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Utilization of Spent Mushroom Substrate and Biocontrol Agents for the Management of *Sclerotinia sclerotiorum* Causing Stem Rot of Chickpea

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KEY WORDS

Chickpea
Pseudomonas fluorescens
Sclerotinia sclerotiorum
Spent mushroom substrate
Stem rot
Trichoderma viride

ABSTRACT Chickpea's low yield is due to its sensitivity to several diseases caused by *Fusarium oxysporum* f. spp. *ciceri*, *Rhizoctonia bataticola*, and *Sclerotium rolfsii*. These pathogens induce dry wilt and collar rot. Similarly, *Sclerotinia sclerotiorum* is a plant pathogenic fungus that causes chickpea stem rot. The pathogen renders significant losses in chickpea, varying from 10 to 100%. The present study has used the spent mushroom substrate (SMS) in combination with biocontrol agents such as *Pseudomonas fluorescens* and *Trichoderma viride* to control this pathogen. It has got an excellent nutritional profile. The combination of *P. fluorescens* and *T. viride* (5 g/kg of soil for both) with the maximum amount of SMS (300 g) efficiently suppressed *S. sclerotiorum* by >50% compared to controls. Chickpea germination was also significantly better (99%) when the treatment level was SMS (300 g) + *P. fluorescens* (5 g/kg soil) + *T. viride* (5 g/kg soil).

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INTRODUCTION

Chickpea (*Cicer arietinum*) is a self-pollinating diploid (2n = 2x =16) pulses crop with a genome of 738 million base pairs (Varshney *et al*., 2013). *Cicer reticulatum* Ladizinsky, a variable wild species originating in different places of South-east Turkey (37.3–39.3°N, 38.2–43.6°E), was the main source of chickpea roughly 11,000 years ago (Mohibullah *et al*., 2020). Chickpea intake is widespread in different parts of India, due to its high nutritious value. Carbohydrates (50–58%), protein (15–22%), moisture (7–8%), fat (3.8–10.20%), and micronutrients (1%) (Wallace *et al*., 2016) make up the chickpea seed medium. Madhya Pradesh, U.P, Rajasthan, Maharashtra, and Andhra Pradesh are the states that produce chickpeas in India. Madhya Pradesh is the largest chickpea-producing state, accounting for around 40% of total production (Sharma *et al*., 2020).

Spent mushroom substrate (SMS) is the material left after growing mushrooms. Mushrooms are ready for harvesting within 2–3 weeks, and the leftover substrate is called SMS. SMS has a nutritional value having 1.51% of N, 3.77% of P, and 0.61% of K. Furthermore, the hydraulic conductivity of SMS was 6.22 m/h. The water holding capacity is 95.03%. The pH of SMS differs, and it is almost between 7.28 and 7.75, and chloride plays an essential role in conductivity (Mortada *et al*., 2020). Soil-borne

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diseases such as *Fusarium* wilt (*Fusarium oxysporum* f. spp. *ciceris*), dry root rot (*Rhizocotonia bataticola*), collar rot (*Sclerotium rolfsii*), black rot (*Fusarium solani*), and stem rot (*Sclerotinia sclerotiorum*) are among the critical diseases that limit chickpea production (Kumar *et al*., 2016).

The major yield-reducing factor is due to disease-causing pathogen *S. sclerotiorum*, which causes stem rot in chickpea. *S. sclerotiorum* causes stem whitening, withering, and stem breakage in chickpeas by infecting them directly from sclerotia in the soil, where the mycelial hat enters host tissue through myceliogenic germination. The emergence of water-soaked irregular patches on fruits, stems, leaves, or petioles is common signs. These patches get more prominent and a cottony mycelium forms a protective layer over the damaged area. As the fungus grows, the plant becomes a slimy and water-soaked mass. After the host dies, the cottony mycelium forms many sclerotia (black seed-like reproductive structures), a reliable indicator of *Sclerotinia* infection. Hence, the present study was designed to evaluate the efficacy of SMS and biocontrol agents against chickpea stem rot disease.

