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Evaluation of *Agaricus bisporus* Lange (Sing.) Strains in the Plains of Punjab, India

Mohini Prabha Singh*, Sabhjeet Kaur and H.S. Sodhi

Department of Microbiology, Punjab Agricultural University, Ludhiana-141004, Punjab, India
**Corresponding author*

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A B S T R A C T

Twelve strains of *Agaricus bisporus* (Lange) Sing. (AVT 01- AVT 06, AVT 07-AVT 11 and U3) were screened for their yield potential with the objective of selecting them to grow best in Punjab conditions. These strains with fifteen white strains (SSI01/12-SSI15/12) were checked for quality parameters (color, texture, stipe length and pileus diameter) to select for post-harvest processing such as canning and pickling. Cultivation was carried on short method compost using wheat straw as substrate. Out of white strains, AVT 02 (18.27kg/100 kg compost) gave maximum yield followed by AVT 06 (14.30kg/100kg compost). From brown strains, AVT 11 gave maximum yield of 10.78kg/100 kg compost followed by AVT 08 giving 8.30kg/100 kg compost. The color index indicated maximum L Value for six strains SSI07/12, SSI09/12, SSI12/12, AVT 02, AVT 04 and AVT 06 with minimum values for brown strains ranging from 30.00 to 31.15. Texture analysis indicated the hardness maximum for white strains SSI08/12 followed by six other strains SSI02/12, SSI06/12, SSI12/12 and U3 while brown strains showed less hardness. The average stipe length varied significantly as short as 2.10 cm in strain SSI12/12 and as long as 4.0 cm in strains SSI03/12 and SSI15/12 (white strains) while among brown strains, it is shortest for AVT-10 (2.40cm) and longest for AVT-08 (3.50cm).There is non- significant difference in the diameter of the pileus for all the white and brown strains of *Agaricus bisporus.* These strains showed a correlation of better yield with good color and textural properties.

Introduction

Agriculture has been a main strength of Indian economy but a struggle is still on to achieve nutritional security demand following secondary agricultural vocations. To meet such challenges, diversification in the agricultural activities which include mushroom production is important to address the problems of quality food, health and environmental sustainability.

Mushrooms represent microbial technology that recycles agricultural, industrial, forestry and household waste into food and manure.

Mushroom farming, today, is being practiced in more than 100 countries and its production is increasing at an annual rate of 6-7%. Presently, three geographical regions-Europe, America and East Asia contribute to about 96% of world mushroom production. China leads in mushroom production and USA is the second largest producer sharing 16% of the world output (Prakasam, 2012). In most countries, mushrooms like *Agaricus bisporus*, *Auricularia* spp., *Lentinus edodes, Pleurotus spp., Volvariella volvaceae,* etc. are commonly acceptable for cultivation (Diez

and Alvarez, 2001). The button mushroom, *Agaricus bisporus*, is one of the most extensively cultivated mushroom in the world. It belongs to phylum Basidiomycota, class Agaricomycetes, order Agaric ales and family Agaricaceae. The most suitable temperature of the mycelium is 24-25°C, while 16-18°C is essential for the formation of fruit bodies. It is cultivated in northern plains as well as coastal plateaus of South India. India produces about 600 million tonnes of agricultural byproducts, which can profitably be utilized for the cultivation of mushrooms. Currently, we are using 0.04% of these residues for producing around 1.2 lakh tonnes of mushrooms of which 85% is button mushroom.

India contributes about 3% of the total world button mushroom production. Two-three crops of button mushroom are grown seasonally in temperate regions with minor adjustments of temperature in the growing rooms, while one crop of button mushroom is raised in north western plains of India seasonally. Mushrooms are popularly grown in Punjab, Uttarakhand, Haryana, Uttar Pradesh, Tamil Nadu, Himachal Pradesh, Orissa, Andhra Pradesh, Maharashtra, Kerala and North eastern regions of India. Punjab alone produces 45-48% mushrooms (Singh *et al.*, 2011).

Productivity and quality of widely cultivated *Agaricus* mushrooms are mainly dependent on the strain and substrates used. Four differentiated commercial mushroom strains exist in the market: 1) pure white strains, 2) off-white strains with large white mushrooms 3) hybrid strains 4) brown strains (Fritsche and Sonnenberg, 1988). The actual cap characteristics of these strains regarding size, shape, and color are strongly influenced by environmental and cultivation conditions (Tschierpe, 1983). In India, a number of commercial strains like S-11, S-130, S-140, S-649, S-791, CM-1, CM-5, CM-10 (hybrid), A-15 (sylvan, hybrid), U-3, X-13, Delta, etc. have been used for cultivation whereas in Punjab, S-11 and U3 are preferably cultivated (Kapoor, 1989).

