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ORIGINAL RESEARCH

Bamboo waste recycling using *Dictyophora indusiata* mycelia cultivation

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Abstract

Purpose Reusing agricultural waste as a substrate material for mushroom cultivation was considered a great alternative to bio-waste disposal. In this study, bamboo waste from a local skewer factory in Lampang Province, Thailand, was used as a substrate, along with the original material for the mycelial growth of *Dictyophora indusiata* mushroom.

Method *D. indusiata* mycelia were grown in two materials in three different combinations: lingzhi pieces, lingzhi pieces combined with bamboo scraps (1:1), and bamboo scraps. A number of days when the substrate was completely covered with mycelia was recorded. After that, the mycelia from all three cultures were used as inoculum for cultivating *D. indusiata* through two procedures, including in-field direct cultivation and spawn production before cultivation. To evaluate the mushrooms productivity, the length of time to fruiting and the weight of the harvested mushrooms were recorded.

Results *D. indusiata* mycelia grew fastest in a lingzhi-based substrate (19.3 days), followed by a combination of lingzhi and bamboo (24.9 days) and bamboo (35.7 days). The highest number of the fruiting body (37.0 mushrooms per plot) and fresh weight (2,310.5 g/0.75 m^2) were observed in treatments in which spawn was produced prior to cultivation and inoculum was derived from lingzhi pieces combined with bamboo scraps. The next two highest mushroom yields were obtained through cultivation using bamboo scraps inoculum, which required more time to develop.

Conclusion Bamboo scraps was a promising material for mycelial growth and cultivation of *D. indusiata*.

Keywords Agro-industrial waste, Edible fungi, Mycelia growth, *Dictyophora indusiata* production

Introduction

Mushrooms belong to the kingdom Fungi, a valuable group of organisms. They contribute to nutrient cycling, symbioses, and soil structure improvement. Mushrooms can degrade and convert lignocellulosic materials into carbohydrates, protein, fatty acids, and other compounds for human consumption. In addition, their growth contributes valuable organic matter, nitrogen, phosphorus, potassium, and other nutrients to

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the soil (Peter et al. 2019; Hu et al. 2021). Due to their nutritional value and medicinal properties, edible mushrooms have become a popular food consumed worldwide. *Dictyophora indusiata* is an edible mushroom belonging to the family *Phallaceae* of the phylum Basidiomycetes. The species, also known as bamboo mushroom or veiled lady mushroom, is extensively employed as a functional food, traditional medicine, and skin care agent (Cheong et al. 2018; Burapapadh et al. 2021). Hu et al. (2021) reported nutrition values of *D. indusiata* with 17.87% protein, 54.98% total carbohydrate, 0.63% crude fat, 11.47% crude fiber and 16.32% total amino acids. Health-promoting activities, including anti-inflammatory, anti-tumor, antioxidant, antimicrobial, anti-obesity, and immunomodulatory activities of this mushroom have been reported (Oyetayo et al. 2009; Ker et al. 2011; Han et al. 2017; Liu et al. 2017; Habtemariam 2019; Wang et al. 2019; Kanwal et al. 2020; Nazir et al. 2021). Developmental stages of the mushroom basidiocarp include pinhead, button, egg, elongation and mature stage (Fig. 1). The in-field *D. indusiata* cultivation process includes culture preparation, spawn production, substrate material preparation, inoculation or seeding, incubation and harvesting which is slightly different from other commercial mushrooms. In Thailand, the cultivation of this mushroom is prominent among local farmers and community enterprises. It is common practice to seed mushrooms in both baskets and soil. Immature mushrooms at late egg stage are usually harvested to collect the viscous mucilage in the peridium before being allowed to grow to maturity outside the field. For *D. indusiata* culture preparation, lingzhi mushroom pieces are regularly used as a substrate, resulting in high production costs.

