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Impact of Spent Mushroom Substrate Enriched with *Trichoderma harzianum* on Damping off Disease in Chilli and Tomato

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Abstract

A study was undertaken to evaluate the impact of soil application of spent mushroom substrate (SMS) and farm yard manure (FYM) enriched with *T. harzianum* on pre-emergency damping off (PED) and post-emergence damping off (POED) in chilli and tomato seedlings in nursery beds containing naturally infested soil with *Pythium aphanidermatum* and *Rhizoctonia solani* in the experimental field area of Department of Plant Pathology, CCSHAU Hisar during 2018-19, 2019-20and 2020-21.The observations on disease incidence (%) were recorded up to 30 days after germination. A control without soil amendment was also maintained. The maximum damping off was at 22.5, 23.4 and 22.9% in controland minimum at 15.7, 15.8 and 16.6% during 2018–19, 2019–20, 2020–21, respectively in chilliwhen SMS enriched with *T. harzianum* was applied. The mean of damping off disease incidence was minimum at 16.0% when SMS enriched with *T. harzianum* was applied and maximum at 22.9% in control. The maximum damping off was at 18.1, 19.4 and 18.7% in control and minimum at 12.2, 12.6 and 14.8% during 2018–19, 2019–20, 2020–21, respectively in tomato when SMS enriched with *T. harzianum* was applied. The mean of disease incidence (%) was minimum at 13.2% when SMS enriched with *T. harzianum* was applied and maximum at 18.7% in control.

Keywords: Chilli, damping off, FYM, SMS, tomato, *Trichoderma harzianum*

1. Introduction

Chilli (*Capsicum annuum* L.) and Tomato (*Lycopersicon esculentum* L.) are two main vegetable crops of the India because of their unique characteristics. Chilli is grown in India for its pungency. It is a rich source of vitamin C, antioxidants, vitamins, minerals etc. and found to prevent diseases like cancers, stomach ulcers, body weight loss, lowering risk of diabetes etc. It is also in demand for export as a raw and in the form of powder. It was cultivated on an area of 0.40 mha with production of 4.2 mt in India, but in Haryana it was cultivated on an area of 13290 ha with production of 0.142 mt during 2021–22 (Anonymous, 2021).

Tomato is another major vegetable crop grown widely in the world. It is considered as a protective food because it has high nutritive value. Its fruit is a rich source of minerals, vitamins and organic acids and has a high degree of lycopene and ascorbic acid content (Kaur and Kapoor, 2008). Its products are now being used to prevent some diseases (Cavene et al., 2005). It was cultivated on an area of 0.83 mha with production of 20.3 mt in India whereas, in Haryana, it was cultivated on an area of 21000 ha with production of 0.44 mt during 2021–22 (Anonymous, 2021).

Both the crops are being affected by soil borne diseases from seed germination to harvest of the crops. Among these PED as well as POED are major soil borne diseases and take heavy toll of seedlings in both crops at seedling stage in Haryana and different parts of India. In PED, the germinated seed die before emergence from soil, where as in POED, the seedlings collapse after emergence from the soil. The chilli and tomato seedlings are attacked by soil borne fungi like *P. aphanidermatum* and *R. solani*, respectively and responsible for damping off of seedlings. Since, it is very difficult to control soil borne pathogens and farmers apply fungicides in raising seedlings. It is not a cost-effective or eco-friendly practice. The *T. harzianum* is a broadly distributed common soil borne fungus found to play important role as bio-control agent against soil borne plant pathogens (Harman et al., 2004, Islam et al., 2010, Sarma et al., 2015). It follows mechanisms like antibiosis, competition and mycoparasitism and inhibits the growth of pathogens by biosynthesis of growth regulators, enzymes, antibiotics and siderophores (Sood et al., 2020). The SMS is a waste after mushroom harvest and its piling up which causes various environmental problems (Beyer, 1996). It has now been considered as a bio-fertilizer to be incorporated in soil to raise many agriculture and horticulture crops. It improves the soil physical, chemical, biological properties, yield of crop and nutritional quality of the agriculture product (Roy et al., 2015, Hairuet al., 2016, Owaid et al., 2017, Paula et al., 2017, Jankowski et al., 2018, Liu et al., 2018, Krystyna et al., 2018, Deshmukh, 2019, Economou et al., 2020, Nmom et al*.,* 2020, Do Carmo et al., 2021, Muchena et al., 2021, Velusam et al., 2021, Dominguez-Gutierrez et al., 2022). It has also been found to possess various microorganisms producing antifungal compounds and effective in reducing soil borne pathogens (Ishihara et al., 2018, Daniel et al., 2021, Othman et al., 2020, Singh et al., 2018). Based on significant results from these studies, it was paramount to study the impact of soil application of SMS as well as FYM alone as well as their integration with *T. harzianum* against PED and POED of chilli and tomato seedlings raised under nursery condition in the experiments conducted consecutively for three years from 2018–19 to 2020–21.