Our environment is conducive for *A. bisporus* cultivation due to abundant availability of straw and plant residues. It is able to degrade the major polymers of woody plant materials; cellulose, hemicelluloses, and lignin **(**at least to some extent). Several formulations can be used for production of *Agaricus* sp. which is usually determined by the availability and cost of substrates (Sanchez and Royse, 2001). Straws (rice, wheat, oat and barley), by-products (sugarcane bagasse), horse and chicken manure are the most important components used as lignocellulosic sources incomposts (Minhoni *et al.*, 2005; Peil *et al.*, 1995).

The preparation of mushroom compost has for many years been divided into distinct phases, phase I during which raw material are mixed, wetted and stacked with considerable dry matter losses, and phase II, which includes pasteurization and conditioning treatment to produce a selective and pathogen free substrate (Ross and Harris, 1983). This process of composting is catalysed by bacteria and fungi (Koschinsky *et al.*, 1998).

The production and quality parameters for mushrooms include: number, size, earliness, freshness, color, shape, firmness, aroma, freedom from debris, the degree of maturity, development stage and visual defects (Carey and O'Connor 1991; Pardo, 1999). Any change in one or more of these factors may affect acceptability by the consumer. Like with most horticultural products, mushrooms have a limited storage life (1 to 3 d at 18°C) and that quality is affected by cultivation techniques, the strain, handling, and storage conditions (Burton, 1989). Freshly harvested

mushrooms have a very high respiration rate and water content which make them prone to microbial spoilage while high tyrosinase and phenolic content makes them susceptible to enzymatic browning (Brennen *et al.*, 2000). This leads to loss in texture, off flavor development and discoloration resulting in poor marketable quality and restricts trade of fresh mushrooms (Mehta *et al.*, 2011).

Therefore, to extend their marketability and availability to the consumers, fresh or processed form is of great significance (Bhupinder and Ibitwar, 2007). Drying, canning and freezing are initially accepted methods of mushroom preservation. Value added products such as pickle, murabba also help in long term storage. Keeping in view the above mentioned points, present study was planned to evaluate the white and brown strains of *Agaricus bisporus* in Punjab region for selecting high yielding strains and to test their color and texture properties for post-harvest processing.

Materials and Methods

Procurement of cultures

Agaricus bisporus Lange (Sing.), strains AVT 01- AVT 06 (white), AVT 07-AVT 11 (brown) and SSI01/12- SSI15/12 (white) were procured from DMR, Solan and strain U3 was obtained from Department of Microbiology, Punjab Agricultural University, Ludhiana. The cultures were maintained on potato dextrose agar (PDA) medium at 4˚C.

Spawn preparation

For cultivation trial, wheat grain spawn was prepared using the standard methodology of Garcha, (1994). Wheat grains were washed and boiled for 35-40 minutes and then excess water was drained. The grains were then mixed with 2% $CaCO_3$ and 4% $CaSO_4$ powder, filled in bottles and steam sterilized at 20 psi for 90 minutes. After cooling overnight, the bottles were inoculated using 14-15 days old culture bit of size 3 x 1 cm, incubated at 25±1°C until the mycelial growth impregnated the grain (@ 20 days incubation).

Composting and spawning

Wheat straw based compost was prepared using short method of composting (Khanna and Kapoor, 2007). The spawning was done using polythene bags (20„x 24„) filling 10 kg compost in each bag and spawned @ 70 g/ bag by thorough mixing. Four replicates of each strain with ten bags for AVT 01-AVT 11 and U3 strains were laid in random block design (RBD).

Casing and harvesting

Casing soil was prepared by mixing well decomposed (2 yrs old) Farmyard Manure (FYM) and spent compost (SC, 2 yrs old) in 2:1 (v/v) ratio adjusted to pH 7.5 with $CaCO_3$. Bags fully impregnated with mycelium were covered with the casing soil to make 4 cm thick uniform layer using 2 kg casing soil per bag. Adequate humidity (=RH 70-90%) was maintained by spraying water on the bags twice a day. Very little or no ventilation was provided until the first appearance of the pinheads. Thereafter, intermittent cross-ventilation was given for total 4-8 hour/ day. The mushrooms were harvested by gentle twisting of the fruit body. A record of total yield, number of opened mushrooms in each harvest and average fruit body weight was made to determine the quality of mushrooms produced.

