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Department of Agricultural Extension
Ministry of Agriculture
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Bangladesh

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Books:

Gomez, K. A. & Gomez, A. A. 1984. Statistical Procedures of Agricultural Research, 2nd ed., John Wiley and Sons, Singapore. p. 21.

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Bangladesh Journal of Mushroom

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Effect of Different Sterilization Techniques on Growth and Yield of Oyster Mushroom

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Abstract

The sterilization technique is crucial in all stages of mushroom production, including pure culture, mother culture, and spawn production. Without proper substrate sterilization, contamination can occur at any of these stages, leading to production losses and negatively impacting entrepreneurs. While many farmers and entrepreneurs continue to use traditional sterilization techniques, there is a growing need for modern and updated methods to improve mushroom yields. In this study, we evaluated four different sterilization techniques: Autoclave, Drum, Stainless Steel (SS) chamber, and Mild Steel (MS) chamber sterilization. Among these, the SS chamber sterilization technique proved to be the most effective, achieving a yield of 197.0 g per packet with a biological efficiency of 65.78%. The highest contamination rate (66%) was observed with the drum sterilization method, with green mold infestation significantly higher (84%) than in other treatments. In our country, most small-scale mushroom farmers and entrepreneurs opt for drum sterilization due to its low cost and minimal technical requirements, whereas autoclave sterilization is more complex and costly. Given these circumstances, SS chamber sterilization emerges as a more cost-effective and efficient option compared to autoclaving. The findings from this study may help inform the choice of suitable sterilization techniques for improving mushroom production.

Keyword: Sterilization, autoclave, drum sterilization, ss chamber sterilization, ms chamber sterilization, contamination.

INTRODUCTION

Pleurotus ostretaus(oyster mushrooms) are the second largest commercially cultivated mushroom in the world (Royse *et al*, 2017). Mushroom cultivation is a possible way to enhance income level in rural area and developing countries with low cost of production (Masarirambi, Mamba & Earnshaw, 2011). Besides mushroom has a good flavor, taste, high nutritional and medicinal properties (Ganeshan *et al.,*1989).

The use of mushroom is upraising and entrepreneurs are also interested for mushroom cultivation. But traditional sterilization technique has been practicing which causes increasing of contaminant and lose productivity. There are many experiments was conducted for betterment of sterilization technique. An experiment was taken place in Nepal, with 3 different treatments such as chemical, steam and hot water sterilization techniques. Where steam sterilization had the shortest period of time for pinhead initiation however chemical treatment (formaldehyde + carbendazim) showed the longest period. (Shrestha *et al.,* 2021).

An experiment was conducted in Sher-e-Bangla Agricultural University, Bangladesh with the help of Mushroom Development Institute, Savar, Bangladesh to investigate the impact of substrate sterilization technique and spawning methods on yield and yield contributing

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character. And the result reveled that hot water treatment with three layer of spawning was the best sterilization technique. (Ahmed *et. al.,* 2020)

An experiment where paddy straw was used as the substrate, was conducted in India. In that study five treatment (hot water treatment, autoclaving, treatment with formaldehyde solution, treatment with bavistin and treatment with normal water) were used for sterilization. It was found that autoclave sterilization was better than bavistin and formaldehyde treatment behaved poorly (Kalita, 2015). This experiment was conducted to identify better sterilization techniques for mushroom production and to calculate the contamination rate in different sterilization techniques.

MATERIALS AND METHODS

The experiment was designed out to find out the effect of sterilization technique on growth and yield of oyster mushroom and was carried out at tissue culture laboratory and culture house of Mushroom Development Institute, Savar, Dhaka during the period from July to September 2023. The study was laid out in Completely Randomized Design (CRD) with 4 replications and four treatment combinations. One oyster mushroom variety PO_2 with 500g size Spawn packet was prepared by using different treatment.

Treatment: Four different sterilization technique such as autoclave sterilization, drum technique sterilization, ss chamber and ms chamber sterilization were prepared with 4 replications and 8 packets per replication. Total (4 replications×8 packets per replication×4 treatments) 128 packets were used for this study. Sawdust spawn packets were tested as basic material. The sawdust spawn packet was prepared with 18cm×25cm polypropylene bags at 500 g/bag. The neck of the bag was prepared by using heat resistant plastic pipe. A hole of about 2/3 deep of the bag was made for space to introduce the mycelium. The neck of each poly bags was plugged with cotton, covered with brown paper or another polythene and tied with a rubber band. The packets were sterilized in –

T_1=Autoclave; sterilization for 2 hours at 121^{0}C under 1.5 kg/cm^2 pressure
T_2=Drum; sterilization for 4 hours at (water steam temp.)
T_3=SS chamber; sterilization for 4 hours at (water steam temp.)
T_4=MS chamber; sterilization for 4 hours at (water steam temp.)

After sterilization, the packets were inoculated separately with oyster mother (PO2) mother at the rate of two tea spoonful per packet. The inoculated packets were incubated at 20±2^{0}C.

Mycelium running in spawn packet: The packets were kept at 20±2^{0}C. temperature until the packets become white with mushroom mycelium. After completion of mycelium running the rubber band, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Then these spawn packets were transferred to the culture house.

Opening the packet: Two end, opposite to each other of upper placed position i.e. on shoulder of plastic bag were cut in 'D' shaped with a blade and opened by removing the plastic sheet.

Cultivation of spawn packet: The moisture of the culture house was maintained 80-85% relative humidity. Spraying water were done 3-5 times a day.

Collection and Analysis of Data: The packets were arranged in culture house following completely randomized design each treatment with 4 replications. Data on Days required to complete mycelium running, Days required opening to first harvest, number of fruiting body and effective fruiting body, length and diameter of stalk, diameter and thickness of pileus, Weight of fruiting body, yield (g/packet), biological efficiency (Be%) and Contamination rate

(%) were recorded. Weight of fruiting body was recorded after removing the lower hard and dirty portion of stipe. The biological efficiency was determined using the following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield } \left(\frac{g}{\text{packet}}\right)}{\text{Total dry weight of the substrate used } \left(\frac{g}{\text{packet}}\right)} \times 100$$

Data were analyzed using MS excel and Statistic 10 computer program.

RESULTS AND DISCUSSION

Days required to complete mycelium running: Days to complete mycelium running showed significant variation. Different treatment showed influence of mycelium growth under the different sterilization techniques. T_4 required (23.86 days) to complete mycelium running which was significantly lowest days among all four treatments (Fig. 1). The highest time (25.8 days) from inoculation to complete running was observed in the T_2 (Fig. 1). All spawn packet was raised in a common culture house and same environmental condition at 20±2°C.

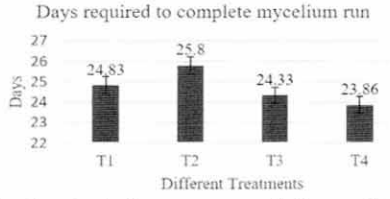

Fig. 1. Effect of different sterilization technique on mycelial growth.

Days required from packet opening to first harvest: Different treatment showed influence on days required from Packet opening to 1st harvest. Significant variation was found in days required for packet opening to 1st harvest. The lowest time (15.87 days) was observed in T_3 while the highest time (20.33 days) was found in T_2 (Fig. 2). In terms of mycelium running to packet opening, Atila, F. (2016) found that hot water sterilization technique was more effective instead of chemical sterilization.

Fig. 2. Effect of different sterilization technique from packet opening to harvesting

Number of fruiting body and effective fruiting body/packet: Variation among treatment showed the highest number of fruiting body (20.25) observed in treatment T_3. And the lowest average number of fruiting body (16.67) was in T_2 treatment (Fig. 3). There was significant difference among different treatment on number of fruiting body and effective fruiting body. The highest average number of effective fruiting body also came from T_3 treatment (9.63) where lowest average number of effective fruiting body also found in T_2 treatment (7.33) (Fig. 3). Experiment showed that T_1 and T_4 had statistically similar in number of fruiting body and number of effective fruiting body (8.6,18.4 and 8.8,18.6 respectively).

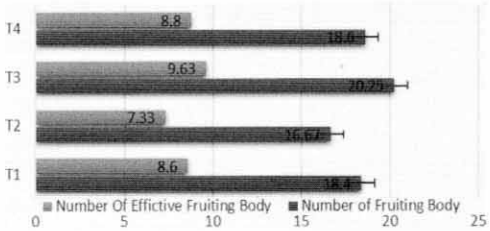

Fig. 3. Effect of different treatment on number of fruiting body per packet

Length of stalk (cm): A significant variation in length of stalk was found among the varieties. The highest length of stalk (4.36 cm) was obtained from T_1 treatment (autoclave sterilization) which was statistically similar to T_2 treatment (drum sterilization) (Table 1). While the lowest length of stalk (3.84 cm) was obtained from T_4 treatment (ms chamber sterilization) which was statistically similar to T_3 treatment (ss chamber sterilization) (Table 1).

Diameter of stalk (cm): A significant variation in diameter of stalk was found among the varieties. Height diameter of stalk (1.50 cm) was found in T_3 treatment (ss chamber sterilization) which was significant than other treatments (Table 1). The lowest diameter of stalk (1.04 cm) was found in T_4 treatment (ms chamber sterilization) (Table 1).

Diameter of Pileus (cm): A significant variation in diameter of pileus was found among the varieties. The largest diameter of pileus (7.10 cm) was obtained from T_4 treatment and the shortest diameter of pileus (5.47 cm) was obtained from T_3 treatment. In this case, T_1 treatment was significantly similar to T_2 andT_3 treatment (Table 1).

Thickness of Pileus (cm): A significant variation in thickness of pileus was found among the varieties. The maximum thickness of pileus (0.45 cm) was found in T_3 treatment (Table 1). The lowest thickness of pileus (0.30 cm) was observed in T_2 treatment which was statistically similar to T_1 treatment (Table 1).