2. Materials and Methods

2.1. Study material and area

The experiment to study the impact of soil application of SMS and FYM enriched with *T. harzianum* and without enrichment on PED and POED in chilli cv. Pusa Jawala (November-December) and tomato cv. Hisar Arun (January-February) was carried out in nursery beds of 1×1 m^2 size containing soil naturally infested with *P. aphanidermatum* and *R. solani* in the experimental field area of Department of Plant Pathology, CCSHAU Hisar (29°08' N latitude,75° 42' E longitude, altitude 215 m MSL), situated in the semi-arid region of North-Western India from 2018–19 to 2020–21.

2.2. SMS and FYM enrichment with T. harzianum

One year naturally weathered button mushroom SMS and two years old well rotten FYM was used for this experiment. The pure culture of *T. harzianum* was collected from Biological Control Lab., Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar and multiplied on oatmeal medium under *in vitro* conditions. The medium containing *T. harzianum* spores at 1×10^9 cfu g^{-1} was mixed @ 0.001% (w/w basis) in thoroughly wet SMS and FYM in shade and covered with gunny bags for 15 days. The water was sprinkled for 15 days on daily basis on gunny bags to favor the multiplication of *T. harzianum* in both substrates. After 15 days each substrate was made uniform by mixing with hands. The SMS and FYM without *T. harzianum* enrichment were also maintained in a similar way.

2.3. Application of substrates in soil and raising of nursery

The soil treatments were carried out as per detail given below. The sowing of chilli was done in Mid-November and tomato in first week of January on raised beds during 2018–19 to 2020–21. The treatments were completely randomized and replicated thrice. The plots without any soil amendment treatment were also maintained and served as a control. The agronomic operations were carried out as per recommended package of practices.

2.4. *Observations and statistical analysis*

The observations were taken on seed germination replication wise in different treatments. The seed germination was compared with control to determine PED (%). The post-emergence mortality was observed on daily basis up to 30 days after germination and combined replication wise in all treatments at the end of 30 days. The PED and POED were summed up to calculate the total damping off in all five treatments in chilli and tomato. The data after angular transformation were analyzed statistically by RBD design.

2.5.*Treatments detail*

T_1= SMS at 25 tonnes ha^{-1} and enriched with *T. harzianum*

T_2= SMS at 25 tonnes ha^{-1} without enrichment with *T. harzianum*

T_3 = FYM at 25 tonnes ha^{-1} and enriched with *T. harzianum*

T_4= FYM at 25 tonnes ha^{-1} without enrichment with *T. harzianum*

T_5= Control (No soil amendment)