Morphological observations

Freshly harvested *Agaricus bisporus* Lange (Sing.), strains AVT 01- AVT 06, AVT 07-

AVT 11 (brown), SSI01/12-SSI15/12 and U3 were subjected to morphological examinations (stipe length, stipe diameter, color, texture) for their acceptance as post-harvest processed products to improve shelf life.

Stipe length and Pileus diameter: The stipe length (cm) and diameter (cm) of freshly harvested mushrooms were measured with metre rule. The mushroom pileus diameter was taken from one end of the pileus to the other passing through the centre of the pileus.

The pileus diameter was obtained on 5 randomly picked mushrooms, from the harvest and then the average pileus diameter was calculated for a given harvest.

The length of stipe was measured by placing the ruler from one end where it was attached to the substrate to the point where the gills on the pileus start on the stipe to get the length in centimetres (cm). The average for that day's harvest was then calculated using 5 readings.

Color analysis: Color of the mushroom pileus was estimated using the CIELAB scale at an observer angle of 10° with a Mini scan XE plus Hunter Lab Colorimeter.

The 'a' value determines greenness ($a < 0$) or redness ($a > 0$) and the 'b' value determines blueness ($b < 0$) or yellowness ($b > 0$). The 'L' value varies between 0 and 100, representing transition from black to white.

Texture analysis: Texture profile analysis (TPA) was done using a texture analyzer (model TA-XT2i; Stable Micro Systems, United Kingdom) with instrument parameters described by Kotwaliwale *et al.*, (2007) with modification of the strain to 75% of sample height and probe (75-mm compression platen).The hardness was calculated as given by Bourne 1982.

Statistical analysis

The yield obtained for various strains of *Agaricus bisporus* Lange (Sing.) with Random Block Design and optical properties observed were statistically analysed through ANOVA to see the critical difference at 5% level of significance between the strains.

Results and Discussion

Cultivation

On short method compost

The cultivation trial was carried out on short method compost for all the *Agaricus bisporus* strains (AVT 01-11, U3). Four replicates each with ten bags for each strain were laid in random block design (RBD) to accommodate 240 bags. Beds were cased after 18-25d of spawning. Pinheads appeared in 17-24d after casing. The first crop was harvested between 17-23d after casing and lasted for4 weeks. AVT 01-11 were compared on short method compost because these are advanced variety trials. From yield data (Table 1), out of white strains, AVT-02 (18.27 kg/100 kg compost) gave maximum yield followed by AVT-06 (14.30 Kg/100kg compost). From brown strains, AVT 11 gave maximum yield of 10.78 kg/100 kg compost followed by AVT 08 giving 8.30 kg/100 kg compost. The average weight of a mushroom ranged between 10.89 to 14.79 g. Kumar and Singh, (2013) reported maximum yield (16.1 kg/100 kg compost) in case of wheat straw based compost. Diamatopoulou and Philippoussis, (2001) observed maximum yield (30.84 kg of edible mushroom biomass) in white strain 207 among five *Agaricus bisporus* strains. Baysal *et al.*, (2007) reported that the highest mushroom yield (1707.2 g) was recorded by wheat straw mixed with pigeon manure with the peat of Caykara and perlite mixture as casing material (Table 2).

Table.1 Cultivation of *Agaricus bisporus* strains on short method compost

Strain no.	Spawn run (d)	Case run (d)	Pinning after casing (d)	First harvest (d)	Last harvest (d)	Yield (kg/100kg compost)	NFB (no./100kg)	Av.wt of a FB (g).	Disease /pest
AVT-01	24	19	20	20	22	5.26	362	14.79	+
AVT-02	23	17	19	19	21	18.27	1374	13.90	-
AVT-03	25	19	20	20	21	9.373	750	12.87	-
AVT-04	25	19	20	20	22	12.49	1058	12.39	+
AVT-05	22	16	18	18	19	8.30	560	14.06	-
AVT-06	24	15	17	17	20	14.30	1038	13.97	-
AVT-07	24	18	NO PINNING NO FRUITING						
AVT-08	19	17	24	23	31.0	8.30	615	13.5	-
AVT-09	20	18	20	22	25.0	5.66	425	13.3	+
AVT-10	21	19	19	21	25.0	6.56	512	12.8	-
AVT-11	18	16.5	19	21	36.5	10.78	823.5	13.08	-
U3	19.5	16.5	20	22.5	38.0	11.94	1632	10.89	-
CD (5%)						0.18	6.58		

