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Mycelial growth and basidiocarp production of edible mushrooms on coconut waste-based substrates

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Abstract

Edible mushrooms are primarily cultivated for culinary purposes using agricultural waste. However, in some areas, these materials may not be readily available. This study explores the potential of coconut wastes as a substrate for mycelial growth and basidiocarp production in edible mushrooms. Coconut water from young and mature coconuts was used to assess mycelial growth performance, while coconut pulp was tested for its efficiency as a spawning material. For fruiting body production, varying formulations of rice straw and coconut coir dust were evaluated to identify the most suitable substrate for optimal productivity. Results revealed that *Cyclocybe cylindracea, Pleurotus djamor, Pleurotus florida,* and *Pleurotus sajor-caju* preferred mature coconut water gelatin. The mycelial growth of *P. florida* was optimal in media with a pH of 5–6, whereas the other species exhibited a broader pH tolerance. Coconut pulp demonstrated a comparable mycelial diameter to rice seeds as a spawning material. All the mushrooms favored substrates with a higher proportion of rice straw, as evidenced by a shorter incubation period and primordia initiation, larger cap sizes, and higher yields and biological efficiency. These findings highlight the efficiency of coconut wastes as viable alternative substrates for mushroom cultivation, demonstrating that optimal fruiting body production can be achieved with the appropriate substrate ratio.

Keywords – biological efficiency – coconut coir – oyster mushroom – *Pleurotus*

Introduction

Mushroom cultivation is an agricultural practice of utilizing agricultural wastes to produce fruiting bodies. These substrates provide nutrition for the mycelia and support the development and maturation of fruits. Carbon and nitrogen are the two primary nutrient requirements for the growth of mushrooms, along with other micronutrients and trace elements (Chang & Miles 2004). The presence and amount of these nutrients vary depending on the source; hence, combining two or more materials is crucial to supply the essential nutrients for the mycelia. Among the many substrates used, sawdust from hardwood trees, often combined with rice bran and rice straw, is the most commonly used substrate for mushroom cultivation in the Philippines (Saravana et al. 2023). Sawdust from the coconut tree (*Cocos nucifera* L.) has proven to be an efficient and highly suitable substrate for several

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species, such as oyster mushrooms (Shah et al. 2004). It is also ideal for cultivating other mushrooms like *Auricularia polytricha, Cyclocybe cylindracea, Lentinus swartzii,* and *Trametes elegan*s (Landingin et al. 2020, Dulay et al. 2021a, b, Aguilar et al. 2024). Besides its suitability as a substrate, coconut coir also enhances the nutritional and bioactive properties of *Pleurotus ostreatus* (Mihai et al. 2022). This highlights the efficiency of coconut waste as a nutrient-rich substrate for mushroom cultivation.

Aside from sawdust, coconut shells, coir, water, and pulp are considered wastes and by-products of the coconut industry (Annamalai et al. 2017). In the Philippines, about 14–15 billion coconuts per year are produced (Hoe 2018). These are utilized in the food and beverage industry, cosmetics, supplements, and agriculture, and are exported in various processed forms (Moreno et al. 2020). On the other hand, a significant amount of waste is generated, which remains underutilized. Hence, these by-products need to be utilized in other fields like mushroom production, where they can serve as a sustainable and viable substrate for food production.

Local growers produce a significant supply of mushrooms in the market, with oyster mushrooms being the most commonly grown (Beetz & Greer 2004). They belong to the genus *Pleurotus*, which is known for its wide adaptability and aggressiveness. Among the many species of oysters, *Pleurotus florida, Pleurotus sajor-caju,* and *Pleurotus djamor* are very popular. They all have a delicate flavor, making them ideal for culinary use, and possess medicinal properties that add to their economic significance (Patel et al. 2012). Their cultivation began in the 1980s involving simple techniques using various materials such as wood wastes, maize stalk, pea residue, banana leaves, paddy straw, wheat straw, apple leaf, Chinar leaf rice straw, banana straw, cotton wastes, sunflower stalk, wheat straw, ground nut straw, banana stalk, Bahia grass, tissue paper, rice husk ash, rubber sawdust, oil palm pressed fiber, and rubber tree sawdust (de Siqueira et al. 2011, Pala et al. 2012, Patil et al. 2012, Pokhrel et al. 2013, Fasehah & Shah 2017, Ab Rhaman 2022, Olasupo et al. 2024). Another species of edible mushroom is *C. cylindracea*, commonly known as Yanagi. This mushroom has a dark brown fruiting body that possesses a ring and has an exceptional taste (Li et al. 2014). Rice straw combined with sawdust, and soybean flour supplemented with wheat straw are the substrates suitable for growing this mushroom (Uhart et al. 2008, Landingin et al. 2020). Introducing other species of edible mushrooms into the market, like *C. cylindracea* is important because it diversifies food options, provides consumers with unique flavors and textures, and helps promote the culinary and nutritional value of lesser-known mushrooms (Rathod 2023).

Despite numerous studies on the cultivation strategies and alternative substrates for these mushrooms, very few have been reported in the Philippines. For instance, *P. sajor-caju* has been cultivated using *Volvariella volvacea* spent mushroom compost combined with sawdust and rice bran (Villaceran et al. 2006), while *P. djamor* has been grown on banana leaves with sawdust (Silva et al., 2018) and on rice straw and cocopeat, enhanced with rice bran (Zurbano et al. 2017). For *P. florida*, rice straw combined with either sawdust or carbonized rice hull has been found suitable (Kalaw & Albinto 2015). However, these previous studies do not involve the use of other substrates like coconut coir. Thus, this study was conducted to determine the efficiency of coconut wastes as alternative substrates for the mycelial growth and fruiting body production of edible mushrooms. Utilization of agricultural wastes for mushroom cultivation promotes sustainability and contributes to waste reduction and environmental management.