3. Results and Discussion

3.1. Chilli

3.1.1. Pre-emergence and post-emergence damping off disease incidence

The incidence of PED (%) in chilli was found significantly low in the all the soil treatments as compared to control during all the three years of experimentation (Table 1). It was found significantly lowest at 5.2, 5.2 and 5.6% when SMS enriched with *T. harzianum* was applied during 2018–19, 2019–20 and 2020–21, respectively and it differed significantly as compared to control. It was followed by 5.4, 5.3 and 5.8% when FYM enriched with *T. harzianum* was applied to the soil and it also differed significantly from control. Similarly, the incidence of POED (%) was also found significantly low in the all the treatments as compared to control during all three years of experimentation (Table 1). It was found significantly lowest at 10.5, 10.6 and 11.0% during 2018–19, 2019–20 and 2020–21, respectively when SMS enriched with *T. harzianum* was applied and it differed significantly as compared to control. It was followed by 11.2, 11.3 and 11.7% when FYM enriched with *T. harzianum* was applied and it differed significantly from control. A total of % disease incidence (PED+POED) was 15.7, 15.8, 16.6 was lowest during 2018–19, 2019–20 and 2020–21, respectively when SMS enriched with *T. harzianum* was applied to the soil. It was followed by 16.6, 16.6, 17.5% during 2018–19, 2019–20 and 2020–21, respectively when FYM enriched with *T. harzianum* was applied. The maximum disease incidence was 22.5, 23.4, 22.9% during 2018–19, 2019–20 and 2020–21, respectively in control. A mean of disease incidence of three years was found minimum at 16.0% when SMS enriched with *T. harzianum* was applied followed

Table 1: Effect of soil application of spent mushroom substrate and farm yard manure enriched with *T. harzianum* on damping off disease in chilli

Treatments	2018–19			Disease control (%)	2019–20			Disease control (%)
	Disease Incidence (%)				Disease Incidence (%)			
	PED (%)	POED (%)	Total disease incidence (%)		PED (%)	POED (%)	Total disease incidence (%)	
SMS alone	6.1 (14.3)	12.0 (20.3)	18.1	19.6	5.8 (15.7)	12.4 (23.1)	18.2	22.2
SMS+ *T. harzianum*	5.2 (13.2)	10.5 (18.9)	15.7	30.2	5.2 (14.9)	10.6 (21.3)	15.8	32.5
FYM alone	7.2 (15.5)	12.9 (21.0)	20.1	10.6	6.7 (16.9)	13.2 (23.8)	19.9	14.6
FYM+ *T. harzianum*	5.4 (13.4)	11.2 (19.5)	16.6	26.2	5.3 (15.0)	11.3 (22.01)	16.6	29.1
Control (No soil amendment)	7.8 (16.2)	14.7 (22.5)	22.5	-	8.2 (18.7)	15.2 (25.7)	23.4	-
CD (*p*=0.05)	(1.03)	(0.64)	-	-	(0.52)	(0.49)	-	-

Table 1: Continue...

Treatments	2020–21				Mean disease incidence (%)	Mean disease control
	Disease Incidence (%)			Disease control (%)		
	PED (%)	POED (%)	Total disease incidence (%)			
SMS alone	6.1 (14.3)	12.6 (20.8)	18.7	18.3	18.3	20.0
SMS+ *T. harzianum*	5.6 (13.7)	11.0 (19.4)	16.6	27.5	16.0	30.1
FYM alone	6.8 (15.2)	13.0 (21.1)	19.8	13.5	19.9	12.9
FYM+ *T. harzianum*	5.8 (13.9)	11.7 (20.0)	17.5	23.6	16.9	26.3
Control (No soil amendment)	8.0 (16.4)	14.9 (22.7)	22.9	-	22.9	-
CD (*p*=0.05)	(0.31)	(0.61)	-	-	-	-

Figures in parenthesis are angular transformed values, PED: Pre-emergence damping off; POED: Post-emergence damping off; Total disease incidence (%): PED(%)+POED(%)

by 16.9% when FYM enriched with *T. harzianum* was applied to the soil. A maximum of disease incidence was observed at 22.9% in control (Table 1).