Average weight of fruiting body (g): A significant variation in fruiting body weight was found among the varieties. The maximum weight of fruiting body (63.30 g) was found in T_3 treatment (Table 1). Whereas the lowest weight of fruiting body (52.20 g) was observed in T_2 treatment which was statistically similar to T_1 and T_4 treatment (Table 1).

Yield (g/packet): A significant variation of yield per packet was found among the varieties. The maximum yield per packet (197 g) was found in T_3 treatment which was statistically similar to T_1 treatment (Table 1). And the lowest yield per packet (171.7 g) was found in T_2 treatment (Table 1). In case of yield per packet T_4 treatment was statistically similar to T_1, T_2 and T_3 treatment (Table 1).

Table 1. Effect of different sterilization technique on fruiting body and yield of oyster mushroom

Treatment	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Average weight of fruiting body (g)	Yield per packet (g)	Biological efficiency %
T_1	4.36 a	1.38 b	5.74 bc	.32 c	54.50 b	186.5 a	62.17 ab
T_2	4.33 a	1.33 b	5.47 c	.30 c	52.20 b	171.7 b	57.23 c
T_3	3.86 b	1.50 a	7.10 a	.45 a	63.30 a	197.0 a	65.78 a
T_4	3.84 b	1.04 c	6.03 b	.40 a	53.33 b	183.3 ab	61.1 bc
$LSD_{(0.05)}$	0.3092	0.0997	0.4606	0.0281	4.2179	13.921	4.6427
CV (%)	4.01	4.03	4.02	4.05	4.01	4	4

Biological Efficiency (Be%): The highest biological efficiency (65.78%) was found in T_3 followed by T_1 (62.17%) treatment. The lowest biological efficiency (53.23%) was found in T_2 treatment (Table 1). Treatment T_4 was statistically similar to treatment T_1 and T_2 (Table 1). Alam

& Singha (2020) and Sarker *et al.* (2012), found that hot water sterilization gave maximum biological efficiency of *V. volvacea.*

Contamination rate (%): There was a significant contamination rate in different treatment. 36% to 66% contamination found in different treatment where T_2 treatment showed 1st contamination in 4th day and 66% contamination in 13 days to inoculation (Fig. 4). T_3 showed lowest contamination (36%) where T_1 and T_4 found 41% and 47% contamination respectively (Fig. 4).

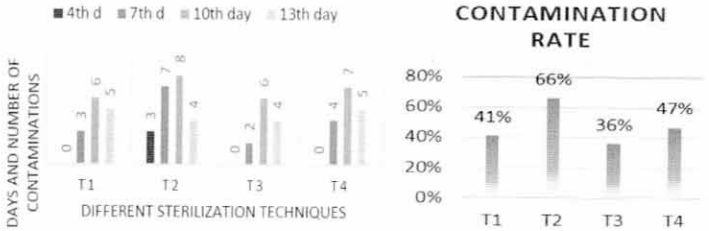

Fig. 4. Effect of different treatment on contamination.

A significant contamination showed different range from 5% to 84% by green mould, orange mould and false mycelium. The height contamination was found by green mould (84%) and lowest contamination rate (5%) was found by orange mould (Fig. 5). False mycelium was also found from different treatment (11%).

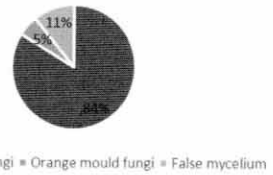

Fig. 5. Contamination of different fungi.

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Bangladesh J. Mushroom. **17**(2): 6-9. 2023 (December)

Yield Performance and Morphological Attributes of Three Strains of Pink Oyster Mushroom (*Pleurotus djamour*) on Sawdust Substrate

Mst. Moka Shefa, Md. Ferdaus Ahmed, Ripon Prosad Saha, Titun Biswas, Abdullah Hel Mafi and Md. Maniruzzaman

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Abstract

This study was conducted at the Mushroom Development Institute to evaluate the yield performance and morphological characteristics of three different strains of *Pleurotus djamour*. Although mushrooms are becoming increasingly popular, many people are still unfamiliar with the different varieties. Although mushrooms are becoming increasingly popular, many people are still unfamiliar with the different varieties. Significant variations were observed in yield and morphological attributes among the strains. POP 3 exhibited the highest number of fruiting bodies (37.13), effective fruiting bodies (12.32), stalk length (2.81 cm), and average fruiting body weight (65.25 g/packet). POP 2, however, showed the largest stalk diameter (1.03 cm) and pileus diameter (12.53 cm). The highest biological efficiency (40.66%) was also recorded in POP 2. The results suggest that both POP 2 and POP 3 strains are promising for future production.

Key words: *Pleurotus djamour,* Pink, Oyster, Mushroom, POP, Color, Shape.

INTRODUCTION

Pleurotus spp. are most popular kind of mushroom under the class of Basidiomycetes (Mondal *et al.,* 2010). Oyster mushroom have significant role in human health (Rathod *et al.,*2021).So world's most leading countries specially China, Southeast Asia, Europe, Africa are enhancing the production of oyster mushroom day by day (Singh *et al.,* 2018).Oyster mushrooms is rich in nutritional value has with dried protein (25-50%), fat (2-5%), sugars (17-47%), mycocellulose (7-38%) and minerals (potassium, phosphorus, calcium, sodium) of about 8- 12% (Sher, 2011). The oyster mushrooms (*Pleurotus* spp.) are in the third place after the white button and shiitake among the world mushroom production (Győrfi *et al.,* 2007).

Pleurotus djamour or pink oyster mushroom is very popular for its color, having outstanding flavor and taste (Sher, 2011). Fruiting body of mushroom is used for consumption which contains high nutrients and medicinal values. Oyster mushroom needs 18-25^{0}C with high humidity 80 to 95% for fruiting body formation and 25-30^{0}C with 80 to 90% humidity for vegetative growth (Onyango *et al.,* 2011 and Nadir *et al.,* 2016).

Mushrooms cultivation is one of the most commercially important steps towards diversification of agriculture. In this study we found the best variety of pink oyster mushroom (*Pleurotus djamour)* according to its morphological character and yield attributing character. Mushrooms can play an important role to reduce poverty and meet the nutritional requirements of the population of Bangladesh.

MATERIALS AND METHODS

The experiment was conducted in the culture house of National Mushroom Development Institute, Sobhanbag, Savar, Dhaka, Bangladesh from September to November, 2023. Three different strains of *Pleurotus djamour* (POP1, POP2 & POP3) were used for experiment. For 100 sawdust packets we used – sawdust:16kg, wheat barn: 8kg, rice husk: 1kg, calcium carbonate: 100g and water: 50% where the weight of each packet was 500g.

Spawn packet preparation: Saw dust spawn packets were prepared in workshop and sterilization was done in autoclave for 2 hours at 121^{0}C under 1.5 kg/cm^2 pressure. Inoculation was done in inoculation chamber with maintained environment at 22±2^{0}C with sterilized equipment and three different pink mushroom varieties.

Experimental condition: All spawn packets were kept in culture house at 22±2^{0}C. After colonization of mycelium was fully completed then packets were taken to another culture house for packet opening and harvesting. Packets were cut in "D shape" style on the shoulder of a packet. Relative humidity and temperature were maintained at 80-90% and 25-30^{0}C respectively. Natural light about 200±5 lux and proper ventilation were maintained in culture house. The yield was obtained from individual number of packet and calculated weight (yield/packet) after removing lower dirty portion.

The biological efficiency was calculated according to the formula:

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

Morphological attributes: Morphological attributes were observed by color and shape of pink oyster mushroom. Differences of color, variation of fruiting body structure and size of fruiting body were prioritized for this study.

Data collection and statistical analysis: The experiment was laid out with 4 replications in Complete randomized design (CRD) design. Data on Days required to complete mycelium running, Days required from pinhead initiation to 1st harvest, number of fruiting body and effective fruiting body, length and diameter of stalk, diameter and thickness of pileus, Weight of fruiting body, yield (g/packet), biological efficiency (Be%) were recorded. Computer program Statistic 10X, MS Excel and MS power point were used for analyze the experimental data.

RESULTS AND DISCUSSION

Days require to complete mycelium running: Different strains showed different time to complete mycelium running. Time variation within 16 days to 19 days from inoculation to complete mycelium running where POP2 showed lowest time (16.68 days) (Fig. 1). The highest time (18.38 days) from inoculation to complete mycelium running was found in POP1 compared to POP 3 (16.75 days) (Fig. 1). All spawn packet was raised in a common culture house and same environmental condition at 22±2^{0}C.

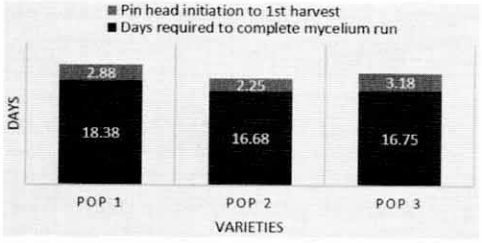


Fig. 1. Days required from inoculation to complete mycelium in different Pink Oyster

Days require from pinhead initiation to 1st harvest: Three different strains showed non significance variation in terms of days require from pinhead initiation to 1st harvest. POP2 showed lowest time (2.25 days) to 1st harvest where POP3 required highest time (3.18 days) compared to POP1 (2.88 days) (Fig. 1). Lowest time for harvesting is important for production and economic benefit.

Number of fruiting body and effective fruiting body: A significant variation in terms of number of fruiting body and number of effective fruiting body was found among different varieties of pink oyster mushroom. The highest number of fruiting body (37.13) was found in POP 3 where lowest (11.94) was found in POP 1 (Table 1). Besides the highest number of effective fruiting body (12.31) was also found in POP 3 whereas POP 1 (6.69) & POP 2 (6.94) was showed statistically similar results in terms of number of effective fruiting body (Table 1).

