



Biochemical estimation and cultivation of *Agaricus bisporus* (Lange) Imbach on different casing materials and bio-inoculant *Pseudomonas putida*

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Abstract: This study was carried out during 2012-2014 to determine the effect of locally available casing materials with association of bio-inoculant *Pseudomonas putida*. Six different combinations of casing mixtures were amended for evaluating its effect on yield, biological efficiency, protein and carbohydrate contents of *Agaricus bisporus*. A mixture of FYM + GLS + Vermi-compost + *P. putida* gave higher mushroom yield, biological efficiency, protein and carbohydrate content. It gave better yield (1306 g), biological efficiency (28.7%), protein (34.07%) and carbohydrate content (5.07%) respectively when compared with other treatments. In addition, waste tea leaves took minimum period (33.00 days) for initiation of pin head when compared with others. Locally available casing materials along with *P. putida* incorporated in the casing soil can be an important factor to obtain maximum and assured yield in mushroom cultivation.

Keywords: *Agaricus bisporus*, *Pseudomonas putida*, Vermicompost, Waste tea leaves

INTRODUCTION

Agaricus bisporus (Lange) Imbach is the most widely cultivated species of edible mushroom and it is a popular cultivar among the artificially grown fungi of the world. It is cultivated around the world for culinary purpose. In addition to its own unique flavor, eating this mushroom may provide important health and nutritional benefits when made a regular part of the diet. *A. bisporus* contains high amounts of protein, minerals, B vitamin group, D and K vitamins, and sometimes A and C vitamins. Against fat amount, its calorie, sodium and cholesterol are low (Saiqa *et al.*, 2008). Button mushroom is a good source of very good quality protein especially rich in lysine and thus can supplement the cereal based Indian diet well.

A. bisporus requires two different substrates to form the fruiting bodies i.e., the compost for nutrition on which it grows vegetatively and the nutrient deficient casing soil in which the suitable physiochemical/ biological conditions stimulate the initiation process of pin head formation for fruit body formation. The casing soil functions to supply and conserve moisture for the mushrooms and their rhizomorphs as well as to act as the transport system for dissolved nutrients (Cho *et al.*, 2008). It is one of the important growing parameters and source of variation in production, quality, and uniformity of commercial cropping. A variety of casing materials have been used worldwide, among these, use of farmyard manure (FYM), as a casing medium for mushroom cultivation, has been in vogue in Indian

subcontinent because of its easy availability and the non availability of peat moss generally used for casing in Europe and USA (Choudhary, 2011). Casing layer supports beneficial microbial population that plays a crucial role in mycelium growth, primordium initiation and development, early completion of cropping stages and the yield. Various species belonging to the genus *Pseudomonas* play key roles in mushroom growth, of which the *Pseudomonas putida* is regarded as the most significant. Several reports are available on the beneficial effects of casing soil microbes, especially *P. putida* and *Alcaligenes faecalis* on *A. bisporus* (Choudhary *et al.*, 2009). Casing soil inoculated with *P. putida* promoted faster growth and more uniform size of primordia (Riahi *et al.*, 2011). Mushrooms are still cultivated on a small scale in small pockets on a specific substrate and yield potential is not satisfied due to specific casing material while the local demand of mushroom is becoming high and claiming. Therefore, there is need to evaluate different casing materials for enhancing better growth behavior, yield potential and nutritional status of button mushroom. The aim of this study was to determine yield, biological efficiency, carbohydrate and protein contents of *A. bisporus* using different locally available casing materials along with *P. putida*.

MATERIALS AND METHODS

The study was carried out during the cropping period from October to February in the Department of Plant

Pathology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad. The compost was prepared by Long method of composting (LMC) proposed by Mantel and Agarwal, 1972 using newly harvested wheat straw. Spawn was procured from the Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (U.P). After the composting process, thorough spawning was done @ 75 g/10Kg compost. The compost filled bags were covered with newspaper sheets to prevent loss of moisture content in mushroom bags. These mushroom bags were placed in the mushroom crop room (Fig. - 1). Casing was done at a thickness of 3 cm. The temperature in the room was maintained at 18°C to 22°C respectively, and humidity was adjusted to 70-80%.