Fig. 1 Developmental stage of *Dictyophora indusiata* mushroom

According to reports, agricultural wastes are a good source for mushroom cultivation. Rice bran, rice straw, wheat straw, cotton straw, sawdust, molasses, maize husks, sugarcane bagasse and empty fruit bunch are common substrates for mushroom production (Jeznabadi et al. 2016; Mohamed et al. 2016; Kamthan and Tiwari 2017; Triyono et al. 2019; Fufa et al. 2021). Local farms and household factories in Thailand generate significant quantities of agricultural waste and agro-industrial residues. The majority of them are burned and discarded, resulting in negative environmental effects. Bamboo is a valuable resource plant that can be used to produce food, medicine, textiles, clothing, furniture, energy, and industrial goods (Li and He 2019). Due to the demand for disposable bamboo products such as skewers and chopsticks which are frequently used at Thai food stalls, production has continued and bamboo waste has increased. In Mae Moh District, Lampang Province, there are 5 local factories producing bamboo

products with a total production capacity of approximately 3 tons per day on average. After the production process, 80% of the raw material that becomes waste is disposed of by incineration. The present study focuses on the conversion of bamboo scraps from a local skewer factory in Mae Moh district, Lampang province into a valuable material source for mycelial growth and cultivation of *D. indusiata* mushroom at Baan Kor Ruak Community Enterprises in Mae Moh district. Although there are numerous reports of using various agricultural wastes in mushroom cultivation, bamboo scraps are still rarely utilized in mycelium and mushroom cultivation. Consequently, the purpose of this study was to assess the performance of *D. indusiata* mushrooms grown on substrates containing bamboo residues.

Materials and methods

Study location

This study was primarily conducted at both the faculty of Science and the faculty of Agricultural Technology of Lampang Rajabhat University in Lampang Province, Thailand. The fruiting body was cultivated at Baan Kor Ruak Community Enterprises in the Jang Nua sub-district of the Mae Moh District in Lampang.

Mushroom strain and identification

D. indusiata strain was provided by Baan Kor Ruak Community Enterprises. Using a genomic DNA extraction kit (RBC Real Genomics), the fungal DNA was extracted from approximately 0.1 g of an egg stage fruiting body for sequence-based identification. The extracted DNA was used as a template for PCR amplification using Excel Taq 5X PCR Master Dye Mix (SMOBIO) and two universal primers including ITS3 (5'-gCATCgATgAAgAACgCAgC-3') and ITS4 (5'-TCCTCCgCTTATTgATATgC-3'). The PCR conditions were executed in accordance with the manufacturer's instructions. Bi-directional sequencing of an approximately 450 bp PCR product was carried out by Gibthai Co., Ltd., and the received sequences were used to perform BLAST searches (Altschul et al. 1990) on the NCBI GenBank database.

Experimental Design

The study was designed and conducted using a Completely Randomized Design (CRD) with six treatment groups (Table 1). Mycelia grew on substrates composed of lingzhi mushroom pieces (T1), lingzhi mushroom pieces combined with bamboo scraps (T2), and bamboo scraps (T3). After complete colonization, each set of mycelia-covered substrates was cultivated using two different procedures: in-field direct mushroom cultivation (T11, T21, and T31) and mushroom spawn production prior to in-field cultivation (T12, T22, and T32).

Table 1 Treatment formulation and procedure

Treatments		Substrates for mycelia growing and composition	Mushroom spawn production	Mushroom cultivation
T1	T11	LZ (100%)	No	Yes
	T12	LZ (100%)	Yes	Yes
T2	T21	LZ (50%) + BB (50%)	No	Yes
	T22	LZ (50%) + BB (50%)	Yes	Yes
T3	T31	BB (100%)	No	Yes
	T32	BB (100%)	Yes	Yes

LZ: lingzhi mushroom pieces; BB: bamboo scraps

Preparation of mycelial substrate and spawn

Mycelia of *D. indusiata* were cultivated in sterilized media containing lingzhi mushroom fragments for stock culture, as is customary in Baan Kor Ruak community enterprises. Periodically, media bottles were maintained at room temperature and subcultured. Inferior lingzhi mushrooms were purchased from Kaset JaoKhun Mushroom Farm Limited Partnership. Bamboo scraps were kindly provided by a skewer household factory in Mae Moh District, Lampang Province. They were soaked in a separate container of clean water overnight, and the excess water was removed using a plastic sieve. Lingzhi mushrooms were cut into small pieces, about 1 cm × 1 cm in size. To facilitate the operation, stick-shaped bamboo scraps were selected and cut to an appropriate length. The culture bottles were filled to a net weight of 60 g with lingzhi and bamboo substrates according to Table 1. The substrates were sterilized using an autoclave for 30 minutes at 121 °C and inoculated with an equal amount of *D. indusiata* stock culture (approximately 3 g) in 24 replicates for each treatment. The inoculated bottles were incubated at room temperature. The number of days that the substrate was completely covered with mycelia was used to calculate the mycelial growth. The quantities of non-expansion and contaminated bottles were monitored. The spawns used in treatment T12, T22, and T32 came from Wiang Hong farm, Mae Moh District, Lampang. Para rubber wood sawdust (100 kg) was used as the primary substrate in the spawn bag. Other components included fine rice bran (6 kg), calcium oxide (1 kg), gypsum salt (1 kg), and sodium sulfate (0.2 kg). Four grams of lingzhi or bamboo substrate containing *D. indusiata* mycelia were transferred aseptically to each spawn bag with 20 replicates. For ramification to occur, they were incubated at room temperature in a dark place while covered with a black cloth. Fungal growth was measured by the number of days the spawn was entirely run.