Length and diameter of stalk (cm): Significant variation among varieties was found in terms of length of stalk and diameter of stalk. POP 3 showed the highest length of stalk (2.81 cm) whereas lowest diameter of stalk (0.65 cm) was also found in POP 3 (Table 1). The lowest length of stalk was obtained from POP2 (1.57 cm) since highest diameter of stalk (1.03 cm) was obtained from POP 2 (Table 1).

Diameter and thickness of pileus: A significant variation in diameter of pileus was found among the varieties. The highest diameter of pileus (12.53 cm) was obtained from POP 2 where POP 3 gave lowest result (10.28 cm) (Table 1). Whereas POP 1 showed the highest result in thickness of pilus (0.69 cm) according to POP 2 (0.59 cm) and POP 3 (0.41 cm) respectively (Table 1).

Average weight (g): In terms of average weight of different straits of pink oyster mushroom the highest weight (65.25 g) obtained from POP 3 which was statistically similar to POP 2 (60.13 g) (Table 1). POP 1 gave the lowest average weight (53.63 g) among three different strains (Table 1).

Table 1. Morphological differences among three different strains of pink oyster mushroom

Variety	NFB	NEFB	LS (cm)	DS (cm)	DP (cm)	TP (cm)	Average weight (g)
POP 1	11.94 C	6.69 B	1.96 B	0.96 A	12.22 A	0.69 A	53.63 B
POP 2	15.75 B	6.94 B	1.57 C	1.03 A	12.53 A	0.59 B	60.13 A
POP 3	37.13 A	12.31 A	2.81 A	0.65 B	10.28 B	0.41 C	65.25 A
$LSD_{(0.05)}$	2.29	0.89	0.21	0.09	1.24	0.06	6.28
CV(%)	4.9	4.58	4.4	4.87	4.71	4.78	4.64

Economic yield: Economic yield per packet is important for producer and farmers. Significant differences found in terms of economic yield per packet. POP 2 found highest economic yield (122 g/packet) where POP 1 gave lowest (105 g/packet). POP 3 had slightly lower yield (118 g/packet) from POP 2 (Table 2).

Table 2. Morphological differences among three different strains of pink oyster mushroom

Variety	Economic yield (g/packet)	Biological efficiency%
POP 1	105 c	35
POP 2	122 a	40.66
POP 3	118 b	39.33

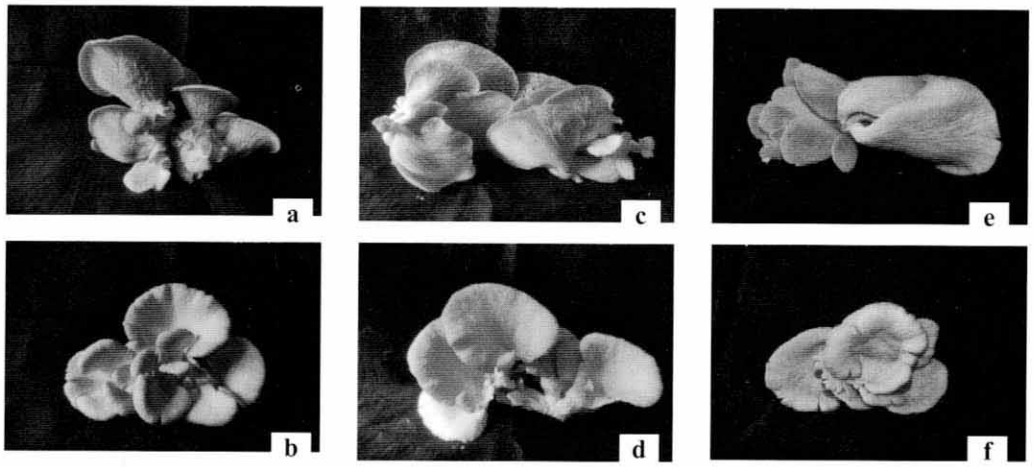
Biological efficiency%: The highest biological efficiency (40.66%) was found in POP 2followed by POP 3(39.33%). The lowest biological efficiency (35%) was found in POP 1 (Table 2). Biological efficiency was calculated from overall yield per packet divided by total dry substrate.

Morphological Attribute: Morphological attribute is important for consumer attraction and preferences. Three different mushrooms of *Pleurotus djamour* (var. POP 1, POP 2 and POP 3) showed significant differences in color and shape.

POP 1: The color of *Pleurotus djamour* var. POP 1 was deep pink. The outside of pilus was curly in shape and the upper portion of pilus was also curly. Color of pilus was evenly distributed (Fig. 2).

POP 2: The color of *Pleurotus djamour* var. POP 2 was light pink. The outside of pilus was straight in shape and the upper portion of pilus was also straight. Color of pilus was evenly distributed (Fig. 2).

POP 3: The color of *Pleurotus djamour* var. POP 3 was light pink. The outside of pilus was straight in shape but the upper portion of pilus was also curly. Color of pilus was light but deep color of pink circle was found in border of pileus (Fig. 2).



POP 1: a) Side view b) Upper view. Colour: Deep pink. Shape: Curly.

POP 2: c) Side view d) Upper view. Colour: Light pink. Shape: Straight.

POP 3: e) Side view f) Upper view. Colour: Light pink. Shape: Straight and curly pilus

Fig. 2. Morphological appearance on color and upper and side view of Pink Oyster.

It is concluded that POP 1 was curly in shape which was less attractive than POP 2 and POP 3. Other hand, POP 2 and POP 3 are similar in shape where POP 2 slightly deep pink color compared to POP 3 pink oyster mushroom. For future, nutritional analysis of three different pink oyster mushroom varieties will require for identify the quality and nutritional properties for fulfillment nutritional needs of Bangladesh.

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Challenges of Mushroom and Mushroom Seed Production in Bangladesh

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Abstract

This study attempts to illustrate the present challenges of mushroom and mushroom seed production in Bangladesh. It is also exploring issues and problems facing the Bangladeshi mushroom farmers by data analysis. The sample size for the study was 90 from 44 districts of Bangladesh. Though, Bangladesh has all the requisites of low-cost labor, favorable climate conditions, supply of raw materials, tribal demandable mushroom market etc. however, the main challenges of mushroom and mushroom seed production in Bangladesh include inadequate amount of fund to buy essential equipment's, lack of adoption of new technology, poor management of disease infestation, traditional sterilization techniques, poor distribution channel of mushroom mother and lack of marketing. The study concludes that there is an urgent need to remove the barriers and overcome the challenges of mushroom production.

Keywords: Mushroom production, Mushroom seed production, Challenges, Problems, Bangladesh.

INTRODUCTION

Mushroom is an edible fungus with high functional and nutritional value. Among 14000 described species of the millions of fungi, approximately 200 species have been successfully grown at laboratory scale (Krik *et al.,* 2008). In terms of edibility, only more than 10 species of mushrooms are considered as safe farming at industrial scale among 7000 species of mushroom (Chang, 2006; Chang and Miles, 2004 and Martins, 2017). Naturally, Bangladesh is diversified climatic condition where *Pleurotus* spp. grows in wide range of temperature (15-30°C) which also varies from species to species. (Sarker *et al.,* 2008).

Undoubtedly mushrooms were used as a glorious food from the ancient time (Aneja, 2001). During 19th century mushroom became popular and mushroom society were formed (Chang. 2006). Now in new ear, global mushroom industry has formed with modern technology (Royse *et al.,* 2017). Besides, healthy eating by cutting down the calories, saturated fat and cholesterol, mushrooms are bound to attract the attention (Zhang *et al.,* 2014). The fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins (Miah *et al.,* 2017).

During 2018 to 2019, Bangladesh produced 4000 MT mushrooms including oyster, rishi, milky, button, straw and shiitake mushrooms with lucrative benefit cost ratio (BCR 1.55-4.25) (Ferdousi *et al.,*2020). Though increasing mushroom production in Bangladesh, there are some problems confronting by the mushroom farmers during cultivation and marketing including lack of cultivation house (Rahman, 2018; Gautam *et al.,* 2014), unavailability of good seed & spawn (Singh & Suresh, 2007), capital shortage, lack of equipment's, lack of available market and promotion in local level (Karthick & Hamsalakshmi, 2016), lack of storage facilities etc. which are needed to be addressed for further development of this sector. There is enormous opportunity of expanding mushroom farming throughout the country.

Considering the country's limited land, over and unemployed population, strengthening the production of mushroom could be one of the sustainable options for the development of

rural economy. Development of this sector would also improve the diversified business and employment opportunities both in the rural and semi-urban areas.

MATERIALS AND METHODS

The present study was conducted in 44 districts of Bangladesh with mushroom and mushroom seed grower. The respondents were classified by problem marking which were related in mushroom production. The problem was identified with snow ball sampling method. The farmers who were involved in mushroom and mushroom seed production were included as respondents.

Total 90 respondents were selected from 44 districts of Bangladesh. The data were collected from the respondents with previously prepared questioner. The interview was conducted in person to person. All the respondents were interviewed during June 2023 to January 2024. Challenges of mushroom and mushroom seed production were studied in some criteria which actually faced by grower. The respondents were recorded as agree or disagree in some of matters, identified using methods, according to availability and demand status. Ranking of the problems were done based on the respondent's response.

RESULTS AND DISCUSSION

Main Problems Faced by Respondents in Mushroom and Mushroom Seed Production: The problem faced by the respondents on mushroom and mushroom seed production were assessed including different aspects such as financial problem, production problem, contamination problem, equipment problem, problems related to seed quality and seed accessibility, marketing problem including people's preferences. It is evident from Fig. 1. that 38% of respondents faced financial crisis in mushroom seed production where production and contamination problem represent 15% and 18% accordingly. 15% respondents faced problem in equipment crisis. Therefore, mushroom and mushroom seed marketing problem identified at 14%.

