SMS extract produced the highest dry weight values of 1.903 g, 0.297 g and 2.200 g for shoot, root and whole plant respectively. The lowest values of 0.086 g, 0.028 g and 0.114 g for shoot, root and whole plant respectively, were observed for the seedlings treated as control (**Table 6**).

Aqueous SMS conc. (%) (w/v)	Fresh weight (g)			Dry weight (g)		
	Shoot	Root	Whole plant	Shoot	Root	Whole plant
20	1.513 ± 0.063	0.388 ± 0.015	1.902 ± 0.078	0.185 ± 0.004	0.048 ± 0.007	0.233 ± 0.004
40	1.612 ± 0.066	0.482 ± 0.007	2.094 ± 0.066	0.212 ± 0.002	0.053 ± 0.008	0.265 ± 0.003
60	1.824 ± 0.072	0.536 ± 0.008	2.361 ± 0.070	0.202 ± 0.008	0.059 ± 0.009	0.262 ± 0.007
80	1.691 ± 0.066	0.458 ± 0.013	2.149 ± 0.070	0.187 ± 0.007	0.050 ± 0.001	0.238 ± 0.007
100	1.400 ± 0.042	0.353 ± 0.008	1.753 ± 0.050	0.155 ± 0.004	0.039 ± 0.009	0.194 ± 0.005
Control	1.544 ± 0.042	0.373 ± 0.009	1.917 ± 0.048	0.171 ± 0.004	0.041 ± 0.001	0.213 ± 0.005

Table 4.
Effect of SMS on seedling growth of black gram.

Aqueous SMS conc. (%) (w/v)	Fresh weight (g)			Dry weight (g)		
	Shoot	Root	Whole plant	Shoot	Root	Whole plant
20	3.182 ± 0.038	0.828 ± 0.014	4.010 ± 0.048	0.189 ± 0.049	0.047 ± 0.018	0.237 ± 0.021
40	3.622 ± 0.031	0.898 ± 0.011	4.520 ± 0.043	0.215 ± 0.006	0.053 ± 0.024	0.269 ± 0.013
60	4.446 ± 0.094	1.108 ± 0.024	5.554 ± 0.119	0.264 ± 0.053	0.065 ± 0.027	0.330 ± 0.016
80	4.048 ± 0.095	1.012 ± 0.025	5.061 ± 0.120	0.240 ± 0.012	0.060 ± 0.038	0.300 ± 0.025
100	2.252 ± 0.258	0.658 ± 0.072	2.910 ± 0.330	0.129 ± 0.096	0.048 ± 0.039	0.177 ± 0.019
Control	1.259 ± 0.011	0.301 ± 0.009	1.560 ± 0.019	0.139 ± 0.001	0.033 ± 0.001	0.173 ± 0.002

Table 5.
Effect of SMS on seedling growth of tomato.

Aqueous SMS conc. (%) (w/v)	Fresh weight (g)			Dry weight (g)		
	Shoot	Root	Whole plant	Shoot	Root	Whole plant
20	5.178 ± 0.201	1.103 ± 0.037	6.282 ± 0.239	1.035 ± 0.040	0.220 ± 0.027	1.256 ± 0.047
40	5.560 ± 0.284	1.288 ± 0.037	6.848 ± 0.266	1.520 ± 0.125	0.257 ± 0.007	1.777 ± 0.129
60	8.887 ± 0.192	1.488 ± 0.028	10.375 ± 0.218	1.903 ± 0.134	0.297 ± 0.015	2.200 ± 0.135
80	4.517 ± 0.219	1.140 ± 0.051	5.658 ± 0.182	1.223 ± 0.098	0.228 ± 0.010	1.452 ± 0.104
100	3.921 ± 0.301	0.418 ± 0.048	4.339 ± 1.166	0.880 ± 0.022	0.083 ± 0.019	0.963 ± 0.254
Control	0.779 ± 0.022	0.253 ± 0.007	1.033 ± 0.029	0.086 ± 0.002	0.028 ± 0.001	0.114 ± 0.003

Table 6.
Effect of SMS on seedling growth of chili.

