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Screening of *Agaricus bisporus* strains and casing variables for improving the yield potential of mushrooms

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ABSTRACT

Eight *Agaricus bisporus* strains, A-15, Delta, 459, U3, AB-P, AB-B, PB-5 and S11, with six casing variables were analyzed in order to study their effect on the yield, texture and colour of mushrooms. Physiochemical properties of casing mixtures were quite variable where highest porosity (%) was observed in FYM+CP with highest potassium (1.16 %) and carbon (12.22%) content. Among all the casing variables, FYM+CP (4:1) showed highest yield as well as number of fruiting bodies after 30 days for all the selected strains that is A-15 (17.3 kg/q), Delta (19.6 kg/q), 459 (21.8 kg/q), U3 (16.3 kg/q), AB-P (14.6 kg/q), PB-5 (14.6 kg/q), AB-B (13.5 kg/q) and S11 (17.5 kg/q). Strains A-15, Delta and 459 showed maximum colour (L-value), while Delta, 459 and AB-B strains showed higher texture (N) value that inferred their good quality parameters. The pileus diameter was maximum in Delta (4.5 cm) and PB-5 (6.0 cm) for the entire casing variables, however, the stipe length was maximum in PB-5 (2.5 cm), U3 (2.5 cm) and S11 (2.9 cm). FYM+CP casing combination were found to be the best for yield potential followed by FYM+VC and FYM+ BC. Strains, 459, followed by Delta, A-15 and U3 were found to be the most potential strains for cultivation under Punjab conditions.

Keywords: *Agaricus bisporus,* casing mixture, potato dextrose agar, coir pith, vermicompost.

INTRODUCTION

Mushrooms are edible fungi that are considered as highly priced asset among vegetables because of their nutritive value, characteristics aroma and flavor. Most of the species of mushrooms are edible. However, the most popular and commercially cultivated mushrooms are white button mushroom (*Agaricus bisporus*), shiitake (*Lentinus edodes*), paddy straw mushroom (*Volvariella* spp.), milky mushroom (*Calocybe indica*) and oyster mushroom (*Pleurotus* spp.). Among these, *A. bisporus* is the most widely cultivated mushroom throughout the world, which provides 35-45% of total world mushroom production, because of high demand among consumers, therefore, fetches higher price in the market. The nutritional value of mushrooms relies on their high protein content, minerals, vitamins and essential amino acids (Giri and Prasad, 3). The amount of protein is adequate in mushrooms and the composition of amino acids is of prime significance and each 100 g of their raw protein has 4.33 g essential amino acids (Chang and Miles, 1). Beside protein, it also contains minerals, vitamins B complex, vitamin D and K. It is considered to be a low calorific food having no fat, no cholesterol and is very low in sodium (Saiqa *et al*., 13). 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 mushroom yield (Singh *et al*., 16). Along with these physico-chemical parameters, casing microflora is also considered to be necessary for its commercial production (Fermor *et al*., 2). A particular casing mixture should be highly porous, abundant water holding capacity, pH 7.2-8.2, total nitrogen (0.1-0.8), low content of soluble organic and inorganic nutrients and disease and pest free (Gulser and Peksen, 4). Although, casing is the indispensable material for button mushroom production, yet little work has been carried out on it as compared to other steps. Therefore, the present study was planned for evaluating different strains of button mushroom with different casing soil mixtures in terms of growth behavior, yield and quality of mushrooms.

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MATERIALS AND METHODS

Agaricus bisporus strains U3, S11, AB-B, AB-P, A-15, Delta, 459 and PB-5 were procured from the Department of Microbiology, Punjab Agricultural University, Ludhiana. These cultures were propagated on potato dextrose agar (PDA) slants at 25 ± 1ºC for 15 days followed by its storage at 4ºC for 1 month. Wheat grain spawn was prepared using the standard methodology. Compost for the cultivation of *A. bisporus* was prepared by short method in two phases in 18 days using the ingredients given in Table 1.

The following schedule was followed for turning of the compost: Phase 1: -6, -4/0, 2, 4, 6, 8d; Phase 2: 6-7d, cool and spawn. The analysis of compost was carried out and the various characters are presented in Table 2.