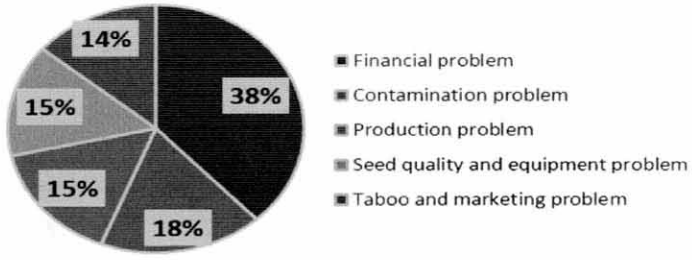

Fig. 1. Distribution of problems in mushroom and mushroom seed production according to respondents' criteria.

Ranking of Financial Problems in Mushroom and Mushroom Seed Production: Ranking of financial problem related to mushroom and mushroom seed production faced by the respondents is presented in Table 1. It is observed that 'Lack of fund to buy essential equipment's to grow mushroom' ranked I where 77% of respondents agreed with this point. There is also 'Lack of government loan facilities for mushroom farming' faced by respondents which is in rank II. In mushroom production there is no 'Government subsidy in Mushroom Production' which is in rank III. As a result farmers need financial support or loan support to start mushroom production. This data was supported previous experiment with Saikia and Bora (2023) and sharma *et al.*

Table 1. Ranking of Financial Problems in Mushroom Seed Production Faced by the Respondents

Sl. no	Statements	Precents of respondents		Rank
1	Lack of fund to buy essential equipment's to grow mushroom	Agree	77%	I
		Disagree	23%	
2	Lack of government loan facilities for mushroom farming	Didn't take loan	83%	II
		Took loan	17%	
3	Non availability of government subsidy for mushroom farmers	Agree	100%	III
		Disagree	0%	

Ranking of Problems Related to Mushroom Production and Contamination Faced by the Respondents: Table 2 shows that Disease infestation is the main problem ranked I. Green mold and orange mold infestation occurred at 13.33% where green mold infestation was high in percent 48.88%. It is noted that various types of contamination problem occurred due to mushroom production and mushroom growers expressed that they would be sufferer mushroom production in future. It is observed that uses of sterilization technique in mushroom and mushroom seed production was traditional and farmers used steel drum along at 81.11% for pasteurization where drum and autoclave both used 13.33% of farmers (Table 2) which was in rank II. Lack of mushroom varieties production in Bangladesh is another problem ranked III. 71.11% of grower cultivated oyster mushroom where Button, shitake and milky mushroom cultivated accordingly 6.66%, 5.55% and 4.44% (Table 2). Seasonal variation of mushroom cultivar was also identified as rank IV problem in mushroom cultivation. All data in line with Sharma *et al,*(2017).

Table 2. Ranking of Problems Related to Mushroom Production and Contamination

Sl. no	Statements	Percents of respondents		Rank
1	Disease infestation in mushroom seed production	Green mold	48.88%	I
		Orange mold	4.44%	
		False mycelium	2.22%	
		Bacteria	5.55%	
		Green and orange mold	13.33%	
		Green, orange and false mycelium	21.11%	
		Others	4.44%	
2	Used of sterilization technique in mushroom and mushroom seed production	1= Drum technique	81.11%	II
		2= Autoclave	3.33%	
		3= MS sterilization	0%	
		4= SS sterilization	0%	
		5= Drum and autoclave	13.33%	
		6= Autoclave and MS/SS	2.23%	
3	Cultivation of different varieties of mushroom and mushroom seed	Oyster	71.11%	III
		Milky	4.44%	
		Button	6.66%	
		Shitake	5.55%	
		All Kind of Mushroom	12.24%	
4	Seasonal variation in mushroom production	1=Summer	25.56%	IV
		2=Winter	28.88%	
		3=All year round	45.56%	

Ranking of Seed Quality and Technical Support Related to Mother/Spawn Production: For mushroom cultivation good quality of mother/spawn is significantly important where respondents identified unavailability good quality (44.44%) of mother/spawn as rank I (Table 3). It is important to noted that mushroom mother/spawn collection point was restricted which is in rank II. 16.66% grower produced their own seed and 46.66% depended to other grower (Table 3). Mushroom Development Institute plays an important role as a seed/mother/spawn collection center (36.68%). For mushroom production, there are many technical equipment require in a mushroom farm. But lack of availability (34.44%) and insufficient amount of technical support (36.66%) this problem ranked as problem III (Table 1). Required of training facilities ranked as IV. Due to lack of those facilities, mushroom grower are demotivated for mushroom production which was also reported by Sharma *et al.* (2017).

Table 3. Ranking of Seed Quality and Technical Support

Sl. no	Statements	Percents of respondents		Rank
1	Unavailability of quality mother/ spawn	Good	44.44%	I
		Medium	54.44%	
		Bad	1.12%	
2	Lack of training in mushroom production	Required training	73%	IV
		Not required training	27%	
3	Unavailability of mushroom mother/spawn collection center	Own produce	16.66%	II
		Collect from - Mushroom Development Institute, Savar	36.68%	
		Collect form others farmer/ grower	46.66%	
4	Lack of technical equipment in local area	1= Available	28.90%	III
		2= Not available	34.44%	
		3= Insufficient	36.66%	

Ranking of Problem Related to Consumption and Food Habit: Consumption and food habit related problem faced by the respondent on Mushroom production presented in the table 4. It is evident that social taboo and misconception about mushroom consumption ranked I (Table 4). But still mushroom is very demandable in market which showed in Table 4. It is very important to maintain supply and demand for mushroom production. Lack of cooking knowledge and food items with mushroom is also problem ranked as III.

Table 4. Ranking of Consumption Related Problem Faced by the Respondents

Sl. no	Statements	Percents of respondents		Rank
1	Social taboo among people about mushroom	Frog umbrella	63%	I
		non-vegetarian food	37%	
2	Consumption and demand in market	Very demandable	51.11%	II
		low demandable	21.11%	
		Sell in different location	27.78%	
3	Lack of knowledge about cooking	Required training	61%	III
		Not required training	39%	

Ranking of Marketing Problem in Mushroom Production by the Respondents: Data on marketing related problem faced by respondents presented in Table 5. It reflects that lack of marketing or publicity in mushroom ranked I, where 63% agreed in that point. Grower sells their mushroom maximum portion 53% in restaurant and fry shop where hand to hand customer was 47% ranked II (Table 5). Lack of packaging quality which might attract customer along with demand of mushroom also ranked III. Local growers and vendors used PP bag 69% as packaging material which was less attractive. 63% of mushroom produces did not use online promotion ranked IV. Lack of transport facilities and demonstration of mushroom product problem ranked V where lack of collaboration with super shop like Agora, Shopno etc. ranked VI.

Table 5. Marketing Problem in Mushroom Production

Sl. no	Statements	Percents of respondents		Rank
1	Lack of marketing / publicity	Agree	63%	I
		Disagree	37%	
2	Lack of engagement in super shop market	Agree	93%	VI
		Disagree	7%	
3	Mushroom sell location	Direct customer	47%	II
		Restaurant/road side shop	53%	
4	Lack of transport facilities for mushroom demonstration or mushroom product	Hand to hand	51%	V
		Carry by bicycle	6%	
		Carry by motor-bike	13%	
		Carry through Van/rickshaw	12%	
		Carry through Bus/currier services	18%	
5	Lack of packaging quality	PP bag	69%	III
		Polyethene bag	30%	
		Plastic bag/box	1%^	
6	Online promotion	No	63%	IV
		Yes	37%	

Overall Ranking of Problem Areas in Mushroom and Mushroom Seed Production: Overall problems in mushroom production is presented in Table 6. The data reflects the most faced problem - Lack of fund to buy essential equipment's to grow mushroom which ranked I followed by Disease infestation & sterilization technique in mushroom production ranked II. Distribution of mushroom mother/spawn collection center is important ranked as III where cooking training facilities and Marketing / publicity ranked accordingly V & IV. It is observed that mushroom farmers are faced most of the problems in those areas.

Table 6. Ranking of Problem Areas

1	Lack of fund to buy essential equipment's to grow mushroom	I
2	Disease infestation & sterilization technique in mushroom production	II
3	Distribution of mushroom mother/spawn collection center	III
4	Marketing / publicity	IV
5	Cooking training facilities	V

CONCLUSION

Sustainability and growth of any enterprise depends on certain facilities and hindering factors. In case of mushroom production in Bangladesh, it is observed that there are lots of challenges like – 'Financial crises, Unavailability of quality seed, Market facilities, Training facilities, Price fluctuation of mushroom, Demand fluctuation due to seasonal change' etc. faced by growers in mushroom and mushroom seed production. The problem faced by the farmers need to be addressed by the government and other concerned authorities. In order to encourage mushroom production in Bangladesh, quality of mother and mushroom growing equipment's must be available. For sustainability and expansion mushroom sector there should be encouragement by providing necessary input support and marketing facilities. Government should build mushroom sub center for mushroom seed/mother/spawn production facilities across the country. Value chain development of mushroom is recommended to concerned authority. Training should be organized by the Govt. Authority to encourage cooking different recipes with mushroom and mushroom products.

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Mycelial Growth of two Different Strains of Button Mushroom (*Agaricus bisporus*) in Various Culture Media

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Abstract

This study was evaluated the mycelium growth of *Agaricus bisporus* on three different culture media i.e. potato dextrose agar media, wheat extract agar media and malt extract agar media. Mycelium density was significantly difference on three different culture media. Moreover, different culture media influenced mycelium growth. malt extract agar media exhibited the fastest mycelium growth (2.1 mm/day) with Ab-in button mushroom variety. Other hand, 560786 button mushroom variety also showed faster mycelium growth (2.0 mm/day) compared to Ab-in on wheat extract media (1.9 mm/day). Besides, mycelium density was found highest on malt extract agar media and lowest on PDA media.

Keywords: Mycelium growth, Button mushroom, *Agaricus bisporus,* Culture media, Malt media, Wheat extract media.