Bacterial isolate*	Inhibition zone (cm)			
	<i>Fusarium</i> sp.	<i>Phytophthora</i> sp.	<i>Alternaria</i> sp.	<i>Aspergillus</i> sp.
B1	0.45	1.17	1.12	1.66
B2	0.77	1.4	1.00	1.51
B3	1.05	1.25	0.75	0.82
B4	0.95	1.02	0.69	1.05

*Bacterial isolates - B1, B2, B3 and B4.

Table 7.
Antifungal activity of bacteria isolated from SMS in dual culture plate assay.

Figure 1.
Antifungal activity of bacteria isolated from SMS in dual culture plate assay. F1 - *Fusarium* sp. F2 - *Alternaria* sp. F3 - *Phytophthora* sp. F4 - *Aspergillus* sp.

4.3 Isolation of microbial flora from SMS

In this study, four different bacteria were isolated from the fresh spent mushroom substrate after *Pleurotus* spp., cultivation. The colony morphological variations among the four bacterial isolates of the bacterial strain 1 (B1) were irregular in shape with a colony size of 1.2 cm dia. The colonies had eros type margin rough or dry texture and were white in color. Cells of bacterial isolate B2 showed a diameter of 0.7 cm. The margin of B2 colonies were entire with smooth texture, mucoid consistency and cream in color. Colony morphology of B3 isolate showed irregular shaped colonies of 0.5 cm dia. B3 isolate colonies showed serrated margin with smooth or glistening texture and cream color. The B4 bacterial isolate had circular shaped colonies of size 0.2 cm with entire margin and yellow in color. The cellular morphology of B1, B2 and B3 isolates was observed as rods while B4 isolate had short rod shaped cells.

The biochemical properties of four bacterial strains isolated from the SMS obtained after *P. eous* cultivation showed significant variations in the biochemical characteristics studied. Based on the observations and results obtained by subjecting the bacterial colonies in different identification techniques, bacterial isolate B1 was identified as *Bacillus* sp., isolate B2 was identified as *Clostridium* sp., isolate B3 as *Pseudomonas* sp. and B4 as *Escherichia coli*. The findings on anti-fungal activity of four bacterial strains isolated from SMS are shown in **Table 7** and **Figure 1**.

5. Discussion

Mushroom growing is an ecofriendly activity as it utilizes the waste from agriculture, horticulture, poultry, brewery etc. for its cultivation. However, piling up of “spent mushroom substrate” released after mushroom crop harvesting may cause various environmental problems, including ground water contamination and nuisance [20, 36].

Production of 1 kg of mushrooms will generate 5 kg of spent residual material called spent mushroom substrate (SMS). An average farm discards about 24 t of SMS per month [37]. In Ireland, approximately 254,000 t of SMS is generated each year [38] and in The Netherlands, more than 800,000 t of SMS is produced per year [39].

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 favorable if SMS is to be recycled and reused. Considering the high organic matter of SMS, rapid advances have been made and the number of scientific research has increased in the past few years.

5.1 Composition of spent mushroom substrate (SMS)

Potting medium is an important factor for the production of crop in containers, and component and properties of the potting media are very crucial for higher and quality yields of potted plants. Chemical properties of growth media are very crucial from the point of view of nutrient availability to the plants. SMS used in our study in **Tables 1** and **2** showed that pH range 6.10 and EC range 1.75 dsm^{-1} are suitable for normal growth of plant. The average moisture content of SMS in our study has been measured as 72% which was also reported. [40].

Ability to provide essential nutrients to plants is one of the most fundamental criteria while judging the suitability of a growth medium [41]. Primary nutrients like nitrogen, phosphorus, and potassium are more available at pH 5.5–6.5 for substrates of organic and mineral origins [42]. Moreover, with the increasing pH, the solubility of many nutrients is reduced and some nutrients are precipitated as solid materials that plant cannot use [43]. In contrast many researchers reported high salinity of SMS, which is mostly responsible for the limited use of SMS as a potting media [9].