MATERIALS AND METHODS

The chickpea stem rot sample was collected from the main field and isolated at the Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab. The isolation was done by the single hyphal tip method (Lakhran *et al*., 2018), and then preparation of mass multiplication was done with the help of sorghum grains, where the sorghum grains were soaked overnight. Then, about 300 g grains were transferred to a flask of 1000 mL and sterilized following standard procedures. After sterilization, grains were inoculated with test pathogen in a sterile condition and kept in an incubator at 27°C. After around 10–15 days, white mycelial growth can be observed (Prajapati *et al*., 2020).

A study on SMS's inhibitory impact was carried out. Air-dried SMS equal to 1, 5, 10, 15, and 20 g were combined with 100 mL of double distilled water to produce SMS concentrations ranging from 0% w/v to 20% w/v. The mixture was vortexed for 10 min and kept at 28°C for 48 h before being vortexed again and filtered through muslin fabric. Half of each concentration was sterilized in an autoclave at 121°C at 15 psi of pressure for 15 min to eliminate the possibility of denaturing any biotic components of the filtrate. Four replicates of PDA medium with a low water content were loaded with those above unsterilized and sterilized filtrates. Control plates were just filled with sterile water. After that, the *S. sclerotiorum* cultures on PDA were put in the center of each plate and incubated at 28°C. The plates were checked every day. Mycelia colony diameters were measured everyday starting on the 2nd day and continued for a total of 12 days. There were a total of 10 treatments in all.

A further pot experiment was done to check SMS's efficacy and bioagents (*Pseudomonas fluorescens* and *Trichoderma viride*) against *S. sclerotiorum.* Chickpea seeds (variety- GNG 469) were treated with the aforementioned biocontrol agents and sown in soil infected with *S. sclerotiorum.* The pathogen was multiplied on sorghum grains and then mixed into the pot @10 g/kg soil. Different amounts of SMS were used, that is, 100, 200, and 300 g, with varying treatment combinations (Saileela *et al*., 2020).

Statistical Analysis

The experiment was carried out in a randomized block design with ten treatments and four replicates for each treatment with a selected quantity of SMS as mentioned above and biocontrol agents (*P. fluorescens* and *T. viride*). Significance was established using ANOVA (Fisher and Yates, 1963).

RESULTS AND DISCUSSION

We observed that the *S. sclerotiorum* mycelia growing on PDA added with autoclaved SMS filtrate fully covered the plates. However, in plates containing unsterilized SMS filtrate, *S. sclerotiorum* growth was reduced and limited. There were no changes in *S. sclerotiorum* colony development comprising PDA with sterilized SMS extracts at various concentrations of 1, 5, 10, 15, and 20% compared to control. *S. sclerotiorum* colony development was significantly reduced on PDA containing unsterilized SMS filtrates (concentrations as above). However, there were no significant variations in *S. sclerotiorum* suppression across the various concentrations of the unsterilized SMS filtrate

Table 1. Radial growth of *Sclerotinia sclerotiorum* altered with varying concentrations of sterilized and unsterilized filtrates of SMS

Treatment (w/v) (%)	Mycelial growth (mm)*	
	Filtered SMS	Unfiltered SMS
1	81.22	54.44
5	85.83	55.52
10	82.80	58.6
15	81.94	48.99
20	81.32	50.14
CD	1.493	0.38
SE (d)	0.47	0.12
SE (m)	0.67	0.17

*Mean of 4 replicates; CD: Critical difference, SE (d): Standard error of difference, SE (m): Standard error of mean

(Table 1). The result was similar to that of Ocimati *et al*. (2021) where in pure culture experiments, media containing unsterilized SMS filtrates with varied concentrations successfully inhibited the *Fusarium* growth.