In a seven-day interval, the rate of mycelial growth through the substrate was monitored by measuring the mycelia growth on every four sides of the mushroom spawn with a ruler.

Preparation of mushroom substrate for the growth of fruiting body

D. indusiata was cultivated on a soil plot in the Baan Kor Ruak Community Enterprises region. By removing approximately 10 cm of soil surface, eighteen plots of 0.75 m × 1.00 m were prepared. Substrate formulas operated by community enterprises for mushroom cultivation (per plot) consisted of removed soil, bamboo scraps (3 kg), sawdust (0.5 kg), rice husks (0.5 kg), dry leaves (0.5 kg), and sugar (1 tablespoon). Bamboo scraps were prepared by soaking in water before use. Before spreading the mushroom seed on top, half of the bamboo scraps were placed in the plot. Four mycelial substrate bottles were used as the seeds for direct mushroom cultivation (T11, T21, and T31), while three mycelial spawns were used for the other procedures (T12, T22, and T32). Sugar, rice husks, and sawdust were dispersedly added. The remaining bamboo scraps were then placed, followed by dry leaves and soil. The fruiting body beds were irrigated twice daily. To evaluate the productivity of *D. indusiata* mushrooms, parameters including fruiting time and harvest weight were recorded. About three months were spent harvesting mushrooms at the late egg or immature stage, which were still rich in viscous mucilage. The yield of mushroom was calculated by the equation as follows (Mkhize et al. 2017):

$$\text{mushroom yield} = \frac{\text{weight of fresh mushroom harvested}}{\text{fresh substrate weight}}$$

Statistical analysis

At a significance level of 0.05, the data was analyzed by one-way analysis of variance (ANOVA), and the

means were compared using the least significant difference (LSD) test using IBM SPSS Statistics (trial version).

Results and discussion

Identification of *D. indusiata*

PCR product of the mushroom sample with the expected size of approximately 450 bp was clearly detected by gel electrophoresis.

After sequencing and blast searching, the nucleotide sequence of mushroom matched 95% *D. indusiata* (accession number HQ414538.1).

Growth of *D. indusiata* mycelia in different substrate

A significant interaction was observed between substrate composition and the number of days required for *D. indusiata* mycelia to complete colonization (Table 2 and Fig. 2). The shortest period of mycelial growth was observed at the lingzhi mushroom substrate (19.3 days), followed by the combination of lingzhi mushroom and bamboo scraps substrate (24.9 days). Bamboo substrate alone resulted in the longest colonization time (35.7 days), and mycelial growth was not observed at 29.2% (7 out of 24 replicates).

The mycelium texture of *D. indusiata* cultivated on lingzhi media was cottony with moderate to high density and abundant growth. Hydrolytic and oxidative extracellular enzymes are required to degrade lignocellulosic substrates like bamboo (Peter et al. 2019). In contrast, the principal constituents of the fungal cell wall are chitin, glucans, and glycoproteins, which are more easily degraded. Lingzhi mushroom is also a rich source of bioactive compounds, nutrients, and fiber (Kumar et al. 2021; Parepalli et al. 2021), affecting the rapid growth of *D. indusiata* mycelia. Contamination was found only in substrates containing bamboo scraps. However, one bottle of substrate contaminated by black mold in T2 on day 12 was overcome by *D. indusiata* mycelia after 4 weeks of incubation.

