

Original Research Article

Evaluation of surface sterilization techniques and different quantities of paddy straw for improving the biological efficiency of paddy straw mushroom

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 oxide @ 2%), Plain water + Bavistin 75 ppm & Formalin 500 ppm and steam sterilization 55°C to 70°C for ½ hour respectively tried on paddy straw substrate. Maximum yield and biological efficiency (1966 g & 15.2%) was obtained from Plain water + 2% Calcium oxide treatment which was 35.59 % more than the Plain water treatment followed by Chemical treatment (Plain water + Bavistin 75 ppm and Formalin 500 ppm). 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. 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, Substrates, Yield, Biological efficiency.

INTRODUCTION

Mushrooms are known to mankind since time immemorial. They are heterotrophs which lack chlorophyll, unable to utilize mineral ions and water like green plants. These organisms derive their nutrients from both inorganic and organic sources, including wood logs, manure composts, and synthetic composts. Mushrooms are commonly favoured due to their palatability, distinctive flavour profiles, and potential therapeutic attributes. Mushrooms possess high-quality proteins, unsaturated fatty acids, minerals, and vitamins. These food items exhibit low levels of fat, carbohydrates, and salts, while being abundant in dietary fibre. They are widely utilised in culinary applications across various gastronomic traditions. Mushrooms, despite not being classified as either meat or vegetable, are commonly referred to as the "meat" of the vegetable kingdom. China is a major edible mushroom producer. 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. Mushrooms possess a protein content ranging from 20% to 35% on a dry weight basis, encompassing all the essential amino acids necessary for human physiological functions. 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°C to 40°C. Oyster mushroom (*Pleurotus* spp.) thrives within a temperature range of 20°C to 30°C, while white button mushroom cultivation is best suited for the winter season, with temperatures

Comment [LH1]: The abstract lacks clarity and conciseness because it does not contain information about the objectives of the study, the place, and period of the study, and the statistical tests used. It is advised that the author rewrite the abstract concisely by including that information as well.

Comment [LH2]: This word should be plural, as it is used the word different to mean many.

Comment [LH3]: This word should be written in lower-case. Please double-check that and use the correct form.

Comment [LH4]: This word should be written in lower-case. Please double-check that and use the correct form.

Comment [LH5]: Please also see the comments above.

Comment [LH6]: Please also see the comments above.

Comment [LH7]: Please consider using lower-cases for these words, except for the names of the products. Please double-check that.

Comment [LH8]: A comma “,” should be used between the number “500 ppm and the word “and” to clearly separate the treatments in this study.

Comment [LH9]: There should be commas “,” between the word “hour” and “respectively” and “tried”

Comment [LH10]: The comma used between these two words should be replaced by the conjunction “and”

Comment [LH11]: “were” should be used instead since the subject is plural.

Comment [LH12]: There should be a comma between this two words.

Comment [LH13]: There should be a comma “,” between these two words. Please double-check that and rewrite it appropriately.

Comment [LH14]: There should be a comma “,” between the number and the word “respectively.” Please double-check that and rewrite it properly.

Comment [LH15]: Please place a comma between these two words.

Comment [LH16]: The whole introduction section was written without any references. Therefore, the author is advised to provide sufficient references to support the statements in this part.

Comment [LH17]: This verb phrase should be rewritten as “have been know” due to the use of preposition “since”

Comment [LH18]: The verb “lacks” should be in the plural form “lack” because of the noun “heterotrophs”

ranging from 15°C to 22°C. The Paddy straw mushroom exhibits a rapid growth rate, possesses a straightforward cultivation technique, and enjoys favourable consumer acceptance. The plant exhibits promising potential and its cultivation has been implemented as a small-scale industry. The necessary raw materials are abundantly available within our nation. In West Bengal it can be adopted for year round cultivation as it requires high temperature and due to the easy availability of basic substrate (Paddy straw). The Government has taken up many initiatives for popularizing the mushroom cultivation but, the production of mushroom is very low in comparison to other states. The cultivation is restricted in the northern hills, adjoining areas of metropolitan cities and coastal parts of West Bengal. Except few cultivars in the hilly region, most of the growers are cultivating oyster mushroom. The mushroom in question stands out from other edible mushrooms due to its exceptional and distinctive flavour, as well as its unique textural attributes. The mushroom in question exhibits a notable protein content, as well as crude fibres and ash, in comparison to other mushrooms. This nutritional profile contributes to its status as a healthful dietary option. Additionally, this particular mushroom possesses a superior composition of diverse elements and amino acids. 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.