The compost was filled @ 5.0 kg (wet weight) per bag in 20”x16” polyethylene bags of 150 gauge and spawned @ 1.0% of wet compost. Each treatment was conducted with 3 replicates with 10 bags per replicate during cultivation trials. Six casing mixtures were prepared from farm yard manure (FYM), coir pith (CP), sawdust (SD), tea waste (TW), biochar (BC), vermicompost (VC) and sandy soil (SS) as a standard casing mixture (control). This standard was prepared by mixing farmyard manure (2 year old) and sandy soil (4:1 v/v). The casing mixture was disinfected with 4% formaldehyde. The formaldehyde treated casing mixture (soil) was kept covered with plastic sheet for 48 h followed by its frequent turning to evaporate traces of formaldehyde. The disinfected casing mixture was used to cover spawn impregnated compost bags at uniform thickness of 2-3 cm. Spray of water was continued directly on cased bags till the end of cropping. These casing variables were prepared from various components in the following ratios:

Sl. No.	Casing mixture	Ratio (v/v)
1.	FYM + CP	4: 1
2.	FYM + SD	4: 1
3.	FYM + TW	4: 1
4.	FYM + BC	4: 1
5.	FYM + VC	4: 1
6.	FYM + SS	4: 1

FYM = Farm Yard Manure, CP = Coir Pith, SD = Saw Dust, TW = Tea Waste, BC = Biochar, VC = Vermicompost, SS = Sandy Soil

Data on spawn run, case run, days taken to pin after spawning, first harvest, last harvest, disease/ pest encountered was recorded. A record of total yield, number of opened mushrooms in each harvest and average fruit body weight was also made to determine the quality of mushrooms produced. Electrical conductivity of the casing soil samples was measured with a conductivity meter ‘Slou bridge’ (Sekhon *et al*., 14). Cation exchange capacity was then measured for CEC as per method outlined in USDA handbook No 60 (Richards, 11). Bulk and particle density of casing soil sample was calculated by Singh *et al*., 17; Prihar and Sandhu, 10. Water holding capacity of casing soil sample was determined as suggested by Singh *et al*. (17).

The pH of sample was determined by the potentiometric method (Sekhon *et al*., 14) on the elico pH meter. Porosity = (1- Bulk density/ Particle density) × 100. Total nitrogen content was determined by Kjeldahl’s technique with slight modification of (McKenzie and Wallace, 8). Phosphorus was estimated at 470 nm using spectrophotometer (Jackson, 6). For potassium estimation, mushroom extract (1 ml) was taken in a 50 ml volumetric flask and the final volume was made to 50 ml with double-distilled water. The solution was fed to the automizer assembly of flame photometer, the galvanometer of which had already been adjusted with standard K solutions and the reading was noted down (Jackson, 5). For the determination of organic carbon, rapid titration method also called as wet digestion method was used, as given by Walkley and Black (18).

Table 1. Compost formulation used in the study.

Ingredient	Quantity (kg)
Wheat straw	1000
Poultry manure	500
Wheat bran	70
Urea	15
Gypsum	40

Table 2. Analysis of composite compost sample (dry weight basis).

Parameter	Value
Initial N (%)	1.7
Final N (%)	2.2
pH	7.1
Moisture (%)	67.0-69.0
Colour	Dark brown
Ammonia smell	Nil
Bulk density	80-90 kg/m^3 bed area

RESULTS AND DISCUSSION

Casing mixtures were analyzed for electrical conductivity, cation exchange capacity, bulk density, particle density, water holding capacity, pH, and porosity. Electrical conductivity of casing mixture ranged between 1.019-1.301 dScm^{-1} with maximum conductivity for FYM + vermicompost and FYM + Biochar followed by FYM + Coir pith, while that of other casing mixtures the electrical conductivity was at par with that of control (FYM + Sandy soil). Cation exchange capacity of casing mixture ranged between 10.81-14.92 meq/ 100 kg with no significant difference between casing mixtures. The particle density ranged between 0.22-0.24 gcm^{-3}, respectively with no significant difference between casing mixtures (Table 3).

Table 3. Physico-chemical properties of casing mixtures.

