

Effects of rice landraces as cultivation media on yield and physiochemical properties of common split gill mushroom (*Schizophyllum commune* Fr.) cultivation

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Abstract

The common split gill mushroom is not only a source of food for humans but also plays a medicinal role. Mushroom cultivation typically uses sawdust as a medium, which often leads to contamination. This study investigated five Thai rice cultivars (*Oryza sativa*): cv. 'Homdokmali' (including regular Homdokmali and parboiled, known as Hang-Homdokmali), 'Riceberry' (including regular Riceberry and parboiled, known as Hang-Riceberry), 'Mun-Pu' 'Jao-Daeng' and 'Jao-Luang' – as culture media for aseptic mushroom cultivation to evaluate the yield and chemical properties of this mushrooms. The experiment was carried out in a completely randomized design (CRD) with three replications. The analyses indicated that cv. 'Jao-Daeng' media significantly influenced the fresh weight, dry weight, yield percentage, and biological efficiency of the common split gill mushroom more than the other media. Moreover, this medium was also effective in producing antioxidant activities in both DPPH and FRAP assays compared to other media. Although mushrooms cultivated with cv. 'Riceberry' media exhibited the highest total glucan and β -glucan contents when evaluated and considered based on dry weight, 'Jao-Daeng' media also demonstrated the highest total glucan. Correlation analysis revealed that yield positively correlated with biological efficiency, while β -glucan content negatively correlated with FRAP reducing potential. The findings of this research show the potential benefits of 'Jao-Daeng' rice landraces for mushroom cultivation, making it an appropriate option to enhance the quality and production of bioactive compounds in cultivated mushrooms.

Keywords: antioxidant activity; fruiting body; glucan content; mushroom cultivation; rice landrace

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Introduction

The common split gill is a small mushroom, 1-4 cm in diameter, fan-shaped, and without flower stalks. It is a globally distributed mushroom species known for its adaptability and ability to grow on a wide range of lignocellulosic substrates. Since this mushroom is high in carbohydrates and protein (Meng *et al.*, 2016), it is consumed as food in South Asian countries like Thailand, Taiwan, Malaysia, Vietnam, Southern China, and Northeastern India (Singh *et al.*, 2021). In some countries, it is used as a medicine because it contains flavonoids, terpenoids, saponin, phenols, and polysaccharides (Teoh and Don, 2013; Meng *et al.*, 2016). The crude extracts from this mushroom can inhibit viruses, mycelia, and cancer cells (Wong and Chye, 2009; Patel and Goyal, 2012; Teon and Don, 2013). Some studies show that the common split gill mushroom can be used to produce high-value products such as cosmetics and pharmaceuticals (Pirshahid *et al.*, 2011; Amin *et al.*, 2022).

The common split gill mushroom is an edible mushroom that naturally grows on decaying wood during the rainy season in all regions of the world except Antarctica, where there is no wood to be used as a substrate (Khatua *et al.*, 2013; Singh *et al.*, 2021). The procedure for common split gill mushroom cultivation is the same as for other mushrooms, except for the media recipe and cultivation techniques, which are slightly different (Muswati *et al.*, 2021). Due to the high nutrient content of the mushroom, it must be treated correctly, as contamination with other fungi will damage the product. Mushroom cultivation usually uses sawdust or wood dust as media. *In vitro* techniques are a good choice for mushroom cultivation to increase mushroom quality and prevent contamination.

Rice is consumed as a grain and is a monocotyledon in the grass family Poaceae or Gramineae. It is classified into two species: *Oryza glaberrima*, which grows only in tropical Africa, and *Oryza sativa*, which grows worldwide. *O. sativa* is further subdivided into three subspecies: javanica, japonica, and indica, which are mostly grown in the tropics (Loko *et al.*, 2021). Rice is a staple crop cultivated worldwide, particularly in regions such as Asia, where it serves as a primary food source. Rice is an important cash crop in Thailand and the main source of income for agricultural exports. In addition, rice is the staple food of the Thai people (Suebpongsang *et al.*, 2020). Thailand grows indica rice, which is classified into two types: non-glutinous rice and glutinous rice (Lei *et al.*, 2021). However, rice cultivation in Thailand is focused on both consumption and export. During the season of 2022-2023, Thailand is expected to produce around 20.2 million tons. The main domestic production is for consumption in the country (about 12 million tons), with the remaining earmarked for export. Some varieties of rice in Thailand are popular for consumption due to their high nutritional value, delicious taste, and aroma. However, there are still some rice cultivars (*i.e.*, landraces or local rice cultivars) that are not grown solely for consumption purposes. Rice landraces, which are locally adapted traditional varieties, exhibit diverse characteristics and genetic variations, making them an interesting option for novel applications beyond food production. By utilizing rice landraces as cultivation media, their unique properties and nutrient profiles can potentially enhance the growth and bioactive content of mushrooms. Some varieties of rice landraces with premium nutritional quality grains can be used for many purposes. Moreover, many landrace varieties are significantly higher in anthocyanin concentration and antioxidative capacity, as well as Fe and Zn concentration (Sreethong *et al.*, 2020). Furthermore, some studies have reported that pigmented rice varieties such as dark purple, brown rice grain, and red rice grain (or dehulled grain) are also important source of vitamins and minerals (Yodmanee *et al.*, 2011; Meng *et al.*, 2005). Black rice contains higher levels of iron, zinc, calcium, copper, and manganese than red rice (Meng *et al.*, 2005).

