

Influence of Different Supplements on the Commercial Cultivation of Milky White Mushroom

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Calocybe indica, known as milky white mushroom, grows and cultivated in the sub-tropical and temperate zones of South Asia. We investigated the most suitable supplements and their levels for the commercial cultivation of milky white mushroom. Rice bran, maize powder, and wheat bran with their different levels (10, 20, 30, 40, and 50%) were used as supplements to evaluate the yield and yield contributing characteristics of *C. indica*. Primordia initiation was observed between 13.5 and 19.3 days. The results indicated that the 30% maize powder supplement was effective for producing viable fruiting bodies. The maximum diameters of the pileus and stalk were observed with 30% maize powder. The highest biological and economic yield and biological efficiency were also obtained with 30% maize powder as a supplement. The results indicate that increasing the supplement level resulted in less biological efficiency, and that 30% maize powder was the best supplement level for rice straw substrate to cultivate milky white mushrooms.

KEYWORDS: Biological efficiency, *Calocybe indica*, Maize powder, Rice bran, Rice straw, Wheat bran

Calocybe indica, a tropical edible mushroom, belongs to the family Tricholomataceae of the order Agaricales [1]. It is becoming more popular, due to its robust size, attractive color, sustainable yield, delicious taste, and unique texture [2]. *C. indica* is rich in protein, lipids, mineral, fiber, carbohydrate, and is abundant with essential amino acids [3, 4]. It is an excellent source of thiamine, riboflavin, nicotinic acid, pyridoxine, biotin, and ascorbic acid [5].

Bangladesh has good environmental conditions for the commercial cultivation of *C. indica*. This mushroom requires a temperature of 30–35°C and a relative humidity of 70–80% for cultivation, which is congenial to the environmental conditions of Bangladesh [6, 7]. A wide range of diverse cellulosic substrates are used for cultivating mushrooms. Rice straw is the most common lignocellulosic substrate, whose major component is cellulose, and it is also the best substrate for cultivating milky white mushroom [2]. Lignocellulosic materials are generally low in protein, which is insufficient for commercially cultivating mushrooms. These materials require different supplements or additives with sufficient amounts of nitrogen, phosphate, potassium, and vitamins for better growth and yield of mushrooms [8]. The ratio of carbon to nitrogen (C : N) plays an important role in spawn running. Nitrogen supplementation is also an important factor for developing fruiting bodies on mushrooms [9]. Supplementing substrates with nutrients increases yields of *Pleurotus sajor-*

caju [10]. In addition, the effect of wheat bran supplement has also been reported in tropical and sub-tropical oyster mushrooms by Gurjar and Doshi [11]. Various additives (urea, ammonium sulphate, gram flour, soybean meal, mustard cake, cotton seed cake, and molasses) are recommended as substrate supplements prior to spawning to enhance oyster mushrooms [9]. According to Moda *et al.* [12], supplementing the substrate is a common method to increase productivity, which is evaluated by biological efficiency. The most common supplements are sources of organic nitrogen such as cereal bran, which are necessary for growth of the mycelial mass but may interfere with the productivity and biological efficiency of the mushrooms. The quantity and kind of bran varies according to the species or strain of mushroom as well as the growth stage. Rice bran is the most popular and available organic substrate additive for growing a number of edible mushrooms in Asia [13]. Supplementing rice straw with cereal bran and oil seed cakes increases the yield and quality of oyster mushrooms [14]. However, no research has been conducted on the effect of different supplements for cultivating milky white mushroom in Bangladesh. This study was designed to evaluate the suitable supplement of rice straw substrate for better growth and yield of *C. indica*.

Materials and Methods

Mushroom strain and supplements. Culture of *C. indica* was obtained from the National Mushroom Development and Extension Center (NAMDEC), Savar, Dhaka,

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Bangladesh. Rice bran, maize powder, and wheat bran of different levels (10, 20, 30, 40, and 50%) were used as supplements, and 0% was used as unsupplemented control. Supplements were collected from the NAMDEC farm.

