Original Research Article

Impact of Spent Mushroom Substrate Integration with *Trichoderma harzianum* on Yield and Gummosis Disease Incidence in Bottle gourd (*Lagenaria siceraria*)

ABSTRACT

Bottle gourd is a commonly grown cucurbitaceous vegetable crop in India. It is affected by various pathogens but gummosis, a soil borne fungal disease is a serious disease of bottle gourd. The symptoms are color change to reddish brown at the collar region. The oozing of brownish gum on affected part is the first sign of disease. The research work carried out in the world has proved that soil application of spent mushroom substrate (SMS) after weathering increased yield and decreased soil borne diseases in different vegetable crops. Keeping in view of its importance, it was felt to study the impact of soil application of SMS enriched with *T. harzianum* on seed germination, yield and gummosis disease incidence of bottle gourd. The twelve months old SMS and well rotten farm yard manure (FYM) were applied in soil alone as well as enriched with *T. harzianum* in the experimental trials conducted from 2019 to 2021. It revealed that soil application of SMS and FYM applied alone or enriched with *T. harzianum* didn't have adverse effect on its germination. The maximum yield at 5752 kg/ha was obtained when SMS enriched with *T. harzianum* was applied followed by 5630 kg/ha when SMS was applied alone in soil. The minimum yield at 4806 kg/ha was obtained in control. A minimum disease incidence of 4.8 per cent was observed when SMS enriched with *T. harzianum* was applied, followed by 6.5 per cent when it was applied alone. The maximum disease incidence of 11.7 per cent was observed in control. Therefore, soil application of SMS enriched with *T. harzianum* before sowing at 25

tonnes/ha significantly increased yield and decreased disease incidence at the maximum rate as compared to other treatments and control.

Keywords: Bottle gourd, disease incidence, farm yard manure, spent mushroom substrate, yield.

1. INTRODUCTION

Bottle gourd is one of the most commonly grown cucurbitaceous vegetable crops in India. It is an important gift from Ayurveda to mankind [1]. Its fruits are being used in the treatment of various human diseases like jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, skin diseases, heart diseases etc. It is rich in iron, vitamins, potassium, flavonoids, saponins, triterpenes etc. It has high yield potential and steady market price. It is widely cultivated in India, Sri Lanka, China, and many parts of the world. In India, it was cultivated on an area of 1.93 lakhs ha with 3.17 million tonnes production during 2020-21 [2]. Among the different states, It is being widely cultivated in Bihar, U.P., M.P., Haryana, Chhatisgarh etc. However, it was cultivated on an area of 20850 ha. with production of 3.03 lakh tonnes during 2020-21 in Haryana.

It is affected by various diseases like gummosis, Alternaria leaf blight, anthracnose, *Cercospora* leaf spot, downy mildew, scab, powdery mildew, *Septoria* leaf spot, *Verticillium* wilt, angular leaf spot, aster yellows, cucumber mosaic virus etc. The gummosis is a serious disease of bottle gourd in Haryana caused by a soil borne fungus *Cladosporium cucumerinum*. The initial symptoms of the disease are change in normal to reddish brown colour at the collar region and plant posture from erect to trailing. The oozing of brownish gum on the discolored stem is the first sign of this disease. The infection spreads upward and ultimately the plant wilts and causes a considerable loss in its production and productivity.

There is a considerable increase in demand of organic manure to reduce the use of inorganic fertilizers, pesticides etc. The SMS is also an organic manure which is considered as a waste after mushroom harvest and its piling up in mushroom farms causes various environmental problems, like ground water contamination, nuisance etc. [3]. The mushroom production is being increased every year and presently an estimate of 13 lakh tonnes of SMS is being produced annually in India. Haryana is also a leading state in mushroom production and produces more than 1.0 lakh tonnes of SMS annually. The study on its use as soil amendments is being conducted throughout the world. The SMS is a rich source of crude protein, fibre and ash contents [4]. In addition, it is light in weight with high cation exchange capacity, slow mineralization rate, 45% water holding capacity [5]). It contains nitrogen at 1.9%, phosphorus at 0.6% and potash at 1.0% after weathering for 8-16 months [6] and an EC (1.9 to 8.3 memos/cm), pH of 7.28 [7]. The removal of soluble salts from it and then weathering before soil application is an important task. Its soil application has been found to give higher yield, improved quality of produce and reduced soil borne diseases in various vegetable crops. The soil application of SMS at 18.5 tonnes/ha gave superior fruit weight, high ascorbic acid content, high dry matter, more total soluble solids (TSS) etc. and further helped in reducing soil borne diseases in tomato plants. The results were also found to be encouraging in chilli (*Capsicum annuum*), pea (*Pisum sativum*), cauliflower (*Brassica oleracea* var *botrytis*), ginger (*Zingiber officinale*), onion (*Allium cepa* L.), brinjal (*Solanum melongena*) etc [8]. The carbon rich compost inoculated with *Trichoderma* strains provided most effective control against soil borne pathogens of tomato [9]. Similarly, the introduction of *Trichoderma atroviride* strain in SMS enhanced biological control of melon wilt caused by *F. oxysporum* f.sp. *melonis* [10]. Keeping in view of its better performance in soil after enrichment with *Trichoderma* sp. in many studies, it was felt imperative