Several studies have explored the nutritional and bioactive properties of rice landraces, highlighting their potential in different applications. For instance, Kunnam *et al.* (2023) demonstrated the high antioxidant capacity and phenolic content of selected rice landraces, suggesting their suitability for functional food development. Moreover, rice landraces contain various bioactive compounds, such as flavonoids, phenolic

acids, and polysaccharides, which highlight their potential as sources of natural antioxidants and immunomodulatory agents. (Rajendran *et al.*, 2018; Thavamurugan *et al.*, 2023).

This study aims to evaluate the effects of different rice landraces as cultivation media on the yield, physical, and chemical properties of *in vitro* common split gill mushrooms. This study examines parameters such as mycelial growth, morphological characteristics, fruiting body development, nutritional composition, and bioactive compound content.

Materials and Methods

Plant materials

Five Thai rice cultivars were examined in this study, including *Oryza sativa* cv. 'Jao-Daeng' (JD), 'Jao-Luang' (JL), 'Mun-Pu' (MP), 'Riceberry' (including regular Riceberry (RB) and parboiled, known as Hang-Riceberry (RB_HA)), and 'Homdokmali' (including regular Homdokmali (HDML) and parboiled, known as Hang-Homdokmali (HDML_HA)). Three samples of rice landraces – *Oryza sativa* cv. 'Jao-Daeng', 'Jao-Luang', and 'Mun-Pu' – were provided by the Sakon Nakhon Rice Research Center, Sakon Nakhon, Thailand. Other samples were collected from the community enterprise of germination organic brown rice, Ban Khok Sawang, Sakon Nakhon province (Figure 1).



Figure 1. Different cultivars of rice (*Oryza sativa*) used as media for aseptic cultivation of common split gill mushroom

From left to right: 'Jao-Daeng'; 'Jao-Luang'; 'Riceberry'; 'Mun-Pu'; 'Riceberry' parboiled; 'Homdokmali' parboiled; 'Homdokmali'

Preparation of mushroom mycelium

The pure mycelium of the *Schizophyllum commune* Fr. was prepared from spores in a piece of fruiting body mushroom. Small pieces of *Schizophyllum commune* Fr. from fresh fruiting bodies were aseptically transferred to Potato Dextrose Agar (PDA) to obtain pure mycelium. The mycelium was incubated at 30 °C for 7-10 days and then isolated by transferring the culture to PDA using a sterile cork borer. The pure mycelium of *Schizophyllum commune* Fr. was used for aseptic cultivation.

Preparation of substrate mixtures for aseptic cultivation

The media mixture was composed of 25 g of rice bran and 375 g of whole grain from each rice cultivar. All ingredients were mixed and added to 40 g of substrate, including 100 g glucose, 50 g yeast, 50 g peptone, 5 g MgSO₄, 5 g KH₂PO₄, 25 g rice bran oil, and filtered potato boiling water (200 g potato/ 1 L water). Each substrate mixture was poured into a glass bottle with dimensions 9×15.5 cm. Ten bottles were used for each treatment. The bottled substrates were sterilized at 121 °C and 15 psi for 45 minutes and then cooled down for 12 hours.

