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Effectiveness of Citronella and Patchouli Waste as Oyster Mushroom Substrates

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ABSTRACT

The essential oil industry produces large amounts of underutilized solid waste containing lignocellulosic compounds that can potentially support the growth of edible mushrooms. This research was conducted to evaluate the potential utilization of solid waste from citronella (*Cymbopogon nardus* (L.) Rendle) and patchouli (*Pogostemon cablin* (Blanco) Benth.) as alternative substrates for oyster mushroom (*Pleurotus* spp.) cultivation. The experimental design employed a completely randomized factorial design incorporating two treatment factors: oyster mushroom varieties (white and brown strains) and eight substrate composition variations, with one control treatment serving as a baseline comparison. Substrate formulations were developed using various combinations of citronella and patchouli waste mixed with conventional materials. The results demonstrated that aromatic waste-based substrates with balanced formulations, specifically M1, M2, and M5, produced significantly higher fresh weights and fruiting body counts, closely approaching the performance of the control treatment (M0B1), which recorded the highest biological efficiency of 42.37% within a cultivation period of approximately 56 days. Conversely, single dominant waste substrates, such as M3 and M6, yielded smaller fruiting bodies and lower total biomass production. Statistical analysis revealed a positive correlation between fruiting body quantity and fresh weight, supporting the use of morphological traits as reliable indicators of cultivation productivity. These findings highlight the significant potential of essential oil industry waste as a sustainable and economically viable alternative cultivation medium for oyster mushroom production, provided that substrates are formulated with appropriate proportional compositions and are properly adapted to their specific chemical characteristics and nutritional properties.

Keywords: biological efficiency, citronella, growth media, patchouli, *Pleurotus* spp.

INTRODUCTION

Indonesia has great potential for developing essential oil-producing plants, with approximately 13 of 40 essential oil plant species being commercially utilized, including citronella (*Cymbopogon nardus*) and patchouli (*Pogostemon cablin*) (DAI 2016). Essential oils are generally extracted from plants using distillation, solvent extraction, or pressing techniques (Permana and Robiah 2018). The main products are essential oil liquids used in the food, cosmetics, and pharmaceutical industries (Nabila *et al*. 2019). These processes generate large amounts of solid waste, despite their high effectiveness. In West Java, citronella production reached 18,659 tons and patchouli reached 9,979 tons during the 2020–2023 period (Directorate of Annual and Seasonal Crops 2024). The patchouli oil yield ranges only between 1.5–3%, with approximately 95% of the distillation results being solid waste, such as leaves and twigs (Kadir *et al*. 2022). Citronella produces essential oil at approximately 0.5–1.5%, while the remainder consists of solid and liquid waste (Usmiati *et al*. 2014).

Solid waste from essential oil plant distillation processes has not been optimally utilized and poses environmental contamination risks through anaerobic decomposition and methane release (Bahri *et al*. 2023). Previous studies have reported that citronella and patchouli distillation residues are rich in lignocellulosic components, including cellulose, hemicellulose, and lignin (Jasmani *et al*. 2023; Bekele *et al*. 2017). These characteristics indicate that essential oil distillation waste has the potential to be explored as a growth substrate for lignocellulose-degrading fungi, including oyster mushrooms.

Traditionally, oyster mushrooms are cultivated using sawdust-based substrates supplemented with rice bran and mineral additives to provide sufficient nutrients for mycelial development and fruiting body formation. However, the increasing demand and limited availability of sawdust have encouraged the exploration of alternative substrates derived from agricultural and industrial residues. Different oyster mushroom varieties may exhibit different growth and production responses owing to variations in genetic

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characteristics, nutrient utilization efficiency, and physiological adaptation to substrate composition.

Oyster mushrooms (*Pleurotus* spp.) are high economic value food commodities that are easily cultivated and efficiently utilize space and cultivation time. These mushrooms can grow on various types of organic waste with lignocellulose structures and convert them into biomass with good biological efficiency. Oyster mushroom growth is influenced by substrate characteristics, such as moisture, aeration, and adequate micronutrient availability. The utilization of solid waste from essential oil plant distillation remains limited and has not been comprehensively investigated. Therefore, this study aimed to evaluate the suitability of citronella and patchouli solid waste as alternative growth media for oyster mushroom (Pleurotus spp.) cultivation.

