J Mycol Plant Pathol, Vol. 44, No.3, 2014

Short Communication

Evaluation of Different Substrate Combinations for Quality Spawn Production and Biological Efficiency of Milky Mushroom in Andaman and Nicobar Islands

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Abstract

Milky mushroom (*Calocybe indica*) is highly suitable for hot and humid tropical climate of Andaman and Nicobar Islands. Two experiments were conducted to exploit locally available substrates with high biological efficiency and to explore suitable substrate combination for quality spawn production of milky mushroom. The highest yield (653.3 g kg^{-1} dry substrate) with high biological efficiency (BE) (65.3 %) was recorded in paddy straw substrate. Among the alternative substrates, cowpea stem alone showed fast spawn run in 13 days, whereas banana leaf in combination with paddy straw (1:3) showed the longest spawn run period of 18.7 days. Okra stem alone was the second best and recorded maximum yield of 366.3 g kg^{-1} of mushroom with BE of 36.6 %. In another experiment, 13 substrate combinations viz., sorghum grains, paddy straw, banana leaf and coconut husk were evaluated individually and in different combinations. Sorghum grains at 60 % moisture resulted in excellent and fast spawn run followed by paddy straw in combination with sorghum grain in the ratio of 1:1, 2:2 and 3:1 and the lowest yield was obtained in coconut husk alone.

Key words: Biological efficiency, *Calocybe indica*, milky mushroom, spawn production

Citation: Kumar K, Ahmed SKZ and Roy SD. 2014. Evaluation of different substrate combinations for quality spawn production and biological efficiency of milky mushroom in Andaman and Nicobar Islands. *J Mycol Pl Pathol* 44(3):294-297.

The milky mushroom (*Calocybe indica*) was recently introduced in Andaman and Nicobar Islands. Since exploration of local species of *Calocybe* and their exploitation is in infancy (Kumar et al 2009a), efforts were made to standardize the suitable mushroom production for the island conditions. This study provides a basis for commercial production for milky mushroom in these Islands. Availability of quality spawn was a major constraint for mushroom production due to high cost of substrates like sorghum and wheat which is mainly imported from mainland India. Milky mushroom is suitable for hot humid tropical climates in these Islands as the highest percentage of biological efficiency was 77.35 followed by arecanut pericap (57.00), coconut leaf (51.25) and in rest of substrates biological efficiency was less than 50 % (Pani 2010). Growing edible mushrooms is the most efficient method of bioremediation of the large quantity of lingocellulosic wastes generated annually through activities of agriculture and allied activities. Though some information is available on the utilization of such substrates for cultivation of milky mushroom (Chakravorty et al 1981; Purkayastha 1982; Doshi et al 1989; Krishnamoorthy and Muthusamy 1997; Chaubey et al 2010) the results are widely varied and contradictory. Keeping this in view the present investigation was undertaken to evaluate the locally available agricultural wastes for spawn production and to assess the biological efficiency for cultivation of milky mushroom under the island conditions.

Material and Methods

Evaluation of substrates and their combinations for biological efficiency. *Calocybe indica* culture were procured from Indian Institute of Horticultural Research, Bangalore were evaluated during 2008-09 and 2009-10 under high humid tropical condition (28-33 C and 75-95 % RH range) of Andaman and Nicobar Islands. Six cheap and locally available substrates (paddy straw, areca nut leaf, coconut leaf, okra stem, banana leaf and cowpea stem) were procured to assess their biological efficiency. Experiment was designed to evaluate the effectiveness of potential substrate and their substrate combinations of paddy straw along with okra stem, cowpea stem, areca nut leaf, and coconut leaf and banana leaf in ratio of 1:1, 1:2, and 1:3 (w/w). All the substrates were chopped into 5 - 8 cm pieces and soaked in water for 12 h in separate plastic drums. The substrates were sterilized by steaming for 1 h and their moisture content was adjusted to optimum level (60 %) by prolonging the drying period. Spawning was done at the rate of 5 % of wet straw in high density polythene bags (60 cm × 40 cm, 100 gauges) following the method described by Pani and Das (1998). The spawned bags were incubated (28-35 C and 80-95 % RH) for spawn run. After incubation, the bags were transferred in to the cropping room for fruiting at 30 to 35 C and 80 to 90 % RH. Sufficient light and controlled ventilation was allowed during the cropping period. Water was sprayed regularly to keep the substrate surface moist. The casing was done with casein

mixture (sand, farmyard manure and garden soil) in the ratio 1:1:1. Approximately 2.0 cm thickness of casing mixture was applied by covering top poly bags of full run bags in each treatment. After casing, the bags were sprayed with water regularly on the casein surface. The spawn run period was recorded for each treatment. The casing medium was gently ruffled, slightly compacted back and sprayed regularly with water.