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. 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. Paddy straw bundles of 0.40-0.75 kg (80 – 95 cm 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. 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°C to 70°C. for ½ hour were tested. 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. Intermittent spawning was conducted at a rate of 2% based on dry weight between each layer, while ensuring a margin of 12 cm - 15 cm 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°C). The Biological Efficiency (B.E.) was computed using Chang's (1978) standard formula.

Comment [LH19]: The word "our" should be replaced with other word to avoiding using the first person in the text. Please double-check that and consider using the right word for this phrase.

Comment [LH20]: A comma "," should be used between the word "Bengal" and "it"

Comment [LH21]: The word "year round" should be rewritten with a comma ion between "year-round"

Comment [LH22]: There should be a comma between the word "cultivation" and "as"

Comment [LH23]: This word should be written in lower-case because it is written in the middle of the sentence. Please double-check that and use the correct form.

Comment [LH24]: What do you mean by "the Government"? Do you mean "The Indian government"? If so, please use the full name like this, so that the reader can understand more clearly.

Comment [LH25]: In this section, the study period should be given, so that the reader may know the time and the duration of the experiment. This information is very important.

Comment [LH26]: There are some points to be considered and addressed properly.
1. In this section, parameters to be measured and measurement techniques should be clearly illustrated, so that the process can replicated by other researchers after reading this paper. Besides that, statistical tests and statistical software for the data analysis were not stated. Therefore, it is advised the author should describe those points in detail for this section, so that the study is much clearer.

Comment [LH27]: The name of the country where the experiment was conducted should be given because this paper may be read by readers from other countries. Besides that, please consider adding the GPS for the experimental location, so that it is much clearer for the reader to track the place.

Comment [LH28]: Do you mean "the tested fungi"? If so, please write the word "tested" instead of "test"

Comment [LH29]: A comma "," should be used between the word "Bhubaneswar" and "and" for clear separation.

Comment [LH30]: Please double-check the way to write a series of nouns connected by the conjunction "and."
- Do we need only one "and" at the end of the list...

Comment [LH31]: There should be a space between the abbreviation "i.e." and "5"

Comment [LH32]: There should be a space between the number and its unit "cm"

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

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°C to 70°C. 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%) was 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. 1.1). 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 volvacea*)

Substrate surface sterilization techniques	Spawn run (in days)*	Average number of sporophores*	Average weight of sporophores (g)*	Total yield (g)*	Average yield (g)*	Biological efficiency (%)
Plain water	12.25	23.38	13.90	1300	325	10.15
Calcium oxide 2%	11.00	20.32	24.80	2016	504	15.76
Formalin 500 ppm + Bavistin 75 ppm	9.75	19.4	23.3	1808	452	14.12
Steam sterilization (55-70°C for ½ hour)	11.25	17.9	22.8	1632	408	12.75
SEM(±)	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

Comment [LH33]: Please consider using the lower-case because the word "To" does not start the sentence.

Comment [LH34]: Please consider using the lower-case because these words does not start the sentence.

Comment [LH35]: Please also see the comments above.

Comment [LH36]: Please also see the comments above.

Comment [LH37]: Please also see the comments above.

Comment [LH38]: Please also see the comments above.

Comment [LH39]: There should be a comma "," between the percentage and the word "respectively"

Comment [LH40]: This phrase should be rewritten as "(Fig. 1)"

Comment [LH41]: Please double-check the space between words. It is found that sometimes more than one single space is used.

Comment [LH42]: There should be a verb "were" after the word "sporophores." Please double-check that and consider adding the verb if required.

Comment [LH43]: A lower-case is correct.