Preparation of spawn and casing material. The substrates were chopped into 3–4 inch lengths. On a dry-weight basis, 0.2% CaCO₃ and the five different levels of rice bran, maize powder, and wheat bran supplements were added to the chopped substrates and mixed thoroughly. Water was added to a moisture level of 65%. Substrate (500 g) was added to polypropylene bags (7 × 10 inch), and the openings of the bags were plugged with cotton and secured with plastic rings. The bags were autoclaved at 121°C and 15 psi for 1 hr, after which they were inoculated with 2 teaspoons of a *C. indica* mother culture. The spawn packet was kept in a dark room and incubated at 30–32°C for approximately 30 to 35 days. Cow dung and loamy soil (3 : 1, v/v) was used as the casing material and was sterilized at 65°C for 4 hr. After mycelial colonization, the mouth of the spawn packet was covered with casing material and maintained with a 3 cm thickness level. It was then transferred to the culture room and maintained at a temperature of 30–35°C and a relative humidity of 70–80%.

Experimental design. The experiment was laid out in a completely randomized design with four replications. The following data were collected; the number of days required for the initiation of primordia, the number of effective fruiting bodies, length and diameter of the stalk, the diameter and thickness of the pileus, biological yield, economic yield, and biological efficiency. The data were analyzed according to standard methods using the MSTAT-C program. Means were compared using Duncan's multiple range test. Biological efficiency was measured using the following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield}}{\text{Total substrate used}} \times 100$$

Results and Discussion

Effect of supplements on primordia initiation and the number of effective fruiting bodies. Primordia formation was observed in all experimental sets. The shortest time (13.5 days) for primordia initiation was recorded in the 40% rice bran supplement, followed by the 20% (14.8 days), and 10% (15.0 days) wheat bran supplement. The longest time (19.3 days) was recorded in 0% level of unsupplemented control rice straw substrate (Table 1). The maximum number of effective fruiting bodies was found in the 30% maize powder supplement (16.8) treatment, followed by the 40% rice bran supplement (16.3), which was statistically similar (Table 1). The results indicate that 30% maize powder supplementation with rice straw substrate was most effective for fruiting body development in *C. indica*. Patra and Pani [15] reported that the time required for *C. indica* primordia initiation on rice straw was 13–16 days. Similar findings were also reported by Amin *et al.* [2] and Jiskani *et al.* [16]. Substrates containing glucose, fructose, and trehalose produced the greatest number of primordia, whereas abnormal fruiting bodies were produced with glycerol, xylose, sucrose, and fructose. Optimal fruiting body production occurred on glucose and fructose containing substrates [17]. Rice bran and maize powder are rich in nutrients, which might have promoted the growth and development of *C. indica* primordia and fruiting bodies.

Effect of supplements on yield-contributing characteristics. The maximum diameter of the pileus (7.1 cm) was obtained with the 30% maize powder supplement, followed by 30% wheat bran (6.9 cm) and 50% rice bran supplements (6.6 cm). The highest thickness of the pileus (2.4 cm) was found with 10% wheat bran supplement, followed by 30% wheat bran (2.2 cm) and 10% rice bran supplements (2.1 cm). The smallest diameter (5.1 cm) and thickness (1.4 cm) of the pileus was observed in the unsupplemented control set (Table 2). The maximum diameter of the stalk (2.8 cm) was obtained with 30% maize powder and 40% rice bran supplements, while the longest

Table 1. Effect of different levels of rice bran, maize powder, and wheat bran supplementation to rice straw substrate on the days to primordia initiation and the number of effective fruiting bodies of *Calocybe indica*

Level of supplement (%)	Primordia initiation (days)			No. of effective fruiting bodies		
	Rice bran	Maize powder	Wheat bran	Rice bran	Maize powder	Wheat bran
0	19.3a	19.3a	19.3a	7.3gh	7.3gh	7.3gh
10	16.8bcd	16.8bcd	15.0fg	7.0gh	12.8b	10.8cde
20	17.3bc	16.3cde	14.8g	12.3bc	10.5cde	11.0bcde
30	17.5b	16.0def	16.5bcde	11.8bcd	16.8a	10.0de
40	13.5h	15.8defg	15.8defg	16.3a	10.0de	8.0fg
50	15.0fg	16.5bcde	15.5efg	11.8bcd	9.3ef	5.5h

The same letters within a column are not significantly different by Duncan's multiple range test at the 5% level.