The pinhead initiation was regularly recorded and mature sporophore was harvested periodically. Mature fruiting body was harvested by twisting them slightly near the base and fresh sporophore weights were immediately recorded. The yield data was recorded and calculated on the basis of fresh mushrooms per kg of dry straw from each bag. The data analyzed for biological efficiency which was calculated as the ratio between the fresh weight of mushrooms and the dry weight of substrate.

Evaluation of substrates and their combination for spawn production. The method for substrate preparation was the same as in the substrate evaluation for mushroom production. Moisture content of the substrate at the time of spawning was also recorded to know the effect of moisture content on the spawn run period. Grading of spawn on the basis of visual quality assessment was graded adopting the scale: Excellent =100 % spawn run, white, no pigmentation; Good = 75 % spawn run, light brown, scanty pigmentation; Fair = 50 % spawn run, brownish, 25 % spawn pigmented and Poor = 25%-50 % spawn run, dark brownish, heavy pigmentation. Spawn run and other quality assessment was recorded up to 20 d at 2-day-intervals just after inoculation of bags. Each experiment was replicated three times in a completely randomly design. The required observations were recorded and the averaged data were analyzed statistically to determine treatment differences.

Results and Discussion

Effect of substrates on milky mushroom production. The pooled data of two cropping seasons (Table 1) showed that paddy straw + coconut leaf (1:1) had the minimum spawn colonization period (12.7 d) followed by cowpea stem + paddy straw (1:1, 1:2), cowpea stem + paddy straw (3:1) was better substrate whereas rest of

Table 1. Evaluation of different substrates and their combination for spawn production of milky mushroom

Substrate/ Combination*	Spawn run period (d)	Pin head initiation (d)	First harvest (d)	Yield (g Kg^{-1} of dry substrate)	Biological Efficiency (%)
PS	13.0	18.0	22.3	653.3	65.3
BL	15.7	24.3	27.0	335.0	33.5
PS + BL (1:1)	16.0	20.3	25.0	85.0	8.5
PS + BL (1:2)	15.0	23.7	27.3	116.7	11.7
PS + BL (1:3)	18.7	23.3	27.3	100.0	10.0
CL	15.7	24.3	28.7	335.0	33.5
PS + CL.(1:1)	12.7	26.0	29.3	322.7	32.3
PS + CL.(1:2)	14.7	21.3	26.0	260.3	26.0
PS + CL.(1:3)	14.3	25.7	29.7	138.3	13.8
AL 3:0	13.3	22.7	26.0	346.7	34.7
PS + AL (1:1)	13.7	20.3	24.3	260.0	26.0
PS + AL (1:2)	15.0	23.6	27.7	116.7	11.7
PS + AL (1:3)	18.7	23.3	26.7	100.0	10.0
CS	13.0	20.0	24.7	323.3	32.3
PS + CS (1:1)	14.7	27.0	31.0	281.7	28.2
PS + CS (1:2)	13.0	22.0	25.7	296.0	29.6
PS + CS (1:3)	13.0	20.0	30.0	240.0	24.0
OS	16.3	23.7	26.7	366.3	36.6
PS + OS (1:1)	13.7	20.7	24.5	336.7	33.7
PS + OS (1:2)	16.3	24.3	28.3	348.3	34.8
PS + OS (1:3)	18.0	24.0	28.3	313.3	31.3
CD ($P \leq 0.05$)	2.41	2.75	2.89	171.36	

*PS = Paddy Straw; BL = Banana Leaf; CL = Coconut Leaf; AL = Arecanut Leaf; CS = Cowpea Stem; OS = Okra

Table 2. Evaluation of different substrate and their combinations for milky mushroom spawn production