The soil application of SMS enriched with *T. harzianum* resulted in a minimum PED and POED incidence in chilli nursery during three years of experimentation. The antagonistic effect produced by SMS microflora and a synergistic antagonism after its enrichment with *T. harzianum* might have affected *P. aphanidermatum* and *R. solani* pathogens in soil and hence, it resulted in a least pre-emergence, post-emergence and total damping off disease incidence consistently in chilli nursery during three years of experimentation. The studies of some researchers are in line with our findings also. The SMS is found to harbor different microflora (Ahlawat and Sagar, 2007, Safianowicz et al., 2018, Moraes et al., 2020, Frac et al., 2021) and this microflora is known to produce antagonism against many soil borne plant pathogens which results in disease incidence reduction in plants. In addition to antagonism by microflora of SMS, its chemical characteristics and nutritional status has also been found to modify the environment of rhizosphere which influence the soil borne pathogens. Ocimati et al. (2021) revealed that addition of SMS filtrate in media suppressed *Fusarium. oxysporum* f. sp. *cubense* growth and its application under field conditions also resulted in reduced wilt disease in banana. Elaamer (2020) found that soil application of SMS had increased the macro and micro nutrients in strawberry rhizosphere which resulted in increased antagonistic microflora like *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., and *Actinomyces* spp. in the rhizosphere of plants. The nutritional status of SMS might have helped in the establishment of existing microflora of soil, microflora of SMS, *T. harzianum* in rhizosphere of chilli seedlings and hence it may be the possible reason of reduced pre-emergence, post-emergence and total damping off disease incidence in chilli nursery in the present study. In a similar study, Daniel et al. (2021) reported that SMS application gave a suppressive effect against *Pythium irregulare* in production of baby leaf lettuce but the incorporation of *T. harzianum* enriched SMS had not influenced the soil borne pathogen. The study partially corroborate with their findings because soil application of SMS alone had reduced damping off disease incidence up to some extent but SMS enriched with *T. harzianum* gave an additional reduction in damping off disease incidence in chilli nursery. The genetic variability of *T. harzianum* isolates, qualitative and quantitative differences in soil or SMS microflora, variations in soil borne plant pathogens may be the possible reasons of contradiction.

3.1.2. Damping off disease control

A maximum of disease control at 30.2, 32.5, 27.5% was achieved during 2018–19, 2019–20, 2020–21, respectively, when SMS enriched with *T. harzianum* was applied in soil. It was followed by 26.2, 29.1, 23.6% during 2018–19, 2019–20, 2020–21, respectively, when FYM enriched with *T. harzianum* was applied in soil. The soil application of SMS without *T. harzianum* enrichment gave 19.6, 22.2, 18.3% disease control during 2018–19, 2019–20, 2020–21, respectively and a minimum of disease control of 10.6, 14.6, 13.5% was observed during 2018–19, 2019–20, 2020–21, respectively, when FYM without *T. harzianum* enrichment was applied. A mean of disease control of three years was found maximum at 30.1% when SMS enriched with *T. harzianum* was applied followed by 26.3% when FYM enriched with *T. harzianum* was applied. A mean of disease control of three years data was at 20.0 and 12.9% when SMS and FYM without *T. harzianum* enrichment were applied in soil, respectively (Table 1).

The *Trichoderma* spp. is found to influence the soil borne pathogens in multiple ways like antibiosis, competition, mycoparasitism, and production of growth regulators, enzymes, antibiotics and siderophores (Sood et al., 2020, Kumar et al., 2021). The microflora on SMS as described earlier and further its enrichment with *T. harzianum* might have produced additional antagonism against the pathogen and had helped in the synergistic control of damping off disease of chilli in nursery. In addition, nutritional status and chemical characteristics of SMS may have been conducive for development of antagonistic microflora including *T. harzianum* and unfavourable for pathogens and it led to a better damping off disease control in chilli nursery. The present study is also in line with the findings of few other workers while working of different crops (Montanari et al., 2004, Elamaar, 2020).

3.2. Tomato

3.2.1. Pre-emergence and post-emergence damping off disease incidence

The incidence of PED (%) incidence in tomato was found significantly low in the all the treatments as compared to control during all three years of experimentation (Table 2). It was found significantly lowest at 4.7, 4.8 and 5.0% where SMS enriched with *T. harzianum* was applied during 2018–19, 2019–20 and 2020–21, respectively and it differed significantly as compared to control. It was followed by 5.0, 5.2 and 5.1% when FYM enriched with *T. harzianum* was applied to the soil and it also differed significantly from control. Similarly, the incidence of POED (%) incidence was also found significantly low in the all the treatments as compared to control during all three years of experimentation (Table 2). It was found significantly lowest at 12.2, 7.8 and 9.9% during 2018–19, 2019–20 and 2020–21, respectively when SMS enriched with *T. harzianum* was applied and it differed significantly as compared to control. It was followed by 8.2, 8.2 and 10.2% when FYM enriched with *T. harzianum* was applied and it differed significantly from control.

