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Harnessing Old Oyster Mushroom Substrate: A Circular Economy Approach to Inky Cap Mushroom (*Coprinopsis radiate*) Cultivation

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Abstract

Mushroom cultivation in Thailand primarily uses rubber tree sawdust as a substrate in plastic bags, resulting in an annual production of approximately 120,000 tons. However, this method generates significant amounts of spent mushroom substrate (SMS), which is often discarded or minimally repurposed as fertilizer, contributing to environmental issues. This study was carried out to evaluate the potential of SMS, derived from oyster mushroom (*Pleurotus ostreatus*), as an alternative substrate for cultivating inky cap mushroom (*Coprinopsis radiata*) within a circular economy framework. Five substrate formulas with varying proportions of rubber tree sawdust (SD) and SMS (100:0, 75:25, 50:50, 25:75 and 0:100) were tested using a completely randomized design. The results showed no statistically significant differences in the number of mushrooms, stem length, or biological efficiency across treatments. However, treatments with balanced ratios of SD and SMS (SD 75%:SMS 25% and SD 50%:SMS 50%) produced the highest fresh weight yields during the initial 1–10 days of cultivation, with yields of 72.05 g and 72.16 g, respectively. The SD100%:SMS0% treatment produced the largest cap width at 1.31 cm. The findings underscore SMS's role in reducing production costs, minimizing waste, and supporting sustainable agriculture. Future research should explore SMS utilization under diverse environmental conditions and for other mushroom species to enhance its commercial viability in mushroom production. This study highlights SMS's contribution to circular economy goals by transforming agricultural waste into a valuable resource.

Keywords: Circular economy; *Coprinopsis radiata;* Inky cap mushroom; Nutrient recycling; Spent mushroom substrate

Introduction

In Thailand, mushroom cultivation primarily involves growing mushrooms in plastic bags, with rubber tree sawdust as the primary substrate. Annual mushroom production is estimated at around 120,000 tons. A significant issue with this method is the lack of disposal for spent mushroom substrate (SMS) after cultivation (Panutat 2018). The majority of mushrooms grown in bags belong to the genus *Pleurotus* spp. SMS, or the spent mushroom substrate, poses environmental challenges due to the large amount of waste generated from mushroom cultivation. Most SMS is repurposed as organic fertilizer, with some left to decompose naturally at cultivation sites (Wu *et al.* 2020a). These disposal methods not only contribute to environmental waste but also overlook SMS's potential for reuse in a circular economy, transforming waste into valuable resources (Medina and Afagh 2023). SMS typically comprises fungal fibers, extracellular enzymes released by mushrooms for breaking down various substances, and unused lignocellulosic materials (Lim *et al.* 2013). These residual by-products contain high nutritional value and can be repurposed in numerous applications (Antunes *et al*. 2020). Utilizing SMS effectively is essential for advancing a sustainable mushroom industry within a circular economy framework. Therefore, examining SMS characteristics is necessary to identify optimal reuse methods (Martín *et al*. 2023). SMS is recognized as a nutrient–rich organic waste with potential for reuse (Li *et al.* 2023).