Results of our study showed that SMS had 0.87% nitrogen, 0.26% phosphorus content, 0.19% of potassium. SMS has been shown to increase the nutrient availability of growth media [44]. In general, most mushroom substrates have low N content, typically in the 1% to 3% range [45]. The overall nutrients of SMS were not enough to support normal plant growth without external fertilizer application. It is well known that physical properties of soil were directly related to crop yield [46]. SMS by maintaining high organic matter content in the soil and by providing the three primary nutrients e.g. nitrogen, phosphorus and potassium helps to provide soil fertility [41, 47].

5.2 Effect of SMS on growth and biomass of selected plants

Growth of root and shoot weight of the seedlings of green gram, black gram, tomato and chili were significantly higher when grown in 60% SMS mended soil than the control (**Tables 3–6**). Higher nutrient availability provided by SMS might have contributed to the better growth in 60% amended soil. Spent mushroom

compost (SMC) of *Pleurotus ostreatus* improved the agronomic characters and yield (pod no, fresh weight and dry weight) when it was added as soil conditioner to soybean at different levels of its concentrations [14].

Reason for poor root and shoot growth of the seedlings of the selected plants in 100% SMS may be that the paddy straw based. SMS may only be used as an amendment and not as a basic growth medium. While growth on straw, *Pleurotus* releases humic acids like fractions which when added to soil would increase its fertility. In addition, humic substances may affect the plant biochemical process [48]. Present findings confirm the efficacy of SMS in growth promotion in terms of seedling shoot and root weight.

SMS from *Agaricus bisporus*, *Hericium erinaceus* and *Pleurotus ostreatus* are effective to the growth promotions of pea, pepper and tomato plants respectively [29, 36].

The results of the present study revealed that the compost has a good impact in promoting better growth and yield. Further in order to promote growth and yield, it becomes imperative to optimize the usage of organic manure according to the crop requirement.

5.3 Characterization of bacterial strains isolated from SMS

Four bacteria species were isolated from spent mushroom substrate used in this study. They were coded isolate B1, B2, B3 and B4. These microorganisms were identified and characterized as *Bacillus*, *Clostridium*, *Pseudomonas*, and *Escherichia coli*.

The isolation of these bacteria from composting agricultural substrates suggests that a form of fermentation had taken place during the composting process. Addition of straw in the soil caused an increase in the number of total bacteria, actinomycetes and fungi of the rhizosphere [49].

Different substrate harbor different kind and number of microorganisms and the variation in microbial population in different substrates is due to nutritional or chemical composition of the substrate.

Similar isolation of *Bacillus* and *Clostridium* species were reported from fermenting cocoa beans [50, 51]. The presence of *Pseudomonas* sp. (Isolate B3) in the fermenting SMS may be related to its ability to survive in vast number of habitats. From the results obtained, it can be concluded that various bacteria genera were involved in the decomposition of further microbial SMS. The pure cultures of these bacteria could be incorporated into agricultural wastes in a controlled fermentation unit.

Effect of rice straw compost on soil microbial population reported that, compost application resulted in marked increase of organic matter content in the soil in relation to initial value of plain sandy soil which affirmatively exaggerated the bacterial and fungal populations and that microbial population increased with the increase in dosage of compost [52].

Similar results were reported in maize by using *Agaricus bisporus* spent mushroom compost [53] using recomposted button mushroom spent substrate with wheat crop and with *Pleurotus florida* spent substrate on tomato crop.

