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Sterilization and Substrate Level Optimization for Improving Yield and Biological Efficiency of Paddy Straw Mushroom

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted to determine the impact of surface sterilization techniques and different quantity of bed substrate on yield and biological efficiency in paddy straw mushroom cultivation. Among the various combinations of sterilization methods i.e., Plain water, Plain water + Calcium

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oxide @ 2%, Plain water + Bavistin 75 ppm & Formalin 500 ppm and steam sterilization 55℃ to 70℃ for ½ hour respectively tried on paddy straw substrate, Maximum yield and biological efficiency (1966 g & 15.2%) was obtained. To evaluate the biological efficiency of different layers and quantity of straw in bed method of cultivation i.e., 5 layers (4.0 kg), 4 layers (3.2 kg), 3 layers (2.4 kg) and 2 layers (1.6 kg) were tested. on biological efficiency four layers bed proved its superiority among all the number of substrate layers experimented, and gave highest biological efficiency (17.6%) followed by three layers and five layers (15.2%) and (14.5%) respectively. Two layers of bed (1.6 kg) were found to be less suitable and gave 13.8% biological efficiency of paddy straw. Maximum average weight of sporophores (24.21 g) was observed from four layers of beds followed by three layers and five layers (20.88 g) and (19.98 g) respectively. Smaller size sporophores were noticed in two layers of beds (14.18 g).

Keywords: Paddy straw mushroom; surface sterilization; paddy straw substrates; sporophores.

1. INTRODUCTION

Mushrooms are known to mankind since time immemorial. They are heterotrophs which lacks chlorophyll, unable to utilize mineral ions and water like green plants. Mushrooms are a widely distributed food resource on earth and have been consumed due of their nutritional value and therapeutic characteristics for over 2000 years. Mushrooms have been found to contribute to human health through their nutrient content, which includes easily digestible proteins, carbohydrates, dietary fibre, vitamins, minerals, and antioxidants, thereby enhancing their palatability and gustatory appeal [1,2]. These organisms derive their nutrients from both inorganic and organic sources, including wood logs, manure composts, and synthetic composts. Mushrooms are commonly favored due to their palatability, distinctive flavor profiles, and potential therapeutic attributes. Mushrooms, despite not being classified as either meat or vegetable, are commonly referred to as the "meat" of the vegetable kingdom. Mushrooms have been farmed for centuries due to their nutritional value and distinct flavor, particularly in the far eastern regions. The protein content in mushrooms is lower compared to that found in mammals, although significantly higher than the protein content in the majority of plants. The food product in question exhibits a low-fat level, a high fibre content, and contains all required amino acids. Additionally, it contains all significant minerals, with the exception of iron [3]. This economically affordable vegetable possesses not only a rich nutrient profile, including vitamin D, but also exhibits qualities that can potentially mitigate the risk of cancer, HIV-1 AIDS, and various other ailments [4]. China is a major edible mushroom producer [5,6,7]. The cultivation of mushrooms ranks as the fifth largest agricultural sector in China [8]. The cultivation of mushrooms presents a significant opportunity for increased protein production per unit of land area, a capability that is not achievable through alternative sources. In India, mushrooms can be cultivated and harvested year-round due to the country's diverse seasonal patterns, which include summer, rainy, and winter seasons. Straw mushroom (*Volvariella spp*.) and milky mushroom (*Calocybe indica*) have the potential for cultivation during the summer and rainy seasons, within a temperature range of 25℃ to 40℃. Oyster mushroom (*Pleurotus spp*.) thrives within a temperature range of 20℃ to 30℃, while white button mushroom cultivation is best suited for the winter season, with temperatures ranging from 15℃ to 22℃. Mushroom cultivation exhibits a high level of environmental sustainability, since it effectively transforms lignocellulosic waste materials into valuable resources such as food, feed, and fertilizers [9,10]. In mushroom cultivation Pasteurization refers to a process wherein substrates are subjected to a specific temperature that effectively eliminates detrimental microbes, while minimizing significant alterations to the chemical composition of the substrate. The process of pasteurization is employed with the aim of mitigating the presence and impact of weeds, diseases, and pests [11]. The mushroom substrate can be pasteurized using hot water treatment with boiling water for 30 minutes, chemical sterilization with formalin, or steam pasteurization with plastic bags in a steam drum filled with 4-5 inches of water and heated at 80 degrees Celsius for one hour [12]. Steam pasteurization is the most effective method for substrate pasteurization, as it produces mycelia development quickly [13]. In light of these considerations, the current study sought to assess the impact of different combinations of surface sterilization techniques, as well as different quantity and layers of paddy

