

## Evaluation of the different substrates for composting in the cultivation of *Agaricus bisporus* under the agro-ecological conditions of West Bengal

Commented [A1]: in

Commented [A2]: for

### ABSTRACT

**Aim:** This study aims to evaluate the effectiveness of different compost substrates in the cultivation of *Agaricus bisporus* (button mushroom) in the lateritic belt of West Bengal, India, by assessing parameters such as spawn run period, pinhead formation, sporophore production, yield, and biological efficiency.

**Study Design:** A Completely Randomized Design (CRD) with three replications per treatment was implemented to ensure the reliability of the statistical analysis. Differences between treatments were assessed using Duncan's multiple range tests at a 5% significance level.

**Place and Duration of Study:** The study was carried out in 2020 at the Mushroom Research Laboratory, within the Department of Plant Pathology, Palli Siksha Bhavana (Institute of Agriculture), situated in Sriniketan, Birbhum district, West Bengal.

**Methodology:** The Long Method of Composting was employed, utilizing various substrates including paddy straw, maize stalks, and their combinations. The experiment measured key cultivation parameters: spawn run duration, time to pinhead formation, number of sporophores, time to first harvest, yield per 8 kg compost bag, and biological efficiency. The presence of any contaminants or infestations, such as *Coprinus* spp., was also monitored.

**Results:** The compost substrate composed of a 1:1 ratio of paddy straw and maize stalks yielded the best results, with a spawn run period of 20.33 days, pinhead formation at 15.33 days, and sporophore production of 92 per 8 kg compost bag. This substrate also facilitated the earliest first harvest at 9.33 days and achieved the highest yield of 1020.00 g per 8 kg bag with a biological efficiency of 12.75%. In contrast, compost made solely from maize stalks resulted in the lowest yield (643.33 g per 8 kg bag) and biological efficiency (8.04%) and was more prone to infestation by *Coprinus* spp.

**Conclusion:** The study identifies a 1:1 mixture of paddy straw and maize stalks as the optimal substrate for *Agaricus bisporus* cultivation in the studied region, offering quicker growth cycles and higher yields, which could benefit local mushroom producers by enhancing production efficiency.

Commented [A3]: It would be better if authors provide weblink of institute where study was performed.

*Keywords: Button mushroom, Composting, Substrates, Yield, Biological Efficiency*

## 1. INTRODUCTION

Mushrooms have a rich cultural significance around the world, often being associated with festivities and revered as valuable sources of nutrition. The term "mushroom" derives from the French "mousse" or "mousseron," which translates to champignon or fungus, reflecting their historical importance in various cuisines. In Romanian tradition, mushrooms are considered "God's flesh" or "God's Food," while the Chinese view them as the "Elixir of Life." The science of fungi, known as mycology, delves into the intricate world of mushrooms, with *Agaricus bisporus*, commonly known as the white button mushroom, holding particular prominence.

In ~~the~~ cultivation of ~~the~~ *A. bisporus*, ~~the~~ substrate or compost plays a crucial role, providing the necessary nutrients for the mushroom mycelium to thrive and eventually produce mushrooms. A considerable amount of agricultural residue, such as straw, leaves, and stems, is left unused and contains abundant lignocellulose, rendering it suitable for growing champignon mushrooms [1]. *A. bisporus*, as a heterotrophic organism, depends entirely on its growth substrate to fulfill its carbon, water, nitrogen, and mineral nutritional needs. Compost preparation stands as a crucial aspect of *A. bisporus* cultivation, typically employing three composting methods: the long method, short method, and indoor method. Various composting methods have been developed to prepare the substrate, each with its own advantages and challenges. Compost preparation involves the breakdown of agricultural wastes such as paddy straw, wheat straw, or maize stalks, often with the addition of nitrogen sources to enhance microbial activity. The long composting method, dating back to earlier times and initially proposed by Atkins [2], involves an outdoor process lasting 4-6 weeks. This method entails various steps such as moistening the ingredients and substrates, blending them together, and regularly turning the mixture until completion, which usually takes around 28 days.