METHODS

Research Location and Time

The research activities spanned five months, commencing in February and concluding in June 2025. The citronella and patchouli waste substrates were collected at PT. The Talasi production facility is situated in Bogor Regency, West Java. Oyster mushroom cultivation and testing were conducted at the Hegarmanah Mushroom House and IPB Biotechnology Center.

Mushroom Cultivation Media Preparation

This study utilized two treatment factors: oyster mushroom spawn types (2 levels) and baglog substrate compositions (8 levels), as follows:

Factor 1: Substrate composition treatment combinations consisting of 8 treatments with 5 replications each:

M0: 82% Sengon Sawdust + 15% Rice Bran + 1.5% Gypsum + 1.5% Dolomite Lime

M1: 60% Sengon Sawdust + 15% Rice Bran + 22% Citronella Waste + 1.5% Gypsum + 1.5% Dolomite Lime

M2: 60% Sengon Sawdust + 15% Rice Bran + 22% Patchouli Waste + 1.5% Gypsum + 1.5% Dolomite Lime

M3: 82% Citronella Waste + 15% Rice Bran + 1.5% Gypsum + 1.5% Dolomite Lime

M4: 82% Patchouli Waste + 15% Rice Bran + 1.5% Gypsum + 1.5% Dolomite Lime

M5: 41% Citronella Waste + 41% Patchouli Waste + 15% Rice Bran + 1.5% Gypsum + 1.5% Dolomite Lime

M6: 22% Sengon Sawdust + 15% Rice Bran + 60% Citronella Waste + 1.5% Gypsum + 1.5% Dolomite Lime

M7: 22% Sengon Sawdust + 15% Rice Bran + 60% Patchouli Waste + 1.5% Gypsum + 1.5% Dolomite Lime

Factor 2**:** oyster mushroom spawn types, consisting of:

B1: White oyster mushroom spawn

B2: Brown oyster mushroom spawn

The experiment was arranged in a factorial completely randomized design with five replicates for each treatment combination. Each treatment was replicated five times, resulting in a total of 80 oyster mushroom baglog units. Statistical analysis employed a Factorial Completely Randomized Design (FCRD) based on the model from Mattjik and Sumertajaya (2013), as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} : Response measurement from the k-th replication exposed to the combination of i-th mushroom type and j-th substrate mixture

μ : Population mean parameter

α_i : Primary effect of the i-th mushroom type treatment

β_j : Primary effect of the j-th substrate composition treatment

$(\alpha\beta)_{ij}$: Combined effect of the i-th mushroom type and j-th substrate composition interaction

E_{ijk} : Experimental error term for the k-th unit under the i-th mushroom type and j-th substrate composition combination

Data analysis was performed using SAS software version 9.3.1 and Microsoft Excel (2021). The effects of the treatments on the observed variables were evaluated using analysis of variance (ANOVA) at a 95% confidence level. When significant differences were detected, Duncan's multiple range test at a 5% significance level was applied to compare the means among the treatments.

Baglog Preparation

Baglog substrates were mixed with 50–60% water for optimal mycelium absorption (Rosmiah *et al*. 2020). The mixture was placed into heat-resistant PP plastic bags (0.8 mm; 18×30 cm), weighed to reach 0.5 kg, coded according to treatments, and tied with rubber bands at the bag ends.

Sterilization

Baglogs were sterilized using steam heat through a steaming method for 12 hours at 90–100°C (Herliyana and Muhyi 2023) and then cooled for 24 hours before inoculation to prevent spawn damage from high temperatures (Hidayah *et al*. 2017).

Inoculation

Inoculation was performed in a laminar airflow (LAF) room to avoid contamination. White and brown oyster mushroom F1 spawns (5 g each) were inserted into baglogs using a spatula through the opened and bunsen-heated ends (Rosnina *et al*. 2024).

Incubation

The bags were incubated in a vegetative growth room until the mycelium covered the entire substrate surface (Irawati *et al*. 2019). They were then transferred to a generative room with a temperature of 22–28°C and humidity of 60–80%, controlled using a thermohygrometer (Utama *et al*. 2013).