The maximum germination was recorded with SMS (300 g) along with *P. fluorescens* (5 g/kg soil) and *T. viride* (5 g/kg soil) (99%), followed by SMS (200 g) with a similar combination of *P. fluorescens* (5 g/kg soil) and *T. viride* (5 g/kg soil) (98.75%) as compared to control (51.75%) (Table 2). It confirms that SMS has to play a key role. The salt concentration of the spent mushroom compost could be an efficient germination-enhancing substrate (Sönmez *et al*., 2016) that improves the seedling quality characteristics. The spent mushroom compost kept under natural conditions in an open field could be a viable seedling medium. Marques *et al*. (2014) observed similar results that substrate, which contained 45% spent mushroom compost, offered the best environment for lettuce seedling germination and growth, as seen by the better biomass and vigor of the 27-day-old seedlings.

Maximum plant height at 30 days after sowing (DAS) was recorded with SMS (300 g) along with *P. fluorescens* (5 g/kg soil) and *T. viride* (5 g/kg soil) (22.5 cm) followed by SMS (200 g) along with *P. fluorescens* (5 g/kg soil) and *T. viride* (5 g/kg soil) (21.25 cm) as compared to control (12.5 cm). After 45 DAS, the plant height recorded under similar treatment conditions was 25.5 and 24.25 cm, respectively, compared to 15.5 cm in controls (Table 2). Maximum plant height after 60 DAS improved further compared to controls (Table 2) and was 8–10 cm more than obtained in the untreated experiments. Roy *et al*. (2015) observed that after 35 days, all of the treated plants exhibited a considerable increase in height, with those treated with fresh oyster mushroom spent substrate, button mushroom leachate, and weathered button mushroom compost showing the highest increase in growth. Khan *et al*. (2019) also observed that seeds germinated, plant height, the number of pods per plant, seed weight per plant, and disease incidence and severity index increased significantly when SMS was added to the soil.

The decrease in disease incidence was observed after 60 DAS when SMS (300 g) along with *P. fluorescens* (5 g/kg soil) and *T. viride* (5 g/kg soil) (18.67%) was used. However, if the amount of SMS was 200 g + *P. fluorescens* (5 g/kg soil) + *T. viride* (5 g/kg soil), the disease severity was slightly higher (22.49%) as compared to control (51.75%) (Table 3). The least disease incidence was recorded after 75 DAS with SMS (300 g) along with *P. fluorescens* (5 g/kg soil) and *T. viride* (5 g/kg soil), followed by SMS (200 g) along with *P. fluorescens* and *T. viride* being at same levels (Table 3). However, there was no further decrease in disease incidence with the extended duration of observation (90 DAS) and remained significantly similar to what was observed after 75 DAS (Table 3).

Smolinska *et al*. (2016) observed that the addition of organic materials inoculated with *Trichoderma* fungus to soil substantially impacts the decrease of *S. sclerotiorum*. The *T. virens* TRS114 overgrown with wheat straw and

Table 2. Effect of SMS along with selected bioagents on germination (%) and plant height (cm) of chickpea

Treatments	Germination (%)	Plant height (cm)*		
		30 DAS	45 DAS	60 DAS
SMS (100 g)+*Pseudomonas fluorescens* (10 g/kg soil)	80.25	14.25	17.25	27.5
SMS (200 g)+*Pseudomonas fluorescens* (10 g/kg soil)	81.5	15.5	18.5	28
SMS (300 g)+*Pseudomonas fluorescens* (10 g/kg soil)	60.75	15.75	18.75	28.25
SMS (100 g)+*Trichoderma viride* (10 g/kg soil)	70.5	16.75	19.75	28.5
SMS (200 g)+*Trichoderma viride* (10 g/kg soil)	61.75	17.75	20.75	29.5
SMS (300 g)+*Trichoderma viride* (10 g/kg soil)	62.5	19	22	30
SMS (100 g)+*Pseudomonas fluorescens* (5 g/kg soil)+*Trichoderma viride* (5 g/kg soil)	80.75	20	23	30.75
SMS (200 g)+*Pseudomonas fluorescens* (5 g/kg soil)+*Trichoderma viride* (5 g/kg soil)	98.75	21.25	24.25	32
SMS (300 g)+*Pseudomonas fluorescens* (5 g/kg soil)+*Trichoderma viride* (5 g/kg soil)	99	22.5	25.5	34
Control	51.75	12.5	15.5	24
SE (m)	0.860	0.305	0.305	0.565
SE (d)	1.216	0.432	0.432	0.799
CD (0.05)	2.50	0.89	0.89	1.64