Table 2 Influence of substrate composition on the mycelial growth of *D. indusiata*

Treatments	Substrates used	Number of days mycelial growth	Non-expansion of mycelia (%)	Contamination (%)
T1	LZ (100%)	19.3 ± 1.0^{c}	0	0
T2	LZ (50%) + BB (50%)	24.9 ± 2.2^{b}	0	4.2
T3	BB (100%)	35.7 ± 3.7^{a}	29.2	8.3

LZ: lingzhi mushroom pieces; BB: bamboo scraps; Means with different letters within the same column represent significant differences at $P<0.05$

There were no significant differences in the number of days the spawn ran utterly between the inoculums obtained from the various substrates. The spawn produced from T1, T2, and T3 substrates (T12, T22, and T32) were fully colonized 60.7, 61.9, and 63.3 days after inoculation, respectively (Table 3). They took less time to grow than Chen (2000) had previously reported. During week 1-7, growth of *D. indusiata* mycelia in T32 appeared to be the slowest (Fig. 3). It grew from the inside out rather than from the top down, so by week 8, the mycelium size was comparable to that of the other experiments.

The yield of *D. indusiata* mushroom cultured by using inoculums grown in different substrate

The fruiting body of *D. indusiata* from direct cultivation treatment (T11, T21, and T31) was observed at 87.7-92.0 days after seeding (Table 3). Since the stock culture of mushroom mycelia was spread directly in the soil plot, they might need more days to reach physical maturation before primordium formation. The

treatment seeded by mycelial spawn (T12, T22, and T32) took 21.3-23.3 days for the appearance of a fruiting body. This period was similar to the normal cultivation of Baan Kor Ruak Community Enterprises (3-4 weeks), but it took less time than reported by Chen (2000) and Bunroj and Rassami (2019). There seemed to be no difference between different substrate compositions on whole days spent for mushroom cultivation, although the treatment using substrate containing bamboo scraps alone took slightly longer to cultivate than other experiments. In the same substrate composition, cultivation through the spawn production appeared to be faster than direct cultivation.