Bag size: 20»x 24» (Polythene, 150 gauge); No. of replicates: 4 each for one strain with 10 bags (10kg compost/bag); Experimental design: RBD; Date of spawning: 6.1.13-8.1.13; Rate of spawn: 0.7% wet compost (70g/bag of 10kg compost); Days of spawn run: 18-25d; Casing: FYM +SC (2:1 v/v); Date of casing: 28.1.13 31.1.13; Days of case run: 15-18 d; Days for pinning: 17-24 d after casing; Days for first harvest : 17-23 d after casing; Days for last harvest: 4 weeks crop data ; NFB: number of fruit bodies.

Table.2 Cultivation of *Agaricus bisporus* strains on short method compost (Kaur *et al.*, 2014)

Strain	Spawn run (d)	Case run (d)	Pinning after casing (d)	First harvest (d)	Last harvest (d)	Yield (kg/100kg)	NFB (no./100kg)	Av. Wt. of a FB (g)
SSI01/12	15	16	18	20	21	13.46	1246	10.8
SSI02/12	15	16	18	20	21	14.80	1203	12.3
SSI03/12	18	17	19	22	21	5.64	440	12.8
SSI04/12	15	19	20	23	21	18.22	1612	11.3
SSI05/12	17	16	18	20	21	5.44	375	14.5
SSI06/12	18	17	18	22	21	13.20	892	14.8
SSI07/12	18	19	20	22	21	10.14	700	14.5
SSI08/12	17	18	20	21	21	17.94	1401	12.8
SSI09/12	21	19	20	21	21	5.40	357	15.1
SSI10/12	18	19	20	22	21	7.20	507	14.2
SSI11/12	18	19	20	22	21	6.54	467	14.0
SSI12/12	18	19	20	22	21	7.70	616	12.5
SSI13/12	17	19	20	23	21	7.62	544	14.0
SSI14/12	16	18	20	22	21	10.64	744	14.3
SSI15/12	18	19	20	22	21	5.04	403	12.5
CD (5%)						2.68	205	

Bag size: 20»x 24» (Polythene, 150 gauge); No. of replicates: 4 each for one strain with 10 bags (10kg compost/bag); Experimental design: RBD; Date of spawning: 6.1.13-8.1.13; Rate of spawn: 0.7% wet compost (70g/bag of 10kg compost); Days of spawn run: 15-18d; Casing: FYM +SC (2:1 v/v); Date of casing: 28.1.13 31.1.13; Days of case run: 16-19 d; Days for pinning: 18-20 d after casing; Days for first harvest : 20-23 d after casing; Days for last harvest: 4 weeks crop data ; NFB: number of fruit bodies.

Table.3 Comparison of color, texture, stipe length and stipe diameter for *Agaricus bisporus* strains

Strains	Color (L Value)	Texture (hardness)	Stipe length (cm)	Pileus diameter (cm)
AVT-01	85.54	1236.25	3.94	4.02
AVT-02	89.00	1818.50	2.74	4.36
AVT-03	83.74	1648.18	2.54	3.08
AVT-04	88.70	1093.00	2.78	3.82
AVT-05	85.55	1235.53	2.74	3.40
AVT-06	89.87	1417.02	2.68	4.02
AVT-07	DID NOT FRUIT			
AVT-08	31.13	1312.9	3.50	4.26
AVT-09	30.00	1066.2	2.78	3.82
AVT-10	30.21	971.90	2.40	3.64
AVT-11	31.15	1489.6	2.54	3.88
U3	78.00	2098.45	3.90	4.36
SSI01/12	84.94	1550.96	2.60	3.08
SSI02/12	84.46	1990.39	3.40	3.82
SSI03/12	83.76	1073.45	4.00	3.90
SSI04/12	86.58	783.42	2.80	3.82
SSI05/12	85.73	792.35	2.68	3.88
SSI06/12	87.19	1910.07	3.90	4.26
SSI07/12	90.85	1525.85	2.40	3.4
SSI08/12	87.44	2575.34	3.94	3.64
SSI09/12	90.78	1545.78	2.60	4.00
SSI10/12	84.52	1234.50	3.40	4.26
SSI11/12	85.95	1158.75	3.50	3.84
SSI12/12	91.26	1924.08	2.10	4.00
SSI13/12	86.56	1453.23	2.80	3.84
SSI14/12	87.25	1125.55	3.10	3.90
SSI15/12	84.64	1123.75	4.00	4.00
CD	3.20	2.99	0.87	NS

