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Isolation of Bio-Agents from Spent Mushroom Substrate and Antagonism Against *Rhizoctonia solani*

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Abstract Fungal isolates predominantly present in spent mushroom substrate (SMS), which were isolated and cultured on potato dextrose agar (PDA) medium and then identified as *Trichoderma harzianum* and *Trichoderma viride*. Antifungal activities of these isolated bio-agents were determined by testing their effect against *Rhizoctonia solani* with dual culture method. The studies depicted 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 3 DAI (27.83 mm) and 4 DAI (16.16 mm), while it is less in presence of *T. harzianum* (23.5 mm and 12.35 mm respectively). Similarly, bio-agent *T. harzianum* showed maximum inhibition of mycelial growth, 33.48% and 72.29%, followed by *T. viride* with 21.22% and 63.68% inhibition over control at 3 DAI and 4 DAI, respectively.

Keywords Spent mushroom substrate, Bio-agents, *Rhizoctonia solani,* Mycelial growth.

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Introduction

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 million tones of SMS is produced [1]. 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.

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. Mushroom industry needs to dispose off more than 50 million tones of used mushroom compost each year. 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. 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. 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. 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. 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. Butt et al. [2] found that organic manure including spent mushroom compost encouraged a population of antagonistic microorganisms such as *Trichoderma* spp., *Bacillus* spp. those interfere with the activity of plant pathogens. The biological analysis of SMS extract 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. The spent mushroom substrate, being a cellulosic agro-waste favored growth of a well-known cellulose degrader, *T. harzianum*. Similarly, it was observed that organic manure like SMS are known to enhance the early growth and establishment of the bioagents including *Trichoderma* spp. [3].

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 studies were undertaken for isolation of beneficial mycoflora from SMS and utilization for diseases management.

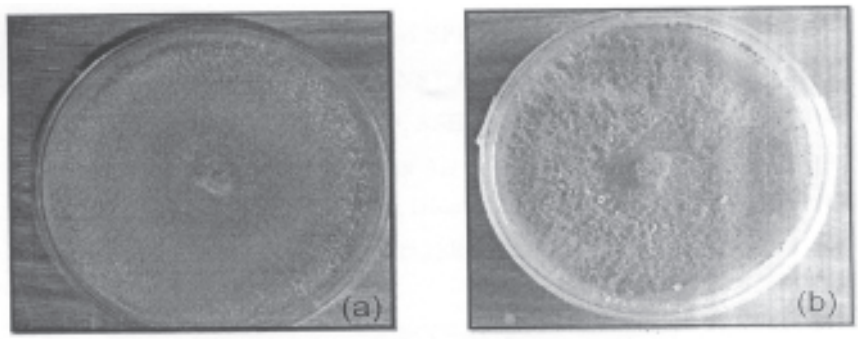
Materials and Methods

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

Spent mushroom substrate was taken from Mushroom Production Unit of CCS Haryana Agricultural University and from this isolation of fungal microorganisms carried out. 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 ^{0}C. The fungal colonies which were abundant and predominantly present, were aseptically transferred to PDA slants separately and kept at 26 ± 1 ^{0}C for further growth. The culture was further purified by single hyphal tip method. The pure culture thus obtained was maintained by sub-culturing it at monthly interval on PDA and stored in a refrigerator for further studies.

Evaluation of isolated fungal bio-agents *R. solani*

Fungal bio-agents isolated from SMS were tested for their antagonism against *R. solani* under *in vitro* condition using dual culture method [4] 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 ^{0}C. Observations on the colony diameter and radial growth of pathogen were taken daily till pathogen occupied the full petriplate 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. Percent growth inhibition of the pathogen was



(a) *T. harzianum* (b) *T. viride*

Fig. 1. *Trichoderma* spp. isolated from spent mushroom substrate.

calculated by the formula given by Vincent [5]

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

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).

Results and Discussion

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 petri-plate with colony growth rate of 8-9 cm in 3-4 days. In early stages of growth, the color of colony was light green; it gradually became yellowish dark green. The culture gave the smell of Malt. The condiation was in ring-like zones with highly branched regular conidiophore brancing with size of 2-3 µm. Based on these characteristics the first fungal isolate was identified as *Trichoderma harzianum* (Fig. 1).

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 color was deep yellow with wavy edges and the culture smell lie coconut. The conidiophores were irregularly and moderately branched with size of 4-5 µm. Based on these observations, the second fungal isolate was identified as *Trichoderma viride.* Ahlawat et al. [6] 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. 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 ^{0}C. These isolates were identified on the basis of cultural and morphological characteristics.

Supported by Park et al. [7] 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.

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. Isolated bio-agents

Table 1. Effect of *Trichoderma* spp. on the growth of *Rhizoctonia solani* in dual culture. *average of six replications.

Growth of *R. solani* against	Radial growth* (mm) 3 DAI	4 DAI	% Growth inhibition 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

namely *T. harzianum* and *T. viride* were tested against *R. solani.*

The results are presented in Table 1 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 3 DAI (27.83 mm) and 4 DAI (16.16 mm) while it is less in presence of *T. harzianum* (23.5 mm and 12.35 mm respectively). *T. harzianum* showed maximum inhibition of mycelial growth, 33.48% and 72.29% followed by *T. viride* with 21.22% and 63.68% inhibition over control at 3 DAI and 4 DAI, respectively.

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. *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. 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% inhibition of mycelial growth of the pathogen followed by *T. viride* (63.68%). The radial growth of pathogen at 3 DAI and 4 DAI was found to be greatly influenced by these bioagents. 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. [8] 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% growth inhibition of the pathogen Bunker and Mathur [9] reported that *Trichoderma* spp. suppress the growth of *R. solani, in vitro* and also reported the effectiveness of *T. viride* and *T. harzianum in vitro* against *R. solani.* Prasad and Gupta [10] 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.*

Conclusion

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. 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% inhibition of mycelial growth followed by *T. viride* (16.16 mm) which showed 63.68% growth inhibition.

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