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Evaluation of casing variables for cultivation of *Agaricus bisporus* strain U3 in Punjab, India

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Abstract: Casing is an important step during cultivation of button mushroom. It promotes a shift of vegetative mycelium to form pinheads leading to mature basidiocarps. Therefore, an experiment was planned at Punjab Agricultural University, Ludhiana in which farm yard manure (FYM), biogas slurry (BS), burnt rice husk (BRH), spent compost (SC), coir pith (CP) and sandy soil (SS) were used in six combinations to evaluate their impact on yield of *Agaricus bisporus* strain U3. Casing mixtures were analyzed for the moisture, water holding capacity (WHC), bulk density, pH, electrical conductivity (EC) and mineral analysis. The moisture content of casing mixtures ranged between 33 to 56.3% with maximum in FYM+CP (1:1) and FYM+BS (1:1) which results in more water holding capacity with 160% and 100% for FYM+CP (1:1) and FYM+BS (1:1) respectively. The bulk density of different casing mixtures ranged between 33.3 to 83.3% and maximum for FYM+BS as well as the control FYM+SS (4:1). No significant variation in pH of casing mixture was observed while EC was significantly higher in FYM+BS (1:1) with 1.7 mmhos cm^{-1}. Microbial count of different casing mixtures was between $3.0\text{-}5.7\times10^{5}$ which was statistically non-significant with respect to casing mixtures. A mixture of FYM+BRH (1:1) gave maximum yield of 15.1 kg with heavy fruit bodies. Number of fruiting bodies in casing mixture FYM+BRH (1:1) were 31.4, 26.4, 148.1,31.4 and 8.1 percent higher over FYM+CP (1:1), FYM+SC (1:1), FYM+BS (1:1), FYM and FYM+SS (4:1,control), respectively. The casing mixture FYM+BRH (1:1) with higher number of fruiting bodies with maximum fruiting bodies weight was the best alternate in mushroom growing industry.

Keywords: Casing, Yield, Farm Yard Manure (FYM), Coir Pith (CP), Biogas Slurry (BS), Spent Compost (SC), Burnt Rice Husk (BRH), Sandy Soil (SS)

INTRODUCTION

India is blessed with varied agro-climatic regions from temperate, tropical to subtropical. This makes India as a suitable place for the production of different types of mushrooms. *Agaricus bisporus* is popularly known as the button mushroom, is extensively cultivated throughout the world and contributes about 40% of the total world production of mushroom. In India, cultivation of white button mushroom started in early 60's. Button mushroom is one of the largely grown mushrooms and has the good demand in the market and world trade too. Keeping this view in mind the choice of the farmers for growing of button mushroom depends on easily and locally available casing mixtures (Chandra *et al*., 2014).

Production of *A. bisporus* to a greater extent depends upon the quality of casing material used. Casing material is used in mushroom to cover a nutritional rich composted substrate colonized with mycelium, and has an essential function in stimulating and promoting the development of sporophores (Noble and Pennington 2005). The casing layer influences yield, quality and uniformity of cropping of the button mushroom. Thus, mushroom productivity, size and mass are directly affected by the casing layer. The casing soil is reported to possess certain physical, chemical and microbiological properties having stimulatory role in *Agaricus* fruiting (Ahlawat 2002). Wei-Ming *et al*., (2009) also reported that the culturable bacterial population was found to be higher in casing soil during mushroom growth.

Nobel and Pennington (2005) observed that in many countries, sphagnum moss and peat were being used as casing medium due to its unique properties to absorb and release water quickly, enough porosity for air exchange and its availability in abundance. But, in India pasteurized or chemically treated soil alone or in combinations with materials having desirable properties were used as casing medium, as peat is either unavailable or too costly to import. Moreover, the increase of peat use worldwide has resulted in a rapid depletion of wetlands, causing the loss of non-renewable resources and becoming a source of the greenhouse effect due to the abundant liberation of CO_2 through the aerobic decomposition of carbon (Bustamante *et al.,* 2008). Evenmore, Peat is not available in some areas. So, these factors created a demand for alternatives to peat in agriculture, making it important to the sustainability

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of mushroom cultivation. Finding a cost-effective alternative material to improve mushroom yield or quality would be profitable to the mushroom farmers. Consequently, Soil can be used as casing soil. The soil is also accessible throughout the year and its low prices have benefits such as easy management (Zied *et al*., 2010). Few substances like FYM, biogas slurry, spent compost, loamy sand soil are used individually or in combination for casing in different regions.