INTRODUCTION

Button mushroom or *Agaricus bisporus* is a basidiomycete mushroom which belongs to the Agaricaceae family (Saravanan *et al.,* 2013). *Agaricus* spp. is very popular and cultivated in over 70 countries (Foulongne-Oriol *et al.,* 2014; Tautorus and Townsley, 1984).

Over 200 years, human consumed button mushroom for diet and for nutritional values (Morin *et al.,* 2013). It is used not only for cooking but also widely used in medical science for its anti-microbial, anti-tumoral, anti-carcinogenic andanti-oxidant properties (Özc¸ elik and Peks¸ en, 2007). It contains high quality of vitamins, protines, amino acids and polysac-charides (Moon and Lo, 2014; Wani *et al.,* 2010). The fruiting bodies of this mushroom havebeen used for centuries as foods and food flavourings (Ma *et al.,*2014).

The ambient temperature needs to be brought down to stimulate sporophore production. Temperatures of 16–19°C are recommended during the fruiting period (Foulongne-Oriol *et al.*, 2014) and 21–25°C during the growth phase (Largeteau *et al.*, 2011). Studies have demonstrated that *Agaricus bisporus* can withstand temperature increases and has adapted to temperatures as 25°C during the fruiting phase (Largeteau *et al.*, 2011), but can be found in all highly humid regions between spring and autumn (Akinyele *et al.*, 2012).

Along with temp. plant growth regulators and media can also play on important role in mycelial colony (Maniruz zaman, 2004). A large number of experiments were conducted through out the world to find out the effect of media on mycelial growth and appropriate media for selected variety. In our country, a little research work has been done on basis of mycelial growth on media for mushroom cultivation. The present research work was undertaken to investigate the growth of mycelium and colony density of *Agaricus bisporus* (two var.- Ab-in and 560786) on PDA media, Wheat media and Malt media. Further investigation will require to identify the best media for mycelial colony.

MATERIALS AND METHODS

Pure culture of *Agaricus bisporus* obtained from the germplasm collection of the Mushroom Development Institute, Sobhanbag, Savar, Dhaka. Using petri plate in three different media i.e. potato dextrose agar media, wheat extract media and malt agar media and two button mushroom variety i.e. Ab-in and 560786 with 3 replications were incubated at 22±2°C temp. to allow mycelium growth.

Preparation of Potato Dextrose Agar Media (PDA): Pure culture was prepared on potato dextrose agar (PDA) medium using 200 g peeled and sliced potato and boiled in 1 liter of water for 30 minutes. Using potato boiled water with 20g agar powder, 20g dextrose and 250mg aspergine per liter. The medium was poured into petri plate at 20 ml/plate. The medium in petri plate was sterilized in an autoclave for 20 minutes at 121°C under 1.5 kg/cm^2 pressure. After sterilization and solidification, the petri plate was packet with tape. All operations were done under sterile condition in a clean bench. The incubation chamber temp. was maintained at 22±2°C with 80-90% relative humidity.

Preparation of Wheat Extract Agar Media (WEA): Wheat extract agar medium was prepared by using 200g of clean and disease free wheat grain. Boiled wheat grain for an hour after washing and cleaning into running water. Remove wheat grain water by using a net and mixed with 20g agar powder, 20g dextrose and 250mg aspergine per liter. Autoclave for 20 minutes at 121°C under 1.5 kg/cm^2 pressure. The medium was poured into petri plate at 20 ml/plate. Temp. was maintained at 22±2°C with 80-90% relative humidity.

Preparation of Malt Extract Agar Media (MEA): Malt extract ager medium was prepared with 50g of malt agar power mixed with 1 liter of boiled water. Hot and boiled water helps to dissolve malt agar powder with water particle. Mixed solution was sterilized in autoclave at 121°C under 1.5 kg/cm^2 pressure for 20 minutes. Clean and dust free incubation chamber was used for inoculation. The room temp. was 22±2°C with 80-90% relative humidity.

Inoculation: After media preparation and sterilization, extract was poured into petri plate and settle down under UV light for 20 minutes. Once fully sterilized, the petri plates were inoculated with different varieties of button mushroom from test tube.

Statistical Design and Density Analysis: The experiment was laid out following the Completely Randomized Design with three replications. Data were analyzed and measured with measuring scale and density of mycelium was observed in visual observation.

RESULTS AND DISCUSSION

Culture media are important source of nutrients for mycelium growth. At present, potato dextrose agar media are being used for culture and mycelium cultivation of mushrooms. In mushroom production, availability, suitability and cost are important consideration for selection of culture media. In this study, we used two different button mushroom varieties i.e. Ab-in and 560786 and three different media i.e. potato dextrose agar media, wheat extract agar media and malt agar media to identify mycelium growth and density of mycelium.

Statistical analysis revealed significant influence of media on mycelium growth. The mycelium growth and density of mycelium in potato dextrose agar media was significantly lower than the malt agar media. Moreover, significant differences identified in different varieties on the same media.

Table 1. Mycelial growth and mycelium density of two different *Agaricus bisporus* variety in three different media

Variety	Media	Mycelium growth (mm/day)	Mycelium Density
Ab in	Malt media	2.1 a	+++
560786	Malt media	2.0 ab	+++
Ab in	Wheat extract media	1.9 b	++
560786	Wheat extract media	1.15 c	++
Ab in	PDA media	0.97 d	+
560786	PDA media	1.0 d	+

Data presented are means of three replications. Means with the same letter are not significantly different at 5% level of significance using Duncan Multiple Range Test (DMRT). (+) low density mycelium, (++) Medium density mycelium, (+++) High density mycelium.

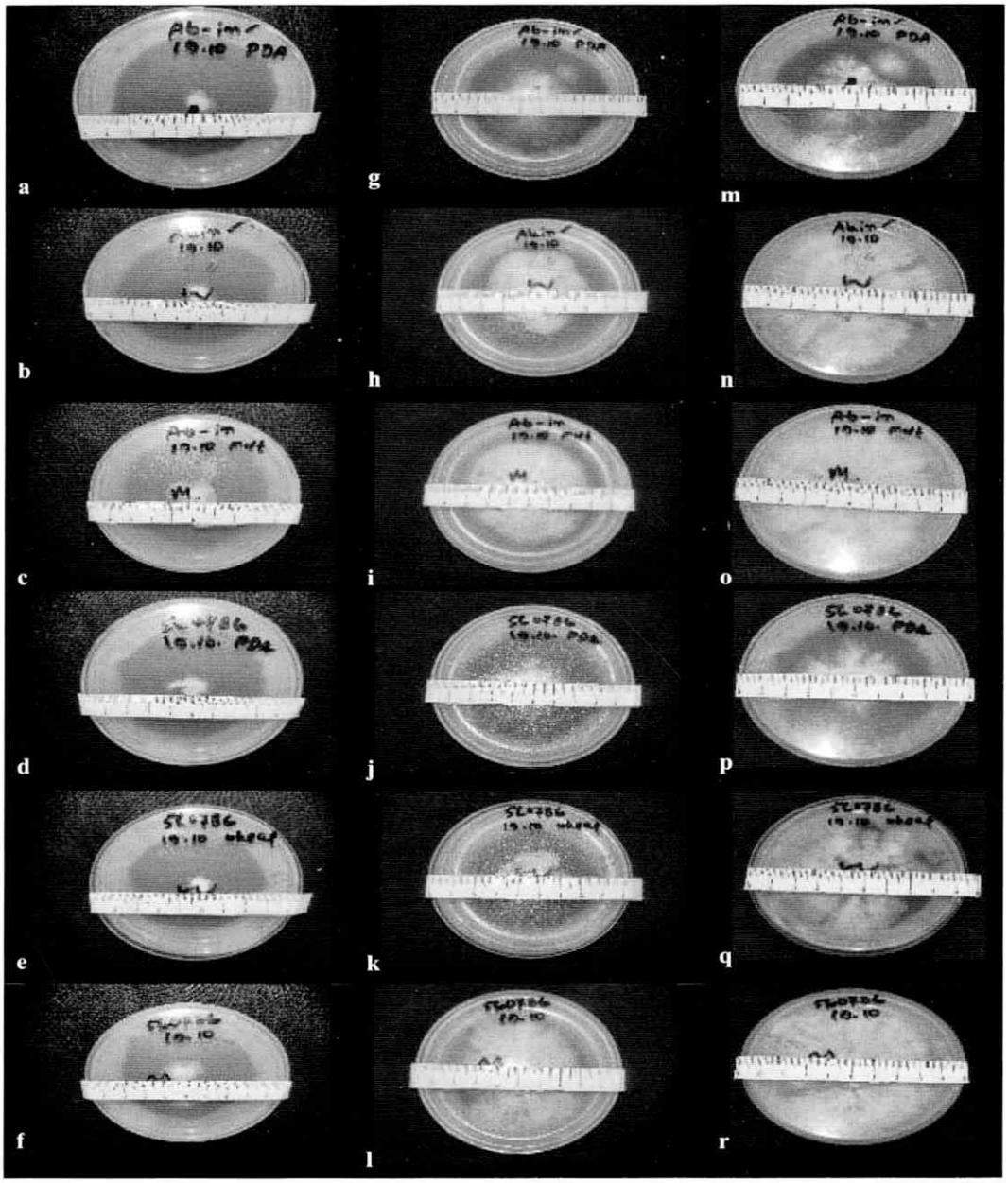

Plate 1: Mycelium growth of *Agaricus bisporus* on (a) Ab-in button mushroom in potato dextrose agar media, (b) Ab-in b utton mushroom in wheat extract media, (c) Ab-in button mushroom in malt media, (d) 560786 button mushroom in potato dextrose agar media, (e) 560786 button mushroom in wheat extract media, (f) 560786 button mushroom in malt media on the 6th day of incubation. (g) Ab-in button mushroom in potato dextrose agar media, (h) Ab-in button mushroom in wheat extract media, (i) Ab-in button mushroom in malt media, (j) 560786 button mushroom in potato dextrose agar media, (k) 560786 button mushroom in wheat extract media, (l) 560786 button mushroom in malt media on the 13th day of incubation and (m) Ab-in button mushroom in potato dextrose agar media, (n) Ab-in button mushroom in wheat extract media, (o) Ab-in button mushroom in malt media, (p) 560786 button mushroom in potato dextrose agar media, (q) 560786 button mushroom in wheat extract media, (r) 560786 button mushroom in malt media on the 21th day of incubation.

