Periodic Research

Evaluation of Substrates for Organic Cultivation of Milky Mushroom (*Calocybe indica*) strain APK-2

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Abstract

Milky mushroom (*Calocybe indica*) also known as *dudh chatta* because of its milky appearance and large sized fruit body. Keeping importance and utility of agricultural wastes for its cultivation, different substrates were organically tested to increase its biological efficiency. Three different combination of substrates *viz*., wheat straw + paddy straw (1:1), wheat straw + paddy straw (1:2), wheat straw + paddy straw (2:1) and two different substrate wheat straw alone and paddy straw alone were evaluated for yield performance. Two year pooled data showed that substrates vary for period taken to spawn run (14-19 days), pin head formation (27 -35 days) and 1st harvest (31 -38 days). The wheat straw alone substrate significantly took minimum days (14 d) for spawn run; pinhead formation (27 d) as well as 1st harvest (31 d) followed by wheat straw + paddy straw (2:1), and wheat straw + paddy straw (1:1). In terms of average production, wheat straw alone substrate found significantly superior than others by giving the maximum (57.36 kg/qt dry substrate) yield followed by wheat straw + paddy straw 2:1 ratio (55.43 kg/qt). The significant lower (35.52 kg/qt dry substrate) yield was obtained from paddy straw alone substrate. However significantly maximum number of fruit bodies (727) were obtained from the substrate wheat straw + paddy straw (2:1), followed by wheat straw alone (630). The average fruit body weight was found maximum (92.22g) in case of wheat straw + paddy straw (2:1), followed by wheat straw alone (91.05g), however minimum weight was obtained in wheat straw + paddy straw 1:1 ratio (70.99g). *Calocybe indica* strain APK-2 maximum average biological efficiency (57.36%) and average mushroom size (12 x12 x4 x 2.5 cm) were recorded in wheat straw alone substrate followed by wheat straw + paddy straw (2:1) combination.

Keywords *Calocybe indica*, Milky mushroom, substrate, organic cultivation

Introduction

Calocybe indica is a tropical domesticated edible mushroom; native to India was first described by Purkayastha and Chandra 1974. It is also known as *dudh chatta* / milky mushroom because of its attractive milky whitish appearance with excellent shelf life and large sized basidiocarp with fleshy stipe and broadly adnate to decurrently gills. It belongs to Kingdom: Fungi, Phylum: Basidiomycota class- Basidiomycetes order: Agaricales and family: Tricholomataceae (Kirk et. al., 2001). The dried sporophores of this mushroom contain 17.69% protein, 4.1% fat, 3.4% crude fiber and 64.26% carbohydrate. Mature sporocarp contains 4% soluble sugar, 2.95% starch and 7.43 % ash. In addition to this, it has most of the mineral salts such as potassium, sodium, phosphorus, iron, calcium and amino acid namely alanine, aspartic acid, glutamine, glutamic acid, glycine, hydroxyproline, histidine, lysine, threonine, tyrosine, valine, arginine and proline **(**Tripathi, 2005; Bhatt, 2007). Due to its alkaline ash and high fiber content it is highly suitable for the people with hyper acidity and constipation. It can be easily grown at a temperature ranged between 25-35^{o}C and its cultivation can be best fitted in early cropping when no other mushroom can grown except *Volvariella* spp. at such a higher temperature. Keeping above importance in view different substrates as well as their combination were organically tested in present investigation to increase its biological efficiency.

Materials and Methods

Inoculum and spawn preparation

The production of inoculum in Petri dishes and its conservation in test tubes was performed according to Singh (2005). Spawn was prepared by cooking wheat grains for 15 minutes, drained and cooled and 1.5% calcium carbonate were added in relation to their mass then transferred into 65 to 75 cm clear polypropylene bags, with a mean thickness of 0.6 mm, and its upper portion was plugged with non absorbent cotton plug and were covered with aluminum foil paper. The bags were sterilized, inoculated, and incubated according to Singh (2005).