Spent mushroom compost as a casing soil material can be used to reduce the cost of production and it can also reduce pollution (Pardo-Giménez and Pardo-González, 2008). Rehman *et al*., (2016) also reported that the use of Lahore compost and FYM + sand + lime was found to be significantly different from the peat soil/control. Moisture content was also found to be higher in Lahore compost and FYM + sand + lime as compared to peat soil with 59.3, 60.3 and 45.9% respectively. Singh *et al.,* (1992) also recommended the use of farm yard manure, spent compost and field soil in different ratios for casing. Traditionally growers use FYM as sole casing medium. Keeping in view the above factors, efforts shall be made to evaluate some locally available casing materials as a part of ideal mixtures. Several materials are reported as casing layers like decomposed cow manure, paper pulp, vermiculite, composted cotton husks, pine sawdust, coffee grounds, composted mushroom stalks, spent compost (Tripathi *et al.,* 1991) and different soil types (O'Donoghue-Maguire and Ryan 1991).

Several intrinsic factors of the casing material could vary the yield potential of button mushroom. The texture, moisture, bulk density, water holding capacity, pH and electrical conductivity are the major physical and chemical parameters that affect the mycelial growth and thus influence the yield (Singh *et al.,* 2000). Along with these physiochemical parameters, casing microflora is also considered necessary for commercial fruiting of *Agaricus bisporus* (Fermor *et al.,* 2000). Although casing is the indispensable material for button mushroom production, yet little work has been carried out on it as compared to other steps. Considering the importance of casing mixtures on the yield of mushroom, therefore, it is important to select locally available casing mixtures for better yield potential of *A. bisporus*.

MATERIALS AND METHODS

Filling and spawning: Polythene bags of size 18"×22" were filled with 5 kg compost and spawned @ 5g/kg wet compost. The spawning was done on 5-01-2012 and total days of spawn run were 12-19 days. The surface was levelled and bags were sealed and then bags were kept in the growing room until complete mycelia impregnation.

Preparation and treatment of casing mixtures: Six casing mixtures were prepared by using six components procured from Mushroom Research Complex, Department of Microbiology, Punjab Agricultural University, Ludhiana. Farm yard manure (FYM), FYM + Sandy soil (4:1) (control), FYM+ Coir pith (1:1), FYM+ Spent compost (1:1), FYM+ Burnt rice husk (1:1) and FYM + Biogas slurry (1:1) were prepared from various components. The six casing mixtures were disinfected with 4% formaldehyde for 100 kg casing soil prepared by taking 600 ml of formaldehyde solution and diluted to six litres with water. This formaldehyde treated casing mixture (soil) was kept covered with plastic sheet for 48 hours followed by its frequent turning to evaporate traces of formaldehyde. The prepared casing was used after 22 days after spawning.

The disinfected casing mixture was applied on 27-01-12 to cover spawn impregnated compost bags at uniform thickness of 1-1.5 inches. Spraying of water was continued directly on cased bags till the end of cropping.

