Journal of Advances in Biology & Biotechnology

Volume 27, Issue 9, Page 199-207, 2024; Article no.JABB.122079
ISSN: 2394-1081

Evaluation of Different Substrates in Composting for Cultivation of *Agaricus bisporus* under Agro-ecological Conditions of West Bengal

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Authors' contributions

This work was carried out in collaboration between both authors. Authors CS and MKB were involved in the conceptualization of the project, study design, and critical inputs. Author CS contributed to the lab work, statistical analysis and wrote the first draft. Author MKB mentored, provided resources, and helped with statistical analysis and editing of the manuscript. Both the authors contributed to the article and approved the submitted version. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i91290

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/122079

Original Research Article

Received: 17/06/2024
Accepted: 19/08/2024
Published: 23/08/2024

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.

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Cite as: *Shanmugaraj, C., and M.K. Biswas. 2024. "Evaluation of Different Substrates in Composting for Cultivation of Agaricus Bisporus under Agro-Ecological Conditions of West Bengal". Journal of Advances in Biology & Biotechnology 27 (9):199-207. https://doi.org/10.9734/jabb/2024/v27i91290.*

Study Design: A Completely Randomized Design (CRD) with three replications per treatment was implemented to ensure the reliability of 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 (https://visvabharati.ac.in/PalliSikshaBhavana.html).

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 quick growth cycles and higher yields, which could benefit local mushroom producers by enhancing production efficiency.

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 cultivation of the *A. bisporus*, 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 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]. Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir has innovated a method to utilize affordable agro-wastes such as linseed straw, maize straw, soybean straw, paddy husk, and fallen leaves from chinar, apple, poplar, and mulberry trees for cultivating button and dhingri mushrooms [9]. Locally accessible substrates, including wheat straw, paddy straw, pea straw, cotton waste, maize straw, sugarcane bagasse, and a mixture of wheat straw with paddy straw, were assessed to determine the most suitable substrate for production of *Calocybe indica* [10]. In the study, a mixture of wheat straw and paddy straw (1:1) emerged as the most effective formulation for button mushroom cultivation, requiring the fewest days to the first harvest (28.02 days) and yielding the highest output (19.98 kg per 100 kg compost) [11]. Six agro by-products, including wheat straw, paddy straw, corn straw, paddy husk, and typha grass, were tested as base substrates for button mushroom production, with all other ingredients kept constant across substrates. Colonization was observed in all compost types. The highest yield (9.76 kg of mushrooms per quintal of compost) was recorded with wheat straw, while the lowest yield (6.69 kg of mushrooms per quintal of compost) was observed with soybean straw [12]. The results indicated that compost prepared with different straws showed the fastest spawn run (13.30 days) in wheat straw, while the fewest days required for pinhead initiation (15.60 days) were observed in the combination of wheat straw and paddy straw. The yield-related traits did not differ significantly, with the number of fruiting bodies ranging from 23.60 to 31.00. The fresh yield of button mushrooms on compost prepared from different straws was significantly highest (560g) with a biological efficiency of 11.2% in the combination of wheat straw and paddy straw [13].

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. [14], underscore the significance of substrate composition in optimizing button mushroom cultivation. 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. Overall, these studies highlight the importance of substrate selection and compost formulation in optimizing mushroom cultivation yields and efficiency.

2. MATERIALS AND METHODS

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 (1:1 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).

2.1 Compost Preparation

The compost preparation in this study was conducted employing the Long Composting method, as illustrated in Figs. 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. 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 [15].