Mushroom Maintenance and Observation

During maintenance, periodic watering was conducted to maintain the substrate moisture. Observations included the vegetative phase (from inoculation until the mycelium covered the entire medium) and generative phase (harvest, fresh weight, biological efficiency, cap number, stem length, and cap diameter) for approximately 56 days. Biological efficiency was calculated using the formula of Stamets (1993), based on the ratio of fresh fruiting body weight to dry substrate weight. Harvesting was performed when the mushrooms reached an optimal size. Proximate analysis of fruiting bodies included moisture, protein, fat, carbohydrate, ash percentage, and total energy using the by difference method (AOAC 2012).

Eight samples were selected from four substrate groups (Control, Citronella, Patchouli, and Combination) multiplied by two oyster mushroom types to evaluate the individual and combined substrate effects. Mushroom maintenance included regular watering to maintain optimal humidity and prevent substrate from desiccating. Watering was performed 2–3 times per day using a hand sprayer, depending on room humidity conditions, without direct contact with the baglog surface to avoid contamination of the substrate.

RESULTS AND DISCUSSION

Environmental Conditions

The mushroom storage room exhibited different temperature and humidity levels during the morning, afternoon, and evening over approximately 56 days of observation. Environmental conditions ranged from 26–30°C temperature and 84–90% humidity throughout the day (Figure 1). Oyster mushrooms require temperatures ranging between 26°–30°C and optimal humidity of 80–95% (Arsella *et al*. 2023). The highest temperature occurred during midday (30°C) with the lowest humidity (84%). Watering processes were conducted to reduce the room temperature, providing natural cooling effects while maintaining humidity within the optimal range for the growth of oyster mushrooms.

Mycelium Growth Rate

The Duncan's multiple range test results presented in Table 1 revealed that substrate M7 exhibited the optimal mycelium growth rate at 0.70 cm/day, whereas white oyster mushroom varieties demonstrated superior performance with a mean growth rate of 0.54 cm/day. The rapid mycelial growth observed on substrate M7 may be associated with the high proportion of patchouli waste, which provides abundant and readily available carbon sources during the early vegetative phase. These observations are consistent with the findings of Owaid *et al*. (2015), who reported that variations in substrate composition and oyster mushroom species yield significantly different mycelium growth responses, although the interaction effects between these factors were not statistically significant. However, rapid mycelial colonization did not necessarily result in superior generative performance, as indicated by the relatively low number of fruiting bodies produced on M7. This phenomenon suggests

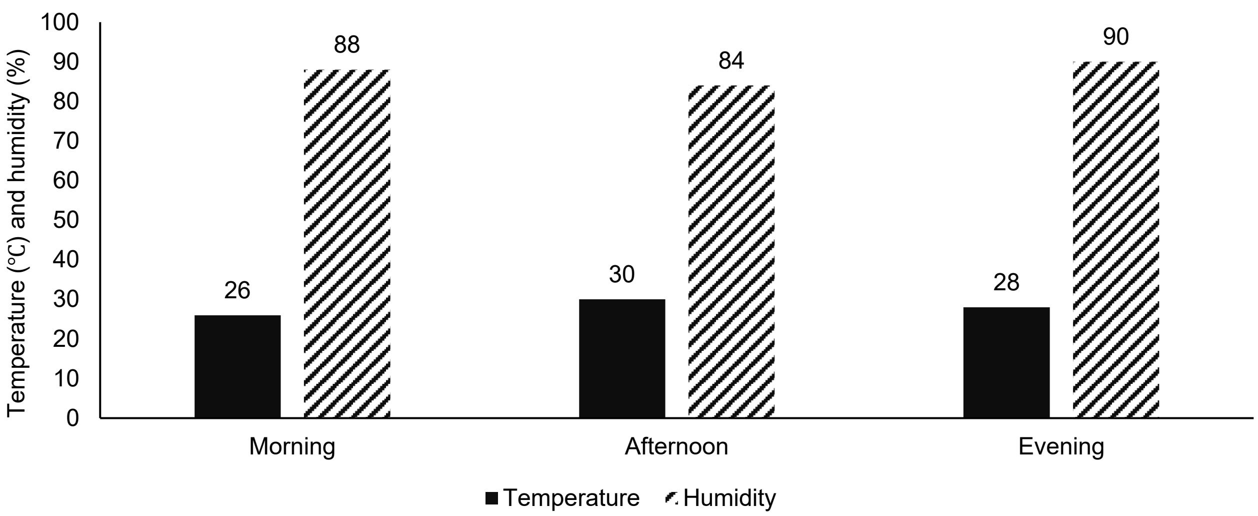

Figure 1 Average temperature and humidity of mushroom storage room.

that excessive concentrations of aromatic compounds and secondary metabolites in patchouli waste may inhibit the transition from the vegetative to the generative phase, despite supporting rapid mycelial growth.