Materials & Methods

Source of culture inoculants

Pure cultures of *Cyclocybe cylindracea* (MC004-0) *Pleurotus djamor* (MC021-0), *Pleurotus florida* (MC023-1), and *Pleurotus sajor-caju* (MC026-1) were obtained from the Center for Tropical Mushroom Research and Development, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Sub-culturing was done using Potato Dextrose Agar (PDA) in plates. The culture plates were used as a source of mycelial discs which were prepared using a cork borer.

Evaluation of culture media and pH

Coconut water from mature (MCW) and young (YCW) coconut were used to evaluate the mycelial growth performance of the four mushrooms. The coconut water was filtered using cheesecloth, then one liter of each was boiled and 20 grams of shredded white gelatin was added. The mixture was continuously stirred until homogenized. The pH of the media was adjusted to pH 6.5 for uniformity then about 200 ml of the mixture was placed in clean bottles, covered with cotton plug, and sterilized for 30 minutes. Approximately 20 ml of the media was poured on sterile 90 mm petri dishes, and allowed to cool. Once solidified, one 10 mm mycelial disc from the seven-day-old mycelia of each mushroom was separately inoculated at the center of the plates. The plates were sealed with cling wrap and stored at 30 ºC. Mycelial diameter was measured daily using a Vernier caliper, and the mycelial density was observed.

The media that demonstrated faster mycelial growth (in terms of diameter) was used to determine the influence of pH. The media was prepared and the pH was adjusted to varying pH levels (5, 6, 7, 8). The culture bottles were covered, sterilized, cooled, pour-plated and then inoculated with a mycelial disc. The mycelial growth was monitored daily, and the density was assessed following the methods of Reyes et al. (2009) and De Leon et al. (2017).

Evaluation of coconut pulp as spawning material

The suitability of coconut pulp as spawning material for the four mushrooms was determined. The pulp was obtained from a mature coconut, and the milk was removed by squeezing. It was then placed on a petri dish (half-filled), covered with clean paper, and placed in a Polypropylene (PP) bag, then secured with a rubber band. The plates were sterilized in an autoclave for 40 minutes, 15 psi. Rice seeds were used as the control. The seeds were washed and boiled until cracked. Then the seeds were placed in a petri dish wrapped with paper and then sterilized. After cooling, the sterilized spawning materials were inoculated with one mycelial disc at the center. The mycelial growth was measured daily, and the mycelial diameter was observed. The experiment for the evaluation of media, pH and spawning material was performed using three replicates.

Evaluation of fruiting body performance

Grain spawn preparation

The spawning material with the faster mycelial growth was used. The spawning material was prepared, and forty grams were placed in a PP bag. PVC ring (15 mm thick) was inserted into the PP bag, secured with a rubber band, and then covered with cotton. The bags were sterilized for one hour, cooled, and incubated with one mycelial disc.

Evaluation of fruiting body performance

The fruiting performance of *C. cylindracea, P. djamor, P. florida,* and *P. sajor caju* grown in the different formulations of rice straw (RS) and coconut coir dust (CCD) was evaluated. The different formulations were prepared following the method described by Aguilar et al. (2024) in a 6×12 PP bag, containing 500 grams of the substrate. Seven parts of RS and three parts of sawdust (SD) were used as the control. The incubation period and the number of days of the first primordia initiation were recorded. The mature fruiting bodies were harvested, weighed, and recorded. The yield was computed as the total weight of fruiting bodies obtained during the entire fruiting period. The biological efficiency (BE) was calculated as follows: total fresh yield divided by the fresh weight of the substrate multiplied by 100 (De Leon et al. 2017). The size of the cap and the stipe was measured using a caliper. A total of five replicates were used in this study.

Statistical analysis

Minitab version 19 was used for the statistical analyses. All treatments were laid out in a Completely Randomized Design (CRD). Analysis of Variance (ANOVA) was used to analyze the

data and the treatment means were compared using Tukey's HSD at a 5% level of significance. Experiments with only two treatments were analyzed using a T–test.

Results

Influence of culture media

The mean mycelial growth per day and the mycelial density of the four mushrooms grown on YCWG and MCWG are shown in Table 1. All mushrooms significantly preferred MCWG, as indicated by the longer mycelial diameter compared to YCWG. Thick mycelia were observed among the cultures.

Table 1 Mean mycelial diameter per day of the four mushrooms grown on YCWG and MCWG.

Mushroom species	Culture Media	Mean mycelial diameter per day (mm)	Mycelial density
C.cylindracea	MCWG	9.76±0.32[a]	+++
	YCWG	8.43±0.51[b]	+++
P. djamor	MCWG	12.99±0.16[a]	+++
	YCWG	9.10±0.57[b]	+++
P. florida	MCWG	11.65±0.75[a]	+++
	YCWG	9.22±1.59[b]	+++
P. sajor-caju	MCWG	11.01±0.95[a]	+++
	YCWG	9.32±2.91[b]	+++

Means with the same letter superscript are not significantly different from each other at 5% level of significance.

Influence of pH

The influence of varying pH of the media on the mycelial growth performance of the four mushrooms was also evaluated, and the mean mycelial diameter per day is presented in Table 2. All the studied mushrooms, except for *P. florida* preferred a wide range of pH concentrations since no significant differences among the mycelial diameters were recorded. On the other hand, the longest mycelial diameter of *P. florida* was recorded at pH 5–6 with a mean diameter of 13.50–13.60 mm. Thick mycelia were produced in all pH concentrations.

Influence of spawning material

The suitability of coconut pulp from mature coconuts as an alternative spawning material was evaluated. Table 3 shows the mean diameter of mycelial growth per day of the four mushrooms grown on coconut pulp and rice seeds. All mushrooms exhibited comparable mycelial growth on both substrates.