*Mean of four replicates, CD: Critical difference, SE (d): Standard error of difference, SE (m): Standard error of mean, DAS: Days after sowing

Table 3. Effect of SMS along with selected bioagents on disease incidence (%) of *Sclerotinia sclerotiorum*

Treatments	Disease incidence (%)*		
	60 DAS	75 DAS	90 DAS
SMS (100 g)+*Pseudomonas fluorescens* (10 g/kg soil)	36.33	33.49	38.52
SMS (200 g)+*Pseudomonas fluorescens* (10 g/kg soil)	40	40.19	42.56
SMS (300 g)+*Pseudomonas fluorescens* (10 g/kg soil)	28.11	34.24	36.09
SMS (100 g)+*Trichoderma viride* (10 g/kg soil)	40.16	38.33	44.38
SMS (200 g)+*Trichoderma viride* (10 g/kg soil)	34.37	34.48	35.37
SMS (300 g)+*Trichoderma viride* (10 g/kg soil)	24.25	26.74	27.33
SMS (100 g)+*Pseudomonas fluorescens* (5 g/kg soil)+*Trichoderma viride* (5 g/kg soil)	33.33	35.33	35.46
SMS (200 g)+*Pseudomonas fluorescens* (5 g/kg soil)+*Trichoderma viride* (5 g/kg soil)	22.49	23.91	25.25
SMS (300 g)+*Pseudomonas fluorescens* (5 g/kgsoil)+*Trichoderma viride* (5 g/kg soil)	18.67	21.74	22.97
Control	54.83	55.33	56.95
SE (d)	0.64	1.68	0.50
SE (m)	0.45	1.19	0.35
CD (0.05)	1.32	3.47	1.04

*mean of four replicates, CD: Critical difference, SE (d): Standard error of difference, SE (m): Standard error of mean, DAS: Days after sowing

apple pomace was very effective. Regardless of the dose, this therapy entirely eradicated *S. sclerotiorum* survival. Ahlawat *et al.* (2022) observed that soils manured with anaerobically recomposted SMS on plants and tomatoes decreased the prevalence of leaf curl virus, fruit rotting microorganisms, and fruit borer. Similarly, Adedeji and and Aduramigba (2016) observed that oyster mushroom SMS in combination with fluorescent *Pseudomonas* spp., *T. viride*, *Bacillus* spp., *Penicillium* spp., and *Aspergillus terrus* was equally effective. *Fusarium* was strongly antagonistic to these fungi isolates. The findings show that SMS extracts might be used as a biological control for tomato wilt caused by *Fusarium* wilt.

Overall, the study showed that the treatment with biocontrol agents is effective in managing the plant pathogen and is environment friendly. *T. viride* and *P. fluroscens* effectively controlled the stem rot of chickpea by inhibiting mycelial growth and using SMS with soil significantly improved the germination and growth of plants. SMS is fertile and can be used for different agricultural purposes such as fertilizer, disease management, mushroom cultivation, etc. SMS's physical and chemical properties are favorable for such purposes. Hence, we can conclude that the treatment that combined *T. viride* and *P. fluorescens* with the higher quantity of SMS (300 g) proved to be the best treatment and could be an easy-to-use and safe strategy for farmers to prevent chickpea diseases (Juliatti *et al*., 2019).

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