Mycelium growth and density on malt agar media: *Agaricus bisporus* is a low temperature mycelium running mushroom. During incubation and coverage, the temperature must be kept below 28^{0}C (Leiva *et al.,* 2015). In this study, petri plate with media and mycelium were kept in a disease free germplasm room at 20±2^{0}C temp. In this temp. two different button mushroom variety showed significant growth and highest density on malt media. *Agaricus bisporus* var. Ab-in showed highest mycelium growth (2.1 mm/day) according to var. 560786 (2.0 mm/day) (Table 1). In terms of mecelial density, both Ab-in and 560786 showed highest mycelial growth (Plate 1).

Mycelium growth and density on wheat extract agar media: Mycelium growth on wheat extract media was significantly lower than malt agar media. For, button mushroom, different variety showed significant growth and density on wheat extract agar media. Ab-in showed higher growth rate (1.9 mm/day) than 560786 (1.15 mm/day) on wheat extract agar media (Table 1). Both variety identified similar density on wheat extract agar media (Plate 1).

Mycelium growth and density on potato dextrose agar media: Potato dextrose agar media is a common media for culture all over the world. It is easy to prepare and products are available in market. But in terms of mycelium growth of mushroom, significant differences are coming on the basis of mycelial colonies. *Agaricus bisporus* var. Ab-in, showed the lowest mycelium growth (1.0 mm/day) compared to var. Ab-in (0.97 mm/day) (Table-1). And mycelial density was lowest found in both varieties on PDA media (Plate 1).

An experiment was conducted on the mycelial growth of banana substrate which showed that 50% banana leaves+50% sawdust exhibited highest yield and biological efficiency (Silva *et al.,* 2018). The result obtained from the present study were significant. *Agaricus bisporus* var. Ab-in and 560786 both showed highest growth rate and mycelial density on malt agar media. On the other hand, regular used PDA media found out the lowest rate of growth and mycelial density for button mushroom varieties.

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Proximate Nutritional Analysis of Different Mushroom Species at MDI

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Abstract

Mushroom cultivation is growing and farmers are interested in Bangladesh. In spite of a great nutritional and medicinal values, many farmers as well as consumers does not know about mushroom nutritional importance. In this study, the nutritional values of different varieties such as- *Pleurotus ostreatus, Pleurotus djamor, Ganoderma lucidum, Calocybe indica, Auricularia auricular-judae, Pleurotus high-king* and *Pleurotus ostreatus* were determined. Those mushrooms were rich in proteins (3~6%) and fibers (2~8% in dry samples) and contained a lower amount of lipid (2 to 17%). The carbohydrate contents ranged from 55 to 66% (on the basis of dry weight). These were also rich in mineral contents (total ash content is 3~10%). The moisture content of mushrooms ranged from 9 to 20% (in dry samples). Data of this study suggest that mushrooms are rich in nutritional value.

Keyword: Ash, Lipid, Protein, Fiber, Moisture, Carbohydrate, Mushroom.

INTRODUCTION

Mushroom has a significant role in human health. It is being widely used as a food from ancient times for food supplements and fulfillment of nutrition (Chang, 1996). There is a common saying that "medicines and foods have a common origin" (Kaul, 2001). Mushroom has a lots medicinal properties and works against life-threatening diseases. Major medicinal properties attributed to mushrooms include anticancer, antibiotic, antiviral activities, immune response stimulating effects and blood lipid lowering effects (Alam *et al.*, 2007).

Mushrooms of *Pleurotus* species are also rich in medicinal values (Jose and Janardhanan, 2000 and Manpreet *et al.* 2004)., *P. ostreatus* possesses antitumor activity (Yoshioka *et al.*, 1985) and hypoglycaemic effects in experimentally diabetic induced rats (Chorvathova *et al.*, 1993). Oyster mushrooms are very effective in reducing the total plasma cholesterol and triglyceride level (Nuhu Alam *et al.*, 2007).

Mushrooms are rich in protein, minerals and vitamins. Besides it contains an abundance of essential amino acids (Sadler, 2003). Nutritional analysis of several mushroom species of different origins had been carried out in many laboratories in the world. But nutritional values of locally cultivated mushrooms remain speculative. Moreover, nutritional composition is affected by many factors; these include differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis (Benjamin, 1995).

Bangladeshi people are still not very aware of nutritional and medicinal importance of mushrooms. The history of mushroom cultivation is very recent in Bangladesh. The aim of this investigation was to analyze the nutritional values of these mushrooms cultivated in Bangladesh, with a goal of increasing awareness of the beneficial effects of edible mushrooms among the consumers.

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MATERIALS AND METHODS

Fresh mushroom was cultivated and harvested from the culture house at Mushroom Development Institute, Savar, Dhaka, Bangladesh from June 2023 to December 2024. Mushroom was carried out for nutritional analysis in the "Quality Control and Quality Assurance" laboratory of Mushroom Development Institute.

Treatments: Nine different mushroom fruiting body were selected for this study. All Mushroom varieties were cultivated on sawdust subtract mother and spawn. Harvested fress mushroom was dried and grained for analysis of protein, lipid, fibre, ash, moisture and carbohydrate.

Determination of moisture content: One gram of well grained mushroom sample was taken to Moisture analyzer (AnD MX-50) to analyze moisture content. Moisture analyzer took average 5-7 minutes for results by heating sample.

Determination of total ash content: One gram of each sample was taken and placed into a crucible and weighed. The crucible was heated by a sprit lamp till the sample were burned and turned into black ash. Then it was taken into a muffle furnace for about 5-6 hours at 600°C. After cooling down of muffle furnace crucible was taken out and weighed. Then total ash was calculated by Raghuramulu *et al.*, as following equation:

$$\text{Ash content} \left(\frac{\text{g}}{\text{100g sample}} \right) = \frac{\text{Wt.of ash}}{\text{Wt.of sample taken}} \times 100$$

Determination of total lipid: Total lipid was determined by using chloroform and alcohol/ methanol mixture with sample. Five gram of grained mushroom sample was dipped into 50ml of chloroform:methanol (2:1) mixture. After 3 days the mixture was filtrated with filter paper and poured into a test tube which was pre weighted. The test tube with filtered mixture was placed into a dryer at 50-55°C. The upper layer of methanol was removed and chloroform was evaporated by heating. The remaining was crude lipid. Total lipid was calculated as following equation:

$$\text{Total Lipid} = \frac{\text{2nd weight -1st weight}}{\text{S}} \times 100$$

Determination of total protein: Five gram of grinded mushroom was taken with 50 ml of 0.1N NaOH and boiled for 30 min. The solution was cooled in room temperature. The supernatant was collected and total protein content was measured according to the method of Lowry *et al.*, (1951).

Determination of crude fiber: Ten grams of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80~100° C and weighed (We) in an electric balance (Keyi: JY-2003; China). The crucible was heated in a muffle furnace (Nebertherm: Mod-L9/11/c6;

Germany) at 600°C for 5~6 hours, cooled and weighed again (Wa). The difference in the weights (WeWa) represents the weight of crude fiber.

Crude fiber (g/100 g sample) = [100 − (moisture + fat)] × (We-Wa) / Wt of sample (Raghuramulu *et al.*, 2003).

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation (Raghuramulu *et al.*, 2003)

$$\text{Carbohydrate} \left(\frac{\text{g}}{\text{100g sample}} \right) = [100\text{-(Moisture+Fat+Protein+Ash+Crude Fiber)}]$$

RESULTS AND DISCUSSION

Several nutritional parameters were measured from different mushroom varieties. Table 1 shows the nutritional parameters of dry mushroom.

Moisture content: Considering moisture content were varied from 8.72% - 20.28% per 100g of dried mushroom. The highest moisture content was found in *Ganoderma lucidum* (GL) (20.28g/100g dry sample) where lowest moisture content was found in *Pleurotus ostreatus* (PO_2) (8.72 g/100 g dry sample) (Table 1). The total ash content found in Au mushroom (*Auricularia auricular-judae*) was 15.72g/100 g dry sample followed by Cid white mushroom (*Calocybe indica*) 15.2g/100 g dry sample (Table 1). Among three varieties of *Pleurotus djamor* (POP), POP 3 found highest moisture content (13.67%) followed by POP 1 (10.93%) and POP 3 (9.71%) accordingly.

Total ash content: Total ash content were varied in different mushroom from 3.0%-10.5% per 100g of dried mushroom. Total ash content was found highest in PO_2 (10.5%) followed by HK 51 (9.48%) and Obig (9.14%). Due to low fiber, ash content was lowest in Au mushroom (3g/100g dry sample) (Table 1). Total ash content in POP1, POP2 and POP3 was found at 9.6g/100g dry, 9.0g/100g dry and 7.7g/100g dry simultaneously (Table 1).

Table 1. Nutritional composition of different mushroom varieties (g/100g of dried sample)

Variety	Moisture	Total ash	Lipid	Protein	Fiber	Carbohydrate
PO2	8.72	10.5	13.16	5.9	2.04	59.68
POP 1	10.93	9.6	8.4	5.9	1.98	63.19
POP 2	13.67	9	3.78	5.3	2.08	66.17
POP 3	9.71	7.7	11.96	4.3	2.16	64.17
HK 51	12.66	9.48	2.9	5.2	7.43	62.33
Obig	14.18	9.14	3.07	4.5	7.77	61.34
GL	20.28	7	3.64	6.08	7.4	55.6
Cid	15.2	9	2.08	4.32	8	61.4
Au	15.72	3	17.2	3.1	1.9	59.08

PO2 –*Pleurotus ostreatus*, POP – *Pleurotus djamor*, GL – *Ganoderma lucidum*, Cid – *Calocybe indica*, Au – *Auricularia auricular-judae*, HK51 – *Pleurotus high-king*, Obig- *Pleurotus ostreatus*.