Fruiting body production in rice straw and coconut coir dust substrate

Incubation period

In this study, *C. cylindracea* fully colonized the substrates within 41.40 to 48.20 days, with the fastest colonization observed in T8. However, statistical analysis showed that the incubation period was comparable across T6 to T11 (Table 4). In contrast, *Pleurotus* species exhibited a faster mycelial run. *P. djamor* completed colonization within 18 to 23.75 days, with T6 to T11 showing the most rapid growth. *P. florida* required 23 to 26.20 days, with T8 to T11 demonstrating significantly faster colonization. Similarly, *P. sajor-caju* took 24.20 to 26.80 days, with T5 to T11 exhibiting the fastest growth.

Table 2 Mean daily mycelial diameter of the four mushrooms grown in MCWG with varying pH levels.

pH Levels	Mean mycelial diameter per day (mm)			
	C. cylindracea	*P. djamor*	*P. florida*	*P. sajor caju*
5	10.48±1.38[a]	13.87±0.10[a]	13.50±0.16[a]	12.90±0.16[a]
6	9.97±0.03[a]	13.97±0.32[a]	13.60±0.02[a]	12.78±0.27[a]
7	9.85±0.28[a]	15.16±0.15[a]	10.76±0.44[b]	12.78±0.12[a]
8	9.95±0.28[a]	14.82±0.24[a]	10.50±0.14[b]	12.67±0.12[a]

Means with the same letter superscript are not significantly different from each other at 5% level of significance.

Primordia formation

Primordia refer to the pinhead-like structures that form in the substrate once mycelia fully colonize it. When the mycelia mature, they thicken and eventually develop into primordia, the initial stage of fruiting body development. In the present study, the primordia formation of four mushrooms grown on different formulations of RS and CCD was recorded (Table 5). For *P. djamor*, primordia appeared after 21 to 26.67 days, with T4 to T11 showing the shortest formation time. *P. sajor-caju* formed primordia between 27 and 30.40 days, with T4 to T11 showing comparable results. *P. florida* required 26.40 to 33.13 days, with T9 to T11 forming primordia in significantly fewer days. In contrast, *C. cylindracea* took 67 to 68.33 days to develop fruiting initials.

Table 3 Mean daily mycelial diameter of the four mushrooms grown in coconut pulp and rice seeds.

Spawning material	Mean mycelial diameter per day (mm)			
	C. cylindracea	*P. djamor*	*P. florida*	*P. sajor caju*
Coconut pulp	11.33±0.01[a]	11.35±0.04[a]	11.36±0.01[a]	10.50±1.45[a]
Rice seeds (control)	11.26±0.13[a]	11.31±0.01[a]	11.41±0.09[a]	11.01±0.27[a]

Means with the same letter superscript are not significantly different from each other at a 5% level of significance.

Table 4 Mean number of days of incubation period of the four mushrooms on different formulations of rice straw and coconut coir dust.

Treatments	RS: CCD ratio	Incubation period (days)			
		C. cylindracea	*P. djamor*	*P. florida*	*P. sajor-caju*
T11	10 RS:0 CCD	42.00±0.00[a]	18.00±0.00[a]	23.10±0.22[a]	24.80±0.45[a]
T10	9 RS:1 CCD	42.00±0.00[a]	18.20±0.45[a]	23.00±0.00[a]	25.00±0.00[a]
T9	8 RS:2 CCD	41.80±0.45[a]	18.00±0.00[a]	23.20±0.45[a]	25.00±0.00[a]
T8	7 RS:3 CCD	41.40±0.89[a]	18.20±0.45[a]	23.20±0.45[a]	24.40±0.55[a]
T7	6 RS:4 CCD	42.60±0.55[ab]	18.20±0.45[a]	26.20±0.45[b]	24.20±0.45[a]
T6	5 RS:5 CCD	42.80±1.10[ab]	18.20±0.45[a]	26.20±0.45[b]	24.20±0.45[a]
T5	4 RS:6 CCD	44.20±1.79[b]	19.60±0.55[b]	26.20±0.45[b]	24.20±0.45[a]
T4	3 RS:7 CCD	46.80±0.45[c]	19.00±0.00[b]	26.00±0.55[b]	26.60±0.55[b]
T3	2 RS:8 CCD	47.20±0.45[c]	23.25±0.96[c]	26.20±0.45[b]	26.60±0.55[b]
T2	1 RS:9 CCD	47.40±0.55[c]	23.00±0.82[c]	26.00±0.00[b]	26.60±0.55[b]
T1	0 RS:10 CCD	48.20±1.63[c]	23.75±0.50[c]	26.25±0.50[b]	26.80±0.45[b]
Control	7 RS:3 SD	42.20±0.45[a]	18.00±0.00[a]	23.00±0.00[a]	24.80±0.45[a]

Means with the same letter superscript are not significantly different from each other at 5% level of significance.

Cap diameter and stipe length

In the present study, *C. cylindracea* exhibited no significant variation in cap diameter and stipe length across all treatments, while a similar trend was observed in the *Pleurotus* species, where smaller fruiting bodies, characterized by reduced cap diameter and shorter stipes, were found in treatments with lower concentrations of rice straw (Table 6). Statistical analysis revealed that the mean cap diameter of *C. cylindracea* (58.33 to 69.03 mm) and mean stipe lengths (90.25 and 96.93 mm) are comparable in all treatments. For *P. djamor*, significantly larger caps were observed in T5–T11 (54.40–60.63 mm), while stipe lengths were comparable in T4–T11 (12.77–19.17 mm). Similarly, *P. florida* produced caps of comparable sizes except for T1, which yielded significantly smaller caps (27.60 mm). Longer stipes were observed in T5–T11, which are also comparable to the control group. *P. sajor-caju* followed a similar trend, with significantly larger caps (59.03–65.67 mm) and longer stipe lengths (48.47–52.50 mm) in T6–T11. The actual photographs of the fruiting bodies of the four mushrooms grown on different formulations of RS and CCD are presented in Figs 1−4.