Substrate preparation

Two different substrate wheat straw (WS) and paddy straw (PS) were evaluated alone and in three different combination *viz.*, WS+PS (1:1), WS+PS (1:2), WS+PS (2:1). The wheat and paddy straw was cut into small pieces (1-2 inch long) to fascinate proper wetting as well as to increase surface area. Then both the substrate were submerged in fresh water for twelve-fourteen hours then to sterilization in hot water at 80-90°C for 2 hours in a 200 liter water capacity container (Bahukhandi and Munjal, 1989; Balasubramanya and Kathe, 1996; Tewari, 2005).After that, straw was taken out, cooled down and drained until a mean moisture of 62-65% calculated by drying 100 g wet substrate in an oven at 60-70°C until constant weight, with three replicates per sample. The pH of the material is adjusted with limestone to about 7.5 or higher to provide selectivity against *Trichoderma* green mold. The sterilized straw substrate combination (WS+PS (1:1), WS+PS (1:2), WS+PS (2:1), wheat, paddy straw alone was manually mixed and packaged into 75 X 45 cm clear polyethylene bags with 5kg wet substrate per bag, together with 250 g spawn (5% in relation to the wet mass of the substrate). Five treatments were taken to a spawn running room/cropping room constructed in rainfall concrete cement roofing and then laid on iron shelves 45 cm above ground. A shade cloth was used to seal the window and door, in order to reduce moisture loss from the environment and avoid the access of insects that could be harmful during cultivation. Temperature in the environment ranged from 25-32°C and relative humidity above 80%. It takes about 14-20 days when substrate is fully colonized and bags are ready for casing. The bags were shifted to cropping room for casing and cropping. Casing material (Soil+ sand (2:1)), with pH adjusted to 7.8-7.9 with using $CaCO_3$, then mixture were steamed at 15 lb psi for 1 hr. Bag's top is made uniform by ruffling top surface of the substrate and sprayed with water. Casing material is spread in uniform layer of 2-3 cm thickness and immediately sprayed with water to saturation level. The temperature (30-35^{o}C) and humidity (80-90%) in the environment were adjusted by regular spraying of water on sanded floor and aeration was controlled by means of an exhaust fan that was turned on 1 hour/day during the mushroom production stage and light provided in day time. Mushrooms were collected during three flushes over a 50 days period (Tewari, 2005).

Observations

Each treatment was replicated twenty times in completely randomized design. Two successive crops were grown for the experiments and observations were recorded for days of spawn run, pinhead appearance, first harvest, and yield and average data were analyzed statistically. The observations on average biological efficiency (ABE), obtained by the ABE of the bags of each treatment, using the expression: ABE % = Total wet mass of mushrooms / dry mass of the initial substrate x 100 (Chang et al., 1981). The total wet mass of mushrooms was obtained by the sum of yields recorded during flushes; dry mass of the initial substrate was calculated by subtracting the mean moisture in the wheat and paddy straw (62-65%) from its wet mass in each treatment.

Results and Discussion

The spawn run on the substrate could be observed from the third day of incubation in the cropping-room, with the formation of light halos around the spawn, indicating the beginning of degradation of the substrate by the fungus. The natural induction of primordial on the substrates occurred between 27-35 days of incubation, and the first flush or harvest occurred after 31-38 days of incubation. The mushrooms sprouted in the form of needle shape white coloured which matured about a week .The second and third flushes occurred 10 and 20 days after the first yield, respectively, and lasted 9-10 days. This behavior was similar in all treatment bags**,** except in those that stopped up producing due to contamination of the substrate by rival microorganisms. Therefore, one harvest was obtained every 12-15 days, totaling a period of 57-70 days between the beginning of mycelium formation and the third flush, after which the substrates were discarded.

The Two year collective data presented in Table 1 depicted that cultivation of *Calocybe indica* strain APK-2 in hot water sterilized substrates vary in favor of period taken to spawn run (14-19 days), pin head formation (27 -35 days), 1st harvest (31 -38 days). The wheat straw alone substrate significantly took minimum days for spawn run(14); pinhead formation (27) as well as 1st harvest (31) followed by wheat straw + paddy straw (2:1), and wheat straw + paddy straw (1:1) but significantly lesser than the period taken by other substrates The period for spawn run was recorded maximum (19days) in case of paddy straw alone. In terms of average production, wheat straw alone substrate found significantly superior than others by producing the maximum (57.36 kg/qt dry substrate) yield followed by wheat straw + paddy straw (2:1) ratio (55.43 kg/qt). The significant lower (35.52 kg/qt dry substrate) yield was obtained from paddy straw alone substrate. However significantly utmost number (727) of fruit bodies were obtained from the substrate wheat straw + paddy straw (1:1), followed by wheat straw (630) alone. The average basidiocarp weight was found maximum (92.22g) in case of wheat straw + paddy straw (2:1),