A total of % disease incidence (PED+POED) was 12.2, 12.6, 14.8% was lowest during 2018–19, 2019–20 and 2020–21, respectively when SMS enriched with *T. harzianum* was applied to the soil. It was followed by 13.2, 13.5 and 15.3% during 2018–19, 2019–20 and 2020–21, respectively when FYM enriched with *T. harzianum* was applied. The maximum disease incidence was at 18.1, 19.4, 18.7% during 2018–19, 2019–20 and 2020–21, respectively in control. A mean of disease incidence of three years was found lowest at 13.2% when SMS enriched with *T. harzianum* was applied followed by 14.0% when FYM enriched with *T. harzianum* was applied. A maximum of disease incidence was observed at 18.7% in control (Table 2).

The soil application of SMS alone as well as enriched with *T. harzianum* resulted in a reduced PED and POED incidence in tomato nursery during three years of experimentation. The antagonistic activity of SMS microflora along with *T. harzianum* might have affected pathogens in soil and it resulted in a least PED, POED and total damping off disease incidence consistently in tomato nursery during experimentation for three years. As described in earlier experiment on PED and POED disease incidence in chilli, the authors speculate that microflora of SMS as reported by some researchers (Ahlawat and Sagar, 2007, Safianowicz et al.2018, Moraes et al. 2020, Frąc et al. 2021) might have produced antagonism against pathogens in the rhizosphere of tomato seedlings. The nutritional status of SMS might have also helped in the establishment of antagonistic microfolora including *T. harzianum* in the rhizosphere of tomato plants which may have affected the pathogens and it resulted in reduced damping off disease incidence in tomato nursery seedlings. The chemical nature of SMS may also be responsible for decreased damping off disease incidence in tomato because in one study Ocimati et al., 2021 application of SMS filtrate in media suppressed pathogen growth and reduced wilt disease in banana when applied under field conditions. The soil application of SMS had also been found to increase the nutrient status in strawberry rhizosphere and it resulted in increased antagonistic microflora in the rhizosphere of plants (Elaamer, 2020). The nutrients of SMS might have helped in the establishment of prevailing microflora including *T. harzianumin* rhizosphere of tomato seedlings in the nursery beds and it might have acted synergistically against pathogens and hence reduced

Table 2: Effect of soil application of spent mushroom substrate and farm yard manure enriched with *T. harzianum* on damping off disease in tomato

Treatments	2018–19 * Disease Incidence (%)			Disease control (%)	2019–20 * Disease Incidence (%)			Disease control (%)
	PED (%)	POED (%)	Total disease incidence (%)		PED (%)	POED (%)	Total disease incidence (%)	
SMS alone	5.4 (13.4)	9.2 (17.6)	14.6	19.4	5.7 (15.6)	9.6 (20.3)	15.3	21.1
SMS+ *T. harzianum*	4.7 (12.5)	7.5 (15.9)	12.2	32.6	4.8 (12.7)	7.8 (18.2)	12.6	35.1
FYM alone	6.0 (14.2)	10.7 (19.1)	16.7	7.7	6.8 (17.0)	11.0 (21.7)	17.8	8.3
FYM+ *T. harzianum*	5.0 (12.9)	8.2 (16.6)	13.2	27.1	5.2 (14.9)	8.2 (18.7)	13.5	30.4
Control (No soil amendment)	6.5 (14.8)	11.6 (19.9)	18.1	-	7.2 (17.5)	12.2 (22.9)	19.4	-
CD (*p*=0.05)	(0.60)	(0.52)	-	-	(0.70)	(0.46)	-	-

Table 2: Continue...