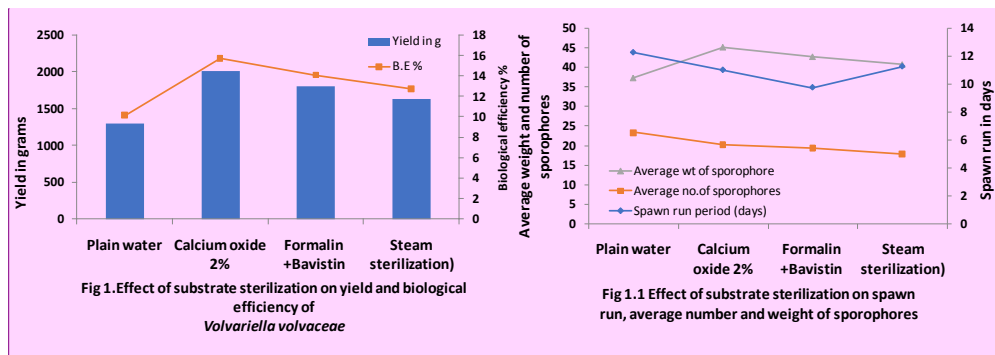
Comment [LH44]: There should be a comma "," between the number and the word "which"

Comment [LH45]: The table description should be written outside the box. Please double-check that against the journal guideline and consider writing the correct format.

Comment [LH46]: Please consider using different alphabetical letters to separate means in case significant differences were detected.

Comment [LH47]: This word seems to be written incorrectly. Please double-check that and write the correct abbreviation.

Comment [LH48]: What do you mean by "CD@"? Please consider using a note below the table to explain all abbreviations.



Comment [LH49]: When the graphs are arranged horizontally like this and they their own graph description below, it is better to number them separately. Let's say Fig. 1. And Fig. 2. - After that, please also modify the figure reference used in the text. For example, if Fig. 1.1 is replaced by Fig. 2, just write down Fig. 2 instead.

Maximum numbers of sporophores were found in plain water treatment. Negative correlations were existed 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 @ 2% 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 (Sarker et al., 2007). Lime is employed in the cultivation of mushrooms to optimise the pH of the substrate, thereby promoting accelerated mycelial growth of the mushrooms (Iqbal & Shah, 1989). (Wajid Khan et al., 2013) also reported comparable outcomes when using a 2% lime treatment. The study conducted by Biswas (2014) 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 utilisation of plain water, which lacks pasteurisation, resulted in the lowest yield and biological efficiency. The process of pasteurisation 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.

Comment [LH50]: This phrase should be rewritten as "500 ppm, respectively, which" this is because commas are needed to separate those words to be grammatically right.

Comment [LH51]: A comma "," should be placed between the word "investigation" and "the."

Comment [LH52]: Please extract the name of the author outside the brackets because it is used as the subject of this sentence.

Comment [LH53]: There should be a space between the word "treatment" and "The"

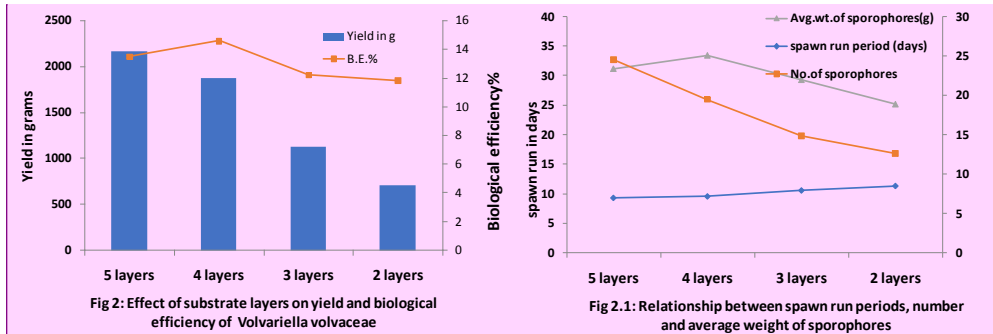
Comment [LH54]: The word "factor" should be in the plural form because it is written after a phrase "one of"

Comment [LH55]: Please refer to the comments on Table 1 above. - Please also address the comments for this table following those in Table 1.