Table 5 Mean number of days of primordia formation of the four mushrooms on different formulations of rice straw and coconut coir dust.

Treatments	RS: CCD ratio	Primordia formation (days)			
		C. cylindracea	*P. djamor*	*P. florida*	*P. sajor-caju*
T11	10 RS:0 CCD	67.00±1.73^{a}	21.00±0.00^{a}	26.80±0.45^{a}	27.60±0.55^{a}
T10	9 RS:1 CCD	67.40±0.55^{a}	21.20±0.45^{a}	26.80±0.45^{ab}	27.90±0.22^{a}
T9	8 RS:2 CCD	67.25±0.50^{a}	21.00±0.00^{a}	27.60±0.55^{ab}	27.80±0.45^{a}
T8	7 RS:3 CCD	67.67±1.16^{a}	21.00±0.00^{a}	28.80±0.84^{b}	27.20±0.45^{a}
T7	6 RS:4 CCD	68.33±1.16^{a}	20.80±0.84^{a}	29.60±0.55^{b}	27.00±0.00^{a}
T6	5 RS:5 CCD	67.67±1.16^{a}	20.80±0.45^{a}	30.80±0.45^{c}	27.20±0.45^{a}
T5	4 RS:6 CCD	n/a	21.40±0.55^{a}	30.80±0.45^{c}	27.10±0.22^{a}
T4	3 RS:7 CCD	n/a	22.00±0.71^{a}	31.00±0.00^{c}	28.80±0.84^{ab}
T3	2 RS:8 CCD	n/a	26.25±0.50^{b}	30.80±0.45^{c}	29.60±0.55^{b}
T2	1 RS:9 CCD	n/a	26.25±0.96^{b}	32.75±0.50^{d}	29.40±0.54^{b}
T1	0 RS:10 CCD	n/a	26.67±0.58^{b}	33.13±0.25^{d}	30.40±0.55^{b}
Control	7 RS:3 SD	67.67±1.16^{a}	21.00±0.71^{a}	26.40±0.89^{a}	27.40±0.55^{a}

Means with the same letter superscript are not significantly different from each other at 5% level of significance.
*n/a− no primordia observed

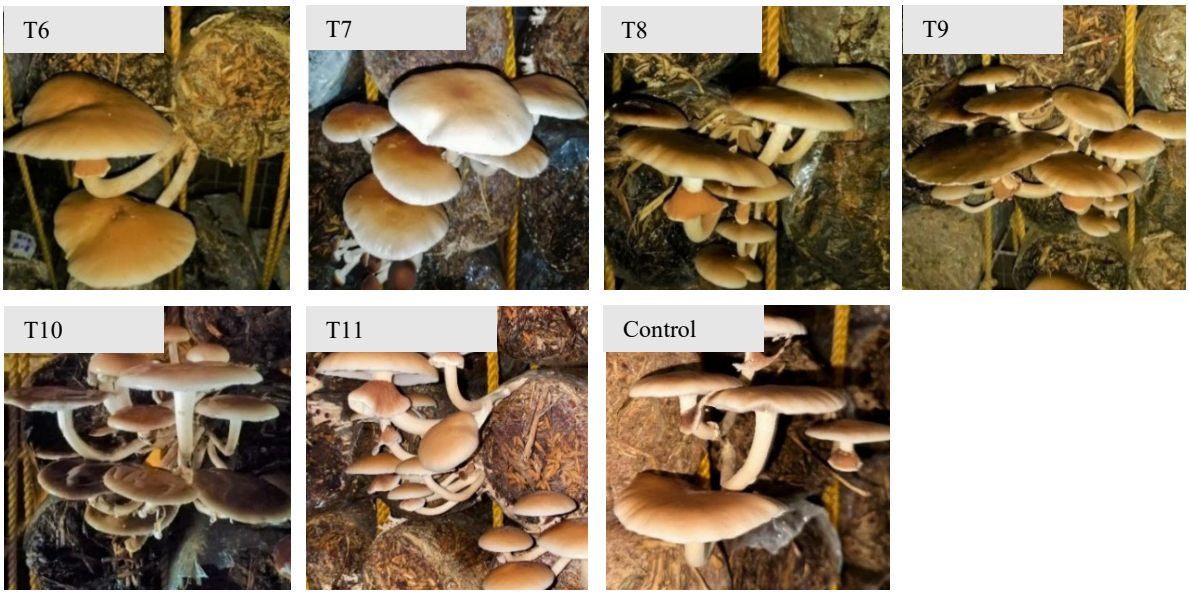

Fig 1 – Fruiting body of *C. cylindracea* grown on different formulations of rice straw and coconut coir dust.

Fig. 2 – Fruiting body of *P. djamor* grown on different formulations of rice straw and coconut coir dust.

Fig. 3 – Fruiting body of *P. florida* grown on different formulations of rice straw and coconut coir dust.