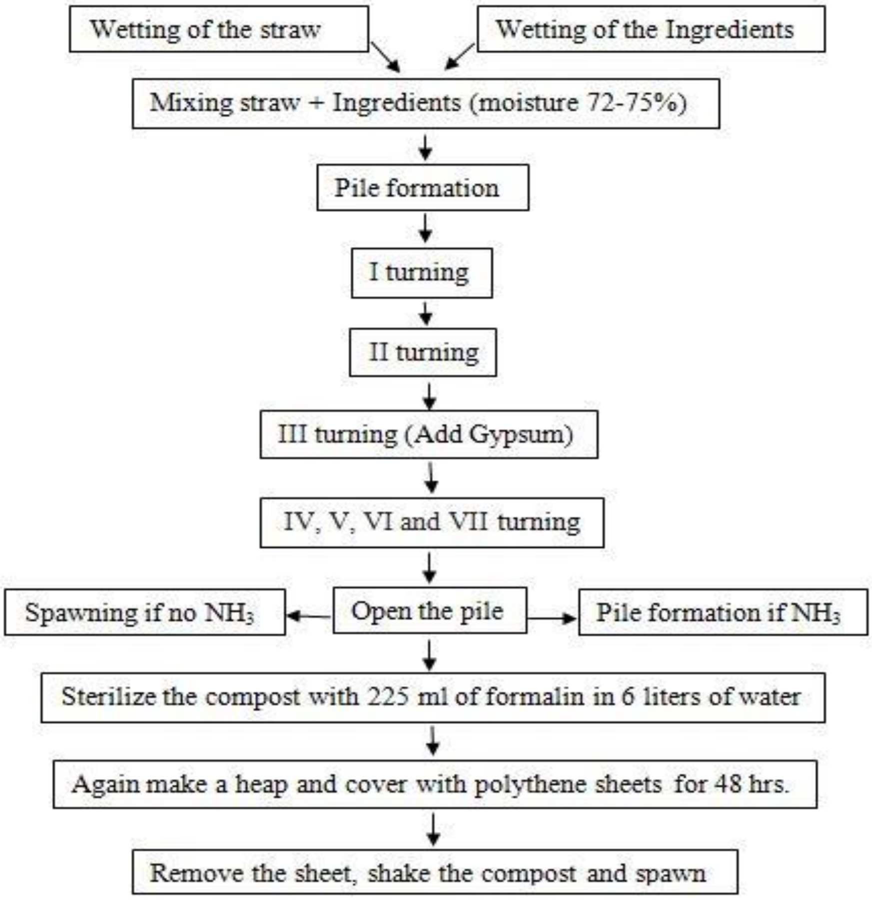

Fig. 1. Flow chart of long method of composting

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*

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.

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.

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:

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

3. RESULTS AND DISCUSSION

The results of all 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 are summarized in Table 1.

3.1 Time for Spawn Run and Primordial Initiation

Paddy straw + maize stalk (1:1) compost exhibited the shortest spawn run period (SRP) of 20.33 days, followed by paddy straw compost with an SRP of 22.67 days, and maize stalk compost with the longest SRP of 27.0 days. The paddy straw + maize stalk (1:1) compost also facilitated early days for pinhead formation (DFPF) at 15.33 days and achieved the first harvest in 9.33 days. In comparison, paddy straw compost showed a DFPF of 17.33 days and days for first harvest (DFFH) of 11.33 days, while maize stalk compost required 20.67 days for pinhead formation and 14.00 days for the first harvest (Fig. 4).

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

Treatments	SRP	DFPF	DFFH	No. FH	Yield(g)	B.E(%)	Remarks
Paddy straw + Maize stalk (1:1) compost	**20.33**	**15.33**	**9.33**	**92**	**1020.00**	**12.75**	-
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	*Coprinus* 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

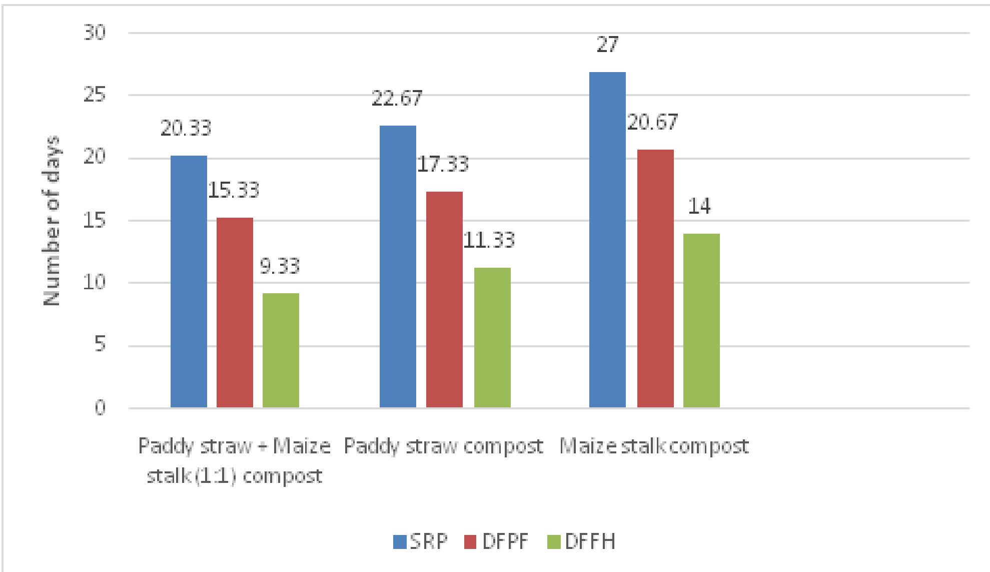

Fig. 4. Effects of different substrates for composting on growth and harvest of *A. bisporus*