Numerous studies have explored different compost formulations and substrates to optimize mushroom cultivation. Studies have explored various compost formulations for the cultivation of *A. bisporus*, with notable findings indicating that a mixture of wheat straw and paddy straw at a 1:2 ratio yields the highest production [3]. Additionally, investigations into substrates for milky mushroom (*Calocybe indica*) cultivation have shown that paddy straw substratum produces the maximum yield and biological efficiency, while maize stalks can

**Commented [A4]:** Use of article THE is not appropriate in many places

enhance yield potential when combined with paddy straw [4]. Research also suggests that alternative materials such as grass and maize stalks can yield significantly higher mushroom production compared to conventional horse manure [5], while Rawal and Doshi [6] evaluated various combinations of wheat and paddy straw for milky mushroom cultivation. Moreover, experiments with corn stover as a substitute for straw-based compost have shown promising results, indicating its potential in regions with limited straw supplies [7]. Furthermore, investigations into substrate combinations for Oyster mushroom cultivation have found that a combination of paddy straw and wheat straw yields the highest production [8]. Several substrates have been investigated for the cultivation of button mushrooms, including paddy straw, maize stalks alone, and various combinations in ratios of 1:1 and 2:1. Researchers observed that compost consisting of a combination of paddy straw and maize stalks in a 1:1 ratio exhibited superior degradation compared to other substrates. Additionally, this combination yielded the highest production, with a recorded yield of 13.6 kg per unit of compost. These findings, as reported by Kaur et al. [9], underscore the significance of substrate composition in optimizing button mushroom cultivation. Overall, these studies highlight the importance of substrate selection and compost formulation in optimizing mushroom cultivation yields and efficiency.

## 2. MATERIALS AND METHODS

### 2.1 Compost preparation

The compost preparation in this study was conducted employing the Long Composting method, as illustrated in Fig. 1 and 2. The formula used for compost composition included 150 kg of chopped straw (substrate) and 7.5 kg of wheat bran. Additionally, nitrogen sources such as urea (1.5 kg) and single super phosphate (1.5 kg), as well as potassium sources like muriate of potash (1.5 kg) and calcium ammonium nitrate (4.5 kg), were ~~added~~<sup>incorporated</sup>. To enhance the physical properties of the compost and aid in nutrient availability, gypsum was added at a rate of 10 kg. This meticulously crafted compost mixture provided the necessary organic matter and nutrients essential for the growth and development of *A. bisporus*, contributing to successful mushroom cultivation.

**Commented [A5]:** Refereces for Long Composting Method

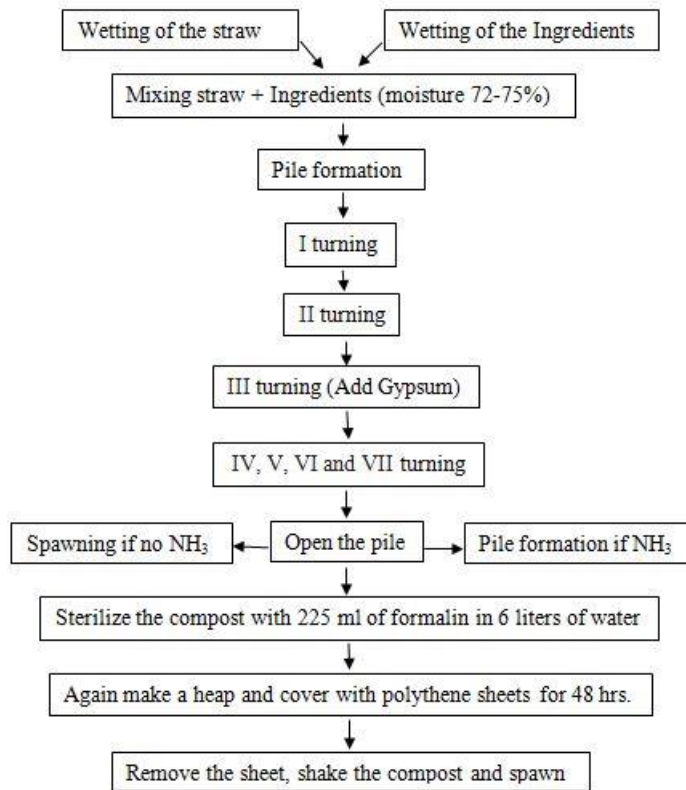


Fig. 1. Flow chart of long method of composting

Commented [A6]: What is the time interval for turning I,II,III and IV



**Fig. 2. Steps involved in Compost preparation. (a)Mixing ingredients with substrate, (b) Pile formation, (c) Addition of gypsum, (d) Turning of compost, (e) Re-pile formation, (f) Final compost**

### 2.2 Evaluation of the different substrates for composting in the cultivation of *A. bisporus*

Paddy straw and wheat straw are commonly utilized as compost materials for mushroom cultivation. This study aims to assess the effectiveness of different proportions of composting substrates, specifically paddy straw and maize stalks. Each treatment consists of three replications, with T1 involving a mixture of paddy straw and maize stalks in a 1:1 ratio, T2 utilizing paddy straw alone, and T3 using maize stalks alone. The composting process for each treatment follows the Long method and takes approximately 28 days to complete and the formalin was used for sterilizing the compost.