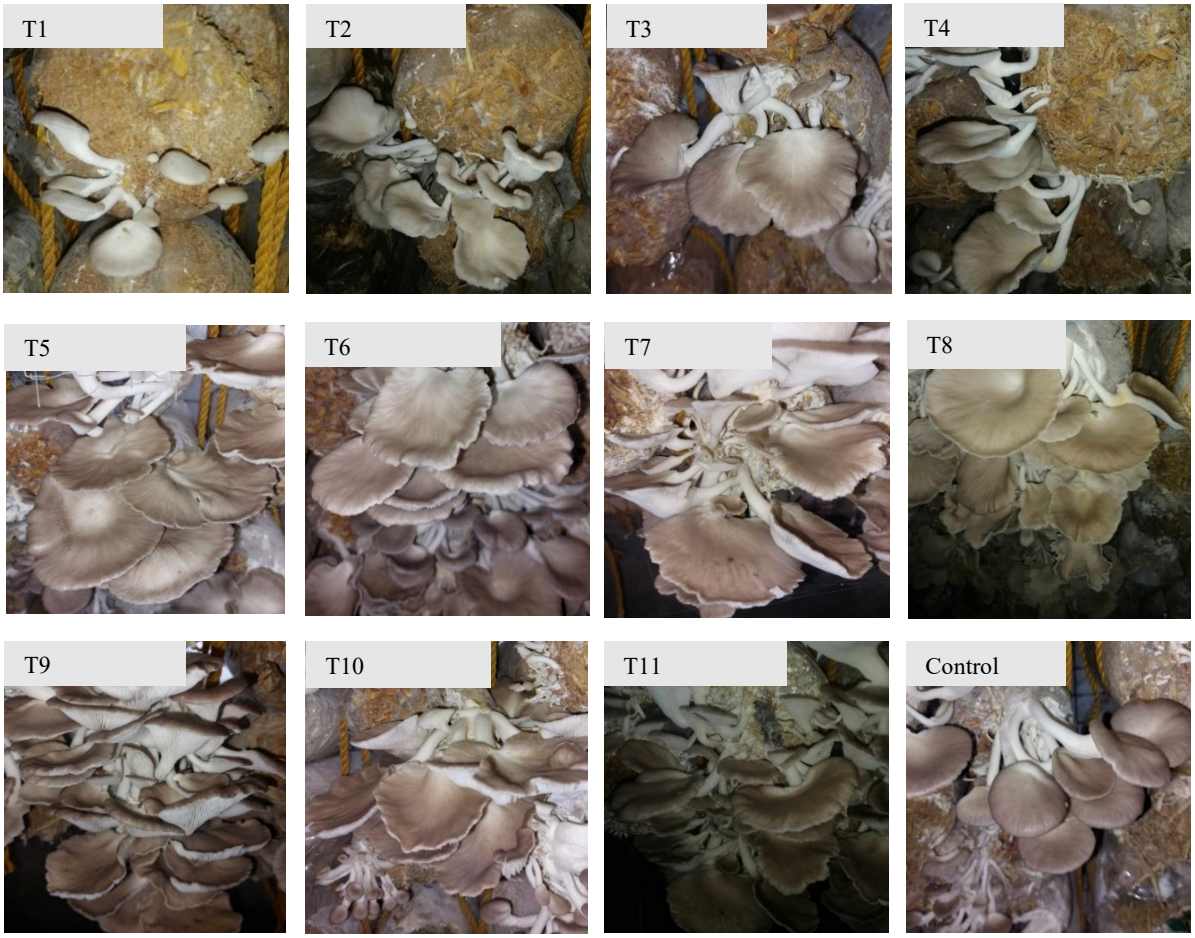

Fig. 4 – Fruiting body of *P. sajor-caju* grown on different formulations of rice straw and coconut coir dust.

Table 6 Mean cap diameter and stipe length of the four mushrooms on different formulations of rice straw and coconut coir dust.

Treatments	RS: CCD ratio	Cap diameter (mm)			
		C. cylindracea	*P. djamor*	*P. florida*	*P. sajor-caju*
T11	10 RS:0 CCD	66.47±5.65^{a}	0.63±5.39^{a}	50.80±14.04^{ab}	59.03±2.84^{a}
T10	9 RS:1 CCD	64.3±18.20^{a}	59.00±8.61^{ab}	62.51±8.71^{ab}	61.80±2.27^{a}
T9	8 RS:2 CCD	66.33±3.95^{a}	60.47±7.94^{ab}	57.27±9.51^{ab}	65.67±8.05^{a}
T8	7 RS:3 CCD	69.03±10.95^{a}	59.13±10.50^{ab}	52.87±9.51^{ab}	60.13±4.02^{a}
T7	6 RS:4 CCD	65.10±8.26^{ab}	58.57±2.50^{ab}	49.80±16.81^{ab}	60.63±2.46^{a}
T6	5 RS:5 CCD	58.33±12.69^{a}	58.93±4.30^{ab}	70.03±3.14^{a}	59.13±3.61^{a}
T5	4 RS:6 CCD	n/a	54.40±2.59^{ab}	38.26±6.61^{ab}	42.47±3.95^{b}
T4	3 RS:7 CCD	n/a	43.50±5.70^{b}	42.64±7.14^{ab}	41.70±3.80^{b}
T3	2 RS:8 CCD	n/a	23.83±2.91^{c}	37.59±2.58^{ab}	32.93±2.11^{b}
T2	1 RS:9 CCD	n/a	24.47±3.20^{c}	42.57±10.55^{ab}	31.43±8.24^{b}
T1	0 RS:10 CCD	n/a	24.37±4.54^{c}	27.60±19.50^{b}	32.77±5.31^{b}
Control	7 RS:3 SD	59.63±5.06^{a}	58.10±4.50^{ab}	56.5±20.80^{ab}	67.97±2.62^{a}
Stipe length *(mm)*					
T11	10 RS:0 CCD	95.63±6.28^{a}	15.40±3.52^{ab}	46.30±4.39^{a}	48.47±2.90^{a}
T10	9 RS:1 CCD	93.30±3.25^{a}	17.63±4.07^{a}	47.14±3.69^{a}	50.42±1.99^{a}
T9	8 RS:2 CCD	90.80±9.04^{a}	15.73±3.40^{ab}	43.02±3.05^{ab}	51.00±3.10^{a}
T8	7 RS:3 CCD	95.97±4.09^{a}	14.80±1.71^{ab}	46.56±4.23^{a}	52.47±3.86^{a}
T7	6 RS:4 CCD	96.13±4.20^{a}	19.17±1.91^{ab}	43.75±3.66^{ab}	49.90±3.18^{a}
T6	5 RS:5 CCD	90.25±5.48^{a}	18.73±3.61^{a}	46.61±7.63^{a}	52.50±3.63^{a}
T5	4 RS:6 CCD	n/a	19.70±1.40^{a}	44.78±7.63^{ab}	40.33±1.12^{bc}

Table 6 Continued.