straw in bed method of cultivation, on the growth parameters and harvests of the Paddy straw mushroom.

2. MATERIALS AND METHODS

The present investigation was carried out at the mushroom farm of Visva-Bharati, Department of Plant Protection, PSB, Bolpur, Birbhum, and West Bengal during 2017-2019. The test fungi were obtained from Centre of Tropical Mushroom Research and Training, Orissa University of Agriculture and Technology, Bhubaneswar and were maintained for further study on Potato Dextrose Agar (PDA) medium. The data obtained from this experiment were statistically analyzed by Completely Randomized Design with 3 replications as per the procedure suggested by Snedecor & Cochran [14].

Bed preparation: Paddy straw bundles of 0.40-0.75 kg (80 – 95cm long & 12.16 cm wide) were prepared from hand threshed paddy. The bundles were soaked in clean water, clean water mixed with 2% calcium oxide and clean water mixed with Bavistin and formalin @ 1 ml and 15 g for 10 litre of water respectively. The bundles were soaked in for 12 – 18 hours in a cemented water tank. The excess of water was drained on by placing the soaked bundles on a raised bamboo platform.

Substrate sterilization: In this study, Plain water and its combinations were experimented to evaluate the suitable substrate sterilization method. To increase the biological efficiency of paddy straw mushroom (*Volvariella volvacea*), various combinations of sterilization methods i.e. Plain water, (Plain water + Calcium oxide @ 2%), Plain water + Bavistin 75 ppm & Formalin 500 ppm and steam sterilization 55℃ to 70℃ for ½ hour were tested.

Different quantity of substrates: To evaluate the biological efficiency of different layers and quantity of straw i.e.5 layers (4.0 kg), 4 layers (3.2 kg), 3 layers (2.4 kg) and 2 layers (1.6 kg) were tested during the cropping season. The bundles were arranged in a parallel manner, with four additional bundles placed in a similar fashion but from the opposite side. This arrangement resulted in the open ends of the bundles overlapping in the middle, forming a single layer consisting of eight bundles. Subsequent layers were constructed in a similar manner, with the number of layers corresponding to the specific treatment. Spawning was conducted at a rate of 2% based on dry weight between each layer, while ensuring a margin of 12cm - 15cm from the edges. The beds were pressed from the top and finally covered with clean transparent plastic sheet for maintaining relative humidity (80 – 85%) and temperature (30 - 35℃). The Biological Efficiency (B.E.) was computed using Chang's [15] standard formula.

$$\text{B. E}(\%) = \frac{\text{Fresh weight of mushroom}}{\text{Air} - \text{dried substrate}} \times 100$$