evaluating the impact of soil application of SMS and FYM enriched with *T. harzianum* on the bottle gourd germination, yield, gummosis disease incidence in the experiments conducted during 2019-2021.

2. MATERIALS AND METHODS

The experiment to study the impact of soil application of SMS and FYM alone and their integration with *T. harzianum* on yield and gummosis disease incidence of bottle gourd cv. Pusa Summer Prolific Long (PSPL) was carried out in soil naturally infested with gummosis pathogen in the experimental field area of Department of Plant Pathology, CCSHAU Hisar from 2019 to 2021. The one year naturally weathered button mushroom SMS and two years old well rotten FYM was used in the 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 as per standard procedure. The oatmeal medium containing *T. harzianum* (1×10^9 cfu/g) was mixed at the rate of 0.001 per cent (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 upon 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* integration were also maintained similarly. The soil application of substrates was carried out as per treatments given below and sowing of bottle gourd cv. PSPL was done on sides of raised beds adjacent to irrigated channel at a spacing of 60 cm from plant to plant in mid February during all the three years. The plot size of treatment was kept as 5 m $\times$ 3.3 m. Each treatment was replicated thrice and randomized completely. The agronomic operations were carried out as per

recommended package of practices. The plots without soil amendments were also maintained as per recommended package of practices and served as control. The observations were taken on seed germination (%), yield (kg/ha) and gummosis disease incidence (%) till the end of crop season. The yield data was compiled replication wise at the end of the season. The gummosis incidence was also recorded replication wise at a weekly interval after germination of seedlings. The experiment was repeated for three consecutive years from 2019 to 2021. The sowing of the bottle gourd was done in the mid February of every year. The data were analyzed statistically by randomized block design through OPSTAT software.

Treatments:

T_1= 25 tonnes SMS amended with *T. harzianum* /ha

T_2= 25 tonnes SMS alone/ha

T_3 =25 tonnes FYM amended with *T. harzianum* /ha

T_4= 25 tonnes FYM alone/ha

T_5= Control (No soil amendment with SMS or FYM)

3. RESULTS AND DISCUSSION

3.1 Yield

The data presented in Table 1 show that none of the treatment affected bottle gourd seed germination. However, soil application of SMS enriched with *T. harzianum* resulted in a significant higher yield of 6125 kg/ha as compared to 5000kg/ha in control during 2019. It remained significantly high during three years of experimental trials in this treatment as compared to control. The maximum average yield was 5752 kg/ha in SMS enriched with *T. harzianum* followed by 5630 kg/ha in soil application of SMS alone and a minimum average

yield of 4806 kg/ha in control (Table 1). In other words, there was an increase in yield of 19.5 per cent in SMS enriched with *T. harzianum* followed by 17.0 per cent when SMS was applied alone. The increase in yield was 10.1 per cent when FYM enriched with *T. harzianum* was applied in soil followed by 7.2 per cent when FYM was applied alone as compared to control (Fig. 1).

3.2 Disease incidence and its control

The gummosis incidence was significantly low in the treatment when SMS enriched with *T. harzianum* was applied in soil during all three years of experimentation as compared to control (Table 1). An average of gummosis disease incidence of 4.8 per cent was found to be lowest when SMS enriched with *T. harzianum* was applied in soil before sowing followed by 6.5 per cent when SMS was applied alone in soil. The maximum average of gummosis disease incidence of 11.7 per cent was observed in control.