Treatments	RS: CCD ratio	Cap diameter (mm)			
		C. cylindracea	*P. djamor*	*P. florida*	*P. sajor-caju*
T4	3 RS:7 CCD	n/a	19.43±1.72^{a}	27.81±5.24^{c}	35.63±3.30^{cd}
T3	2 RS:8 CCD	n/a	13.27±2.08^{b}	30.29±2.46^{bc}	30.43±1.67^{d}
T2	1 RS:9 CCD	n/a	12.77±1.11^{b}	26.92±5.96^{c}	19.07±1.59^{e}
T1	0 RS:10 CCD	n/a	13.10±1.15^{b}	25.95±5.92^{c}	20.80±1.66^{e}
Control	7 RS:3 SD	96.93±6.28^{a}	17.13±1.72^{a}	50.33±4.06^{a}	46.56±3.26^{ab}

Means with the same letter superscript are not significantly different from each other at 5% level of significance.
*n/a− no fruiting body observed

Yield and biological efficiency

The mean yields and BE of four mushroom species grown on different formulations of RS and CCD are presented in Table 7. The highest yield of *C. cylindracea* was observed in T8 to T11, ranging from 69.55 to 81.20 grams. Significantly lower yields were recorded in T7, T6, and the control, with the lowest yield observed in T6 (6.84 grams). Similarly, the optimal yield of *P. djamor* was recorded in T8–T11, which showed comparable results to the control. These substrate formulations demonstrated a BE ranging from 21.23% to 25.39%. Likewise, *P. florida* exhibited significantly higher yields in T8–T11, with a BE of 20.17% to 24.93%. Meanwhile, *P. sajor-caju* achieved the highest yields in T6–T11, with a BE ranging from 21.52% to 28.05%. In terms of number of harvests, *C. cylindracea* produced one to three flushes, while the *Pleurotus* species yielded up to five flushes.

Table 7 Mean yield per bag and biological efficiency of the four mushrooms on different formulations of rice straw and coconut coir dust.

Treatments	RS: CCD ratio	Yield (grams)			
		C. cylindracea	*P. djamor*	*P. florida*	*P. sajor-caju*
T11	10 RS:0 CCD	79.85±7.76^{a}	112.40±8.01^{ab}	124.64±11.29^{a}	116.3±21.1^{a}
T10	9 RS:1 CCD	81.20±7.05^{a}	126.93±5.15^{a}	123.88±14.22^{a}	128.6±18.9^{a}
T9	8 RS:2 CCD	80.51±1.95^{a}	106.14±12.08^{ab}	102.92±3.83^{ab}	140.30±23.9^{a}
T8	7 RS:3 CCD	69.55±11.83^{a}	109.54±10.04^{ab}	100.85±9.46^{ab}	109.87±2.96^{ab}
T7	6 RS:4 CCD	36.63±2.37^{b}	100.50±17.50^{b}	86.28±5.50^{bc}	107.59±11.31^{ab}
T6	5 RS:5 CCD	6.84±0.14^{c}	73.31±3.38^{c}	73.0±16.10^{bcd}	111.40±5.29^{a}
T5	4 RS:6 CCD	n/a	67.70±4.85^{c}	84.50±6.26^{bc}	68.08±2.75^{cd}
T4	3 RS:7 CCD	n/a	56.02±2.03^{c}	83.72±16.38^{bc}	46.07±6.75^{cde}
T3	2 RS:8 CCD	n/a	25.63±3.69^{d}	66.67±4.10^{cd}	42.49±3.67^{cde}
T2	1 RS:9 CCD	n/a	11.87±1.46^{d}	35.9±17.3^{de}	36.27±2.14^{de}
T1	0 RS:10 CCD	n/a	3.06±0.03^{d}	10.43±1.49^{e}	17.80±3.08^{e}
Control	7 RS:3 SD	22.00±7.29^{bc}	112.54±6.10^{ab}	107.90±20.10^{ab}	74.86±11.37^{bc}
Biological efficiency (%)					
T11	10 RS:0 CCD	15.97±0.15^{a}	22.48±1.60^{ab}	24.93±2.26^{a}	23.25±4.22^{a}
T10	9 RS:1 CCD	16.24±1.41^{a}	25.39±1.03	24.78±2.84^{a}	25.72 ±3.78^{a}
T9	8 RS:2 CCD	16.10±0.39^{a}	21.23±2.42^{ab}	20.58±0.77^{ab}	28.05 ±4.77^{a}
T8	7 RS:3 CCD	13.91±2.37^{a}	21.92±2.01^{ab}	20.17±1.89^{ab}	21.97±0.59^{ab}
T7	6 RS:4 CCD	7.32±0.47^{b}	20.09±3.49^{b}	17.26±1.10^{bc}	21.52±0.57^{ab}
T6	5 RS:5 CCD	1.37±0.03^{c}	14.66±0.68^{c}	14.61±3.22^{bc}	22.28±1.06^{a}
T5	4 RS:6 CCD	n/a	13.54±0.97^{c}	16.90±1.25^{bc}	13.62±0.55^{cd}
T4	3 RS:7 CCD	n/a	11.20±0.41^{c}	16.74±3.28^{bc}	9.22±1.35^{cde}
T3	2 RS:8 CCD	n/a	5.13±0.74^{d}	13.33±0.82^{c}	8.50±0.74^{cde}
T2	1 RS:9 CCD	n/a	2.37±0.29^{d}	4.78±4.81^{d}	7.25±0.43^{de}
T1	0 RS:10 CCD	n/a	0.61±0.01^{d}	2.09±0.30^{d}	3.56±0.62^{e}
Control	7 RS:3 SD	4.00±0.30^{bc}	22.51±1.22^{ab}	21.57±4.02^{a}	14.97±2.27^{bc}

Means with the same letter superscript are not significantly different from each other at 5% level of significance.