Generative Phase Duration

Based on Duncan's test results in Table 2, substrate M5 extended the generative phase to 35 days, which was significantly different from M0 and M6 (20 days), while white oyster mushrooms showed a longer duration (33 days) compared to brown oyster mushrooms (28 days). The extended generative phase observed on substrate M5 indicates a more stable and sustained fruiting process than that of the other substrate compositions. This condition was accompanied by a higher number of fruiting bodies and greater fresh weight production, suggesting that substrate M5 effectively supports the generative development.

Tesfay et al. (2020) reported that differences in fruiting performance among substrates are closely related to nutrient balance, lignocellulosic composition, and water retention capacity, which influence the transition from the vegetative to generative phases. Additionally, Akter *et al*. (2022) reported that polyphenolic compounds present in aromatic plant–based substrates may exert inhibitory effects on fungal development, particularly during the transition to fruiting body formation. These findings demonstrate that substrate formulation plays a critical role in enhancing the generative performance of oyster mushrooms, highlighting the potential of combined essential oil waste substrates as effective alternative growth media.

Morphological Characteristics

Treatments M3 and M6 produced the lowest stem lengths (3.80 and 3.59 cm) and relatively small cap diameters (2.71 and 2.18 cm), presumably due to the excessively high concentrations of citronella waste containing aromatic compounds that can disrupt metabolism and inhibit oyster mushroom cap formation. The reduced morphological development observed in these treatments indicates that substrates dominated by single aromatic waste components may

Table 1 Results of Duncan's further test on the effect of substrate composition and mushroom type on mycelial growth

Treatments	Mycelium growth (cm/day)
Media Composition	
M0	0,57^{b}
M1	0,50^{b}
M2	0,60^{ab}
M3	0,37^{c}
M4	0,38^{c}
M5	0,71^{a}
M6	0,36^{c}
M7	0,70^{a}
Types of Mushrooms	
White Oysters	0,54^{a}
Brown Oysters	0,50^{a}

Note: Numbers followed by different letters in the table indicate significant effects at the 5% level based on Duncan's extended test. M0–M7 refer to substrate composition treatments described in Factor 1 (Substrate composition treatments) in the Methods section.

Table 2 Results of Duncan's further test on the effect of growth substrate composition and mushroom type on the generative phase

Treatments	Generative phase (day)
Substrate Composition	
M0	20^{b}
M1	30^{ab}
M2	23^{ab}
M3	20^{ab}
M4	24^{ab}
M5	35^{a}
M6	20^{b}
M7	21^{ab}
Types of Mushrooms	
White Oysters	33^{a}
Brown Oysters	28^{b}

Note: Numbers followed by different letters in the table indicate significant effects at the 5% level based on Duncan's extended test.

limit the nutrient balance required for optimal fruiting body formation. Regarding the cap quantity parameters, treatment M5 demonstrated the highest productivity (Table 3). The higher number of caps produced under substrate M5 reflects a more efficient allocation of nutrients toward generative growth than substrates with an unbalanced aromatic waste composition. These findings are consistent with those of Mardiana *et al.* (2018), who reported that aromatic plantation waste materials can support oyster mushroom growth when the substrate composition is appropriately balanced and nutritionally complementary. Overall, morphological characteristics, such as stem length, cap number, and cap diameter, were strongly influenced by substrate composition, highlighting the importance of balanced aromatic waste formulations in improving mushroom quality.