Characterization of casing soil: The casing soil mixtures were characterized for physical, chemical and microbiological components. Under physical characterization; moisture content, water holding capacity and bulk density of casing soil sample was determined by gravimetric method (Singh *et al.,* 1986). Chemical characterization was carried out for pH, electrical conductivity and mineral analysis. The pH of sample was determined by the potentiometric method (Sekhon *et al.,* 1986) on the elico pH meter. Electrical conductivity of the sample was measured with a conductivity metre known as solu-bridge (Sekhon *et al.,* 1986). The elements Ca, K, Mg, P, S, Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn were estimated from the casing soil samples, using Inductively Coupled Argon Plasma Atomic Emission Spectrophotometer (Thermo Electron *i*CAP 6300) by the method of Arora and Singh (2004). In microbiological characterization; total plate count of casing mixtures was taken during first week of casing and at termination of the crop. One gram of soil was taken with the help of the core sampler inserted into the entire depth of casing layer, suspended in 9 ml sterilized water blank and shaken well. Serial dilutions up to 1×10^{-5} were prepared in sterilized de-ionized water. 1 ml of each dilution of the casing mixtures was poured on nutrient agar plates. Petri plates were incubated at 25°C ±1°C for 24 hours. Total bacterial colonies were counted for minimum three replicates in each set of casing mixtures.

Yield data: Yield data for total number and total weight of fruiting bodies per bag was recorded upto a period of 5 weeks following appearance of pin head and percentage yield was calculated. To calculate the weight of fruiting bodies, average weight of 10 fruiting bodies were taken.

Statistical analysis: Various data were statistically analysed as per the procedure given by Gomez and

Gomez (1984) and by using CPCS1, software developed by the Department of Mathematics and Statistics, PAU, Ludhiana to test for significant differences between the treatments at 5% probability level.

RESULTS AND DISCUSSION

Physical, chemical and microbial characterization of different casing mixtures: Biogas slurry (BS), burnt rice husk (BRH), coir pith (CP), farm yard manure (FYM), sandy soil (SS) and spent compost (SC) were used in this study to prepare five casing mixtures to compare with the recommended mixture (FYM+SS, 4:1). Casing mixtures were analyzed for the moisture, water holding capacity (WHC), bulk density, electrical conductivity (EC), pH and minerals (Table 1). Peyvast *et al*. (2011) reported that the required physical and chemical properties of a good casing should be high porosity and water holding capacity (WHC), 7.2-8.2 pH, low content of soluble inorganic and organic nutrients, and free of disease and pests. The maximum WHC of 160 % was recorded for FYM+CP (1:1) which leads to higher moisture content of 56.3 % in FYM+CP (1:1). However, FYM+CP (1:1) was statistically at par with FYM+BS (1:1) with 55.0 % of moisture content. Further, the data revealed that the casing mixtures with higher moisture content had low electrical conductivity (EC) values. Gier (2000) suggested that addition of salts led to increase in the EC value of the casing soil making it more difficult for mushroom to extract water from the soil. He also stated that this resulted in a firm mushroom with a high dry matter content and whiter colour. According to Gier (2000) an EC value of more than 7 to 9 mho had a negative influence on the quality and especially quantity of mushrooms. Singh *et al.,* (2000) used spent compost with 91.7% water holding capacity and farm yard manure with 191% water holding capacity but when used in combination of 1:1, 2:1, 1:3 of FYM and spent compost, the WHC was 141%, 109%, 121% respectively. The water holding capacity of 80-160% was close to the water holding capacity of peat as observed by Noble and Pennington (2005).

The bulk density ranged between 33.3 to 83.3% and FYM+BS (1:1) had significantly higher bulk density of 83.3 % but it was statistically at par with the control FYM+SS (4:1) which had a bulk density of 76.9 % (Table-1). Singh *et al.,* (2000) had used six casing mixtures with 0.60-0.88 g cm^{-3} of the bulk density. The pH for different casing mixtures was found to be non significant with a range of 7.5-7.9 (Table 1).

Microbial count of different casing mixtures was between $3.0\text{-}5.7\times10^5$ which was statistically non-significant with respect to casing mixtures (Table 1). The bacterial microflora of casing mixture is considered essential for commercial fruiting as reviewed by Fermor *et al.,* (2000). The productivity,

Table 1. Physical (Water holding capacity, Bulk density and Moisture), Chemical (pH and Electrical conductivity) and microbial (Microbial count) characterization of casing mixtures.