Discussion

The nutritional composition of the medium is crucial to the development of mycelia. Coconut water contains 0.79–0.81% ash, 0.11–0.14 % fatty acid, 2.79–2.86% potassium, 0.46–1.21% phosphorus, 0.31–0.38% sodium, 0.21–0.31 % calcium, and traces of iron, copper, magnesium, zinc, vitamins, and manganese (Lazim et al. 2015). Apart from these, cytokinin, particularly trans-zeatin riboside, is found in coconut water, an enzyme that promotes cell division and stimulates mycelial biomass production of mushrooms (Lazim et al. 2015). The influence of cytokinin, including trans-zeatin, on *P. ostreatus* physiology was found to be linked to tRNA degradation products that stimulate colony diameter expansion, biomass accumulation, and morphological changes (Grich et al. 2025). However, the amount of these nutrients could vary depending on the age of the fruit, thereby influencing its potential as a growth medium for mushroom mycelia. Water from mature coconuts contains higher amounts of minerals and carbohydrates than young coconuts, which favors the enzymatic activity and cell metabolism of the four mushrooms (Halim et al. 2018). A similar response was observed for other *Pleurotus* species like *Pleurotus citrinopileatus, Pleurotus djamor,* and *Pleurotus salmoneostramineus,* which showed faster mycelial growth on mature coconut water gelatin (Jacob et al. 2015). Aside from coconut water, coconut shell solution combined with potato dextrose agar was also suitable for both mycelial growth and biomass production of *P. ostreatus* (Lindao-Perez et al. 2022).

The growth and metabolic processes of mushrooms are also affected by the pH of the growth medium. Adjusting the pH will help improve the absorption of nutrients, leading to faster and more efficient mycelial growth (Bellettini et al. 2019). In general, the four mushrooms exhibited a broad preference for pH, proving their aggressiveness. However, findings on pH requirement of these mushrooms vary; for instance, the optimal mycelial growth of *Cyclocybe cylindracea* was observed at pH 6–7 in potato sucrose agar (Landingin et al. 2020). *Pleurotus sajor-caju* grew best at pH 6.5–7.5 (Gorai & Sharma 2018) while *P. djamor* demonstrated a more specific pH preference, growing best at pH 7.5 (Singh & Singh 2018). Almost similar trend was observed for *Pleurotus florida,* which exhibited optimal growth at pH 6 (Kaur et al. 2023) and produced the highest biomass at pH 5 and 6.5 (Rawte & Diwan 2011, Gbolagade et al. 2006). The strain of the mushrooms, culture media, and the environmental factors might influence this variability in the pH preferences.

Coconut pulp is rich in carbohydrates, proteins, minerals, ash, and fats, making it a suitable substrate for growing mycelia (Adoyo et al. 2021). Aside from that, its fibrous texture allows it to retain moisture, which is favorable for mushrooms. As of now, there is no available data on the use of coconut pulp for growing mushroom mycelia; however, other studies have demonstrated the suitability of pulp for cultivating oyster mushrooms like *P. ostreatus*, which successfully grew on coffee, sugar beet, carrot, and sugarcane pulp (Garcia-Oduardo et al. 2006, Jafarpour et al. 2010, Rivera-Omen et al. 2013). Similarly, pulp of apples, cherries, and plums are efficient substrates for mushrooms like *Pleurotus* species, *Ganoderma lucidum, Ganoderma frondosa,* and *Lentinus edodes* (Petre et al. 2013, 2014). This result suggests that, similar to other fruit pulps, coconut pulp can be an efficient alternative spawning material for mushrooms.

The incubation period refers to the duration from the inoculation of grain spawn to the full mycelial colonization of the fruiting bags. The shorter incubation period observed for *Pleurotus* mushrooms indicates their aggressiveness, making them thrive in a wide range of substrates. This may be due to their ability to degrade substrates more efficiently compared to *C. cylindracea* and other mushrooms that require a longer period to fully colonize the substrate. The findings in this study on *Pleurotus* species support the reported average duration for mycelial colonization of most mushrooms, which ranges between 21 and 31 days (Ejigu et al. 2022). Compared to other studies on oyster mushrooms, almost similar incubation period was recorded. For instance, *P. ostreatus* required 18.40 to 20.80 days in substrates composed of varying ratios of sawdust, rice bran, and coir dust (Rafaya & Jeyagowri 2025). Similarly, *P. florida* exhibited varying incubation periods depending on the substrate used, taking 21 to 24.75 days on banana leaves and rice straw (Mondal et al. 2010) and 18.33 to 25.0 days on wheat straw, corncob, newspaper, and sugarcane waste (Bhatt et al. 2024). However, a shorter incubation period was recorded on wheat straw, where *P. florida* fully colonized

the substrate in 13 days, while *P. sajor-caju* required 15 days under controlled temperature and humidity conditions (Singh et al. 2012). Tangjang et al. (2022) found that soaking paddy straw overnight before sterilization shortened the mycelial colonization time of *P. sajor-caju* to nine days. In contrast, it required a longer incubation period of 37 days to colonize *V. volvacea* mushroom spent substrate (Villaceran et al. 2006). Meanwhile, *P. djamor* exhibited different colonization rates depending on the substrate, taking 17.67 days to fully colonize pure coir pith, 11.33 days in pure rice straw (Jegadeesh et al. 2018), and 20.3 to 21.3 days to colonize sawdust from different tree species (Kilic 2020). Interestingly, the incubation period obtained for *C. cylindracea* closely aligns with the findings of Landingin et al. (2020), who reported a colonization period of 42.3 to 49.53 days. Meanwhile, it required more than 55 days for full mycelial colonization in pure sawdust (Uhart et al. 2008). Given that similar environmental conditions are employed during incubation, variations in the rate of mycelial colonization can be attributed to the nutritional composition of the substrates. Additionally, substrate decomposition can affect mycelial colonization; a well-decomposed substrate allows for easier nutrient utilization, as the nutrients are fully broken down into reusable forms with the help of extracellular enzymes (Kurt & Buyukalaca 2010). This enables the mycelia to colonize the substrate more quickly, indicating their ability to efficiently extract the available nutrients. A shorter incubation period is better as it speeds up the process of the fruiting stage, reducing the overall cultivation time.