Treatments	2020–21 Disease Incidence (%)			Disease control (%)	Mean disease incidence (%)	Mean disease control (%)
	PED (%)	POED (%)	Total disease incidence (%)			
SMS alone	5.8 (13.9)	10.7 (19.1)	16.5	11.8	15.5	17.4
SMS+ *T. harzianum*	5.0 (12.9)	9.8 (18.2)	14.8	20.9	13.2	29.5
FYM alone	6.5 (14.8)	11.0 (19.4)	17.5	6.4	17.3	7.5
FYM+ *T. harzianum*	5.1 (13.0)	10.2 (18.6)	15.3	18.2	14.0	25.2
Control (No soil amendment)	7.1 (15.4)	11.6 (19.9)	18.7	-	18.7	-
CD (*p*=0.05)	(0.45)	(0.39)	-	-	-	-

Figures in parenthesis are angular transformed values, PED: Pre-emergence damping off; POED: Post-emergence damping off; Total disease incidence (%): PED(%)+POED(%)

reduction in pre-emergence, post-emergence and total damping off disease incidence in tomato nursery seedlings in the present study.

3.2.2. Damping off disease control

A maximum of damping off disease control in tomato seedlings at 32.6, 35.1, 20.9% was achieved during 2018–19, 2019–20, 2020–21, respectively, when SMS enriched with *T. harzianum* was applied in soil. It was followed by 27.1, 30.4, and 18.2% during 2018–19, 2019–20, 2020–21, respectively, when FYM enriched with *T. harzianum* was applied. The soil application of SMS without *T. harzianum* enrichment gave 19.4, 21.1, 11.8% disease control during 2018–19, 2019–20, 2020–21, respectively and a minimum of disease control of 7.7, 8.3, 6.4% was observed during 2018–19, 2019–20, 2020–21, respectively, when FYM without *T. harzianum* enrichment was applied. A mean of three years data on disease control was found maximum at 29.5% when SMS enriched with *T. harzianum* was applied followed by 25.2% when FYM enriched with *T. harzianum* was applied. A mean of three years data on disease control was at 17.4 and 7.5% where SMS and FYM without *T. harzianum* enrichment were applied in soil, respectively (Table 2).

The soil application of SMS enriched with *T. harzianum* resulted in a minimum pre-emergence and post-emergence damping off disease incidence in tomato and the results were consistent in the experiments conducted for three years consecutively. The results in present study are in line with other workers while working on different crops (Montanari et al., 2004, Ghazanfar et al., 2019, Elamaar, 2020). The presence of microflora in SMS including *T. harzianum* and their combined antagonism towards soil borne pathogens might led to increased disease control as discussed earlier in the present manuscript. Similarly, the role of chemical nature of SMS can also not be ignored because Ocimati et al. (2021) have already documented that addition of SMS filtrate reduced banana wilt disease incidence in an experiment.

The SMS is a cheap, abundantly available good source of nutrients, rich in antagonistic microflora and very suitable for multiplication and establishment of bio-control agents. Therefore, more experiments need to be conducted for eco-friendly management of soil borne diseases in vegetables, fruit plants and field crops after SMS enrichment with different antagonistic microorganisms. The soil application of SMS enriched with *T. harzianum* had not only helped in synergistic control of damping off disease in chilli and tomato under nursery conditions, but it also helped in transmission of enriched bio-control agent to the field with the transplants. In addition to diseases management, it also improves carbon content, texture, structure, water holding capacity and essential nutrients in soil required for plant growth which leads to improvement in yield and quality of the produce. The authors opine that the researchers should analyze the physical, chemical and biological characteristics of SMS before its application in soil for better interpretation of results.

4. Conclusion

The soil application *T. harzianum* enriched SMS at 25 tonnes ha^{-1} gave a minimum disease incidence at 16.0% as compared to 22.9% in control in chilli seedlings. In tomato seedlings, disease incidence was as low as 13.2% when *T. harzianum* enriched SMS at 25 t ha^{-1} was applied as compared to 18.7% in control. Its incorporation in soil is cost-effective, eco-friendly and improves physical, chemical and biological characteristics. It is a good substrate for integration of bio-control agents for soil borne disease management in plants.

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