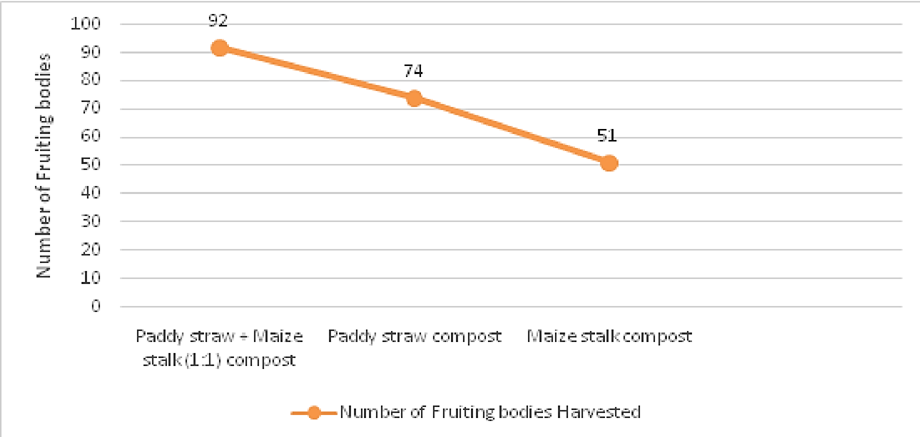

Fig. 5. Effects of different substrates for composting on sporophores production of *A. bisporus*

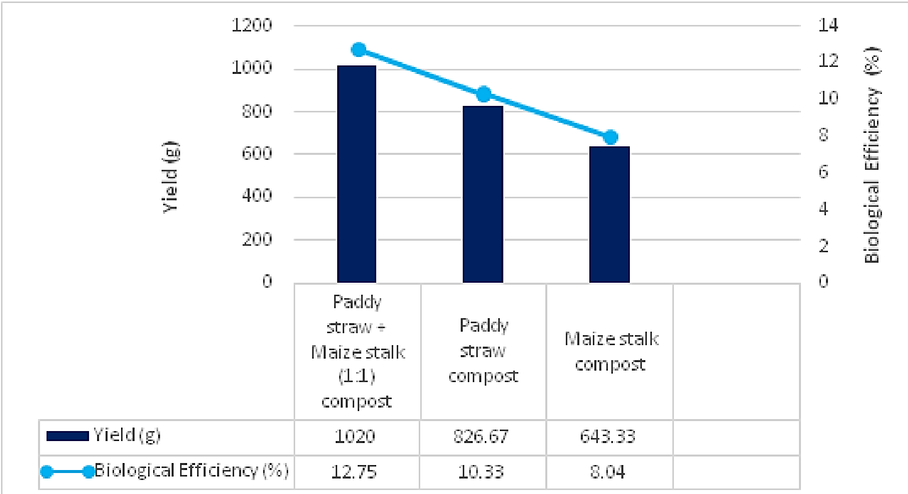

Fig. 6. Effects of different substrates for composting on yield and biological efficiency of *A. bisporus*

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).

3.3 Performance on 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).

The findings of the current study clearly demonstrate that a combination of paddy straw and maize stalks (1:1) is the most effective for compost preparation, resulting in a shorter spawn run period, earlier pinhead formation, and the highest production of sporophores, yield, and biological efficiency, followed by compost made solely from paddy straw. This investigation aligns with the research of Kaur et al. [14], which underscores the potential of a high-yielding compost formulation based on paddy straw and maize stalks as a viable alternative to the conventional wheat straw-based compost. Yield data revealed that the paddy straw and maize stalk (1:1) compost produced the maximum yield (13.6 kg per quintal of compost) and 1563 fruit bodies per quintal of compost. It was observed that the paddy straw and maize (1:1, w/w) compost decomposed more effectively than the paddy straw and maize stalk (2:1, w/w) compost and the paddy straw compost alone. The study concluded that the paddy straw and maize stalk (1:1, w/w) compost is the best formulation for large-scale mushroom production, particularly benefiting small and marginal farmers. The use of paddy straw and maize stalks as a substitute for wheat straw has been shown to deliver yields comparable to wheat straw compost, as observed by Tewari and Sohi [16]. They found that synthetic compost made from equal parts maize stalks and paddy straw produced a yield of 145.5 kg per ton of dry matter, which was comparable to that of wheat straw compost. Milky mushrooms (*Calocybe indica*) were cultivated using various substrates, including paddy straw, sorghum stalks, sugarcane bagasse, maize stalks, soybean hay, blackgram hay, groundnut haulms, sawdust, paddy straw compost, and coir pith compost. Among these substrates, paddy straw and maize stalks yielded significantly higher outputs (356.5 g and 354.3 g per bed, respectively), followed by sorghum stalks [17]. The paddy straw and maize stalk (1:1) substrate for the cultivation of *Calocybe indica* has also been recommended by other researchers [18]. Mixing maize stalks with other substrates creates effective composting materials; however, maize stalks alone are not ideal as a substrate, particularly for button mushroom composting. This is because they slow down the decomposition process due to their high lignin content and they gave 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENTS

The authors are thankful to the Palli Siksha Bhavana, Visva-Bharati for providing support, encouragement and facilities during the course of this investigation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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