Notably, *C. cylindracea* did not form primordia in T1 to T5, suggesting that it relies on nutrients present in rice straw, and the lower rice straw concentration in these treatments possibly hindered the process of primordia formation. The results of this study showed similarity with previous findings, for instance, primordia initiation in *P. djamor* was observed after 17 to 22 days using paddy straw, raggi straw, corn straw, coir pith, sugarcane bagasse, and the mixture of all these substrates (Jegadeesh et al. 2018). Paddy straw substrate pre-treated with Bavistin + Formalin, cold lime, autoclaving, or hot water resulted in a shorter primordia formation time of 14 to 21 days for *P. sajor-caju*. (Tangjang et al. 2022). On the other hand, the findings for *P. florida* showed a longer duration for primordia formation compared to that grown on rice straw, newspaper, coconut husk, sugarcane bagasse, wood residue, and sal leaves, which exhibited a shorter period of 17.67 to 24.33 days before primordia formation (Kundu et al. 2024). For *C. cylindracea*, a shorter duration of 52 to 59 days was required for primordia formation when grown on substrates with varying formulations of rice straw and sawdust (Landingin et al. 2020). Meanwhile, the suitability of coconut coir as an alternative substrate for mushrooms was demonstrated in *P. ostreatus* which took 24 days to form primordia when grown on pure coconut coir enhanced with rice bran, chalk powder, gypsum, and corn flour while a shorter duration was observed as the percentage of coconut coir decreased (Purnomo et al. 2023). The rapid and early pinhead formation observed in substrates with a higher rice straw content can be attributed to its nutrient composition and improved aeration due to its fibrous structure, which may be preferred by the mushrooms. In contrast, the compactness of substrates made of pure coconut coir dust or those with lower rice straw content may have restricted gas exchange, slowing down the colonization process and delaying the formation of fruiting initials (Bumanlag et al. 2018).

The size of the fruiting body is another crucial parameter in assessing the efficiency of substrates in mushroom cultivation, as larger cap sizes are often associated with better growth and higher yield (Kortei et al. 2018). These findings align with previous studies, but with variations due to differences in substrate composition. Alvarez & Bautista (2021) reported a *P. djamor* pileus diameter of 49.70–86.86 mm and stipe length of 19.75–68.35 mm, while *P. sajor-caju* had a cap diameter of 25.82–92.40 mm and a stipe length of 23.47–70.59 mm. Similarly, *P. florida* demonstrated cap diameters ranging from 33.36 to 83.51 mm and stipe lengths of 26.32–60.69 mm. However, a study by Kundu et al. (2024) recorded notably smaller cap diameters (6.58–10.05 mm) and stipe lengths (2.69–4.02 mm) for *P. florida,* indicating a significant impact of substrate composition on fruiting body development. In contrast, Landingin et al. (2020) documented *C. cylindracea* with smaller cap diameters (28.01–36.81 mm) and stipe lengths (26.52–41.97 mm) compared to those observed in the present study. This suggests that the coconut coir dust combined with rice straw provided a more favorable nutrient environment for cap and stipe development.

Accordingly, these results emphasize the importance of substrate composition in optimizing fruiting body size. The proper nutrient balance in the substrate supports full cap expansion and stipe elongation, whereas lower rice straw content may result in nutrient deficiency, leading to smaller and underdeveloped fruiting bodies (Aguilar et al. 2024).

Yield refers to the total quantity of fruiting bodies produced from a given amount of substrate over the entire fruiting period, while BE measures the ability of the mushroom to convert a specific amount of substrate into fruiting bodies. Both parameters are critical indicators of productivity and are influenced primarily by the nutrient composition of the substrate, as well as environmental factors such as temperature, light, humidity, and aeration. These results among *Pleurotus* species show a notable trend, wherein higher RS content in the substrate resulted in increased yield and BE compared to substrates with a higher proportion of CCD. This suggests that substrate with a higher proportion of RS provides a more favorable nutrient composition for these mushrooms. A similar preference was observed for *A. polytricha*, which produced optimum yield in substrates with a higher proportion of rice straw compared to coconut sawdust (Aguilar et al. 2024). Comparing these results with previous studies, *P. sajor-caju* cultivated on pre-treated paddy straw yielded 232.33 to 286.0 grams, with a BE of 76% to 94% (Tangjang et al. 2022). A higher yield of *P. sajor-caju* (141.19−233.42 grams) was reported when grown on a combination of paddy straw and flower waste (Agarwal et al. 2016). Meanwhile, *P. florida* grown on coconut husk yielded 205.42 grams, with a BE of 20.54% (Kundu et al. 2024). Regarding alternative substrates, Alvarez & Bautista (2021) utilized used paper, banana peel, and leaf litter for three *Pleurotus* species. Compared to the present study, their findings showed lower yields for *P. djamor* (19-90.77 grams), *P. sajor-caju* (18.27-132.83 grams), and *P. florida* (15.82-75.52 grams). Regarding BE, other studies on *P. florida* using corn cob and sawdust reported values ranging from 16.5% to 36% (Kinge et al. 2016). Moreover, its BE reached up to 90% when cultivated on paddy straw mixed with 25% waste paper (Nisha et al. 2022), which is relatively higher than the BE obtained in the present study. Meanwhile, previous reports on *C. cylindracea* showed yields ranging from 78.38 to 106.94 grams, with a lower BE of 10.34% to 12.89% when cultivated on rice straw and sawdust (Landingin et al. 2020). Accordingly, CCD can serve as a viable alternative to sawdust, supporting comparable productivity.

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