Six treatment combinations *viz.* Farm Yard Manure (FYM) + Garden loam soil (GLS) (2:1), Farm Yard Manure (FYM) + Garden loam soil (GLS) + *P. putida* (2:1), FYM + GLS + Waste tea leaves (2:1:1), FYM + GLS + Waste Tea Leaves + *P. putida* (2:1:1), FYM + GLS + Vermi-compost (2:1:1), FYM + GLS + Vermi-compost + *P. putida*. (2:1:1) were taken (Fig. 2). These

treatments were replicated seven times and collected data were analyzed using CRD.

Inoculum preparation: The culture of bacterial inoculum (*P. putida*) was procured from the Division of Plant Pathology, IARI, New Delhi, India. The inoculum was prepared by growing the selective strains in King's B broth medium. After incubation at 30°C for 72hrs, the densities of culture were determined. Then, the cultures were diluted further in King's B broth until the final bacterial cell numbers were 1×10^8 cells/ml. Bacterial suspension (77 ml/bag) was sprayed in different treatments at the time of casing.

The harvesting was done when buttons were fully-grown (but not yet open), and total harvest was recorded in each bag (Fig. 4).

Biological efficiency: Biological efficiency (BE) of the compost at each location was calculated using the formulae (Gupta and Sharma, 2008).

BE% = Total weight of fresh mushroom harvested / Dry weight of substrate $\times 100$

Protein content: The protein content of fruit bodies of *A. bisporus* was estimated by Lowry et al, 1951.

Carbohydrate content: In this study, carbohydrate



Fig. 1. Mushroom bags in mushroom crop room.



Fig.2. Different casing materials used.



Fig. 3. Initiation of pin head and fruiting of *A. bisporus*.



Fig. 4. Harvested fruiting bodies of *A. bisporus*.

Table 1. Effect of casing mixtures on different parameters of white button mushroom (*Agaricus bisporus*) during 2013-2014.

Casing mixtures*	Initiation of pin head (days) (2013)	Protein (%) (2013)	Carbohydrate (%) (2013)	Biological efficiency (%) (2013)	Yield(g) (2013)	Initiation of pin head (days) (2014)	Protein (%) (2014)	Carbohydrate (%) (2014)	Biological efficiency (%) (2014)	Yield(g) (2014)
Farm Yard Manure (FYM) + Garden loam soil (GLS) (2:1)	39.57	24.6	3.07	19.9	705	39.85	25.54	3.05	19.8	718
Farm Yard Manure (FYM) + Garden loam soil (GLS) + <i>Pseudomonas putida</i> (2:1), FYM	38.28	26.57	3.09	20.8	1080	38.7	27.52	3.11	20.3	1027
FYM + GLS + Waste tea leaves (2:1:1)	34.14	29.8	4.30	22.2	895	34.28	28.7	4.28	22.1	879
FYM + GLS + Waste Tea Leaves + <i>Pseudomonas putida</i> (2:1:1)	33.00	31.18	4.80	24.6	1190	33.14	31.80	4.89	24.4	1065
FYM + GLS + Vermicompost (2:1:1)	37.42	31.11	5.30	26.5	971	37.57	31.15	5.32	26.18	985
FYM + GLS + Vermicompost + <i>Pseudomonas putida</i> . (2:1:1)	36.14	33.5	5.90	28.7	1290	36.28	34.07	5.70	27.8	1306
SEd±	1.143	0.023	0.016	0.029	4.707	1.226	0.104	0.000	0.041	4.546
C.D. (P=0.05)	2.317	0.038	0.039	0.067	9.543	2.484	0.193	0.081	0.043	9.229

* All the treatments are seven replications

was determined by anthrone method. The anthrone reaction is the basis of a rapid and convenient method for the determination of carbohydrates, either free or present in polysaccharides (Hedge and Hofreiter, 1962).