Lipid content: Different mushroom varieties contains different lipid content range about 2.08-17.2 g per 100 g of dried sample. The highest lipid content (17.2g/100g) was estimated in Au mushroom compared to PO2 (13.6g/100g) and POP3 (11.96g/100g) respectively (Table 1). And the lowest lipid content was found in Cid (2.08g/100g) compared to HK 51 (2.9g/100g), Obig (3.07g/100g), GL (3.64g/100g) and POP 2 (3.78g/100g) (Table 1).

Protein content: The protein content was found highest in GL (6.08g/100g dry sample) followed by PO2 (5.9g/100g), POP 1 (5.9g/100g), POP 3 (5.3g/100g) and HK 51 (5.2g/100g). Lowest protein content was estimated in Au mushroom (3.1g/100g dry sample). Among the three pink oyster mushroom, highest protein was found in POP 1(5.9g/100g) followed by POP 2 (5.3g/100g) and lowest found in POP 3 (4.3g/100g dry sample) (Table 1).

Fiber content: The fiber content was varied from 1.9g to 8g per 100g of dried sample. The fiber content was found highest in Cid mushroom (8g/100g dry sample) followed by Obig (7.77g/100g), HK 51 (7.43g/100g) and GL (7.4g/100g). And lowest was found in Au mushroom (1.9g/100g). POP 1 found lowest fiber content (1.98g/100g) among POP 2 (2.08g/100g) and POP 3 (2.16g/100g) respectively.

Carbohydrate content: The highest carbohydrate was found in POP 2 (66.17g/100g) followed by POP 3 (64.17g/100g) and POP 1 (63.19g/100g). Carbohydrate content includes fiber, such as the structural polysaccharides beta-glucans, chitin, hemicelluloses and pectin substances. The lowest carbohydrate content was found in GL (55.6g/100g).

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Phenotypic Evaluation of the Wild and Commercial Genotypes of Chinese Bailinggu (*Pleurotus tuoliensis*)

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Abstract

Pleurotus tuoliensis is known as Bailinggu as its trade name in China. Bailinggu is a precious edible mushroom containing high nutrient and medicinal value. The present study was conducted to evaluate the important agronomic traits of 15 wild strains comparing with one commercial variety (CCMSSC00489). The radial vegetative growth rate in PDA media ranged from 3.00 to 10.14 mm/day and the linear vegetative growth rate in cotton seed hull substrate varied from 2.40 to 3.66 mm/day. The length of cap ranged from 16.62 cm to 20.76 cm, wide of cap was 12.91 cm to 18.15 cm and thickness of cap was 6.31 cm to 10.27 cm. The range of stipe length was 3.69 cm to 13.05 cm, while stipe thickness varied from 3.15 cm to 6.63 cm. The yield per packet varied from 226.24g to 538.79g and the range of biological efficiency was 37.31 % to 89.80 %. These wild strains could be used as the source of desired characters in breeding schemes. Moreover, two wild strains CCMSSC02622 and CCMSSC03595 showed significantly higher yield & biological efficiency than the cultivated strain. So the findings of this research will help to improve Bailinggu mushroom production.

Key words: *Pleurotus tuoliensis*, Wild genotype, Phenotypic evaluation, Agronomic traits.

INTRODUCTION

The increase in human population results in an increasing demand for food. Among the most available healthy food ingredients, mushrooms are considered as sources of important nutrient including dietary fiber, minerals and vitamins, in particular vitamin D (Ren *et al.*, 2012). Mushrooms have been consumed by human since ancient times, not only as part of a healthy diet but also as a delicacy because they have a highly desirable aroma and taste (Colak, 2004). *Pleurotus* mushroom are a great renewable and easily accessible source of functional foods or nutraceuticals and pharmaceuticals with antioxidant, antimicrobial, antiinflamatory, antitumor and immunomodulatory effects (Correa *et al.*, 2016). *Pleurotus tuoliensis* is known as Bailinggu as its trade name in China. In the beginning, the scientific name of Bailinggu was ambiguous. *P. nebrodensis, P. ferulae*, and *P. eryngii* var. *tuoliensis* were used as its scientific name (Zhang *et al.*, 2006; Huang *et al.*, 2011; Kawai *et al.*, 2008; Mou *et al.*, 1987). A taxonomic study showed that *P. tuoliensis* is an independent species rather than a variety or subspecies of *P. eryngii* (Zhao *et al.*, 2016), although they are closely related (Kawai *et al.*, 2008).

Bailinggu is a high nutrient and medicinal value containing white-coloured precious edible mushroom (Guo *et al.*, 2007; Lv *et al.*, 2009). It has a white fruiting body with a crisp texture, good taste and excellent flavour. Bailinggu is considered as one of the costly mushroom

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species in the market due to its pure white colour, particular flavour and also having high nutritional value. However, the profit of Bailinggu cultivation is moderately low compared to the king oyster mushroom (*P. eryngii*). The price of Bailinggu is high, but small profits mainly resulted from the high production cost which is due to the longer production period and low yield. Low yield and longer production period are related to the agronomic traits such as vegetative growth rate, size of fruiting body, weight of fruiting body, etc. So these traits represent targets for breeding schemes designed to improve the yield and earliness. Due to the wide range of genetic variability, natural germplasms extensively used as valuable resources of favourable genes for traits of interest in crop genetic improvement (Fermi *et al.,* 2006). As the unique origin and significant producer, China harbours abundant natural germplasm resources of *P. tuoliensis* mainly in the collection of CCMSSC (China Center for Mushroom Spawn Standards and Control) (Zhao *et al.*, 2016).

Analysing the phenotypic variation in Bailinggu natural population is a great practical significance for breeding schemes. The present study was carried out - i) to evaluate the important agronomic traits such as vegetative growth rate, yield, biological efficiency etc of wild strains. ii) to select better quality wild genotype as new cultivar/s compared with commercial variety.

MATERIALS AND METHODS

Strains and growth conditions: The experiment was conducted in the Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China. The strains used in this study were presented in Table 1. All the strains were provided by the China Center for Mushroom Spawn Standards and Control (CCMSSC), respectively. The population consisted of one commercial cultivar (CCMSSC 00489) and 15 wild Bailinggu strains which were collected from Yumin, Tuoli, Qinghe, Mulei and Shihezi of the Xinjiang Autonomous Region in China at an altitude of 790-1400 m^2. The strains were cultured and maintained on potato dextrose agar medium at 25 ^{0}C in the Key Laboratory of Microbial Resources, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China.

Table 1. List of Bailinggu genotypes (strains) used in this experiment

Source/origin	Strain name CCMSSC	Strain number
Qinghe County, Xinjiang	00932, 00943, 00951	3
Chen Qiang Xinjiang	02480, 02622	2
Yizhong Biotechnology Co., Ltd	02835	1
Gobi Desert, Qinghe County, Xinjiang	03090, 03091, 03123, 03144, 03587, 03595	6
Yumin County, Xinjiang	03180, 03197,	2
Toli County, Xinjiang	03230,	1
Commercial/ Cultivated strain	00489	1

Trait evaluation

The mycelium growth rate on PDA: The mycelium growth rate of the tested Bailinggu population was studied on potato dextrose agar (PDA) medium (Difco). To determine the mycelium growth rate, a piece of 5 mm diameter mycelium was inoculated into the center of a Petri dish (90 mm in diameter) containing 20 ml PDA medium. All samples were incubated at 25 °C in the dark. The mycelium growth rate was calculated at day 7th after inoculation from germination as the radial extension of each mycelial colony per day (Larraya *et al.*, 2002). Three repetitions were performed for each strain.

Vegetative growth rate in substrate: The dikaryotic mycelium growth rate of the Bailinggu strains were studied using following medium substrate formula: cotton seed hull 75%, wheat bran 15%, gypsum 1% and water 60±3%. The substrate containing glass tubes (2 cm in diameter and 16 cm in length) were sterilized at 126°C for 120 minutes. A piece of mycelium (1.5 cm in diameter) was inoculated into the top of glass tube containing 36 g of mixed substrate. Then the glass tubes were kept vertically in an incubator at 25 °C in the dark for sufficient mycelium growth. The mycelium growth was measured three times (9th day, 17th day, and 25th day after inoculation) in each tube at eight days interval from the distance of mycelium colony growth into the medium (Larraya *et al.*, 2002). Three repetitions were performed for each strain.

Cultivation: Cultivation spawn bags were prepared using the substrate formula same as vegetative growth rate measurement. 1.5 kg mixed substrates were loaded in each polyethylene plastic bag. The substrate containing spawn bags were sterilized at 126o C for 120 minutes. After cooling, the bags were inoculated with one stick (length 15cm, wide 7mm and thickness 4mm) spawn into each bag. 15 culture bags were prepared for each strain and the bag was incubated at 25° C in the dark after inoculation. Relative humidity was maintained 40-60%. After completion of mycelium running, the spawn bags were opened and 3-6 cm2 on the surface area were scratched to stimulate primordia. Then the spawn bags were incubated for 5 days to recover the mycelium. After mycelium recovering the spawn bags were kept at 1-3° C temperature (cold stimulation) for a period of 10 days. Then the spawn bags were transferred into culture house and the temperature were (3-18° C) along with the changing environmental conditions. When the primordium grows to 1-2 cm, keeping the best one others were removed from spawn bags. The fruiting body was harvested when the edge of the cap in the back was open and flat.