Parameter	Casing mixture						
	F+VC	F+BC	F+CP	F+SD	F+TW	Control	CD 5%
Physical							
EC (dS cm^{-1})	1.032	1.301	1.209	1.281	1.021	1.019	0.70
CEC (meq/100 kg)	13.26	13.93	11.82	10.81	11.32	14.92	NS
Bulk density (g cm^{-3})	0.81	0.88	0.79	0.32	0.53	0.91	NS
Particle density (g cm^{-3})	0.220	0.231	0.239	0.235	0.230	0.240	NS
WHC (%)	58.0	75.0	116.0	67.0	82.0	75.0	4.2
Porosity (%)	86.36	52.03	90.90	63.2	60.3	37.5	10.8
pH	7.5	7.3	7.4	7.5	7.0	7.1	0.24
Chemical							
Nitrogen (%)	0.82	0.46	0.72	0.53	0.42	0.33	0.06
Phosphorus (%)	0.33	0.49	0.42	0.31	0.37	0.32	0.02
Potassium (%)	0.69	1.63	1.16	0.86	0.92	0.85	0.13
Carbon (%)	10.73	8.30	12.22	12.18	6.02	10.92	0.22

Where F = farmyard manure; VC = vermicompost; CP = coir pith; SD; saw dust; TW = tea waste; control = farmyard manure + sandy soil

The bulk density of casing variables ranged between 0.32-0.91 gcm^3 with no significant difference between the casing mixtures. Water holding capacity of the casing mixture was observed between 58-116 % with maximum WHC for FYM + coir pith. It was also found that FYM + coir pith showed maximum porosity in comparison to control. The pH was observed between 7.0-7.5 for all mixtures. The maximum nitrogen content was found in FYM + vermicompost (0.82%). The FYM + Biochar showed maximum phosphorus (0.49%) and potassium (1.63%), while organic carbon was found to be maximum in FYM + Coir pith (12.22%) and FYM + Sawdust (12.18%). Kaur *et al*. (7) evaluated different casing variables which include farm yard manure, biogas slurry, burnt rice husk, spent compost, coir pith and sandy soil used in combination for the cultivation of *Agaricus bisporus* U3 strain and checked the relationship of physical and chemical properties of these casing mixtures on the cultivation of *Agaricus bisporus*. They revealed that pH showed no significant effect while other factors like water holding capacity, bulk density, moisture and electrical capacity showed significant effect on the cultivation of *Agaricus bisporus* as observed in the present study.

A. bisporus strain A-15 showed maximum yield (17.3 kg/q of compost) with casing mixture of FYM+ CP. The number of fruiting bodies was also recorded higher than those harvested from the control. Maximum yield (19.6 kg/q compost) and number of fruiting bodies (1889 No./q) were recorded from the bags cased with FYM + CP in strain Delta. The maximum yield in *A. bisporus* strain 459 was obtained with the bags cased with FYM + CP (21.8 kg/q compost). The number of fruiting body harvested from FYM + CP and FYM + VC were at par with one another. In *A. bisporus* strain U3, the yield potential was maximum (16.3 kg/q) when FYM + CP was used as casing mixture (Table 4). The spawn run for all *A. bisporus* strains took 14-22 days. Early spawn run period was observed in strain Delta (14 days), while maximum time taken for spawn run was observed in strain S11. The casing mixture, FYM+TW (4:1) was highly contaminated which resulted in sparse spawn run with no primodial formation. The yield data recorded for all *A. bisporus* strains ranged between 2.72-21.8 kg/q compost, Maximum yield was observed in strain 459 with FYM + CP (4:1) as casing mixture. The average number of fruiting bodies ranged between 166 and 1889 (Table 5). Strain delta showed maximum number of fruiting bodies (1889) on casing mixture FYM + CP (4:1). The casing mixture FYM + SD (4:1) did not perform well in all the strains. Although, strain 459 had yielded maximum with FYM + CP (4:1) yet with all the *A. bisporus* strains this casing mixture was found better than the control (FYM + SS (4:1). Effect of different casing treatments has been studied on the production of button mushroom by various researchers. Casing mixture containing farmyard manure produced maximum number of fruiting bodies in comparison to other treatments (Peat soil, clay + sand + lime, Lahore compost) as recorded by (Rehman *et al*., 12). Different casing

Table 4. Yield potential of different *A. bisporus* strains (kg/ 100 kg compost).

Treatments	A-15	Delta	459	U3	Strains AB-P	PB-5	AB-P	S11
FYM + VC (4:1)	13.7	16.4	18.2	14.6	6.9	12.6	10.4	14.8
FYM + BC (4:1)	10.8	10.8	15.4	8.2	9.0	10.7	8.8	4.9
FYM + CP (4:1)	17.3	19.6	21.8	16.3	14.6	14.6	13.5	17.5
FYM + SD (4:1)	6.4	2.72	3.2	6.3	5.2	8.3	6.9	11.2
FYM + SS (C)	16.2	16.9	19.8	15.5	10.7	14.7	11.3	11.3
CD at 5%	0.38	0.32	0.45	0.61	0.45	0.38	0.57	0.52

Table 5. Fruiting bodies of different *A. bisporus* strains (No. of mushrooms/ 100 kg compost).