5.4 Anti-fungal activity of bacterial strains isolated from SMS extracts

Spent mushroom compost from *Pleurotus* sp., used in this study harbored bacterial population including, *Bacillus*, *Clostridium*, *Pseudomonas* and *E. coli*. This is in support of the findings on the microbial composition of spent mushroom compost of *Pleurotus* sp. [53]. SMS used for soil amendment has been found to be more efficient than commercial fungicides and nematicide in controlling soil borne pathogens like *Meloidogyne* sp. in tomato, *Venturia inaequalis* in apple [53]. Some of these microorganisms have been reported to possess antagonistic property in several

studies [54] and this was confirmed in this study from the result obtained from the dual culture assay involving the SMS microbial isolates and plant pathogenic fungi.

Fungal pathogens such as *Fusarium oxysporum* and *Phytophthora* cause severe plant diseases, limiting plant yields as well as the quality of the products. Moreover, they have wide host spectra, causing diseases in economically important agricultural crops worldwide [55]. These fungal phytopathogens are difficult to control not only because of their wide host spectra, but also because of their soil borne nature [56]. *Aspergillus flavus* is the most deleterious fungus in stored rice grains, and it receives particular attention because of its ability to produce potent carcinogenic aflatoxins [57].

Alternaria solani, *Alternaria alternata*, *Fusarium solani*, *Phytophthora megasperma* and *Verticillium dahlia* are ubiquitous and cosmopolitan phytopathogens causing severe diseases in wide range of crops [58, 59].

The findings on antifungal activity of four bacterial strains *Bacillus* sp., *Clostridium* sp., *Pseudomonas* sp. and *E. coli* isolated from SMS are shown in **Table 7** and **Figure 1**. Using antibiotic producing bacteria to control plant fungal diseases is a popular topic and has extensively been studied [60]. Compared with chemical biocides, many antibiotics produced by antagonistic strains have the advantage of being easily decomposed in nature, leaving no harmful residues behind. The results, of the present study on the **in vitro** sensitivity of phytopathogenic fungi to antagonistic bacteria revealed that the isolates of *B. subtilis* were suppressive, though with different degrees, to the tested isolates of phytopathogenic fungi, are consistent with those obtained by others [61, 62].

Bacillus and *Pseudomonas* sp. show antifungal effect against soil borne plant pathogenic fungi *c*, *Fusarium solani* and *Fusarium oxysporum* [63].

Bacillus subtilis showed strong ability against many common plant fungal pathogens **in vitro** [61]. The investigation of bio control activity of the strains in this study revealed that they could produce extracellular secondary metabolites with antifungal activity against the tested fungi. These strains inhibited the mycelial growth of the fungus in dual-culture assays. The *Pseudomonas* isolates obtained from soil were shown to reduce growth of *Aspergillus niger*, *Fusarium* sp., *Alternaria solani*, *Drechslera oryzae* etc., [64].

Our results on the antifungal activity of the microbes isolated from SMS (**Table 7** and **Figure 1**) which showed that the culture filtrates of bacterial strains, *Bacillus megaterium* KU143, *Microbacterium testaceum* KU313, and *Pseudomonas protegens* significantly inhibited the growth of *A. flavus*.

The SMS isolate of *Pseudomonas* sp. was tested positive for antifungal activity against *Phytophthora* sp. (**Table 7** and **Figure 1**). Similarly this bacterium was found efficient in inhibiting the mycelial growth and the antifungal compounds extracted were found inhibitory to the growth of *Rhizoctonia* sp., *Phytophthora parasitica*, *P. palmivora* and *Fusarium solani*.

The inhibitory properties of spent mushroom substrate remained unaffected even after autoclaving and filter sterilization of extract [54]. Unsterilized spent mushroom compost had a better inhibition potential than sterilized compost, this suggested that the pathogen inhibitory properties of spent mushroom compost could be more due to the biotic components than the abiotic components, i.e., more due to the activities of the inherent microorganisms rather than the chemical properties or the organic matter content. Similar views were reported [65].

The results of the present study confirm that spent mushroom substrate contains a large number of indigenous beneficial microbes capable of suppressing soil-borne pathogens. This character of the SMS can be utilized as an alternate substrate for the in mass production of biocontrol agents for field application which may lead to suppression of diseases leading to increased crop productivity.

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