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

Number of substrate layers	Spawnrun n(in days)*	Average number of sporophores*	Av.wgt. of sporophores (g)*	Total body 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
SEm(±)	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



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) weretested 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. All the treatments produced fair quantity of paddy straw mushroom.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.no.2).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 was taken by the beds prepared from 2 layers substrate (11.25 days). The relationship between various methods in term of spawn run period was found to be significant (Fig.2.1).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, CO₂ concentration etc. and there was 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 beds 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. The bed must reach the temperature of 40°C to 45°C which enhance the mushroom production (Alicbusan and Ela 1967). Because of less compactness in beds proper temperature and relative humidity were not maintained and hence leads to less production in 2 layers beds.

Comment [LH56]: What can be seen here is that the two graphs have their own title description below. So, it can be suggested that the author number them separately, for example, Fig. 3 and Fig. 4, as the previous two graphs above are recommended to be Fig. 1 and Fig. 2.

Comment [LH57]: The names of y axes for the graphs should be rewritten like this:
 - In graph 1, Yield (g)
 - In graph 2, Biological efficiency (%)

Comment [LH58]: A space is needed between "i.e." and "5"

Comment [LH59]: Please consider shortening these two sentences to avoid unnecessary writing repetition.

Comment [LH60]: There should be a comma "," between the word "efficiency" and "four"

Comment [LH61]: There should be a comma "," between the percentage and the word "followed"

Comment [LH62]: There should be a comma "," between the percentage and the word "respectively"

Comment [LH63]: The number and its unit (kg) should have a space.

Comment [LH64]: Please reduce the spacing.

Comment [LH65]: The number and its unit (kg) should have a space.

Comment [LH66]: Please also see the comments above.

Comment [LH67]: There should be a comma "," between the number and the word "respectively"

Comment [LH68]: Please rewrite this as "(Fig. 2)"

Comment [LH69]: There should be a comma "," between the number in brackets and the word "respectively"

Comment [LH70]: Please consider changing the Figure number according to the suggestion above.

Comment [LH71]: There should be a comma "," between the number in brackets and the word "respectively"

Comment [LH72]: There should be a comma "," between the word "investigation" and the word "four"

Comment [LH73]: Please reduce the space between the word "of" and the word "four"

Comment [LH74]: A full word of CO₂ should be written first; then, the abbreviation can be used. (...)

Comment [LH75]: This phrase should be "there were" because it is followed by a plural noun (...)

Comment [LH76]: Please consider changing the word "Whereas" by a conjunctive adverb that ha (...)

Comment [LH77]: Please consider rewriting this sentence to be easily understandable.

CONCLUSION

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. Minimum yield and biological efficiency of mushroom (1156 g & 8.9%) was observed in plain water treatment. 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 differences in spawn run period of various treatments found to be significant. Among all the different number of substrate layers experimented, four layer bed gave highest biological efficiency (17.6%) followed by three layers and five layers (15.2%) and (14.5%) respectively. 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. 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 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. Therefore, Plain water + 2% Calcium oxide for substrate sterilization and four layers bed method may be recommended for farmer to get attractive fruit body as well as high yield in commercial production of *Volvariella* spp. and also for other paddy straw mushrooms.

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Comment [LH78]: Please consider reducing the spacing between the word "by" and "chemical"

Comment [LH79]: There should be a comma between the number in brackets and the word "which"

Comment [LH80]: Please leave a space between the number and the unit "g"

Comment [LH81]: Please consider using the word "and" rather than using the symbol.

Comment [LH82]: There should be a comma between the percentage and the word "respectively"

Comment [LH83]: Please reduce the spacing between the word "ppm" and the word "took"

Comment [LH84]: Please consider reducing the spacing between the word "produced" and the word "sporophores"

Comment [LH85]: This noun phrase should be rewritten as "four-year beds"

Comment [LH86]: There should be a comma between the percentage and the word "respectively"

Comment [LH87]: There should be a spacing between the number and its unit.

Comment [LH88]: Please consider reducing the spacing between the number and the word "was"

Comment [LH89]: Please also see the comments above.

Comment [LH90]: There should be a comma between the number in brackets and the word "respectively"

Comment [LH91]: Please consider replacing the comma "," by the conjunction "and"

Comment [LH92]: Please use a comma between the number and the word "respectively"

Comment [LH93]: Please consider placing a comma "," between the number and the word "respectively"