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UTILIZATION OF SPENT MUSHROOM SUBSTRATE AS CARRIER FOR BIOCONTROL AGENT AND BIOFERTILIZER

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ABSTRACT

Spent mushroom substrate (SMS) is a by-product of the edible mushroom (*Agaricus bisporus*) industry. Traditionally, SMS was discarded as wastes, creating an environmental nuisance. The pH, Ec, organic carbon, organic matter and ash content of the SMS was 7.56, 0.84 ds/m, 28.78%, 49.63% and 44.37% respectively. It was found to contain 1.82 %, 0.64 % and 1.98 % NPK, respectively. The maximum water holding capacity of SMS was 98.87%. Five substrates combination were screened for mass multiplication of *Trichoderma viride* and *Rhizobium japonicum*. Among these carriers, Talc gave maximum propagules of *T. viride* (13x106 cfu/g) . However, SMS found to be good carrier for shelf life and survival of *T. viride* (2 x 10^6cfu/g) and *Rhizobium* (20.66x10^8cfu/g) at 180 days as compared to other carrier combination. However, SMS may serve as a carrier material for the production of *T.viride* and *Rhizobial japonicum*.

INTRODUCTION

Spent mushroom substrates (SMS) is the substrate left after harvesting mushroom fruit bodies. Global production of mushroom is greater than six million tones and has approximate value of at least $ 14 billion (Rinker, 2002). In 2007, the production of edible mushrooms in Japan was estimated to be 4, 23,224 tones and it is expected that this amount will increase in the future due to market demand. Despite the evident benefits of mushrooms, the exponential increase in their consumption worldwide is also generating a high volume of spent mushroom substrate (SMS). It has been reported that about 5 kg of substrate are needed to produce 1 kg of mushroom (Williams *et al*., 2001; Uzun, 2004 and Finney *et al*., 2009), and about 17 million tones of SMS are produced each year. Consequently, one of the main problems faced by mushroom production companies is finding a way to properly dispose of the SMS without contaminating the water and soil. In fact, the lack of a sustainable waste management solution for SMS is the most significant barrier to the future development of the mushroom industry (Finney *et al*., 2009).

SMS has good physical properties. It includes the water holding capacity, soil pH, soil porosity, salt content *i.e.* electrical conductivity and also important properties. Addition of SMS will add the great amount of macronutrient like nitrogen, phosphorous and potassium (NPK) and tie up nutrients but it is in little quantity (Kim *et al.,* 2011). Many workers have suggested peat, lignite and charcoal (Gaur and Gaind, 1984) as the efficient carrier for *Trichoderma* and *Rhizobium*. Problems associated with the above carriers in India are little availability of peat soil (Thomas *et al.,* 1974) and high cost of talc and lignite.

The primary goal of our research was to broaden the scope of application for SMS to stimulate its demand, and thereby reduce field inventories. Specifically, we are exploring the possibility of SMS as carrier for biocontrol agents and Biofertilizers. This approach will provide an alternative outlet for SMS. Unlike most other recycling methods, SMS don't have to be weathered in the field for months to be reused as carrier for bio control agent and bio fertilizer.

MATERIALS AND METHODS

Determination of chemical and physical properties of spent mushroom substrate

During late summer 2012, fresh mushroom substrate samples were collected from mushroom farms particularly the mushroom-growing regions in Akola. The samples were acquired as the material was being removed from a production facility and were not allowed to stock pile or age outdoors. Each sample was placed in a plastic container and was securely shipped to the Soil and Agril. Chemistry Laboratory (PDKV University (M.H.) India) for processing and analysis. Laboratory tests measured the following properties: Available nitrogen present in SMS was determined by Alkaline Permanganate method. Available phosphorous present in SMS was determined by Olsen's method. Available potassium present in SMS was estimated by Neutral Ammonium Acetate method.

Micronutrients present in SMS were estimated by using Di-acid digestion method. Maximum water holding capacity of SMS was estimated by using Keen Reczkowski box method.Salt content (electronic conductivity) of SMS by Elico soil bridge conductivity meter method. Available Organic carbon, Organic material and mineral (ash) content in sample of SMS was estimated by Ignition method. Bulk density was determined ''as is'' (*i.e.*, as received at the laboratory, and not oven-dried) on a wet volume and particle size also was determined ''as is'' on a wet weight basis. Data from all samples were compiled to determine mean value (Singh *et al*., 2007).