Morphological and optical parameters

Twelve strains of *Agaricus bisporus* (Lange) Sing. (AVT 01- AVT 06, AVT 07-AVT 11 and U3) along with fifteen white strains (SSI01/12- SSI15/12) were checked for quality parameters (color, texture, stipe length and pileus diameter) to select strains for post-harvest processing such as canning and pickling.

Color and texture analysis

The color determination showed that L Value was maximum for six strains SSI07/12, SSI09/12, SSI12/12, AVT 02, AVT- 04 and AVT- 06 with minimum values for brown strains ranging from 30.00 to 31.15. Texture analysis indicated the hardness as a main quality parameter which was maximum for strain SSI08/12 (2575g).When compared with white strains, brown strains showed less hardness (Table 3). The strains showed a correlation of better yield with good color and textural properties. Among white strains, SSI04/12 (86.58), SSI08/12 (87.44), AVT-02 (89.00) and AVT-06 (89.97) showed better color index which was close to the highest value of L (90.85) while brown strains had the similar L Values ranging from 30.00 to 31.15. The hardness was maximum for SSI08/12 (2575g) followed by AVT-02 (1818.50g)

among white strains while in case of brown strains AVT-11 showed maximum texture (1489.60g) followed by AVT-08 (1312.90g). These strains also gave better yield. The results were similar to the study done by Kumar and Suman, (2014) where significant variations in the morphological traits of various strains, like color and toughness of a fruit body was seen. Monolopoulou *et al.*, (2007) also found that the mushroom whiteness assessment allowed the characterization of most strains as well as their acceptance among wholesalers.

Stipe length and pileus diameter

The stipe length varied significantly from strain to strain. The average stipe length recorded as short as 2.10 cm in strain SSI12/12 and as long as 4.0 cm in strain SSI03/12 and SSI15/12 (white strains) while among brown strains, it is shortest for AVT-10 (2.40cm) and longest for AVT-08 (3.50cm). Stipe length is the most important character of button mushroom, as shorter the stipe length, better the quality. SSI04/12 (2.80cm) and AVT-02 (2.74cm) have small stipe length which is also correlated with their high yield obtained through short composting. There is non-significant difference in the diameter of the pileus for all the white and brown strains of *Agaricus bisporus.* The average range of pileus diameter varies from 3.08 to 4.36. These observations were similar to the data obtained from Prakasam and Singh, (2008), where the average stipe length recorded as short as 17.60 mm in strain NCB-13 and as long as 37.30 mm in strain X-13 while no significant differences in pileus diameter among the strains were obtained. The maximum diameter was observed in strain Delta (35.20 mm) and minimum was observed in strain CM-10 (26.20mm). The observations were also in agreement with the work carried by Kumar and Suman (2014) where strain DMRA-7, in addition to being high yielding also exhibited better morphological quality traits followed by strains DMRA-37, DMRA-102 and U3. Different test strains exhibited different morphological qualities in addition to their yield. Strain DMRA-7 exhibited best morphological qualities, while other strains gave one or more acceptable qualities, but failed to exhibit all the best qualities. There were significant variations in the morphological traits of various strains, like stipe length, stipe diameter, pileus diameter, color, toughness and average weight of fruit body.

On the basis of results obtained, it can be concluded that AVT 02 (White strain) and AVT 11(Brown strain) gave maximum yield on wheat straw based compost through short method of composting. The strains showed a correlation of better yield with good color and textural properties. AVT 02 and AVT 06 showed better color index which was close to the highest value of L (90.85). AVT 02 among white strains and AVT 11 among brown strains showed maximum texture. Length of stipe is correlated with yield as shorter the stipe length, better the quality. SSI04/12 and AVT-02 with small stipe gave high yield. These strains, thus, have been further selected for post-harvest processing such as canning and pickling.

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