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Farmers in Thailand predominantly use rubber tree sawdust as a substrate in plastic bags. However, rubber tree sawdust prices have increased by over 50% in recent years, with a truckload now costing around 830–890 USD, or more depending on transport distance (Nukpook *et al*. 2019). Selecting suitable agricultural or agro–industrial waste substrates for mushroom cultivation is essential for achieving economically viable production (Banasik *et al*. 2017). Inky cap mushroom (*Coprinopsis radiate*) is a nutritionally rich mushroom with economic importance in many countries. It is a valuable source of protein, vitamins, minerals, and beneficial bioactive compounds (Miles and Chang 2004). This mushroom has a short cultivation cycle, allowing farmers to generate quick income. Quality substrate is vital for producing high-quality yields, with natural materials like rice straw, sawdust, and rice husks commonly used. Substrate selection influences growth and yield by affecting aeration, water retention, and nutrient availability for mushrooms (Akinyele and Atanda 2008; Sánchez 2010). Studies have investigated SMS's use as a substrate for various mushroom species, focusing on popular mushrooms such as oyster mushroom (*Pleurotus ostreatus*) and hairy jew's ear (*Auricularia polytricha*) (Hoa *et al*. 2022; Zied *et al*. 2024). The SMS from *Pleurotus* spp. has been repurposed to cultivate mushrooms like grey oyster mushroom (*Pleurotus sajor-caju*) (Sharma and Jandaik 1992a, hairy jew's ear (Sharma and Jandaik 1992b) and wine cap stropharia (*Stropharia rugosoannulata*) (Rinker 2002, 2017). Specifically, oyster mushroom SMS has been reused for cultivating oyster mushroom (Pardo-Giménez and Pardo-González 2009; Picornell *et al*. 2016) and white oyster mushroom (*P. florida*) (Ashrafi *et al.* 2014). Despite these applications, there is no standardized method for reusing spent oyster mushroom sawdust blocks as substrate for cultivating inky cap mushroom.

The primary degradation role of *Pleurotus* spp. in decomposition allows these mushrooms to rapidly extend their mycelium, decomposing plant tissues effectively. Each mushroom species produces unique enzymes to degrade lignin–cellulose structures in plant cells. Once one species has fully decomposed these structures, other saprophytic microorganisms use their enzymes to further break down the material (Stamets 2000) using oyster mushroom. SMS for inky cap mushroom presents unique challenges due to specific growth patterns and nutritional requirements. Prior research indicates that nutrient dynamics in SMS are complex due to microbial interactions, affecting nutrient access and thus growth variability (Gómez 1984; Hawkins 2018). The limited understanding of SMS's effectiveness as a substrate for inky cap mushroom highlights the need for targeted research to enhance substrate efficiency in mushroom cultivation systems. The aim of this study was to evaluate the potential of SMS, derived from oyster mushroom, as an alternative substrate for cultivating inky cap mushroom within a circular economy framework.

Materials and Methods

Experimental details

This experiment was conducted at the Department of Agriculture, Faculty of Agricultural and Industrial Technology, Phetchabun Rajabhat University, Phetchabun, Thailand, between April and October 2024. The mushroom spawn was obtained from Lanlalin Biotech Company Limited, certified under the code of practice for mushroom culture (TAS 2507-2016). The following steps describe the process of cultivating Inky cap mushrooms in plastic bags. All ingredients for each substrate formula were combined in a mixing tray and thoroughly blended. Water was added according to the formula's requirements, and mixing was continued until the moisture content reached 70-75%. A portion of the prepared substrate was set aside to measure moisture content and checks nutrient levels. Heat–resistant plastic bags (15 × 28 cm) were filled with approximately 600 g of the substrate, pressed firmly into solid blocks. A plastic bottle neck was placed on each bag, sealed with cotton wool, and covered with a plastic lid. Water was added to a sterilization container, covering the bottom to a depth of 15–20 cm. A metal rack was placed above the water, and a burlap sack was laid over the rack and container edges to prevent direct contact between the mushroom bags and container walls. The mushroom bags were stacked in layers, covered with another burlap sack then the container was sealed with a lid and steamed for 4 h at a temperature of 80–100°C. After steaming, the heat was turned off, and the bags were left to cool overnight before inoculating with prepared millet spawn. The mushroom bags were arranged in rows for easy handling. Hands and tools were sterilized with alcohol. Cotton spawn was broken up in a basin to ensure even distribution and 3-5 g of spawn was inoculated into each bag. The inoculated bags were placed in a room at ambient temperature, away from direct sunlight, and incubated for 5-7 days until the mycelium fully colonized the substrate. The bags were left until the mycelium had bound the substrate firmly, indicating readiness for fruiting. After 5 days of incubation, the mushroom bags were transferred to a black plastic-covered grows room. Excess cotton spawn was removed to prevent contamination by green mold. The bag surfaces were misted lightly to stimulate mushroom development, and water-filled plastic cups were placed beside the bags to maintain humidity. On dry days, water was sprayed around the grow room to support the moisture levels required by inky cap mushrooms.