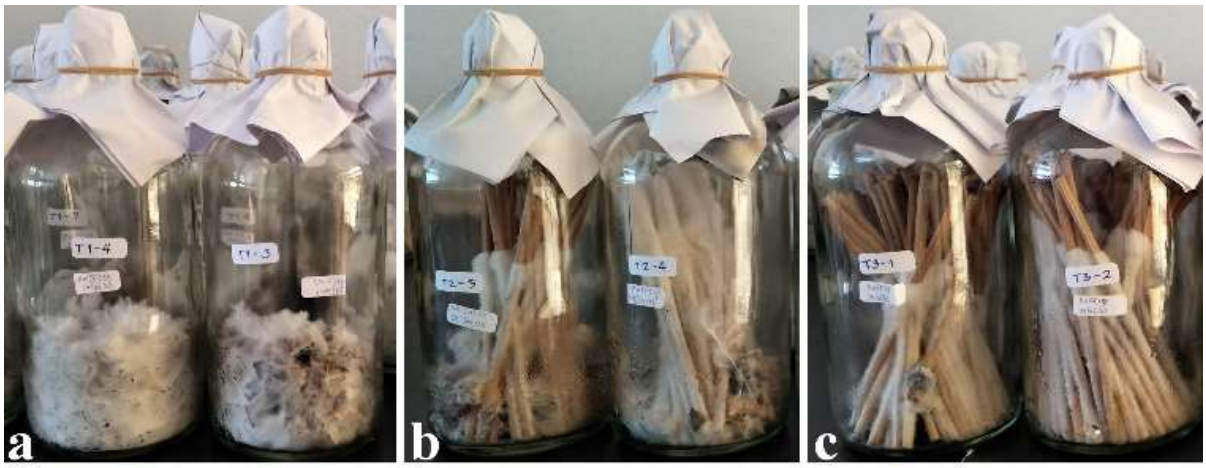


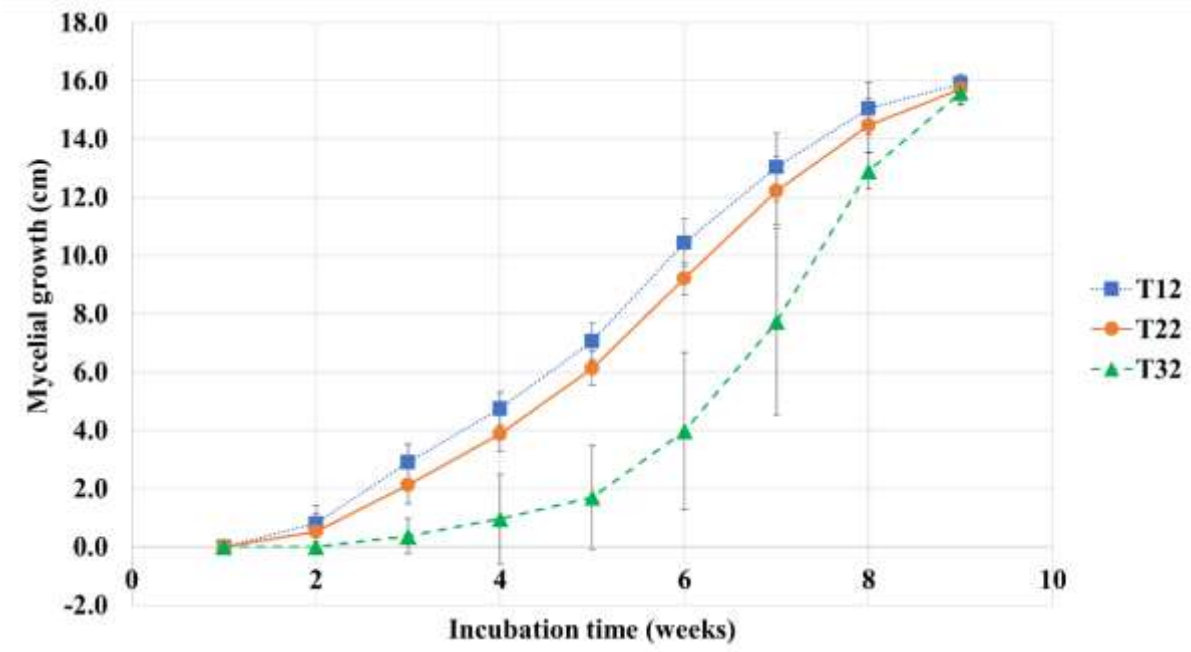
Fig. 2 Growth of *D. indusiata* mycelia cultured in different substrates for 24 days; (a) lingzhi mushroom, (b) lingzhi mushroom combined with bamboo scraps, and (c) bamboo scraps

The T22 treatment produced the highest number of fruiting bodies per plot (37.0), fresh weight of fruiting bodies (2,310.5 g/0.75 m^2), and mushroom yield (0.51) (Table 4). Mycelial substrate for this experiment consisted of a combination of lingzhi mushroom and bamboo scraps. The treatments of bamboo scraps alone (T31 and T32) also produced a high number of fruiting bodies. The yield of *D. indusiata* mushroom varied between 1,042.3– 2,310.5 g/0.75 m^2. This productivity was comparable to that of Baan Kor Ruak Community Enterprises and appears to be greater than that of other reports. *D. indusiata* cultivation with bamboo chips and sugarcane bagasse yield 124.8 g/m^2 and 104.3 g/m^2, respectively (Hu et al. 2021).

Table 3 Duration time of mushroom production using inoculum cultured from different substrate composition and different cultivation procedure

Treatments	Days for mycelia growth	Days for fully colonized spawn	Days for fruiting body formation	Days for mushroom cultivation
T11 (LZ_nS)	19.3 ± 1.0^{c}	-	91.0 ± 1.7^{ab}	110.3
T12 (LZ_S)	19.3 ± 1.0^{c}	60.7 ± 4.1^{b}	23.3 ± 2.5^{c}	103.3
T21 (LZ+BB_nS)	24.9 ± 2.2^{b}	-	92.0 ± 2.0^{a}	116.9
T22 (LZ+BB_S)	24.9 ± 2.2^{b}	61.9 ± 3.8^{ab}	21.3 ± 2.5^{c}	108.1
T31 (BB_nS)	35.7 ± 3.7^{a}	-	87.7 ± 2.5^{b}	123.4
T32 (BB_S)	35.7 ± 3.7^{a}	63.3 ± 3.4^{a}	22.0 ± 1.0^{c}	121.0

LZ: lingzhi mushroom pieces; BB: bamboo scraps; nS: no spawn production; S: spawn production
Means with different letters within the same column represent significant differences at $P<0.05$