3. RESULTS AND DISCUSSION

In the present study, to increase the biological efficiency of paddy straw mushroom (*Volvariella volvacea*), various combinations of sterilization methods i.e., Plain water, (Plain water + Calcium oxide @ 2%), Plain water + Bavistin 75 ppm & Formalin 500 ppm and steam sterilization 55℃ to 70℃. for ½ hour were tested. The data obtained on various parameters have been presented in Table 1. Plain water + Calcium oxide @ 2% produced fair quantity of yield of *Volvariella volvacea*. Maximum yield and biological efficiency (1966 g & 15.2%) were obtained from Plain water + 2% Calcium oxide treatment which was 35.59 % more than the Plain water treatment (1156 g & 8.9%) followed by Chemical treatment (Plain water + Bavistin 75 ppm and Formalin 500ppm) which gave 1673g & 12.9% respectively. Both of the treatments differ significantly with plain water treatment in terms of yield and biological efficiency. Minimum yield and biological efficiency of mushroom (1156 g & 8.9%) was observed in plain water treatment Fig. 1. Treatment of substrate with plain water along with Bavistin 75 ppm + formalin 500 ppm took minimum time (9.75 days) for completing the spawn run and produced sporophores earlier than plain water treatment (12.25 days). The treatment of substrate with plain water + calcium oxide @2% though produced maximum yield, took (11.0 days) for spawn run. Steam sterilization treatment took 11.25 days to complete the spawn run. The differences in spawn run period of various treatments found to be significant (Fig. 2). The number and average weight of sporophores also taken as parameters for comparison. Among the different treatments experimented, Plain water + Bavistin 75 ppm + formalin 500 ppm produced maximum average weight of fruiting bodies (29.50 g) which differ significantly from all other treatments followed by plain water + calcium oxide @2% (24.80g) and steam sterilization treatment (22.8 g). Smaller size of sporophores (13.90g) was noticed in plain water treatment.

Table 1. Evaluation of different substrate sterilization methods for increasing the biological efficiency of Paddy Straw Mushroom (*Volvariella volvaceae*)

Substrate surface sterilization techniques	Spawn run (in days) *	Average number of sporophores*	Average weight of sporophores (g)*	Total bed yield (g)*	Average bed yield (g)*	Biological efficiency (%)
Plain water	12.25	23.38	13.90	1300	325	10.15
Calcium oxide 2% in Plain water	11.00	20.32	24.80	2016	504	15.76
Formalin 500 ppm+ Bavistin 75 ppm in Plain water	9.75	19.4	23.3	1808	452	14.12
Steam sterilization (55-70^{0}C for ½ hour)	11.25	17.9	22.8	1632	408	12.75
SE m (±)	0.57	1.51	2.24		0.88	0.88
CD@	1.81	4.84	7.17		2.81	2.81
CV%	10.28	16.22	19.71		14.06	14.06

**Average of three replications; S. E= Standard Error; CD= Critical Difference; CV= Coefficient of Variation*

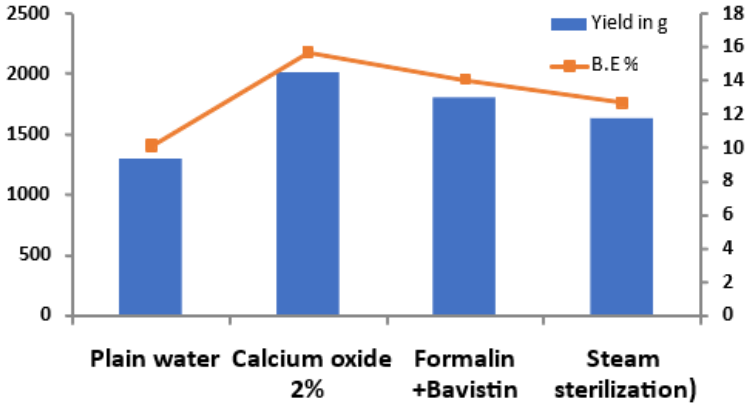

Fig. 1. Effect of substrate sterilization on yield and biological efficiency of *Volvariella volvaceae*

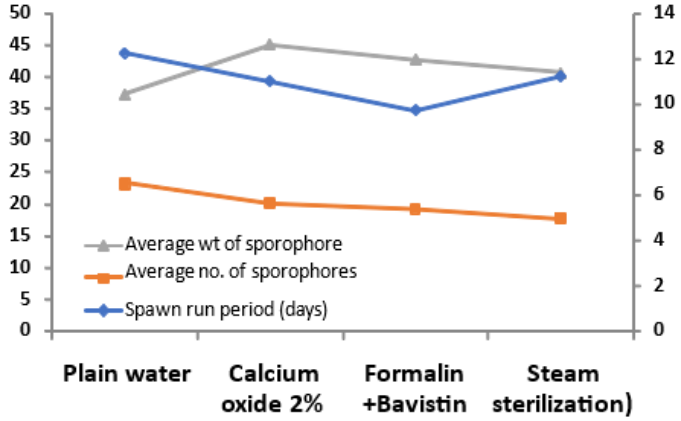

Fig. 2. Effect of substrate sterilization on spawn run, average number and weight of sporophores