Preparation of carrier materials

Carrier materials taken for study *viz* spent mushroom substrate was shade dried up to 3 per cent moisture. The sample was homogenized by hand, which was not given any metallic contamination and grinded in Plant sample grinder to a fine powder and sieved at 60 mesh. Talc was procured in ready condition (120 meshes).

Preparation of carrier mixture and sterilization of carriers

Two materials were tested as carrier for *Trichoderma* and *R. japonicum*, with five combinations *viz* spent mushroom substrates (SMS) + Talc in proportions of 75:25, 50:50 and 25:75, spent mushroom substrate (SMS) and talc alone. The CMC (Carboxy Methyl Cellulose) used @ 15 g/kg of carrier material. pH of carriers was adjusted to 7 with calcium carbonate. They were sterilized two times at 15 lbs pressure at 121.6ºC for 15 minute in autoclave for two successive days.

Inoculation of carriers with *Trichoderma viride* culture

Harvested 10 days old *T. viride* inoculum on PDB medium (Fungal biomass). In each 100 g substrate, 50 mL of broth containing mycelial mat of *T. viride* was added and mixed under aseptic conditions. *Trichoderma* grown in the PDB medium was mixed with talc powder in the ratio of 1:2 and dried to 8% moisture under shade. These mixtures were equally mixed and packed, distributed into three number of Polythene bags (after adjusting moisture to 50 per cent), sealed and incubated at room temperature 27± 2ºC.

Assessment of *Trichoderma viride* in the carriers by Dilution Plate Technique using PDA

T. viride carrier based cultures were stored at room temperature 27 ± 2ºC and shelf life study was carried out at monthly interval. A sample of one gram of the product drawn from each carrier before packing as an initial (0 day) and later at 30, 60, 90, 120, 150, and 180 days of storage. The population was estimated by serial dilution plate technique (at 10^6 dilutions). This study was initiated for development of a cheap and suitable system of the most suitable and cheap carrier for mass multiplication of the *T. viride*.

Inoculation of carriers with *Rhizobium japonicum*

Five days old broth culture of *R. japonicum* was used for inoculation with the neutralized, sterilized carrier material in the proportion of 2:1(carrier: broth) *i.e.* to the moisture content 50 per cent. The initial *Rhizobial* populations in each inoculated test carrier were quantified by following usual dilution plate method using CRYEMA medium and observations were recorded.

Statistical analysis

Experiments were design as CRD with four replications. All data analysis was done which included 5 carriers replicate for biocontrol agent and biofertilizer. The statistical analysis of the data was done by statistical method as suggested by Panse and Sukhatme (1978). 'F' test of significance was used to know whether observed treatment effects were real or not from the data in which the treatment effects were significant. The standard error (SE) and critical difference (CD) at 1% level of probability were calculated. Values of critical difference were used to interpret the result. The data have been illustrated graphically at appropriate place in the text.

RESULTS AND DISCUSSION

Fresh mushroom substrate had an average pH of 7.56 (Table 1). Kumbhar (2012) reported an average pH of 7.3 for fresh mushroom substrate. Only three samples were used in that study from a single mushroom farm compared with 21 samples from multiple farms in this investigation and the amount and quality of materials used to make mushroom substrate is

Table 1: The physio-chemical characteristics of SMS pH and C: N RATIO

Parameter measured		Amount present
pH of SMS		7.56
Electrical conductivity of SMS (ds/m)		0.84
Organic carbon content in SMS (per cent)		28.78
Micronutrients content in SMS(mg/kg)	Zinc (Zn)	0.76
	Copper (Cu)	0.45
	Iron (Fe)	35.20
	Manganese (Mn)	1.84
Macronutrients content in SMS(per cent)	Nitrogen (N)	1.82
	Phosphorous (P)	0.64
	Potassium (K)	1.98
Maximum water holding capacity (MWHC) (cm/hr)of SMS		98.87
Organic material/ matter in SMS(per cent)		49.63
Mineral matter/ Ash in SMS (per cent)		44.37
Organic carbon in SMS (per cent)		28.78
Bulk density lb/yard3		482.47
Moisture content (per cent)		64.89
C: N Ratio		15.81: 1

Table 2: Effect of different carrier materials on population of *T. viride* (x10^{6}cfu/g)