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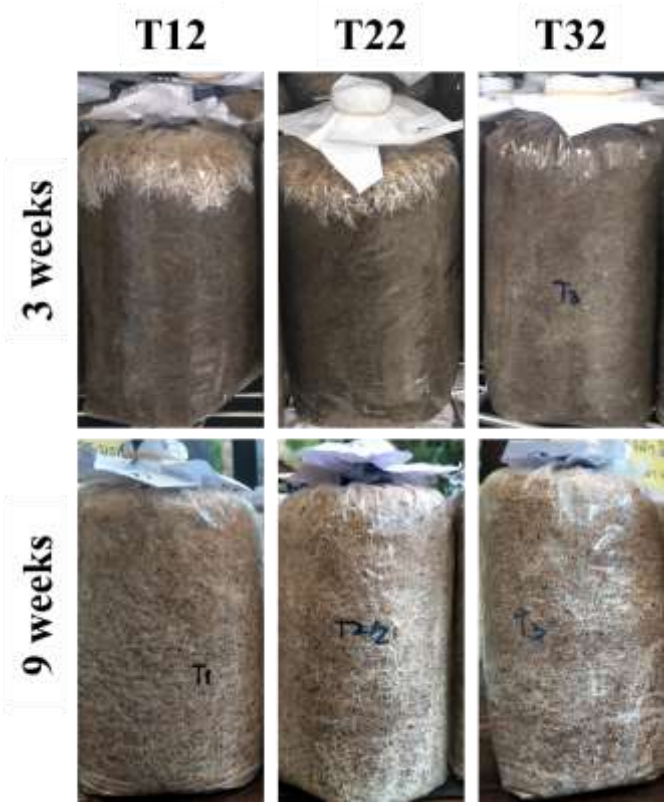

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Fig. 3 Mycelial growth in mushroom spawn produced by different substrate inoculums, including lingzhi mushroom (T12), lingzhi mushroom combined with bamboo scraps (T22), and bamboo scraps (T32); (a) growth of *D. indusiata* mycelia measured during week 1-9, and (b) mycelia ran in spawn at week 3 and 9

The highest yield was achieved by bamboo mushrooms grown beneath the bitter guard's grip on durian leaves, with a yield of 348.3 g/m^2 (Bunroj and Rassami 2019). In these works, the mushrooms may have been harvested at a mature stage where they lose the mucilage, making them lighter. In the current study, they were harvested in the late egg stage as this is the usual procedure for farmers operating in community enterprises. Mushrooms weighed more because of the viscous mucilage that was abundant at this stage. A

brick-block planting plot with rice straw and coconut husks as substrates yielded about 4 kg/m^2 of bamboo mushrooms, according to Chaima et al. (2017) (Table 5). There are a number of variables that affect the yield of mushrooms, including the type of growth media, environment, climate, and cultivation systems (Triyono et al. 2019). The cultivation of mushrooms through mushroom spawn or seeding production is still an appropriate procedure. Two sets of direct cultivation without spawning (T11 and T21) appeared to produce lower yields and take longer time than that of the spawning method (T12 and T22). Another set was consistent only in terms of cultivation time; the productivity from direct cultivation (T31) was slightly higher than that of spawning treatment (T32). Insects and snails were partially responsible for the occurrence of atrophy in all sample plots (Fig. 4), particularly in experiments where lingzhi was used as the substrate inoculum (T11 and T12). As a result, only modest amounts of mushroom were produced.

Table 4 Influence of substrate formulation and procedure on the growth and productivity of *D. indusiata* mushroom

Treatments	Number of the fruiting body per plot*	Fresh weight of the fruiting body (g/plot*)	Mushroom yield
T11 (LZ_nS)	18.0 ± 7.9^{b}	1,042.3 ± 387.5^{c}	0.23
T12 (LZ_S)	25.0 ± 9.5^{ab}	1,484.9 ± 529.1^{abc}	0.33
T21 (LZ+BB_nS)	22.0 ± 7.5^{ab}	1,286.0 ± 416.0^{bc}	0.29
T22 (LZ+BB_S)	37.0 ± 7.5^{a}	2,310.5 ± 523.1^{a}	0.51
T31 (BB_nS)	32.7 ± 10.0^{ab}	2,017.4 ± 596.7^{ab}	0.45
T32 (BB_S)	27.7 ± 4.9^{ab}	1,729.4 ± 162.8^{abc}	0.38

LZ: lingzhi mushroom pieces; BB: bamboo scraps; nS: no spawn production; S: spawn production
*Area plot = 0.75 m^2; Means with different letters within the same column represent significant differences at P<0.05

Table 5 Yield of *D. indusiata* mushroom in soil cultivation with different substrate material