Treatments

The experiment was conducted using a completely randomized design (CRD) with five treatments and 20 replications per treatment, totalling 100 experimental units. Each treatment contained different proportions of rubber tree sawdust (SD) and spent mushroom substrate (SMS) as follows: Treatment 1 consisted of SD 100% and SMS 0%; Treatment 2 consisted of SD 75% and SMS 25%; Treatment 3 consisted of SD 50%and SMS 50%; Treatment 4 consisted of SD 25% and SMS 75%; and Treatment 5 consisted of SD 0% and SMS 100%. Each treatment batch, weighed 5 kg, was supplemented with 200 g of fine rice bran, 10 g of Epsom salt ($MgSO_4 \cdot 7H_2O$), 100 g of granulated sugar, 50 g of calcium carbonate and 50 g of urea (46–0–0) Water was added to adjust the moisture content to 70–75%.

Data recording

Growth and yield data of mushrooms were collected daily over a 30-day period. Data regarding harvest frequency, number of mushrooms, cap width, stem length, fresh weight and biological efficacy were recorded. The accumulated data were used to calculate the biological efficiency. Biological efficiency was determined using the formula:

$$\text{Biological Efficiency (\%)} = \frac{\text{Weight of Fresh Fruiting Body (g)}}{\text{Dry Weight of Substrate (g)}} \times 100$$

Statistical analysis

The collected data values were presented as the mean ± standard deviation. Differences between the means of individual groups were assessed using a one-way ANOVA followed by Duncan's New Multiple Range Test at a 95% confidence level.

Results

The cap width results show that the amount of fresh sawdust and spent mushroom substrate significantly affects cap width, with a statistically significant difference ($P < 0.05$), as shown in Table 1. Treatment 1 (SD100%:SMS0%) had the highest cap width at 1.31 ± 0.09 cm, followed by Treatment 4 (SD25%:SMS75%), Treatment 5 (SD0%:SMS100%), Treatment 3 (SD50%:SMS50%), and Treatment 2 (SD75%:SMS25%), with cap widths of 1.28 ± 0.14, 1.24 ± 0.10, 1.23 ± 0.11 and 1.20 ± 0.14 cm, respectively. This indicates that the amount of fresh sawdust and spent mushroom substrate influences cap width.

For stem length, however, the results indicate no significant effect of fresh sawdust and spent mushroom substrate on stem length, as there was no statistically significant difference ($P < 0.05$) as shown in Table 1. Regarding biological efficiency (BE), there was no significant difference in the biological efficiency of Inky Cap mushroom production among the treatments ($P < 0.05$), as shown in Table 1.

The total fresh weight results show that the amount of fresh sawdust and spent mushroom substrate significantly affects the fresh weight yield ($P < 0.05$), as shown in Table 2. During days 1–5, the SD75%:SMS25% and SD50%:SMS50% treatments yielded the highest fresh weights at 72.05 and 72.16 g, respectively. The SD100%:SMS0% and SD0%:SMS100% treatments yielded the lowest fresh weights, with SD100%:SMS0% having the lowest average at 41.76 g. In the 6–10 days period, the SD50%:SMS50% treatment yielded the highest fresh weight (91.92 g), while the (SD0%:SMS100%) treatment yielded the lowest fresh weight (80.18 g). From days 11 to 30, the SD100%:SMS0% treatment produced the highest fresh weight, while the (SD0%:SMS100%) treatment consistently yielded the lowest fresh weight during this period. It can be observed that the ratios of SD75%:SMS25% and SD50%:SMS50% resulted in the highest yield during the initial period (1–10 days). A decline in yield occurred across all ratios, with the most rapid decrease observed between days 6-10. After 20 days, the mushroom yield decreased to the point where there was little difference between the various ratios. Since inky cap mushrooms have a short harvesting period, it is advisable to use the ratios of SD75%:SMS25% or SD50%:SMS50% for cultivation, as they provide the highest yield during the early stages of cultivation (Table 3).