Inoculation and harvesting

The sterilized substrate bottles were inoculated with a 10 mm diameter piece of agar containing *Schizophyllum commune* Fr. mycelium under aseptic conditions. The inoculated bottles were transferred to a clean and disinfected incubation room with alternating light and dark conditions at ambient temperature for approximately 30-40 days to promote mushroom mycelia ramification. The fresh and dry weights of the complete mushrooms from 10 bottles of each substrate mixture were recorded and compared. The yield percentage was calculated using the formula in Equation 1. The biological efficiency (BE) was calculated using the formula in Equation 2 (Devi and Sumbali, 2021). The experiment was set up using a completely randomized design (CRD) with three replications.

$$\text{Yield (\%)} = (\text{dry weight} / \text{fresh weight}) \times 100 \quad (1)$$

$$\text{BE} = (\text{mean yield} / 80 \text{ g weight of substrate}) \times 100 \quad (2)$$

Determination of glucan content

The glucan contents were examined with dry carpophore from each treatment using the Mushroom and Yeast Glucan Assay Kit (K-YBGL 09/2009; Megazyme International Ireland Ltd.; Bray, County Wicklow, Ireland).

Evaluation of antioxidant activity

The DPPH assay: The antioxidant capacity was expressed as milligram of Trolox (Sigma-Aldrich, Germany) equivalent per gram dry weight (mg TE/g DW). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) scavenging capacity was measured using a modified method following Brand-Williams *et al.* (1995). The reaction was initiated by mixing 0.5 mL of diluted sample with 0.5 mL of 0.1 mM DPPH in methanol. The mixtures were incubated at room temperature and kept in the dark for 30 minutes. The antioxidant activity was immediately recorded using spectrophotometer (Shimadzu, UV 1800) with absorbance at 517 nm. The radical scavenging capacity was calculated using the following equation:

$$\text{DPPH free radical scavenging capacity (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (3)$$

Where:

(A₀) = the absorbance of control sample

(A₁) = the absorbance of test sample

The FRAP assay: this method was assayed using the ferric reducing antioxidant potential (FRAP) method with a modification of Benzie and Strain (1996) protocol. The FRAP reagent was a mixture of 300 mM acetate buffer pH 3.6, 20 mM Ferric chloride, and 10 mM TPTZ (2,4,6 Tris (2-pyridyl-s-triazine)) (Sigma-Aldrich, Germany) in a ratio of 10:1:1. The reaction was initiated with 50 µL of diluted sample mixed with 1.5 mL FRAP reagent. The mixture was incubated at room temperature for 4 minutes and then measured for absorbance at 593 nm with a spectrophotometer (Shimadzu, UV 1800). The FRAP value was exhibited as milligram of Trolox equivalent per gram dry weight (mg TE/ g DW).

Statistical analysis

The experiment was carried out in a completely randomized design (CRD) with three replications. The variance and mean differences of data were statistically analyzed using ANOVA, and the means were compared using the Least Significant Difference (LSD) test with Statistix 8.0 program (Analytical Software; Tallahassee, FL, USA) at the significant level $p \leq 0.05$. Principal component analysis (PCA) was examined by PAST program ver. 4.12 (Hammer *et al.*, 2001) to determine correlations between the rice media and phytochemical properties of *Schizophyllum commune* Fr.

Results and Discussion*Effects of rice media on mushroom cultivation yield and biological efficiency*

The development of the common split gill mushroom carpophore in various media after one month is illustrated in Figure 2. The results indicate that the fresh weight of the fruiting bodies ranged between 13.19 and 24.29 grams. Moreover, the yield percentage and biological efficiency ranged between 27.80-34.86% and 34.75-43.58%, respectively (Table 1). There were significant differences ($p \leq 0.05$) in fresh weight, yield percentage, and biological efficiency of common split gill mushrooms, with *Oryza sativa* cv. 'Jao-Daeng' media recording the highest value. Overall, substrates that resulted in higher yields also exhibited higher biological efficiency (BE). Several studies have investigated the effect of different substrates on the growth and yield of various mushroom species, providing valuable insights into optimizing cultivation conditions. For instance, a study by Hoa *et al.* (2015) examined the effect of different substrates on the yield and biological efficiency of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Similar to our findings, they reported significant variations in mushroom yield and biological efficiency based on substrate composition.



Figure 2. Fruiting body (carpophore) development of common split gill mushroom under different rice media, depending on cultivars: (a) 'Homdokmali'; (b) 'Homdokmali' parboiled; (c) 'Riceberry'; (d) 'Riceberry' parboiled; (e) 'Mun-Pu'; (f) 'Jao-Daeng'; and (g) 'Jao-Luang'

Table 1. Mean of yield and biological efficiency of common split gill mushroom cultivated from different rice media