Treatments	A-15	Delta	Strains 459	U3	AB-P	PB-5	AB-B	S11
FYM + VC (4:1)	1240	1504	1802	1224	574	1063	906	1334
FYM + BC (4:1)	1112	1114	1573	886	786	1226	772	468
FYM + CP (4:1)	1086	1889	1804	1648	1381	1357	1230	1082
FYM + SD (4:1)	685	494	166	492	745	741	661	1133
FYM + TW (4:1)	***	***	***	***	***	***	***	***
FYM + SS (C)	1643	1582	1602	1730	1226	1448	887	952
CD at 5%	129.51	103.58	118.54	100.34	103.74	134.97	111.16	84.02

variables were evaluated by Kaur *et al*. (7) 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 combination for the cultivation of *A. bisporus* U3 strain. They revealed that casing mixture FYM + BRH (1:1) showed the maximum number of fruiting bodies.

The fruit bodies of *A. bisporus* strains were evaluated for pileus diameter and stipe length harvested from different casing mixtures. The pileus diameter of strain U3 was more in FYM + Vermicompost (4.4 cm), FYM + Biochar (4.0 cm) and FYM + coir pith (4.0 cm) in comparison to control (3.5 cm). The stipe length was more in case of FYM + Coir pith (2.5 cm). No significant difference in the pileus diameter and stipe length of *A. bisporus* strain AB-P was observed. In *A. bisporus* strain Delta, pileus diameter (4.3 cm) and stipe length (1.0 cm) was less in treated casing mixture (FYM + saw dust) in comparison to control (pileus diameter 5.0 cm and stipe length 1.5 cm), while treatments FYM + vermicompost, FYM + biochar and FYM + coir pith showed maximum fruiting body length as compared to control. In *A. bisporus* strain 459, pileus diameter (4.4 cm) and stipe length (1.5 cm) was observed to be maximum in FYM + coir pith while pileus diameter of harvested mushrooms from FYM + biochar (4.3 cm) and stipe length from FYM + vermicompost (1.4 cm) was at par with that of FYM + coir pith (1.5 cm). In *A. bisporus* strain S11, the pileus diameter of mushroom harvested from FYM + vermicompost and FYM + biochar was higher than that of control but stipe length was shorter in mushroom harvested from different casing mixtures. In *A. bisporus* strain A-15, pileus diameter was found to be maximum in mushrooms harvested from FYM + vermicompost. *S*train PB-5 showed larger pileus diameter in all casing mixtures with respect to control while stipe length was longer in harvested mushroom from FYM + biochar casing mixture (Table 6) Netam *et al*. (9) conducted an experiment on the production of *Agaricus bisporus* S11 strain and revealed that the average stipe length was 3.02 cm with girth of 1.7 cm. Moreover, the average pileus length and width was 2.1 cm and 3.37 cm, respectively however, average yield was 19.1 Kg with 960 fruit bodies from 100 kg of compost mixture.

The fruit bodies of *A. bisporus* strains were evaluated for colour and texture harvested from different casing mixtures. The colour of *A. bisporus* strain U3 was observed maximum in FYM + coir pith (82.3) which was followed by FYM + biochar (80.2). However, control L value (78.5) was statistically higher than FYM + vermicompost (77.1), FYM + saw dust (75.3). Consequently, texture was recorded maximum in FYM + coir pith (41.46), followed by FYM + biochar (35.60), FYM + vermicompost (31.77), control (28.88) and FYM + saw dust (19.63), respectively. There was significant difference observed in colour and texture of *A. bisporus* strain U3. The colour of *A. bisporus* strain Delta was observed maximum in FYM + saw dust (86.1) in comparison to control (85.3). However, all

Table 6. Pileus diameter and stipe length of harvested mushrooms from different *A. bisporus* strains.