A maximum of gummosis disease control was 45.9 per cent when SMS enriched with *T. harzianum* was applied in soil followed by 36.0 per cent when SMS was applied alone. The FYM enriched with *T. harzianum* gave an average of 29.4 per cent gummosis disease control and a minimum of gummosis disease control of 11.7 per cent was observed in FYM enriched with *T. harzianum* and applied in soil before sowing. The soil application of SMS enriched with *T. harzianum* gave consistently good and uniform results throughout three years of experimentation providing maximum yield and gummosis disease control in bottle gourd.

Table 1. Effect of soil application of spent mushroom and farm yard manure enriched with *T. harzianum* on germination, yield and gummosis incidence in bottle gourd cv. PSPL

Treatments	*Germination (%)				*Yield (kg)/ha				*Per cent increase in yield				*Gummosis Disease incidence (%)				*Per cent Disease Control			
	2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean
SMS + TH	90	90	90	90	6125	5970	5161	5752	22.5	21.5	14.5	19.5	05.8	05.8	2.9	4.8	63.1	61.1	13.7	45.9
SMS alone	90	90	90	90	6000	5837	5054	5630	20.0	18.8	12.2	17.0	08.0	08.6	2.9	6.5	49.0	46.9	12.1	36.0
FYM + TH	90	90	90	90	5525	5447	4925	5299	10.5	10.5	9.3	10.1	09.2	09.6	3.1	7.3	41.4	40.7	6.1	29.4
FYM alone	90	90	90	90	5450	5323	4671	5148	09.0	09.0	3.7	7.2	11.3	12.3	3.2	8.9	28.0	24.1	3.0	18.4
Control	90	90	90	90	5000	4913	4505	4806	-	-	-	-	15.7	16.2	3.3	11.7	-	-	-	-
C.D. at 5%	NS	NS	NS	-	135.8	87.4	67.7	-	-	-	-	-	0.57	0.44	0.5	-	-	-	-	-