**Commented [A7]:** Text is more relevant to introduction section.

### 2.3 Mushroom bed preparation and spawning

After the compost preparation process, it is transferred into polyethylene bags, with a typical spawning rate ranging from 0.5% to 2%. Spawning can be carried out using either mixed or layer spawning methods, utilizing spawns prepared in bottles or bags. In this particular study, the S-11 strain spawn was utilized at a 2% spawn rate through layer spawning. Subsequently, the bags filled with compost are moved to the cropping room and maintained at a temperature

of 24°C with a relative humidity of 90%. Once the spawn run is completed, typically within a span of 15 days, the bags are prepared for casing.

#### 2.4 Casing material

During the spawn run phase, the necessary casing materials are collected. Components such as garden soil are sourced from the Experimental Farm within the Department of Agronomy at Visva-Bharati, Sriniketan. Farmyard manure is obtained from the Model Dairy and Poultry Farm associated with the Department of Animal Science at Visva-Bharati, Sriniketan.

Commented [A8]: Geographical location?

#### 2.5 Casing of the beds

Upon completion of the spawn run, indicated by the uniform white mycelial growth of *A. bisporus*, the sterilized casing materials are evenly spread over the bed surface to a depth of 4 cm. Following this, the beds are maintained at a temperature range of 16-18°C with a relative humidity of 90% aimed at evaluating their effects on the yield and biological efficiency of button mushroom cultivation.

#### 2.6 Yield and Biological Efficiency

The internationally recognized standard for harvesting button mushrooms is characterized by a closed membrane, a stem length not exceeding 2 cm (3/4 inch), and a cap diameter ranging from 2.5 to 6 cm (1-2.5 inches). Harvesting is ideally carried out when the cap diameter is twice the length of the stem. Biological efficiency is calculated using the formula:

$$\text{Biological Efficiency (\%)} = \frac{\text{Weight of Fresh Mushroom (g)}}{\text{Weight of Compost (g)}} \times 100$$

### 3. RESULTS AND DISCUSSION

To evaluate the most effective composting substrate for Button mushroom cultivation in the lateritic belt of West Bengal, different substrates including a mix of paddy straw and maize stalks (in a 14:1 ratio), pure paddy straw, and pure maize stalks were subjected to long composting method. The study aimed to assess their impact on various growth parameters such as spawn run period, duration of pinhead formation, days to first harvest, number of fruiting

bodies harvested, average weight of a fruiting body, yield, and biological efficiency (Fig. 3). The results of all parameters are summarized in Table 1.

Commented [A9]: Not related to results and discussion

**Table 1. Evaluation of the different substrates for composting in the cultivation of White Button mushroom (*A. garicus bisporus*)**

Treatments	SRP	DFPF	DFFH	No. FH	Yield (g)	B.E (%)	Remarks
Paddy straw + Maize stalk (1:1) compost	<b>20.33</b>	<b>15.33</b>	<b>9.33</b>	<b>92</b>	<b>1020.00</b>	<b>12.75</b>	-
Paddy straw compost	22.67	17.33	11.33	74	826.67	10.33	-
Maize stalk compost	27.00	20.67	14.00	51	643.33	8.04	<i>Coprinus</i> Sp.
SE(m)(±)	0.72	0.67	0.54	1.29	6.60	0.08	
CD @ 1%	2.49	2.31	1.88	4.47	22.83	0.29	
CV %	5.35	6.50	8.16	3.09	1.38	1.39	

SRP – Spawn Run Period, DFPF – Days for Pinhead Formation, DFFH – Days for First Harvest, No.FH – Number of Fruiting bodies Harvested, B.E – Biological Efficiency