Substrate/ Combination*	Substrates		Full run spawn period (d)	Grade Quality***	Bag Contaminated (%)
	Moisture gained (g)**	Moisture gained (%)			
SG 4:0	1600	60	11	Excellent	0
SG 4:0	1550	55	12	Excellent	0
SG 4:0	1500	50	11	Excellent	0
PS 4:0	2010	101	12	Good	0
PS 4:0	2000	100.0	14	Good	0
PS 4:0	2015	101.5	12	Good	0
PS+SG (3:1)	1732	73.2	13	Fair	0
PS+SG (3:1)	1750	75.0	15	Fair	0
PS+SG (3:1)	1800	80.0	13	Fair	0
PS+SG (2:2)	1870	87.0	14	Good	0
PS+SG (2:2)	1790	79.0	13	Good	0
PS+SG (2:2)	1800	80.0	14	Good	0
PS+SG (1:3)	1822	82.2	15	Fair	0
PS+SG (1:3)	1790	79.0	14	Good	0
PS+SG (1:3)	1803	80.3	14	Fair	0
BL 4:0	2816	181.6	13	Fair	0
BL 4:0	2016	101.6	14	Fair	0
BL 4:0	2100	110.0	14	Fair	0
BL+SG (3:1)	2208	120.8	12	Good	0
BL+SG (3:1)	2200	120.8	12	Good	0
BL+SG (3:1)	2150	120.8	11	Good	0
BL+SG (2:2)	2208	120.8	12	Good	0
BL+SG (2:2)	2241	120.8	14	Good	0
BL+SG (2:2)	2230	120.8	14	Good	0
BL+SG (1:3)	2201	120.8	13	Excellent	0
BL+SG (1:3)	2209	120.8	11	Excellent	0
BL+SG (1:3)	2219	120.8	13	Excellent	0
H 4:0	3600	260.0	15	Poor	40
CH 4:0	3700	270.0	14	Poor	40
CH 4:0	3650	265.0	15	Poor	40
CH+SG (3:1)	2600	160.0	14	Fair	0
CH+SG (3:1)	2650	165.0	13	Good	0
CH+SG (3:1)	2630	163.0	12	Good	0
CH+SG (2:2)	2650	165.0	15	Fair	0
CH+SG (2:2)	2600	160.0	14	Fair	0
CH+SG (2:2)	2600	160.0	15	Fair	0
CH+SG (1:3)	2626	162.6	14	Poor	0
CH+SG (1:30	2621	162.1	15	Poor	0
CH+SG (1:3)	2619	161.9	15	Poor	0
CD ($P \leq 0.05$)	210.39	20.94	1.45		

*SG = Sorghum Grain; PS = Paddy Straw; BL = Banana Leaf; OD = Okra Stem; CS = Cowpea Stem; CH = Coconut Husk. **Initial weight of each substrate = 1000 g; *** Quality grading: Excellent=100 % spawn run, white in colour, no pigmentation, Good = 75 % spawn run, light brownish in colour, scanty pigmentation; Fair = 50 % spawn run, brownish in colour, 25 % spawn pigmented and Poor = 25 % - 50 % spawn run, dark brownish in colour, heavy pigmentation.

the substrate combinations took after 13 d for colonization. The time taken for spawn run in bags prepared different combinations of various substrates varied from 13 to 24 d. Cowpea stem alone showed the least spawn run period of 13 d whereas banana + paddy straw (1:1) showed the highest spawn run period of 16 d.

The period of spawn colonization was observed maximum in case of paddy straw + areca nut leaf (1:3). Early pin head initiation was recorded in paddy straw (3:0) in 18 d followed by cowpea stem, paddy straw + cowpea stem (1:3) in 20 d whereas in rest of the combinations pin head initiation was recorded in more than 20 d. It has been established that early pin head initiation reduce the days to first harvest resulting in reduction of crop cycle.

Paddy straw was the best among the substrates as it produced the maximum yield of 653.3 g with 65.3 % BE (Biological Efficiency) followed by okra stem (36.6 %), paddy straw + okra stem (1:2) with 34.8 % BE. Significantly, lower BE (8.5 %) was obtained from paddy straw + banana leaf (1:1). The next best treatment was okra stem with maximum yield of 366.3 g of mushroom and 36.6 % BE and the lowest yield was observed in banana leaf + paddy straw (1:1) with 8.5 % BE. Similar observations were also reported by Krishnamoorthy and Muthusawmy (1997), Bhatt et al (2007) and Kumar et al (2009b). Our study suggested that crop residue of less important substrate with appreciably high significant biological efficiency could be utilized as substrate combination with paddy straw for milky mushroom production in these Islands.

Effect of substrates on spawn production. Among the different substrates and their combinations tested for milky mushroom spawn production showed that sorghum grain alone was the best substrate followed by its combinations in all three ratios with banana leaf (1:3, 2:2 and 1:1) (Table 2). Banana leaf+sorghum grain (1:3) substrate combination was graded excellent substrate combination whereas 3:1 and 2:2 ratios were graded lower quality spawn. Of the 13 treatments evaluated significant differences were found in different substrate combination. The results suggested that substitution of banana leaf in small pieces could reduce the cost of spawn production as sorghum grain imported from mainland @ Rs. 40 kg^{-1} which results in higher cost of production. To our knowledge there is no such report on evaluation for milky spawn production, though sorghum grains, saw dust, rice bran and wheat bran have been used for the *Lentiula edodes* spawn production (Thiribhuvanamala et al 2005).

Acknowledgements

Authors thank the ex-Head, Division of Field Crops, Central Agricultural Research Institute, Port Blair for his valuable suggestion, valuable guidance and help during experimentation.

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Received: 18 Jun 2014 Accepted: 11 Aug 2014