Maximum numbers of sporophores were found in plain water treatment. Negative correlations noted between the treatments in terms of number and average weight of sporophores. Fewer numbers of sporophores 20 and 14 were observed from Plain water + 2% Calcium oxide, and Plain water + Bavistin 75 ppm + formalin 500 ppm respectively which gave bigger size of sporophores. In the present investigation the substrate treated with calcium oxide (lime) @ 2% in plain water gave maximum yield and biological efficiency. The pH level plays a crucial role in facilitating optimal growth of paddy straw mushrooms, which thrive within a pH range of 8 to 9. The mycelium of fungi, specifically mushrooms, acquires nutrients from substrates within a particular pH range [16]. Lime is employed in the cultivation of mushrooms to optimize the pH of the substrate, thereby promoting accelerated mycelial growth of the mushrooms [17]. Wajid Khan *et al*., [18] also reported comparable outcomes when using a 2% lime treatment. The study conducted by Biswas [19] reported comparable outcomes regarding the minimum spawn run when using Bavistin at a concentration of 75 ppm in combination with Formalin at a concentration of 500 ppm. The utilization of plain water, which lacks pasteurization, resulted in the lowest yield and biological efficiency. The process of pasteurization results in a reduction of microscopic competitors present in a substrate. This gives mushroom mycelium an advantage over the harmful organisms and it allows to grow mycelium by overcoming the growth of competitor organisms into the substrate & eventually produce mushrooms. Here the unsterilized substrate is one of the factor which leads to minimum yield, biological efficiency and for maximum spawn running period.

To evaluate the biological efficiency of different layers and quantity of straw i.e.5 layers (4.0 kg), 4 layers (3.2 kg), 3 layers (2.4 kg) and 2 layers (1.6 kg) were tested during the cropping season and the data obtained on different parameters have been presented Table 2. To evaluate the biological efficiency of different layers and quantity of straw i.e.5 layers (4.0 kg), 4 layers (3.2 kg), 3 layers (2.4 kg) and 2 layers (1.6 kg) were tested during the cropping season and the data obtained on different parameters have been presented Table 2. It was evident from the table that layers and quantity of straw had different responses in terms of yield, biological efficiency and spawn run period. However, on biological efficiency four layers bed proved its superiority among all the number of substrate layers experimented, and gave highest biological efficiency (17.6%) followed by three layers and five layers (15.2%) and (14.5%) respectively. Two layers of bed (1.6 kg) were found to be less suitable and gave 13.8% biological efficiency of paddy straw. Maximum average yield/kg substrate (146g) was recovered from four layers of substrate (3.2kg), followed by five layers, three layers and two layers (135g), (122g) and (118g) respectively (Fig. 3). Spawn run period was found minimum (9.25 days) in five layers of spawning followed by four layers and three layers (9.50 days), (10.50 days) respectively. Maximum time for spawn run was taken by the beds prepared from 2 layers substrate (11.25 days) (Table 2). The relationship between various methods in term of spawn run period was found to be significant (Fig. 4). Maximum average weight of sporophores (24.21 g) was observed from four layers of beds followed by three layers and five layers (20.88 g) and (19.98 g) respectively. Smaller size sporophores were noticed in two layers of beds (14.18 g). In the present investigation four layers of substrate gave maximum biological efficiency. In the beds of four layers substrate appropriate conditions were maintained inside the bed in terms of temperature, relative humidity exchanges of gases, Co_2 concentration etc. and there were proper procurements of nutrients of substrates by mushroom mycelium. Whereas, in case of five layers substrate in spite of having favorable growing conditions inside the bed's inappropriate utilization of nutrients of substrate by the mushroom mycelium could be reason for less biological efficiency. Two layers of substrate (6 kg) beds gave minimum yield and biological efficiency. Size and compactness of bed are the basic factors for temperature maintenance in beds. The bed should be pressed tightly to increase the temperature inside the bed as the bed must reach the temperature of 40 ℃ to 45 ℃ which enhance the mushroom production [20,21]. Because of less compactness in beds proper temperature and relative humidity were not maintained and hence leads to less production in 2 layers beds.