**Fig. 3. Steps involved in production of sporophores of *A. bisporus*. (a) Spawning of compost, (b) Bag filling of compost, (c) Incubation, (d) Spawn run initiation, (e) Spawn run completed bag, (f) Casing of beds, (g) pinhead initiation, (h) Developing pinheads, (i) Matured sporophores**

### **3.1 Time for spawn run and primordial initiation**

Paddy straw + Maize stalk (1:1) compost showed an early spawn run period (20.33 days), followed by paddy straw compost (22.67 days) and Maize stalk compost (27.0 days). Paddy straw + Maize straw compost (1:1) compost beds initiated early pinhead formation (15.33 days) and gave the first harvest on (9.33 days) followed by paddy straw compost (17.33 and 11.33 days) and maize stalk compost (20.67 and 14.00 days) (Fig. 4).

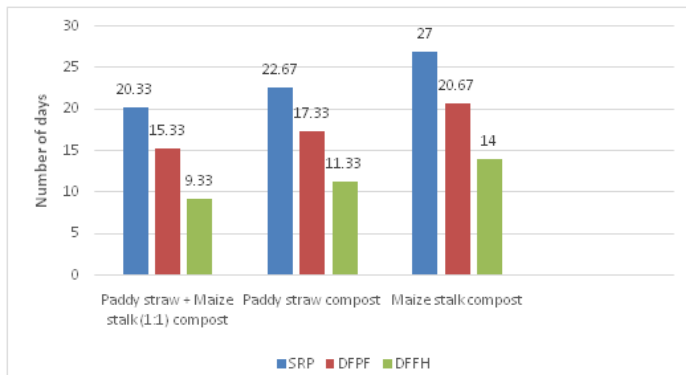


Fig.

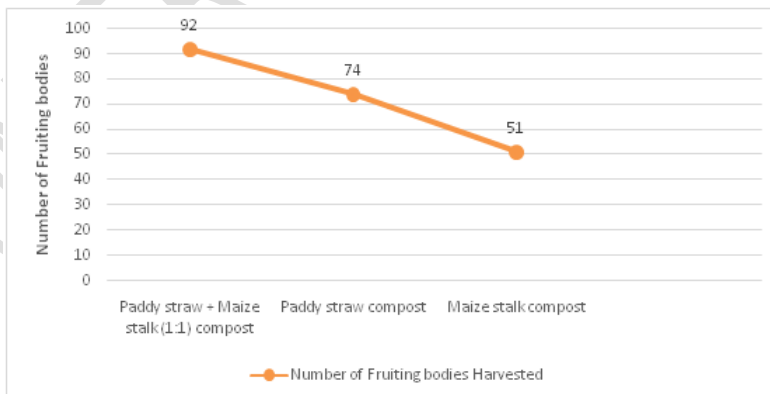
4.

**Effects of different substrates for composting on growth and harvest of *A. bisporus***

Commented [A10]: Not explained well

### 3.2 Production of sporophores

The effect of different composting substrates were measured by the number of sporophores they produced and the maximum number of sporophores (92/8 kg compost bag) was obtained from paddy straw + maize stalks (1:1) compost followed by (74/8 kg compost bag) from paddy straw compost. The average number of sporophores obtained from the maize stalks compost was found to be minimum (51/8 kg compost bag) (Fig. 5).