Discussion

This study demonstrated that the use of spent mushroom substrate significantly affects the cap width and fresh weight of inky cap mushroom aligning with previous research such as the one that showed that SMS from king oyster mushroom (*P. eryngii*) and maple oyster mushroom (*P. cystidiosus*) can effectively replace fresh sawdust in cultivating hairy jew's ear (Wu *et al.* 2020b). Similarly, that improving substrate composition impacts the growth and quality of oyster mushroom and maple oyster mushroom (Hoa *et al.* 2022). This study is significant as it specifically focuses on using SMS as a substrate for inky cap mushroom, aligning with circular economy principles that emphasize sustainable resource utilization (Wu *et al.* 2020b; Hoa *et al*. 2022).

The experimental results indicated that the formula using 100% fresh sawdust (SD100%:SMS0%) provided the best outcome in terms of mushroom cap width, with an average of 1.31 ± 0.09 cm. This may be attributed to the porous structure and organic content of fresh sawdust, which supports mushroom growth. However, formulas with high SMS proportions, such as (SD25%:SMS75%) and (SD0%:SMS100%), also produced mushrooms with large caps, suggesting that an appropriate level of SMS can adequately meet the nutritional needs of inky cap mushroom for growth and may reduce reliance on fresh sawdust (Sripheuk 2007). This finding is consistent reported that SMS retains essential nutrients such as nitrogen and

Table 1: Frequency of harvests number of fruiting bodies cap width, stem length and biological efficiency of inky cap mushroom fruiting bodies in varying ratios of rubber tree sawdust and spent mushroom substrate

Treatment	Harvest frequency (times)	Number of fruiting Bodies	Cap width (cm)	Stem length (cm)	Biological Efficiency (%)
1 rubber tree sawdust 100% and spent mushroom substrate 0%	9.95 ± 1.70a	44.95 ± 6.70	1.31 ± 0.09a	3.85 ± 0.55	93.51 ± 28.22
2 rubber tree sawdust 75% and spent mushroom substrate 25%	7.95 ± 2.01b	43.75 ± 10.06	1.20 ± 0.14b	3.84 ± 0.33	92.83 ± 13.23
3 rubber tree sawdust 50% and spent mushroom substrate 50%	8.30 ± 1.92b	47.50 ± 5.33	1.23 ± 0.11ab	3.62 ± 0.81	89.95 ± 7.10
4 rubber tree sawdust 25% and spent mushroom substrate 75%	8.65 ± 2.05ab	46.35 ± 6.25	1.28 ± 0.14ab	5.51 ± 9.23	90.49 ± 32.70
5 rubber tree sawdust 0% and spent mushroom substrate 0%	9.95 ± 2.69a	44.95 ± 7.07	1.24 ± 0.10ab	3.46 ± 0.65	89.49 ± 22.34
F-Test	*	ns	*	ns	ns
C.V.%	23.50	15.96	9.75	16.01	24.94

Note: (*) Significant, (ns) = Non-significant. Statistically significant differences at a 95% confidence level ($P < 0.05$), with mean comparisons made using Duncan's new multiple range test (DMRT)

Table 2: Fresh weight (g/treatment) at each interval of inky cap mushroom with different ratios of rubber tree sawdust and spent mushroom substrate