S. N.	*Casing mixtures	Water holding capacity (%)	Bulk density (%)	Moisture (%)	pH	Electrical conductivity	Microbial count (1^{st} week) (1×10^5)	Microbial count termination (1×10^5)
1	FYM+CP(1:1)	160	33.3	56.3	7.6	1.2	3	5
2	FYM+SC(1:1)	60	41.7	33.3	7.5	1.2	3.3	1.7
3	FYM+BS(1:1)	100	83.3	55.0	7.9	1.7	5	3
4	FYM+BRH(1:1)	80	66.7	36.1	7.8	1.5	1.2	2.5
5	FYM	50	71.4	34.2	7.6	1.4	5.7	5.3
6	FYM+SS(4:1) (control)	60	76.9	34.2	7.7	1.1	4	5.7
	C.D. (p=0.05)	18.5	8.03	5.06	N.S.	0.19	N.S.	N.S.

*Farm Yard Manure (FYM), Coir Pith (CP), Biogas Slurry (BS), Spent Compost (SC), Burnt Rice Husk (BRH), Sandy Soil (SS).

Table 2. Elemental analysis of casing mixtures for potassium, magnesium, manganese, sodium, nickel, phosphorus, lead, sulphur and zinc (mg/kg).

S.N.	*Casing mixtures	K	Mg	Mn	Na	Ni	Ph	Pb	S	Zn
1	FYM+CP(1:1)	2256	467	23.8	343	2.1	860	1.5	420	18.2
2	FYM+SS(4:1)	1209	403	24.1	196	1.9	829	1.6	364	17.5
3	FYM+SC(1:1)	1484	457	37.0	205	2.4	1256	3.5	695	23.6
4	FYM+BS(1:1)	2302	808	41.2	339	2.9	1923	2.4	791	33.5
5	FYM+BRH(1:1)	2008	517	42.4	244	2.2	1293	2.2	530	31.4
6	FYM(control)	1544	476	27.1	238	2.00	1101	1.8	494	21.0
	C.D. (p=0.05)	**43.6**	**20.9**	**2.0**	**22.1**	**0.36**	**18.7**	**0.43**	**19.1**	**1.5**

*Farm Yard Manure (FYM), Coir Pith (CP), Biogas Slurry (BS), Spent Compost (SC), Burnt Rice Husk (BRH), Sandy Soil (SS).

product quality and uniformity of mushroom were also influenced by the bacteria in casing soil as reported by Choudhary (2011).

Different minerals were estimated in all the casing soil mixtures which are given in Table 2 and Table 3. The presence of Ca, K, Mg, Na and P indicate their possible role in upgrading the nutritional status of mushroom and fruit bodies formation. The significantly higher amounts of minerals were observed in FYM + BS (1:1) which was due to the high organic matter content of biogas slurry (BS). Flegg (1998) has identified ten mineral elements essential for growth of mushroom mycelium which included five micro elements Cu, Fe, Mn, Mb and Zn. Furthermore, it was reported that the involvement of mineral elements in the metabolism of the mushroom as a part of enzyme system (s). Potassium is connected with the metabolism of carbohydrates, calcium with production of calcium oxalate and in fruit body formation while sulphur is important constituent of some amino acids, vitamins and proteins. Phosphorus is a constituent of phospholipid, nucleic acid and is greatly implicated in the transfer of energy with in mushroom cells. Presence of copper and aluminium encourage the growth of mushroom mycelium in compost and iron is known to show the stimulating effect in fruit body initiation. Sodium, mercury and cadmium are not essential for the mushrooms but mushroom can absorb these elements. Singh *et al.,*(2000) observed that casing mixture of FYM alone showed higher levels of elements such as N, P, Fe, Cu and Zn followed by FYM + SC (2:1) and (3:1) which showed higher levels of K and Mn.