Table 2. Evaluation of appropriate substrate quantity for paddy straw mushroom cultivation

Number of substrate layers	Spawn run (in days) *	Average number of sporophores*	Av.wgt. of sporophores (g)*	Total bed yield (g)*	Average Yield (g)*	Biological efficiency (%)
5 layers (16 kg)	9.25	24.54	22.00	2160	135.00	13.5
4 layers (12.8 kg)	9.50	19.48	24.00	1870	146.00	14.6
3 layers (9.2 kg)	10.50	14.87	18.88	1123	122.00	12.2
2 layers (6 kg)	11.25	12.64	14.00	708	118.00	11.8
SE m (±)	0.49	2.10	1.87		1.85	1.24
CD @ 1%	1.51	6.47	5.76		5.69	3.82
CV %	9.67	19.71	18.89		11.62	16.84

**Average of three replications; S. E=Standard error; CD=Critical Difference; CV=Coefficient of Variation*

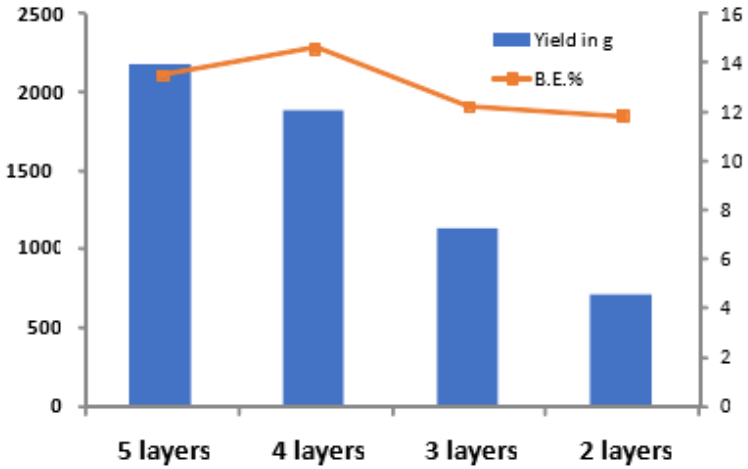

Fig. 3. Effect of substrate layers on yield and biological efficiency of *Volvariella volvaceae*

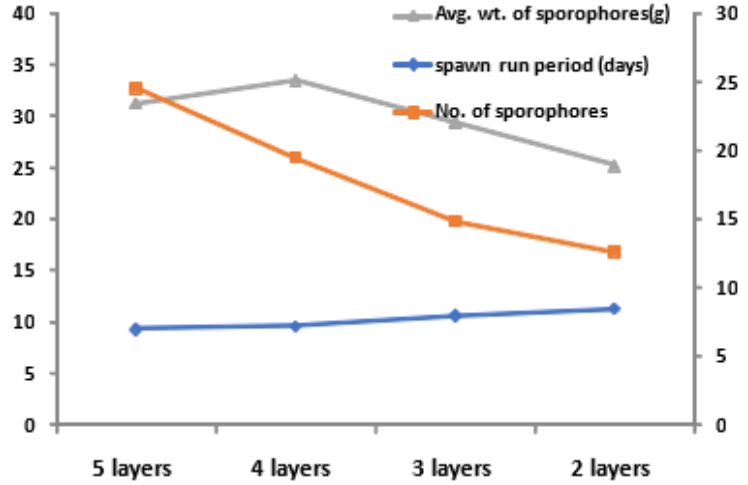

Fig. 4. Relationship between spawn run periods, number and average weight of sporophores

4. CONCLUSION

The current study highlights the impact of different combinations of surface sterilization techniques, as well as different quantity and layers of paddy straw in bed method of cultivation, on the growth parameters and harvests of the Paddy straw mushroom. The protein content in mushrooms is lower compared to that found in mammals, although significantly higher than the protein content in the majority of plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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