Treatment	A-15	Delta	459	U3	AB-P	PB-5	AB-B	S11
				Pileus diameter (cm)				
FYM + VC	4.5	5.2	4.2	4.4	4.5	6.0	3.7	2.9
FYM + BC	3.5	5.3	4.3	4.0	4.4	5.5	3.5	2.7
FYM + CP	4.0	4.9	4.4	4.0	4.2	4.5	3.0	2.5
FYM + SD	3.2	4.3	3.9	3.4	4.2	6.0	3.5	2.6
Control	3.5	5.0	4.0	3.5	4.0	3.0	3.4	2.5
CD at 5%	0.6	0.6	0.2	0.2	NS	0.7	0.2	0.1
				Stipe length (cm)				
FYM + VC	1.5	1.2	1.4	2.0	1.5	2.0	2.5	3.4
FYM + BC	1.0	1.5	1.0	1.5	1.0	2.5	2.0	3.2
FYM + CP	1.5	1.6	1.5	2.5	1.8	1.5	1.5	3.1
FYM + SD	2.0	1.0	0.8	1.0	1.5	1.0	1.8	2.9
Control	2.0	1.5	0.8	1.3	2.0	2.0	2.3	3.5
CD at 5%	0.3	0.3	0.3	0.8	0.4	0.2	0.5	0.2

casing mixtures were statistically lesser than control. Consequently, texture was recorded maximum in control (51.20) while minimum in FYM + saw dust (35.26), respectively. *A. bisporus* strain A-15 colour was observed maximum in FYM + Biochar (90.2) which was followed by control (87.4). Consequently, texture was recorded maximum in FYM + Sawdust (29.45), followed by FYM + Vermicompost (22.08). In strain 459, colour was recorded maximum in FYM + Coir pith (84.5) which was followed by FYM + Biochar (83.7). Consequently, texture was recorded maximum in control as compared to all casing mixtures. There was significant difference observed in colour and texture of *A. bisporus* strain 459. *A. bisporus* strain AB-B colour was recorded maximum in control (83.2) as compared to other casing mixtures. Consequently, texture was recorded maximum in FYM + coir pith (51.05) and minimum in FYM + Saw dust (39.51). Colour in AB-P strain was observed maximum in FYM + Vermicompost (85.3) which was followed by FYM + coir pith and control (82.3). Consequently, no significant difference in the texture of *A. bisporus* strain AB-P was observed. *A. bisporus* strain PB-5, colour was observed maximum in FYM + vermicompost (78.3) while in FYM + coir pith (72.3). Consequently, texture was recorded maximum in control (26.56) and less in FYM + saw dust (18.48), respectively. There was significant difference observed in colour and texture of *A. bisporus* strain PB-5. In strain S11, colour was recorded maximum in FYM + coir pith (78.3) which was followed by FYM + saw dust (77.8). Consequently, texture was recorded maximum in FYM + vermicompost as compared to that of control. There was significant difference observed in colour and texture of *A. bisporus* strain S11 (Table 7). Singh *et al*. (15) evaluated twelve different strains of *Agaricus bisporus* along with the quality parameters like colour, texture, stripe length and pileus diameter and revealed that colour, texture and stipe length showed significant effect while pileus diameter was recorded non significantly different among all the treatments.

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Table 7. Colour (L-value) and texture (N-value) of different *A. bisporus* strains**.**

Treatment	A-15	Delta	459	U3	AB-P	PB-5	AB-B	S11
			Colour (L-value) of *A. bisporus* strains					
FYM+VC	85.6	84.2	83.5	77.1	85.3	78.3	80.7	76.1
FYM+BC	90.2	79.5	83.7	80.2	80.2	76.8	79.5	73.5
FYM+CP	83.5	82.2	84.5	82.3	82.3	72.3	76.6	78.3
FYM+SD	86.3	86.1	80.2	75.3	75.6	73.2	80.6	77.8
Control	87.4	85.3	82.2	78.5	82.3	76.3	83.2	72.3
CD (5%)	2.0	0.24	0.45	0.66	0.65	0.78	4.4	0.69
			Texture (N-value)					
FYM+VC	22.08	48.35	32.04	31.77	31.05	19.06	42.60	23.67
FYM+BC	16.96	38.54	32.28	35.60	29.31	21.65	41.28	16.24
FYM+CP	18.24	42.08	34.62	41.46	35.29	23.41	51.05	19.35
FYM+SD	29.45	35.26	35.21	19.63	34.05	18.48	39.51	18.57
Control	21.54	51.20	36.14	28.88	38.25	26.56	46.32	21.05
CD (5%)	2.3	2.4	2.3	3.6	NS	1.5	3.0	1.44

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