Fresh Weight Production

Based on the results in Figure 2, white oyster mushrooms produced the highest fresh weight on substrate M0B1 (36.11 g), followed by M5B1 and M2B1 (32.25 g and 30.63 g, respectively). The higher fresh weight observed in these treatments indicates more efficient biomass accumulation during the generative phase of the plant. These results were consistent with the cap quantity results (Table 3), supported by research by Rambey *et al*. (2019), indicating that increased fruiting body formation tends to increase fresh weight due to the water percentage stored in each cap. This relationship confirms that fresh weight production is closely associated with morphological development, particularly with cap number and size. Conversely, low fresh weight can result from small fruiting bodies with a consequently low water percentage per fruiting body, as observed in composition M3. This suggests that substrate compositions dominated by single aromatic waste components may restrict water retention and nutrient availability, thereby limiting the production of fresh weight. Overall, fresh weight production was strongly influenced by substrate composition, highlighting the importance of balanced essential oil waste formulations for optimizing oyster mushroom yield.

Table 3 Duncan's test results for stalk length, number of caps, and mushroom cap diameter

Treatments	Stem length (cm)	Number of hoods	Hood diameter (cm)
Substrate Composition			
M0	5,98^{a}	5,90^{ab}	4,65^{a}
M1	6,74^{a}	6,50^{a}	4,49^{a}
M2	6,36^{a}	7,40^{a}	3,44^{abc}
M3	3,80^{b}	3,20^{bc}	2,71^{bc}
M4	5,21^{ab}	5,92^{ab}	2,58^{bc}
M5	5,39^{ab}	7,60^{a}	4,00^{ab}
M6	3,59^{b}	5,70^{ab}	2,18^{c}
M7	5,98^{a}	2,00^{c}	3,22^{abc}
Types of Mushrooms			
White Oysters	4,49^{b}	5,05^{a}	3,34^{a}
Brown Oysters	6,27^{a}	6,03^{a}	3,48^{a}

Note: Numbers followed by different letters in the table indicate significant effects at the 5% level based on Duncan's extended test

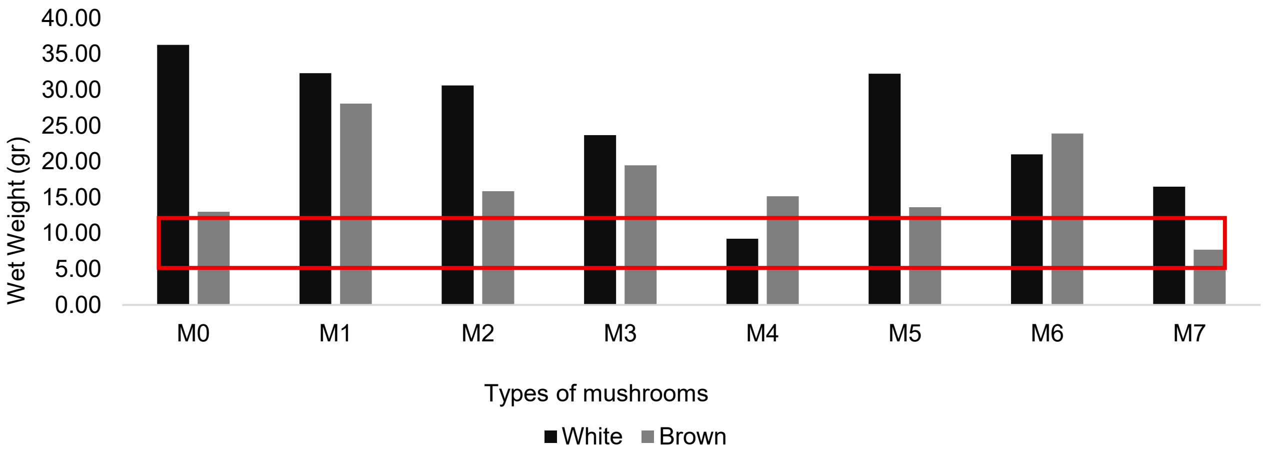

Figure 2 Average wet weight of oyster mushrooms

Proximate Composition of Oyster Mushrooms

The results in Table 4 show that the brown combination substrates produced the highest protein percentage (38.16%) among the alternative substrates, approaching the brown control levels (39.61%). The ash percentage in aromatic plant waste substrates (1.31–1.71%) exceeded white control levels, (0.77%), indicating optimal mineral transfer. Carbohydrate percentage variations (3.74–11.46%) and significant differences between white and brown oyster mushrooms demonstrated the influence of substrate and genetic factors on mushroom proximate composition. Tiupova *et al*. (2025) reported that oyster mushrooms contain protein concentrations ranging from 15–30%, with a complete essential amino acid profile, thereby establishing them as superior non-animal protein sources.