Yield of *A. bisporus*: Early emergence of pin heads were occurred on FYM+SC (1:1) (18 days) followed by FYM+SS (4:1) (control treatment) i.e. 19 days (Table-3). Delayed pin head formation was recorded in FYM+BRH (1:1), FYM+CP, FYM+BS and FYM with total number of 23 days after casing. The overall spawn run period ranged between 12-19 days and first harvest of mushroom fruiting bodies was ranged 24-29 days after casing. The crop lasts after first harvest for 27-37 days for different casing variables. Maximum yield of 15.1 kg per 100 kg compost was harvested from the bags cased with FYM+BRH (1:1) with maximum number of fruiting bodies i.e. 1340 per 100 kg of compost (Table-3). The average weight of each fruit bodies ranged between 9.6 to 11.2 grams in all the casing variables but it was statistically at par with each other. Ram and Holkar (2009) reported that maximum fruit bodies were recorded in coconut coir pith + vermi compost + FYM + saw dust + sand casing mixture. Combinations of coconut fiber pith and spent mushroom substrate of 4:1 and 3:2 (v/v) gave biological efficiencies of 92.9 and 82.6 kg per 100 kg compost, respectively (Pardo-Gimenez and Pardo-Gonzalez 2008). It is clear from the results of this experiment that in respect of time required from

Table 3. Elemental analysis of casing mixtures for aluminum, arsenic, boron, calcium, cadmium, chromium, copper and iron (mg/kg)

S.N.	*Casing mixtures	Al	As	B	Ca	Cd	Cr	Cu	Fe
1	FYM+CP(1:1)	1309	0.79	5.11	633	0.03	0.83	2.92	800
2	FYM+SS(4:1)	1267	0.91	1.79	552	0.04	2.28	2.13	808
3	FYM+SC(1:1)	1568	1.0	4.72	1233	0.05	0.94	5.15	1034
4	FYM+BS(1:1)	1783	1.08	6.05	1280	0.06	0.29	2.49	1212
5	FYM+BRH(1:1)	1430	0.89	4.47	1034	0.05	1.07	7.49	875
6	FYM(control)	1299	0.90	6.61	797	0.04	2.85	2.50	835
	C.D. (p=0.05)	37.3	NS	1.2	43.1	0.18	0.26	0.65	27.9

*Farm Yard Manure (FYM), Coir Pith (CP), Biogas Slurry (BS), Spent Compost (SC), Burnt Rice Husk (BRH), Sandy Soil (SS).

Table 4. Spawn run, pinning after casing, first harvest, last harvest, yield, number of fruit bodies and average weight of *Agaricus bisporus* strain U_3 as affected by different casing mixtures.

S. N.	*Casing mixtures	Spawn run (d**)	Pinning after casing (d**)	First harvest (d**)	Last harvest after first harvesting (d**)	Yield (Kg/100 Kg)	No. of fruit bodies /100 Kg	Average weight (g)
1	FYM+CP (1:1)	18	23	26	35	10.4	1020	10.2
2	FYM+SC (1:1)	17	18	24	34	10.8	1060	10.2
3	FYM+BS (1:1)	19	23	29	30	5.2	540	9.6
4	FYM+BRH (1:1)	12	23	29	27	15.1	1340	11.2
5	FYM	15	23	26	35	10.8	1020	10.6
6	FYM+SS (4:1) (control)	16	19	24	37	12.1	1240	9.8
C.D. (p=0.05)						0.73	11.2	NS

*Farm Yard Manure (FYM), Coir Pith (CP), Biogas Slurry (BS), Spent Compost (SC), Burnt Rice Husk (BRH), Sandy Soil (SS). d** = number of days

spawning to pinhead appearance, yield and average weight of fruit body, FYM+BRH (1:1) was the best substrate for cultivation of *A bisporus* as compared to other treatments. All the treatments having different casing materials were significantly different from each other for the production of the fruiting bodies and the results of our study are well supported by the findings of Taherzadeh and Jafarpour (2013).

Conclusion

The results obtained from different casing variables demonstrate the viability of re-incorporating new casing mixtures in mushroom cultivation. This would be an important alternative in commercial operations to fully replace peat, especially in the light of growing problems associated with availability and cost. In Punjab farm yard manure is easily available and being cheap option in addition with burnt rice husk (FYM + BRH), as Punjab is a dominant rice growing area. So, this mixture can be used as casing variables for cultivation of mushroom.

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