Table 2. Effect of different levels of rice bran, maize powder, and wheat bran supplementation to rice straw substrate on the diameter and thickness of the pileus of *Calocybe indica*

Level of supplement (%)	Diameter of pileus (cm)			Thickness of pileus (cm)		
	Rice bran	Maize powder	Wheat bran	Rice bran	Maize powder	Wheat bran
0	5.1de	5.1de	5.1de	1.4e	1.4e	1.4e
10	5.7bcd	4.1e	5.6bcd	2.1abc	1.6de	2.4a
20	6.4abc	6.5ab	5.9abcd	1.9bcd	1.9bcd	1.8bcd
30	6.1abcd	7.1a	6.9ab	1.7de	1.8bcde	2.2ab
40	5.7bcd	5.8bcd	6.2abcd	1.7de	1.7cde	1.5de
50	6.6ab	5.2cde	5.8bcd	1.7de	1.6de	1.8cde

The same letters within a column are not significantly different by Duncan's multiple range test at the 5% level.

Table 3. Effect of different levels of rice bran, maize powder, and wheat bran supplementation to rice straw substrate on the diameter and length of the stalk of *Calocybe indica*

Level of supplement (%)	Diameter of stalk (cm)			Length of stalk (cm)		
	Rice bran	Maize powder	Wheat bran	Rice bran	Maize powder	Wheat bran
0	1.7f	1.7f	1.7f	5.5f	5.5f	5.5f
10	2.4bcd	2.6ab	2.4bcd	7.3de	7.2de	8.7bc
20	2.4bcd	2.4bcd	2.5bc	8.5bc	7.4de	8.4bc
30	2.7ab	2.8a	2.1de	10.3a	8.8bc	8.7bc
40	2.8a	2.2cd	1.8f	7.0de	7.8cd	8.9b
50	2.5bc	1.9ef	2.1de	10.1a	6.8e	6.4ef

The same letters within a column are not significantly different by Duncan's multiple range test at the 5% level.

Table 4. Effect of different levels of rice bran, maize powder, and wheat bran supplementation to rice straw substrate on the biological and economic yield of *Calocybe indica*

Level of supplement (%)	Biological yield (g/packet)			Economic yield (g/packet)		
	Rice bran	Maize powder	Wheat bran	Rice bran	Maize powder	Wheat bran
0	173.8k	173.8k	173.8k	171.3k	171.3k	171.3k
10	205.5j	291.0fg	211.0j	203.3j	288.5fg	207.8j
20	236.8i	303.8f	266.8h	233.8i	301.3f	263.8h
30	272.3gh	459.3a	391.0b	269.8gh	457.0a	389.0b
40	388.8bc	370.3c	322.8e	386.5bc	368.0c	320.3e
50	344.8d	268.3h	260.3h	342.3d	265.8h	257.8h

The same letters within a column are not significantly different by Duncan's multiple range test at the 5% level.

stalk length (10.3 cm) was recorded with 30% rice bran supplement (Table 3). The results revealed that 30% maize powder and rice bran supplementation to rice straw substrate was significantly effective for contributing to the yield of *C. indica*. These findings are comparable with those of previous studies using *C. indica* [18, 19]. It is likely that rice straw substrate supplemented with maize powder has a high C:N ratio, which resulted in enhanced development of the pileus [20].