Treatments	Carriers (Talc : SMS)	Population of *T. viride* * (x10^{6}cfu/g) at Initial 0 day	30 days	60 days	90 days	120 days	150 days	180 days
1.	Talc alone (100:00)	114.60	101.00	79.60	75.80	48.20	25.70	13.00
2.	Talc+SMS (75:25)	115.90	110.80	76.20	64.70	40.20	17.06	8.20
3.	Talc+SMS (50:50)	116.20	116.30	74.60	66.70	40.70	20.70	5.80
4.	Talc+SMS (25:75)	113.40	118.20	74.50	66.40	37.60	11.20	3.70
5.	SMS alone (00:100)	110.20	122.80	76.40	56.60	22.30	8.20	2.00
	SE(m)+	3.77	3.57	2.64	3.04	1.21	0.87	0.38
	CD at (p=0.01)	-	14.89	11.00	12.68	5.07	3.65	1.59

*Average of four replications

Table 3: *Rhizobial* population (×10^{8}cells/g) in different carriers

Treatments	Carriers (Talc :SMS)	Population of *R. japonicum* * (×10^{8}cells/g) at Initial 0 day	30 days	60 days	90 days	120 days	150 days	180 days
1.	Talc alone (100:00)	74.06	68.25	61.00	57.66	54.33	43.00	31.00
2.	Talc+SMS (75:25)	74.89	72.00	68.33	66.33	47.66	37.00	27.33
3.	Talc+SMS (50:50)	76.44	68.33	62.00	59.33	51.33	35.33	26.00
4.	Talc+SMS (25:75)	72.92	66.04	58.66	55.66	30.66	38.00	24.00
5.	SMS alone (00:100)	73.67	72.19	51.00	46.33	38.86	37.68	20.66
	SE(m)+	2.46	2.23	2.64	1.88	2.89	1.95	1.24
	CD at (p=0.01)	-	9.30	11.00	7.85	12.07	8.14	5.20

* Average of four replications

dramatically different in Akola (M.S.) India. The C:N ratio of fresh mushroom substrate in this study averaged 15.81: 1 (Table 1), within the desired range of 10:1 to 16:1 for ideal substrate (Fidanza *et al*., 2005).

Physico-chemical characteristics of SMS

The average EC of fresh mushroom substrate was 0.84 ds/m (Table 1). Fidanza *et al*. (2010) have reported 13.30 mmho/cm (wet weight basis) average soluble salt content of SMS.

Mean bulk density averaged at 482.47 lb/yard3 (wet volume), with over half of the weight attributed to water (Table 1). Fidanza *et al*. (2010) reported an average bulk density of 574.73 lb/yard 3 for fresh mushroom substrate and again this lower value reflects a difference in the amount and quality of materials used to make mushroom substrate.

Organic matter content of fresh mushroom substrate was 49.63% (dry weight) and an average carbon content of 28.78% (dry weight) (Table 1). The organic matter in mushroom substrate consists of decomposed plant, animal and fungal residues and materials (Davis *et al*., 2006).

Average available N content of fresh mushroom substrate was 1.82% (dry weight). The majority of this N is in the organic form, with a very small amount in the ammonium form (Table 1). In general, substrates have low N content, typically in the 1% to 3% range (Polat *et al*., 2009). Average P content was 0.64% (dry weight). Average K content was 1.98% (dry weight). Overall, the average amounts of primary and secondary macronutrients determined from samples tested did not show extreme minimum or maximum values, thus indicating similar methods used by mushroom farms for producing this material in India.

The micronutrients Zn, Cu, Fe and Mn were detected in fresh mushroom substrate at a very low average range of 0.45 to 1.84 mg/kg (dry weight) (Table1). This information is consistent with previous findings on the micronutrient content of SMS contain 0.76, 0.45, 35.20 and 1.84 mg/kg Zinc, Copper, Iron and Manganese respectively (Table 1). Medina *et al*. (2009) who reported that the total available levels of the micronutrients i.e. Fe, Zn, Cu and Mn were 3117.0, 145, 29.0 and 302 mg/kg, respectively.

On average, 89% of fresh mushroom substrate particles measured e"3/8 inch in diameter, with 10% at 3/8 to 5/8 inch, and <1% at 5/8 to 1 inch. No particles were measured >2 inch in diameter. Overall, this material is of a consistent and uniform size and is easy to handle and distribute to dry and grind for making carrier material. Water holding capacity of SMS was 98.87 cm/hr (Table1). These data correlated with Fidanza *et al*. (2010) who investigated all the macronutrient content i.e. N (2.65%), P (0.69%), K (2.44%), Organic matter (60.97%) and Organic carbon (33.42%).