followed by wheat straw (91.05g) alone, though minimum weight was obtained in wheat straw + paddy straw (1:1) (70.99g). *Calocybe indica* strain APK-2 maximum average biological efficiency (57.36%) and average mushroom size (12X12X4X2.5 cm) were recorded in wheat straw alone substrate followed by wheat straw + paddy straw (2:1) combination (55.43%, 11X11X3X2.5cm, respectively). Approximately similar observations were reported by Sharma et.al, 1997; Krishanamoorthy et.al, 1998; Jadhav et.al. 2002; Bhatt et.al, 2007) but were using chemical substrate sterilization. It has been well established that physical and chemical state of substrate largely decides their suitability for mushroom growing (Zadrazil, 1978). Different types of substrates have been used to grow *Calocybe indica* in several researches. The intrinsic variability of the biological material, its phenotypic plasticity, and the type of substrate used could also have influenced the results. Similar in present substrates performance study, hot water sterilized wheat straw alone was found promising for the production of this mushroom followed by wheat straw + paddy straw (2:1) then paddy straw and other substrate combinations.

References

1. Bahukhandi, D. and Munjal, R.L. 1989.Cultivation of *Pleurotus* species on different agricultural residues. *Indian Phytopathol*: 42:492-495.
2. Balasubramanya, R.H. and Kathe, A.A. 1996. An inexpensive pretreatment of cellulose materials for growing edible oyster mushrooms. *Bioresource Technology*. 57:303-305
3. Bhatt, P., Kushwaha, K.P.S. and Singh, R.P. 2007. Evaluation of different substrate and casing mixture for production of *Calocybe indica*. *Indian Phytopath*. 60(1):128-130.
4. Chang, S.T.; Lau, O.W. and Cho, K.Y. 1981. The cultivation and nutritional value of *Pleurotus sajor-caju*. *European J. Appl. Microbiol. Biotech.*12:58-62.
5. Jadhav, S. W., Wange, S.S., and Gaikwad, J.G. 2002. Studies on cultivation of *Calocybe indica. Indian J. Mycol. Pl. Pathol.* 15(1):338
6. Krick, P.M, Cannon, P.F., David, J.C. and Stalpers, J.A. 2001. *Ainsworth and bisby's dictionary of the fungi.* Biddles, London.655 pp
7. Krishnamoorty, A.S., Muthusamy, M., Marimuthu, T., Narasimhan, V. and Muthusankaranayanan, A. 1998. Milky mushroom APK-2. Bulletin on new mushroom variety release. Regional Research Station, TNAU, pp16
8. Sharma, S.S., Doshi, A., Trivedi A., and Kothari, K.L. 1997. Evaluation of different casing materials for C*alocybe indica. Mushroom Res.* 6(2):81-82.
9. Singh, S.K. 2005. Modern spawn production technology (2005). *In Frontiers in Mushroom Biotechnology.* Eds: RD Rai, RC Upadhyay and SR Sharma, NRCM Solan (HP) pp. 56-71.
10. Tewari R.P. 2005. Cultivation and economics of milky mushroom (*Calocybe indica). In Frontiers in Mushroom Biotechnology.* Eds: RD Rai, RC Upadhyay and SR Sharma, NRCM Solan (HP) pp. 165-169
11. Tripathi, D.P. 2005. Cultivation of specialty mushroom. In Mushroom Cultivation. Oxford & IBH Pub. CO.PVT.LTD. pp.250-253
12. Zadrazil, F. 1978. Biology and cultivation of edible mushroom (S.T. Chang and W.A. Hayes Ed.) Academic Press, New York pp. 521-557.

Table 1:
Effect of different substrates on yield performance of Milky mushroom (*Calocybe indica*) strain APK-2

SN	Treatment	Spawn Run days	Pinhead appearance days	Days of 1st harvest	Avg. Mushroom yield (kg/qt)*	Avg. Mushroom No.*	Avg. Fruit body wt. (g)*	Biological efficiency (%)*	Avg. Size (cm)*
1.	**Wheat Straw alone**	14	27	31	57.36	630	91.05	57.36	12X12X4X2.5
2.	**Paddy Straw alone**	19	35	38	35.52	406	87.48	35.52	8X8X4X3.5
3.	**Wheat Straw + Paddy Straw (1:1)**	17	32	36	51.60	727	70.99	51.60	10X10X3X2.5
4.	**Wheat Straw + Paddy Straw (1:2)**	18	35	37	46.76	515	90.79	46.76	7X7X4X3.5
5.	**Wheat Straw + Paddy Straw (2:1)**	15	30	35	55.43	601	92.22	55.43	11X11X3X2.5
				CD 5%	1.07	84.68			
				CD 1%	1.89	112.50			
				CV %	4.84	5.41			

*** Average Mean of 20 replications in each treatment**
*** Yield and number based on per quintal dry substrate**