Effect of supplements on biological yield, economic yield, and biological efficiency. A significant variation was observed in the different levels of rice bran, maize powder, and wheat bran supplementation to rice straw substrate on biological yield, economic yield, and biological efficiency of *C. indica*. The maximum biological (459.3 g) and economic yield (457.0 g) was obtained with the 30%

maize powder supplement. The lowest biological (173.8 g) and economic yield (171.3 g) was found with the unsupplemented control set (Table 4). The biological efficiency results for the different levels of rice bran, maize powder, and wheat bran supplements are presented in Figs. 1~3. The Fig. 1 results indicate that the maximum biological efficiency (77.8%) was recorded with 40% rice bran. The highest biological efficiency (91.9 and 78.2%) was with 30% maize powder and wheat bran supplements (Figs. 2 and 3). Increasing the amount of supplement resulted in an increase in biological efficiency up to 30% and then the efficiency decreased again. Such a reduction in efficiency may be due to the compactness or poor aeration of the substrates, which results from insufficient utilization of nutrients. This may also be due to the limitation of spaces or surfaces for developing fruiting bodies [21]. The cereal bran and maize powder supplementation promoted the

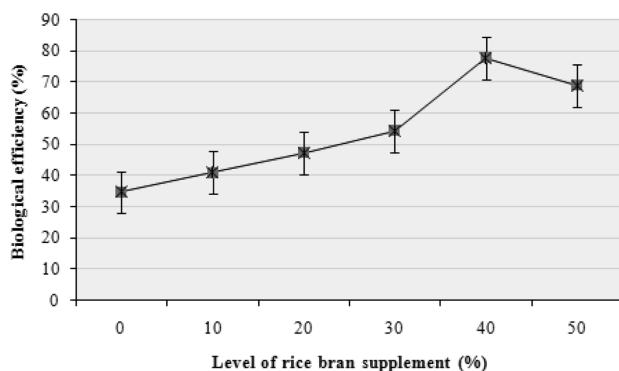


Fig. 1. Effect of different levels of rice bran supplementation to rice straw substrate on the biological efficiency of *Calocybe indica*. Vertical bars represent the standard error.

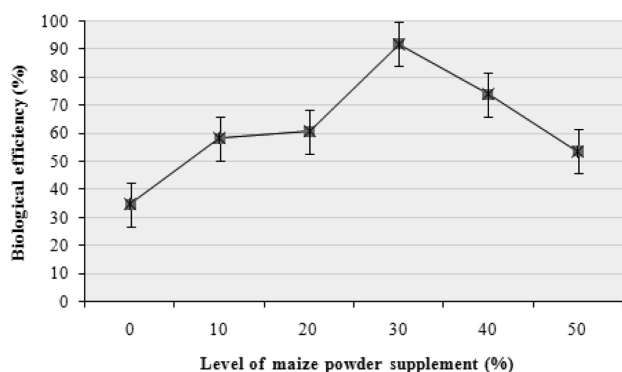


Fig. 2. Effect of different levels of maize powder supplementation to rice straw substrate on the biological efficiency of *Calocybe indica*. Vertical bars represent the standard error.

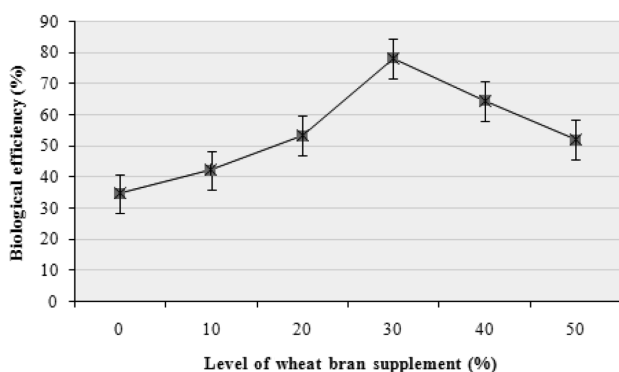


Fig. 3. Effect of different levels of wheat bran supplementation to rice straw substrate on the biological efficiency of *Calocybe indica*. Vertical bars represent the standard error.

secretion of cellulase(s), hemicellulase(s), and laccases which involved in the degradation of cellulose, hemicellulose and lignin, respectively [22, 23]. The degraded carbohydrates serve as energy sources for constructing the

structural components of the fruiting body. In view of the above, it can be concluded that the yield, yield contributing characteristics, and biological efficiency in the supplemented sets increased as compared to the unsupplemented control set, whereas a reduced yield was noted in the higher levels of supplements. The 30% maize powder supplement was the best supplement of rice straw substrate for cultivating milky white mushroom.

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