Effect of different carrier material on population of *T. Viride*

Data in Table 2 revealed that viability of *T. viride* in different carriers up to180 days. Talc was found best carrier material among the other carrier materials to retain maximum number of propagules of *Trichoderma* sp. up to 180 days. However, on 30 days onwards significant variations were observed in all the five carriers up to 180 days of storage indicating the decrease in the number of propagules. After 30 days maximum viable propagules were recorded in SMS alone (122.80x 10^{6}cfu/g).Data in Table 2 revealed that viability of *T. viride* was found in different carriers up to 180 days. Talc was found best carrier material among the other carrier materials to retain maximum number of propagules of *Trichoderma* sp. up to 180 days. SMS alone (00:100) formulation can be used up to 120 days as it will be the cheaper source for *Trichoderma* formulation (Chaudhari *et al*., 2011).

However, on 30 days onwards significant variations were observed in all the five carriers up to 180 days of storage indicating the decrease in the number of propagules. After 30 days maximum viable propagules were recorded in SMS alone (122.80x 10^{6}cfu/g).

At 180 days talc recorded maximum propagules (13.00 x 10^{6}cfu/

g) and was found significantly superior over all other carriers. Minimum propagules were recorded in SMS alone (00:100) (2 x 10^6cfu/g),these findings were correlated with Bheemaraya *et al*.(2011) who reported that the viable propagule of bioagent (*Trichoderma viride*) can be observed even after 180 days of storage in the both vermicompost (14.17 × 10^6cfu/g) and talc (18.50 × 10^6cfu/g and 135.50 × 10^6cfu/g), respectively.

Effect of different carrier material on population of *Rhizobium japonicum*

The data presented in Table 3 reveal that there were significant differences on *Rhizobial population* at all the intervals except 0 days. Maximum population was attained in SMS alone (72.19 x10^8 cells/g) and it was found significantly superior over all other combination at 30 days. Among SMS and talc combinations maximum *Rhizobial* population was noticed in talc (75:25)(31.00 x10^8cells/g) at 180 days and T_3 and T_4 were found at par with T_2. At 30 days there was gradual decrease in count in all the treatments. Similar trend was also noticed at 60, 90, 120, 150 and 180 days. It indicates that combination of SMS can be done with Talc as a carrier.

Temprano *et al*. (2002) reported the survival of *Rhizobium* sp. in non-refrigerated inoculants with an initial increase in population followed by a steady decrease to a final density of 10^7*Rhizobial*. Khavazi *et al*. (2007) investigated that among perlite, malt residue, sugarcane baggase, coal and rice husk all these carrier materials were capable of maintaining *Rhizobium japonicum* population in excess of 1 x 10^9 *Rhizobial* inoculent for at least 6 months. Bahl and Jauhri (1987) reported the suitability of spent compost, a waste of mushroom cultivation as carriers for *Rhizobium* and *Azotobacter* inoculants.

In the present investigation talc recorded maximum *Rhizobial* colonies (31x10^8 cfu/g), followed by talc + SMS (75:25) (27.33 x10^8/g) at 180 days. This may be due to more water holding capacity of carrier material. These findings are correlated with Rebah *et al*. (2007) who reported that some industrial and agricultural by-products (e.g. cheese whey, malt sprouts) contain growth factors such as nitrogen and carbon which can support growth of *Rhizobia*. Other agro-industrial wastes (e.g. plant compost, filtertermud and fly-ash) can be used as a carrier for *Rhizobial* inoculant. More recently, wastewater sludge a worldwide recyclable waste has shown good potential for inoculant production as a growth medium and as a carrier (dehydrated sludge).

Fresh mushroom substrate should be considered a viable, recycled agricultural product useful as a carrier for bio agents and Biofertilizers. The methods used to grow white button mushrooms are very similar among the farms in India. Therefore, results of the analysis of fresh mushroom substrate in this report should be representative of the chemical and physical properties throughout that material as produced in India. The spent mushroom substrate has desirable chemical and physical properties like pH, Electrical conductivity, water holding capacity, organic carbon, organic matter, mineral matter, macronutrients (NPK), micronutrients (Zn, Mn, Cu, Fe). SMS retain viable propagules up to 180 days, as it is very cheap and locally available, SMS may serve as a carrier material for the production of *Trichoderma viride* and *Rhizobial japopnicum*. Minimum propagules were recorded in SMS alone (00:100) (2 x 10^6cfu/g).

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