*Average of three replications

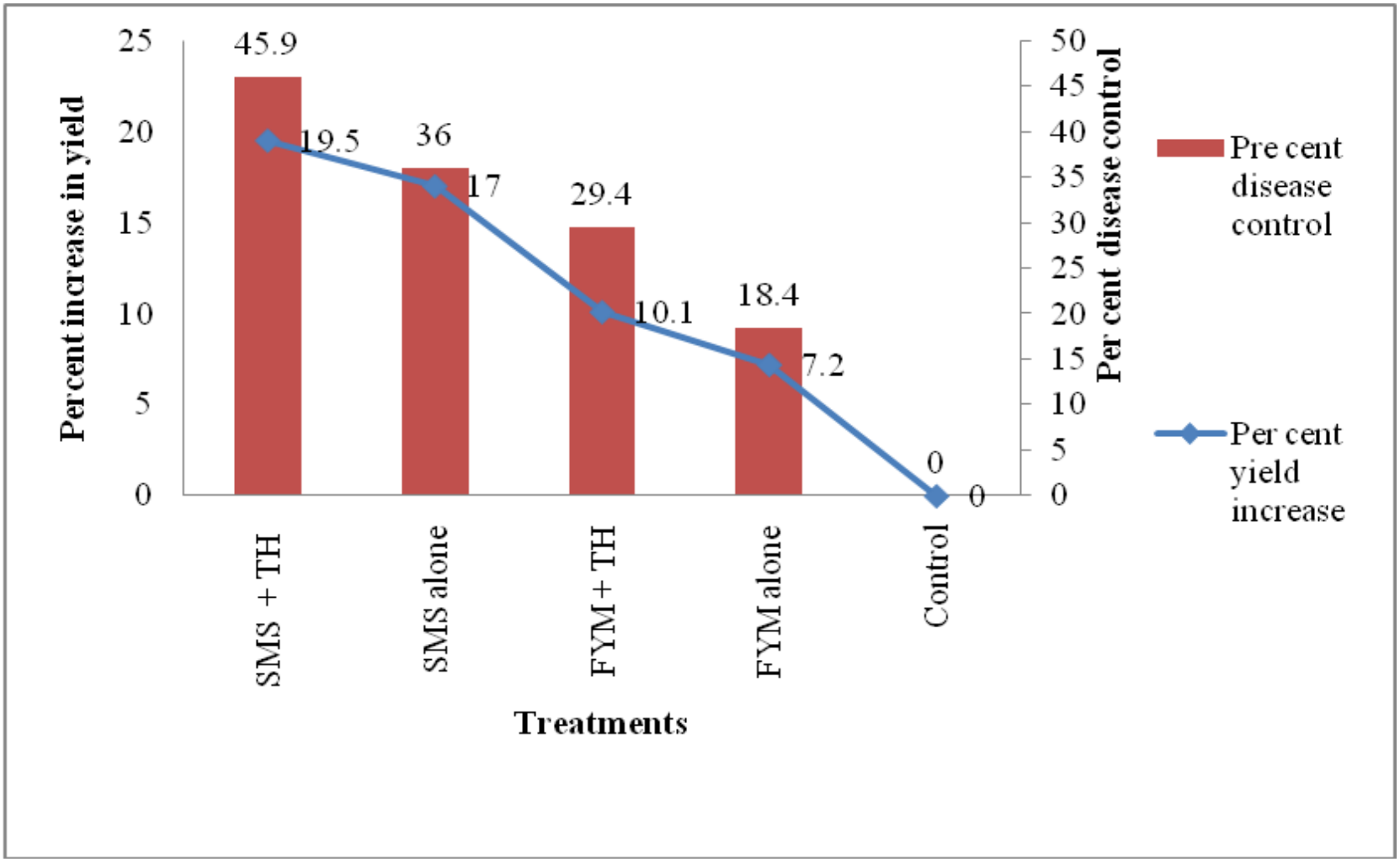

Fig. 1. Impact of soil application of SMS and FYM mixed with *T. harzianum* on yield and gummosis disease control in bottle gourd

Many of the following researchers have also observed an increase in yield of different crops by soil application of SMS and their studies are in agreement with our findings. The introduction of SMS in soil after proper composting produced higher red baby leaf lettuce yields [11]. Similarly, an increased yield and biomass production in straw berry was observed when fresh SMS was applied in soil [12]. The SMS application in soil upto 30 tonnes/ha significantly increased the fresh yield and quality of baby spinach [13]. The soil application of SMS led to increase in growth of *Capsicum annuum* L. as well as high carotenoid, protein content over control [14]. There was an increase in yield of capsicum, tomato, cauliflower, ginger, garlic, potato, pea, wheat, maize, paddy and apple by soil application of SMS[15]. The amendment of soil with SMS at 18.5 tonnes/ha alone led to a higher yield at 746q/ha in tomato as compared to 456.53q/ ha in the treatment when FYM was applied in soil [8]. The soil application of SMS at 50 tonnes/ha in onion crop revealed that it not only increased bulb yield but also improved P, K, Ca and Mg contents in onion bulbs [16].

The nutrients in SMS might have influenced the yield directly by providing more nutrients to the plants. It might have helped in establishment of antagonistic microflora in the rhizosphere of the plant also and thus, may have resulted in reduced soil borne diseases. The researchers have not observed the status of plant disease after soil application of SMS in different crops. But, it can't be ignored that SMS also affect the antagonistic microflora in rhizosphere of plant which might have helped in minimizing soil borne disease and hence a higher yield was possible to be obtained. In a study on nutritional status of SMS, it was found that it contains ash (6.58%), C:N ratio (116.29), phosphorus (57.14 ppm), calcium (7366.67 ppm), magnesium (1230.83 ppm), iron (85.18 ppm), copper (3.75 ppm), nitrogen (0.38%), potassium (706.67 ppm), zinc (16.90 ppm), manganese (68.65 ppm) and pH (5.92) in SMS [17]. The soil application of SMS