Main raw material	Yield	Harvested mushroom stage	References
Bamboo chips	124.8 g/m^2	Mature stage	Hu et al. (2021)
Sugarcane bagasse	104.3 g/m^2	Mature stage	Hu et al. (2021)
Durian leaves	348.3 g/m^2	Mature stage	Bunroj and Rassami (2019)
Rice straw and coconut husks	4,109.4 g/m^2	Late egg stage	Chaima et al. (2017)
Bamboo scraps	3,080.7 g/m^2	Late egg stage	This study

One of the lignocellulosic materials useful for edible mushroom production was waste from bamboo plants. Bamboo sawdust was used as an oyster mushroom cultivation substrate. In comparison to conventional media, bamboo media had a shorter total growth period and bamboo media with rice bran had higher yields and fruiting bodies (Yamauchi et al. 2019). *Pleurotus ostreatus* thrived in a mixture of bamboo sawdust and microorganisms (EM4) (Taskirawati et al. 2020). For Indian oyster production, Pongthornpruek and Kraiwutthinan (2021) used bamboo sawdust mixed with fermented chopped straw. The mycelium of *Volvariella volvacea* was able to grow on bamboo shoot shells, and the fruiting body yield was 1.52-fold higher than straw cultivation (Li et al. 2021). It was composted before being used as a substrate material in most of these works. In this study, good yielding mushrooms were found to grow on unfermented bamboo scraps.

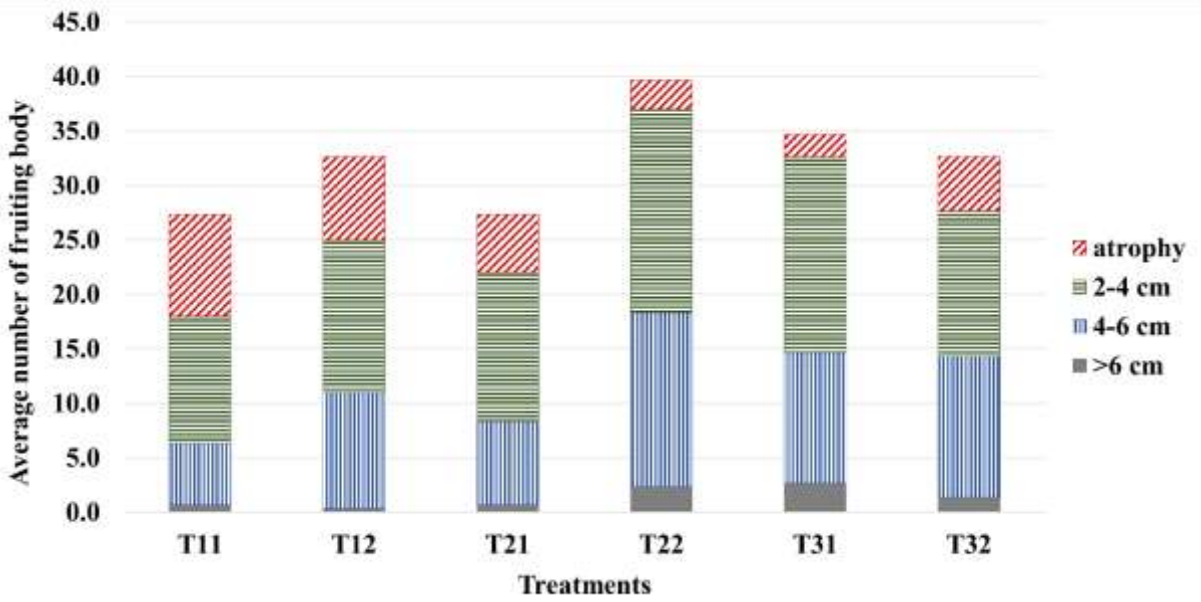

Fig. 4 Average number of the fruiting body of *D. indusiata* mushroom produced by different seeding and procedure. The number of harvested mushrooms organized by size and atrophy mushroom were shown.

Conclusion

D. indusiata can grow its mycelium and produce mushrooms from bamboo scraps.

Bamboo scraps replace lingzhi pieces in the substrate, extending mycelial growth time but maintaining high productivity at a lower production cost. *D. indusiata* production can still be done using the traditional method of spawn production. An important finding of this research is that converting local agricultural waste into a marketable and useful product is feasible.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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