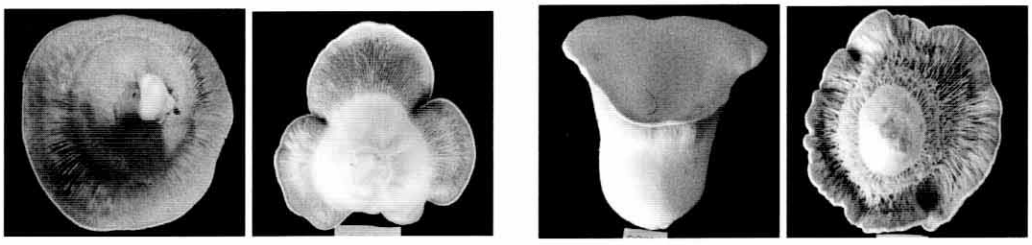

Plate 1. The harvested fruiting body of cultivated and wild Bailinggu strains

The experiment was conducted following completely randomized design (CRD) with three replications and five bags for each strain in each replication. Phenotypes were measured from six fruiting bodies of each strain. The target agronomic traits such as Cap length (CT), Cap wide (CW), Cap thickness (CT), Stipe length (SL), Stipe thickness (ST), were measured using a digital calliper. Yield per packet (Y) were measured using an electronic balance with 0.01g precision. At the end of the harvesting period, the Biological efficiency (BE) was calculated based on the following formula: BE (%) = Weight of fresh harvested mushroom per bag / dry weight of substrate per bag × 100 (Wang *et al.*, 2018).

Statistical analysis: The agronomic trait data of Bailinggu population were analyzed by following the statistical procedures using the packages of Statistical Package for Social Sciences (SPSS, version 23 for Windows, SPSS Inc., Chicago, IL, USA). The degree of phenotypic variability for each trait was estimated using the coefficient of variation (CV).

RESULTS AND DISCUSSION

Trait performances of the accessions: Most of the studied strains showed high variability for most of the traits based on the analysis of variance (ANOVA, $P<0.01$) (not shown). Results of the agro-morphological traits recorded from the wild genotypes and significant variation was observed in all the traits (Table 2, 3 & 4). The radial vegetative growth rate in PDA media ranged from 3.00 to 10.14 mm/day and the linear vegetative growth rate in cotton seed hull substrate varied from 2.40 to 3.66 mm/day. The length of cap ranged from 16.62 cm to 20.76 cm, wide of cap was 12.91 cm to 18.15 cm and thickness of cap was 6.31 cm to 10.27 cm. The range of stipe length was from 3.69 cm to 13.05 cm, while stipe thickness varied from 3.15 cm to 6.63 cm. The yield per packet varied from 226.24g to 538.79g and the range of biological efficiency was 37.31 % to 89.80 %. The coefficient of variation (CV) reflected the varying degrees within the traits. The values of CV estimated for the agronomic traits ranged from 1.09% to 13.21%.

Promising Bailinggu genotypes with desirable traits: Bailinggu was first successfully cultivated in the 1980s and commercial cultivation has been started since 1997 in China (Feng et al., 2010; He et al., 2016; Zhao et al., 2016). Due to the recent and concise cultivation history, research on Bailinggu is inadequate, and production issues are general such as long cultivation period, low yield, a high amount of deformed fruiting bodies production and strain degeneration (Zhang *et al.*, 2010). In addition to the necessity of cold stimulation for the maturation of mycelia, the main trouble is the requirement of new, high-quality strains.

Table 2. Comparison of mycelium growth rates of cultivated and wild Bailinggu strains

Strains CCMSSC	Radial mycelium growth rate in PDA media (mm/day)	Linear mycelium growth rate in substrate (mm/day)
00489	8.48b	3.27bc
00932	6.62e	3.66a
00943	3.00f	3.11cde
00951	6.64e	3.49ab
02480	7.93bc	2.95def
02622	7.79bc	3.00def
02835	8.09bc	3.31bc
03090	6.78de	2.65gh
03091	10.14a	3.47ab
03123	8.54b	2.80fg
03144	9.36a	3.18cd
03180	7.47cde	2.87efg
03197	7.64bcd	2.49h
03230	7.78bc	2.97def
03587	7.65bcd	2.64gh
03595	7.98bc	2.40h
CV	5.86	3.54

[a] Means in each column followed by the same letters are not significantly different at $P < .05$ according to Duncan's multiple range tests.

Table 3. Comparison of Cap dimensions of cultivated and wild Bailinggu strains

Strains (CCMSSC)	Cap length (cm)	Cap width (cm)	Cap thickness (cm)
00489	19.08 ± 0.75 abcde	15.82 ± 0.73 abcd	7.91 ± 0.98 bcd
00932	16.62 ± 0. 57 e	13.46 ± 0.75 d	9.72 ± 0.66 ab
00943	19.12 ± 1.15 abcde	17.65 ± 0.87 a	10.27 ± 0.23 a
00951	13.97 ± 0. 50 f	13.41 ± 0.39 6d	8.15 ± 0.43 bcd
02480	18.80 ± 0.67 abcde	13.03 ± 1.00 d	7.13 ± 1.07 cd
02622	19.57 ± 0.83 abc	15.39 ± 1.16 abcd	8.62 ± 0.29 abc
02835	16.73 ± 0.69 de	14.34 ± 0.43 bcd	6.31 ± 0.37 d
03090	20.76 ± 0.63 a	16.85 ± 0.51 ab	9.28 ± 0.64 ab
03091	17.69 ± 0.35 bcde	12.91± 1.03 d	8.19 ± 0.33 bcd
03123	20.71 ± 0.38 a	18.15 ± 0.79 a	7.11 ± 0.55 cd
03144	18.92 ± 0.58 abcde	14.37 ± 1.44 bcd	7.84 ± 0.76 bcd
03180	20.21 ± 0.36 ab	17.26 ± 0.52 ab	9.06 ± 0.42 abc
03197	18.04 ± 1.14 bcde	13.39 ± 0.42 d	8.45 ± 039 abc
03230	17.34 ± 0.78 cde	14.51 ± 1.09 bcd	8 51 ± 0.51 abc
03587	16.99 ± 1.05 cde	13.71 ± 1.28 cd	8.21 ± 0.63 bcd
03595	19.27 ± 1.18 abcd	16.63 ± 1.28 abc	9.62 ± 0.56 ab
CV	1.09	1.40	1.99

Commercial and wild Bailinggu strains were compared based on the yield and yield contributing traits. The yield per bag and biological efficiency of the commercial strain CCMSSC00489 were 462.44g and 77.07% respectively which was significantly lower than the wild strains CCMSSC02622 and CCMSSC03595. The radial mycelium growth rate in PDA media of wild strains CCMSSC03091 & CCMSSC03144 were significantly higher than the cultivated strain. The linear vegetative growth rate in substrate of the commercial strain recorded in this study was significantly lower than the wild strain CCMSSC00932. CCMSSC00943 displayed the highest thickness of cap where as CCMSSC02622 showed the longest stipe and CCMSSC02835 performed the highest thickness of stipe.

Table 4. Comparison of Stipe dimensions & yield of cultivated and wild Bailinggu strains

Strains (CCMSSC)	Stipe length (cm)	Stipe thickness (cm)	Yield per packet (g)	Biological efficiency(%)
00489	7.26 ± 0.73 cdef	4.45 ± 0.13 def	462.44 ± 17.16cde	77.07± 2.86cde
00932	7.10 ± 1.06 cdef	3.15 ± 0.33 g	226.24 ± 24.93g	37.71 ± 4.15g
00943	6.25 ± 0.42 ef	5.61 ± 0.18 bc	507.69 ± 14.68abc	84.61± 2.45abc
00951	7.49 ± 0.88 bcdef	4.52 ± 0.32 def	362.19 ± 23.99f	60.36 ± 3.99f
02480	3.69 ± 0.53 g	5.24 ± 0.28 bcd	482.84 ± 30.35abcd	80.47± 5.06abcd
02622	13.05 ± 0.90 a	4.82 ± 0.26 cde	538.79 ± 31.95a	89.80 ± 5.32a
02835	6.75 ± 0.46 def	6.63 ± 0.25a	527.10 ± 5.21abc	87.85± 0.86abc
03090	7.84 ± 0.71 bcde	4.63 ± 0.18 de	483.68 ± 12.11abcd	80.61 ± 2.02abcd
03091	9.34 ± 1.03 bc	5.03 ± 0.51 bcde	430.69 ± 13.72de	71.78 ± 2.29de
03123	5.38 ± 0.33 efg	5.75 ± 0.10 b	472.48 ± 2.95bcde	78.75 ± 0.38bcde
03144	6.88 ± 0.83 cdef	3.72 ± 0.18 fg	408.97 ± 17.73ef	68.16 ± 2.95ef
03180	6.12 ± 0.75 ef	4.91 ± 0.29 bcde	465.74 ± 15.45cde	77.62 ± 2.57cde
03197	5.01 ± 0.51 fg	4.64 ± 0.18 de	476.41 ± 15.80abcd	79.40 ± 2.63abcd
03230	9.23 ± 0.57 bcd	4.95 ± 0.26 bcde	469.13 ± 18.28bcde	78.19 ± 3.05bcde
03587	7.11 ± 0.62 cdef	4.56 ± 0.40 de	468.84 ± 25.66bcde	78.14 ± 4.28bcde
03595	9.77 ± 1.37 b	4.14 ± 0.10 ef	535.02 ± 26.71ab	89.17 ± 4.45ab
CV	2.80	1.46	13.21	13.21

CONCLUSIONS

The results have demonstrated that the wild Bailinggu strains can represent a promising resource for the traits of interest, such as vegetative growth rate, cap length, cap width, cap thickness, stipe length and stipe thickness. These wild genotypes could be used as donor parents in breeding schemes for generating new genotypes with desired characters. Moreover, wild strains CCMSSC02622 and CCMSSC03595 showed significantly higher yield & biological efficiency than the cultivated strain. These two wild strains have a clear advantage over the cultivated strain from a commercial point of view, thus the findings of this study will help to improve Bailinggu mushroom production.

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