RESULTS AND DISCUSSION

Table.1 shows the yield, biological efficiency, protein, and carbohydrate contents of *A. bisporus* cultivated using different casing materials. In this study, mushroom bags were completely colonized by mushroom mycelium within 17 to 25 days of spawning. The bags cased with casing mixture FYM + GLS + WTL + *P. putida* took a minimum of 33.00 days to initiate pin heads while FYM + GLS took a maximum of 39 days (Fig.- 3). These casing mixtures deferred non-significantly among themselves. Similar results were reported by Ram and Kumar (2010) that casing mixture Vermicompost + FYM + Saw dust + Sand took minimum 37 days for initiation of pin head. Lakshmi-pathy *et al.* (2011) observed on different substrate that the spawn of *Calocybe indica* took 17-23 days to initiate primordia. Amin *et al.* (2010) observed that different agricultural wastes took 13-19 days to initiate primordia in *C. indica*. The probable reason for this finding may be that waste tea leaves having high water holding capacity appears to be directly related to porosity and bulk density. These factors would have directly affected microbial build up and initiation of pin heads of *A. bisporus*.

The casing mixture FYM + GLS + Vermicompost + *P. putida* showed the highest protein content (34.07%) and was significantly at par, while the lowest was obtained from FYM + GLS (24.6%). Similar results were reported by Coskuner and Ozdemir (1997) that the protein level of mushroom varies from 19 to 35% and Ebadi *et al.* (2012) reported that the level varies from 35 to 40%. Han (1999) studied protein content of *A. bisporus* cultivated on a mixture of horse manure, straw, chicken manure, gypsum and water was 32.92 to 34.08%. Colak *et al.* (2007) reported that the protein content of *A. bisporus* was within the range of 19.13 to 26.94%. However, protein values in our study were higher than Colak's findings. It may be due to the difference in nature of the substrate, atmospheric conditions and stage of development of mushrooms. The highest carbohydrate content was obtained with the mixture of FYM + GLS + Vermicompost + *P. putida* (5.70%) while the lowest content was obtained from FYM + GLS (3.05%). It had no significant difference between the substrate. Colak *et al.* 2007 reported that carbohydrate values of *A. bisporus* vary from 3.05 to 5.385%. Boda *et al.* 2012 determined the carbohydrate content of *A. bisporus* 4.85g/100g from the Kashmir valley of India. The probable reason for this finding may be that *P. putida* improved mushroom quality through increasing nutrient uptake. Vermicompost is rich in microbial life which converts the nutrients al-

ready present in the soil into available forms. Hence, it enhanced the quality of mushroom.

Biological efficiency varied significantly between the substrates. The highest (19.8%) and lowest (28.7%) biological efficiencies were observed from FYM + GLS + Vermicompost + *P. putida* and FYM + GLS, respectively. The findings of this study are comparable with those of previous studies recorded by Udugama *et al.* (2005) that biological efficiency of compost prepared by LMC ranged between 25 to 30%. The highest yield was obtained from the mixture of FYM + GLS + Vermicompost + *P. putida* (1306 g/7kg) while the lowest yield was obtained from the mixture of FYM + GLS (705g/7kg). Babu *et al.* (2004) reported that strain S₁₁ of *A. bisporus* produced 0.99 to 1.32 kg of mushrooms depending on the casing material under natural conditions at Sita Eliya. Udugama *et al.* (2005) recorded 0.31 to 1.82 kg/bag kg of white button mushroom from different locations. The probable reason for this finding may be that *P. putida* through basidium development and acceleration of mycelium growth resulted in increase in yield and mushroom biological efficiency. Vermicompost is a nutrient rich organic fertilizer therefore, it directly affects the biological efficiency and yield of *A. bisporus*. Choudhary *et al.* (2009) reported that the yield and biochemical contents of mushroom was effected by the nature of casing materials like bulk density, porosity, water holding capacity and pH value. Besides this, bacterial association present in the substrate significantly enhanced the yield, protein and carbohydrate contents of *A. bisporus*.

Conclusion

The most suitable casing material obtained in this study was vermicompost mixed with *P. putida*, FYM and GLS. Waste tea leaves mixed with *P. putida*, FYM and GLS also gave good result. So, locally available casing materials can be an important factor to obtain maximum and assured yield in the mushroom cultivation. *P. putida* can be incorporated along with the casing soil. It plays an important role in the initiation of primordia ultimately resulting in high yield, biological efficiency and quality. Supplementing substrate at casing is a relatively easy and low-cost cultural practice that may successfully be used to enhance the yield, BE and maximize the utilization of the substrate.

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