enhanced the macro and micro nutrients in strawberry rhizosphere which resulted in increased antagonistic microflora like *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., and *Actinomyces* spp [18]. The SMS is a good substrate for growth of *Trichoderma* and in addition *Trichoderma* produces enzymes like cellulase, xylanase, amylase and β-glucosidase in SMS [19]. However, in the present study also, SMS enriched with *T. harzianum* applied in soil at pre sowing stage led to a synergistic increase in yield; probably it may be due to its better nutritional status which might have helped in providing better nutrition to the plant as well as it might have helped in influencing the antagonistic microflora and better establishment of *T. harzianum* in the rhizosphere of the plant. There may be synergistic increase in antibiosis by *T. harzianum* and other antagonistic micrfolora in the plant rhizosphere against gummosis pathogen. It may be the reason of low disease incidence and hence resulted in higher yield of bottle gourd. Some of the following studies have been taken into consideration for elaborating our findings. The soil application of SMS produced suppressive effect against *Pythium irregulare* but incorporation of *T. harzianum* did not affect against *P. irregulare*. But in contrast they reported that enrichment of *T. harzianum* did not have an additional effect against pathogen [11]. It may be due to genetic variations in *T. harzianum* isolate used in their study as compared to isolate used in this study. It may also be due to differences in physical, chemical, biological composition of the SMS because establishment of *T. harzianum* and other antagonistic microflora is also affected by its composition. In a similar study, the wet root rot of chickpea caused by *Rhizoctonia solani* was reduced effectively by soil application of FYM enriched with *Trichoderma* [20]. A minimum of losses by soil borne disease in tomato, chilli, peas, cauliflower and ginger were observed by soil application of SM. The SMS harbored microflora like *Pseudomonas, Bacillus, Trichoderma* sp., *Aspergillus* sp., *Mucor* sp., actinomycetes and these might have produced antagonistic effect

against several soil borne pathogens which led to reduced disease incidence in vegetable crops [8].

3.3 Nutritional status of SMS

The analysis of macro nutrients of one year old naturally weathered SMS was conducted at Department of Soil Science, College of Agriculture, CCS Haryana Agricultural University, Hisar. The data presented in Table 2 revealed that SMS and FYM are approx. at par in P and K concentrations; however, N content is more in FYM (2.5%) as compared to SMS (1.28%).

Table 2. Nutrients status of spent mushroom substrate and farm yard manure

Substrate(s)	**N (%)**	**P (%)**	**K (%)**
Spent Mushroom Substrate	1.28	0.89	1.25
Farm Yard Manure	2.50	0.75	1.56

The covered and uncovered SMS were analyzed for physical and chemical characteristics after storage for 12 months and found no consistent differences in uncovered or covered SMS. The content of nitrogen (N) and manganese (Mn) was significantly lower in uncovered SMS, while the content of iron (Fe) and copper (Cu) was significantly higher. The nitrogen-phosphorus-potassium concentrations per kg wet weight were all higher in SMS that was stored under cover. Its pH ranged 7.8–8.1 and hence may be useful in acidic soils [21]. Since, SMS used in this experiment was weathered naturally under open environment conditions, therefore, N content was found low in SMS. Therefore, the researchers are may cover the SMS while weathering to increase macro nutrients concentration. It has been reported earlier that pH of SMS was minimum at 6.72 in 9 months weathered as compared to 7.50 with 48 months weathered SMS. The electrical conductivity was highest at 6.22 mS cm^{-1} in 6 months and lowest at 0.24 mS cm^{-1}

in 48 months weathered SMS. The N, P, C, Ca content decreased by increasing weathering duration. The nitrate content in SMS also decreased from 12.80 to 1.95 ppm when it was weathered for 6 and 48 months, respectively [8]. Therefore, many physical, chemical and biological properties like pH, electrical conductivity, carbon content, micro nutrients, heavy metals, microflora etc. of SMS is needed before the conduct of trials, which may further help in substantiating the results.

Since SMS is a cheapest, abundantly available, environment friendly waste and has been found to increase in yield, quality of produce and suppressing soil borne pathogens in many studies, therefore, experiments may be conducted on other vegetables, horticultural and field crops for sustainable crop production by soil application of SMS enriched with antagonistic microorganisms.

4. CONCLUSION

The soil application of SMS enriched with *T. harzianum* before sowing at the rate of 25 tonnes/ha gave a maximum yield of 5752 kg/ha as compared to 4806 kg/ha of bottle gourd in control. This treatment also gave a 45.9 per cent gummosis disease control as compared to control. It is concluded that SMS enrichment with *T. harzianum* and its soil application before sowing resulted in 19.7 per cent higher yield and 45.9 per cent gummosis disease control in bottle gourd as compared to control. Therefore, it is recommended that soil application of SMS enriched with *T. harzianum* at the rate of 25 tonnes/ha at pre sowing stage gave higher yield and lower gummosis disease incidence in bottle gourd. It is further suggested that experiments on pre sowing soil application of SMS enriched with *T. harzianum* or other beneficial

microorganisms may also be conducted on other vegetable crops to maximize yield and eco-friendly management of soil borne diseases.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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