Treatment	1-5 Days	6-10 Days	11-15 Days	16-20 Days	21-25 Days	26-30 Days
1 rubber tree sawdust 100% and spent mushroom substrate 0%	41.76 ± 11.45c	88.10 ± 13.77ab	110.61 ± 10.88a	118.78 ± 9.98a	122.97 ± 9.63a	125.39 ± 9.74a
2 rubber tree sawdust 75% and spent mushroom substrate 25%	72.05 ± 11.11a	87.77 ± 11.23ab	96.87 ± 11.88bc	102.05 ± 11.63bc	102.45 ± 11.58b	102.58 ± 11.58b
3 rubber tree sawdust 50% and spent mushroom substrate 50%	72.16 ± 6.74a	91.92 ± 4.72a	99.99 ± 6.75b	105.93 ± 7.03b	106.65 ± 7.16b	106.69 ± 7.12b
4 rubber tree sawdust 25% and spent mushroom substrate 75%	66.46 ± 15.99a	90.30 ± 17.13a	98.30 ± 18.02b	103.77 ± 17.13bc	104.33 ± 17.16b	104.40 ± 17.17b
5 rubber tree sawdust 0% and spent mushroom substrate 0%	54.17 ± 14.14b	80.18 ± 10.22b	89.46 ± 8.42c	97.14 ± 8.74c	100.20 ± 9.02b	101.22 ± 9.29b
F-Test	*	*	*	*	*	*
C.V.%	20.05	13.84	11.95	10.84	10.65	10.64

Note: (*) Significant. Statistically significant differences at a 95% confidence level ($P < 0.05$), with mean comparisons made using Duncan's new multiple range test

Table 3: Nutrient composition before and after cultivating inky cap mushroom with different ratios of rubber tree sawdust and spent mushroom substrate

Nutrients	N (mg/kg)	P (mg/kg)	K (mg/kg)	pH	Temp (°C)	EC (μS/cm)
Before Cultivating						
1 rubber tree sawdust 100% and spent mushroom substrate 0%	337.41 ± 46.52b	478.86 ± 51.28	970.05 ± 125.83b	9.0 ± 0.0	27.5 ± 2.42	4587.64 ± 1026.66b
2 rubber tree sawdust 75% and spent mushroom substrate25%	661.53 ± 201.5ab	835.77 ± 292.86	1488.57 ± 287.03a	9.0 ± 0.0	27.62 ± 1.61	9121.2 ± 2527.09ab
3 rubber tree sawdust 50% and spent mushroom substrate 50%	718.45 ± 311.6a	949.92 ± 490.44	1568.97 ± 377.06a	9.0 ± 0.0	28.6 ± 2.85	10672.76 ± 5011.49a
4 rubber tree sawdust 25% and spent mushroom substrate 75%	340.07 ± 69.88b	528.29 ± 152.19	1025.63 ± 265.93b	9.0 ± 0.0	27.08 ± 1.29	4911.48 ± 1106.32b
5 rubber tree sawdust 0% and spent mushroom substrate 0%	421.0 ± 89.1ab	581.78 ± 117.1	1188.58 ± 235.31ab	9.0 ± 0.0	26.6 ± 1.35	5047.95 ± 551.68b
F-Test before	*	Ns	*	Ns	Ns	*
C.V. % before	46.42	46	27.95	0	7.1	51.12
After Cultivating						
1 rubber tree sawdust 100% and spent mushroom substrate 0%	83.78 ± 10.88b	118.69 ± 14.3b	239.41 ± 27.8b	8.58 ± 0.59	30.81 ± 0.56	1199.15 ± 141.72b
2 rubber tree sawdust 75% and spent mushroom substrate25%	217.81 ± 64.51ab	316.72 ± 94.74ab	616.53 ± 238.8ab	8.9 ± 0.21	30.31 ± 0.64	2903.76 ± 625.22ab
3 rubber tree sawdust 50% and spent mushroom substrate 50%	296.09 ± 122.33a	413.17 ± 160.26a	843.32 ± 341.23a	8.97 ± 0.06	31.02 ± 0.65	4138.90 ± 1622.47a
4 rubber tree sawdust 25% and spent mushroom substrate 75%	347.18 ± 98.28b	563.91 ± 152.78b	1072.52 ± 295.89b	8.61 ± 0.57	31.24 ± 0.5	5865.26 ± 1931.22b
5 rubber tree sawdust 0% and spent mushroom substrate 0%	584.29 ± 243.33a	835.49 ± 361.57a	1607.29 ± 667.35a	8.7 ± 0.41	31.28 ± 0.55	8241.51 ± 3268.56a
F-Test after	*	*	*	ns	ns	*
C.V. % after	67.88	67.47	66.18	4.76	2.07	67.27

Note: (*) Significant, (ns) =Non-significant. Statistically significant differences at a 95% confidence level ($P < 0.05$), with mean comparisons made using Duncan's new multiple range test (DMRT)

phosphorus after the initial mushroom cultivation (Medina and Afagh 2023).