Biological Efficiency

Based on Figure 3, the highest biological efficiency was achieved by M0B1 at 42.37%. This result indicates that the conventional substrate provided optimal conditions for biomass conversion during the cultivation period. These results indicate that optimal performance was achieved within a relatively short period of approximately 56 days compared to the standard oyster mushroom cultivation period of up to 4 months (±120 days). The shortened cultivation period suggests efficient substrate utilization and rapid biomass conversion under favorable conditions. These findings align with the statement by Gume *et al.* (2013) that all substrates achieving biological efficiency exceeding 40% can be recommended for oyster mushroom cultivation. Accordingly, the biological efficiency obtained in this study confirms the feasibility of using alternative substrates derived from the essential oil industry waste. Other substrates, such as M4B1 (34.11%), M3B1 (33.74%), and M6B2 (29.19%), showed moderate potential while remaining viable for development. Although the control substrate exhibited the highest biological efficiency, several waste-based substrates demonstrated competitive performance, emphasizing the potential of properly formulated essential oil waste as an alternative cultivation medium.

CONCLUSION

Citronella and patchouli solid waste can be utilized as alternative growth media for oyster mushroom cultivation when combined with conventional substrate components. Substrates formulated with balanced proportions of aromatic waste supported mycelial growth and generative performance comparable to that of conventional substrates, whereas substrates dominated by single aromatic waste components

Table 4 Results of proximate tests of oyster mushrooms

Numbers	Sample code	Ash	Fat	Protein	Carbohydrate
				%	
1	White control	0,77	0,57	27,77	5,47
2	Brown control	0,94	1,17	39,61	3,74
3	White lemongrass	0,82	0,93	36,58	4,02
4	Brown lemongrass	0,93	0,68	36,78	5,51
5	White patchouli	1,71	1,41	33,47	11,46
6	Brown patchouli	1,31	0,06	35,39	7,04
7	White L+P	0,99	0,40	36,00	4,63
8	Brown L+P	1,35	0,61	38,16	5,92

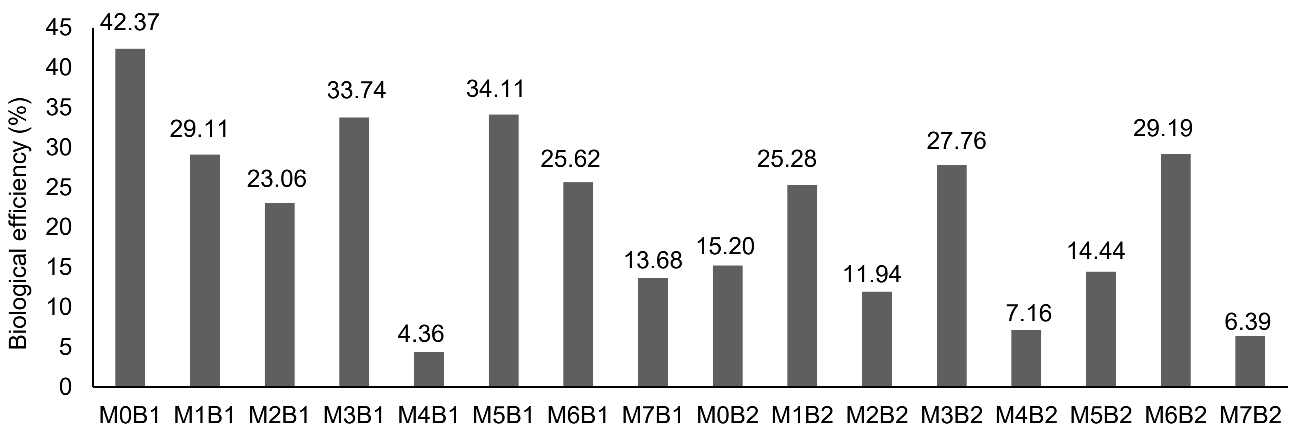

Figure 3 Results of the biological efficiency of oyster mushrooms.

resulted in lower productivity. These findings indicate that an appropriate substrate formulation is a key factor in optimizing oyster mushroom production. Overall, this study demonstrates the potential of essential oil industry waste as a sustainable alternative cultivation medium, contributing to waste valorization while maintaining acceptable levels of mushroom productivity and quality.

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