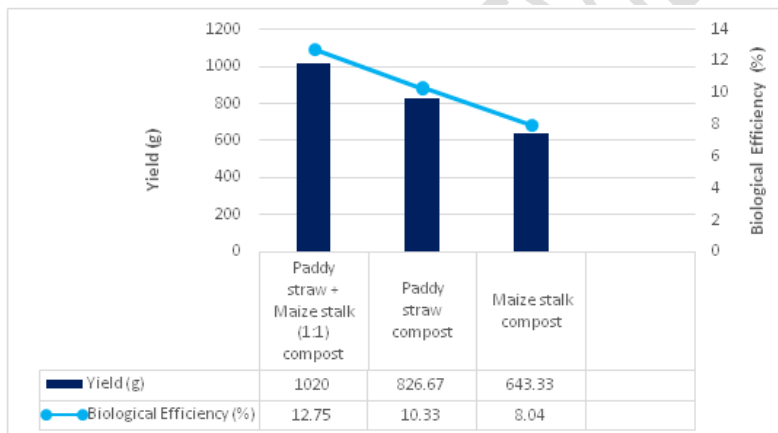


**Fig. 5. Effects of different substrates for composting on sporophores production of**

## *A. bisporus*

### 3.3 Performance on yield and biological efficiency

The key criterion for testing the effect of the various substrates used for composting in the cultivation of *A. bisporus* was yield and biological efficiency. The maximum yield and biological efficiency were observed from Paddy straw + Maize stalk (1:1) Compost beds (1020.00 g/8 kg bag and 12.75 %) followed by Paddy straw compost beds (826.67 g/8 kg bag and 10.33 %). Minimum yield and biological efficiency was obtained from Maize stalk compost beds (643.33 g/8 kg bag and 8.04 %) which also invites the weed fungus, *Coprinus* spp. in beds. All the treatments differed significantly from each other (Fig. 6).



**Fig. 6. Effects of different substrates for composting on yield and biological efficiency of *A. bisporus***

The results of the present studies clearly showed that Paddy straw + Maize stalks (1:1) combination is best for making compost which gives lesser days for spawn run, early pinhead formation, and highest number of sporophores production, yield and biological efficiency followed by Paddy straw compost. The present investigation collaborated with the findings of by Kaur et al. [9]. Paddy straw + maize stalk (1:1) substratum for the cultivation of *Calocybe indica* was suggested by few other researchers also [10]. Maize stalks compost is not suitable for the cultivation of button mushroom under the agro-climatic condition of the lateritic belt of West Bengal because they gave

Commented [A11]: Should in conclusion

less yield and biological efficiency and also invites the various competitor moulds and weed fungus viz. *Coprinus* spp.

#### 4. CONCLUSION

In summary, the investigation into *A. bisporus* cultivation using the long method of composting with various substrates has demonstrated that the combination of paddy straw and maize stalks in a 1:1 ratio yields the most favorable outcomes. This substrate showcases shorter spawn run periods, earlier pinhead formation, increased sporophores production, higher yield, and biological efficiency compared to alternative substrates. Consequently, Paddy straw + Maize stalk (1:1) compost emerges as the preferred choice, highlighting its potential as an optimal substrate for *A. bisporus* cultivation in the lateritic belt of West Bengal, India.

#### REFERENCES

1. Zandrazil F. Conversion of different plant wastes into food by basidiomycetes. Eur J Appl Microbiol. 1978; 9:243-248.
2. Atkins FC. Mushroom Growing Today. Faber and Faber: London; 1966.
3. Kaur H, Khanna PK. Physiochemical and microbiological characteristics of paddy straw based compost for *Agaricus bisporus* production. Indian Journal of Mushrooms. 2002;19:15-20.
4. Pani B. Evaluation of some substrates for cultivation of white summer mushroom. Res J Agric Sci. 2010;1:357-359.
5. Kariaga MG, Nyongesa HW, Keya NC, Tsingalia HM. Compost physico-chemical factors that impact on yield in button mushroom, *Agaricus bisporus* (Lange) and *Agaricus bitorquis* (Quel) Saccardo. J Agric Sci. 2012;3(1):49-54.
6. Rawal P, Doshi A. Evaluation of substrate for organic cultivation of *Calocybe indica* strain APK-2. Periodic Research. 2014;2:28-30.
7. Pecchia JA, Beyer DM, Li X. The use of corn stover to replace straw in compost formulations for the production of *Agaricus bisporus*. International Society for Mushroom Science. 2016;19(1):article-35.
8. Sinha N. Effect of different substrates on the growth and yield of oyster mushrooms. Int J Pure App Biosci. 2018;6(3):456-461.

**Commented [A12]:** Possible reason for less yield and biological efficiency from Maize stalk compost?? Is there any special nutrient in maize stalk compost that invite weed fungus??

**Commented [A13]:** Unfortunately I could not see the reason why combination of paddy straw and maize stalks in a 1:1 ratio yields the most favorable outcomes??

9. Kaur S, Kaur M, Devi R, Kapoor S. Paddy straw and maize stalks compost for cultivation of *Agaricus bisporus*. *Int J Curr Microbiol Appl Sci*. 2019;8(1):2418-2428.
10. Chakraborty B, Chakraborty U, Barman S, Roy S. Effect of different substrates and casing materials on the growth and yield of *Calocybe indica* in North Bengal, India. *J Appl Nat Sci*. 2016; 8(2):683-690.

UNDER PEER REVIEW