Regarding stem length, the results showed no statistically significant differences among the treatments, suggesting that the SD–to–SMS ratio may not directly influence stem length. This finding in terms of biological efficiency, no significant differences were observed across all treatments. This may be due to the short harvesting period of inky cap mushroom, resulting in similar overall efficiencies. These findings are consistent that short harvesting periods require balanced nutrient availability in the initial growth phase (Wu *et al.* 2020b). Fresh weight yield results revealed that the (SD75%:SMS25%) and (SD50%: SMS50%) treatments produced the highest yields during the first 1–10 days, yielding 72.05 and 72.16 g, respectively. This suggests that an optimal SD-to-SMS ratio can maximize early production. This observation aligns that balanced substrate mixtures improve mushroom yield during the early stages of cultivation (Zied *et al.* 2024). However, during days 6–10, a sharp decline in yield was observed across all treatments, likely due to nutrient depletion in the substrate after the initial intense absorption phase. Furthermore, differences in substrate ratios may influence aeration. Rapidly growing mushroom mycelia require good aeration to ensure adequate oxygen supply, which promotes rapid mycelial growth (Royse *et al*. 2004). Additionally, fast-growing mushrooms can quickly absorb nutrients from the substrate, enabling them to form fruiting bodies in a short period. For example, straw mushrooms can form fruiting bodies within 7–10 days after the mycelium fully colonizes the substrate (Miles and Chang 2004). Nutrient depletion in the substrate may limit mushroom growth in later stages (Medina and Afagh 2023). Therefore, adding supplemental nutrients during cultivation could help maintain stable yields in later stages.

Nutrient levels and environmental conditions in the substrate before and after cultivating inky cap mushrooms, focusing on nitrogen, phosphorus, potassium, pH, temperature, relative humidity, and electrical conductivity. Initially, Treatment 3 showed the highest nutrient concentrations, while Treatment 1 had the lowest. After cultivation, all treatments exhibited a significant decrease in nutrient levels, particularly nitrogen, as the mushrooms absorbed these nutrients for growth. However, Treatments 4 and 5 retained slightly higher nutrient levels compared to other treatments, possibly due to the breakdown of stored nutrients in the spent mushroom substrate, continued microbial activity, and the recycling of remaining nutrients back into the substrate. The electrical conductivity values also decreased, especially in Treatment 1, indicating nutrient depletion and varying nutrient uptake efficiency among treatments.

A key recommendation from this study is to use SD-to-SMS ratios of SD75%:SMS25% or SD50%:SMS50% for cultivating inky cap mushroom, as these ratios provided the highest yields during the early harvesting period. This approach is particularly suitable for mushrooms with short harvesting cycles. Moreover, using SMS reduces production costs, decreases dependence on fresh sawdust, and promotes the sustainable reuse of waste from the mushroom industry. This practice supports sustainable agriculture and circular economy principles (Wu *et al*. 2020a; Medina and Afagh 2023; Zied *et al.* 2024).

Conclusion

This study confirmed that spent mushroom substrate (SMS) is an effective and sustainable alternative for cultivating inky cap mushroom. The results showed that balanced ratios of fresh sawdust and SMS (SD75%:SMS25% and SD50%:SMS50%) provided the highest yields during the early harvesting period. SMS demonstrated potential as a cost-effective nutrient source, supporting sustainable agriculture and circular economy principles. Future research should focus on enhancing SMS properties and optimizing its long-term use in mushroom cultivation.

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Author Contributions

KP and IT planned the experiments and interpreted the results; KP and CP made the write up statistically analyzed the data and made illustrations.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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