Treatment (cultivar)	Fresh weight of fruiting body (g)	Yield (%)	Dry weight of substrate (%)	Biological efficiency (%)
Homdokmali	17.62 ± 3.87 ^{bc}	30.56 ± 3.53 ^{bc}	38.51 ± 8.37	38.20 ± 4.42 ^{bc}
Homdokmali parboiled	14.53 ± 6.46 ^{bc}	30.33 ± 3.87 ^{bc}	39.35 ± 5.17	37.92 ± 4.84 ^{bc}
Riceberry	13.19 ± 4.82 ^c	30.97 ± 2.30 ^{abc}	42.36 ± 4.04	38.71 ± 2.88 ^{abc}
Riceberry parboiled	19.42 ± 7.02 ^{ab}	33.07 ± 1.93 ^{ab}	43.46 ± 2.57	41.34 ± 2.41 ^{ab}
Mun-Pu	15.60 ± 1.79 ^{bc}	27.80 ± 4.36 ^c	38.13 ± 3.36	34.75 ± 5.45 ^c
Jao-Daeng	24.29 ± 6.93 ^a	34.86 ± 2.93 ^a	43.55 ± 1.57	43.58 ± 3.66 ^a
Jao-Luang	19.54 ± 6.29 ^{ab}	32.61 ± 4.39 ^{ab}	42.07 ± 3.14	40.76 ± 5.49 ^{ab}
Mean	17.26	31.18	40.83	38.97
F-Test	*	*	ns	*
CV	31.70	11.20	11.58	11.20

Data were expressed as mean ± SD, and shown as an average value of the three replications. Different letters indicated statistically significant differences based on LSD at $p \leq 0.05$

Effect of rice media on glucan content

The fruit bodies of mushrooms were examined for glucan content. It was found that different rice cultivars used as mushroom media affected glucan contents. The total glucan, α -glucan, and β -glucan contents of common split gill mushrooms grown in different media were significantly different. The Riceberry media showed the highest total glucan and β -glucan content in common split gill mushroom, at 24.46% and 22.91% w/w, respectively (Table 2). Media containing *Oryza sativa* cv. 'Homdokmali' parboiled, known as 'Hang-Homdokmali' showed the highest α -glucan content (4.12 %w/w). Furthermore, Razak *et al.* (2018) reported that common split gill mushrooms cultivated using traditional mushroom media and extracted with 100% methanol exhibited the highest total glucan content at 11.85% w/w. However, this value was lower than that observed for common split gill mushrooms cultivated in different rice media in our study. Moreover, when the total glucan content was compared to the dry weight obtained from fruiting bodies of mushrooms cultivated in different rice media, it was observed that the highest total glucan content was exhibited by those cultivated from *Oryza sativa* cv. 'Jao-Daeng' media (Figure 3). Glucans are polysaccharides known for their various health benefits, including immune-modulating and antioxidant properties. Therefore, the choice of rice media can be fitted to the desired glucan composition, depending on the specific health-promoting effects one wishes to target. Many studies have demonstrated the impact of cultivation media on the composition of bioactive compounds in mushrooms. These results align with the study by Siwulski *et al.* (2019), which explored the effect of different substrates on the chemical composition and nutritional profile of six cultivated mushroom species. They reported significant variations in the amino acid and mineral contents of mushrooms grown on different substrates, indicating that substrate selection influences the nutritional composition of mushrooms. This finding supports our observation that the appropriate option of rice media can influence the glucan content and antioxidant activity of common split gill mushrooms.

Table 2. Content of total glucan, α -glucan, and β -glucan of common split gill mushroom from different rice media

Treatment (cultivar)	Total glucan (%w/w)	α -glucan (%w/w)	β -glucan (%w/w)
Homdokmali	19.27±0.49 ^b	1.99±0.10 ^c	17.28±0.52 ^{bc}
Homdokmali parboiled	22.38±0.65 ^{ab}	4.12±0.20 ^a	18.25±0.45 ^{bc}
Riceberry	24.46±2.45 ^a	1.55±0.19 ^d	22.91±2.33 ^a
Riceberry parboiled	21.75±1.03 ^{ab}	1.86±0.08 ^c	19.89±0.96 ^{abc}
Mun-Pu	21.66±4.00 ^{ab}	1.48±0.12 ^d	20.18±3.90 ^{ab}
Jao-Daeng	19.36±1.39 ^b	2.55±0.21 ^b	16.81±1.60 ^c
Jao-Luang	21.03±0.54 ^b	2.47±0.18 ^b	18.56±0.39 ^{bc}
Mean	21.42	2.29	19.13
F-Test	*	*	*
CV	8.99	7.14	9.84

Data were expressed as mean \pm SD, and represent the average of three replications. Different letter notations indicate statistically significant differences based on the Least Significant Difference (LSD) test at $p \leq 0.05$.

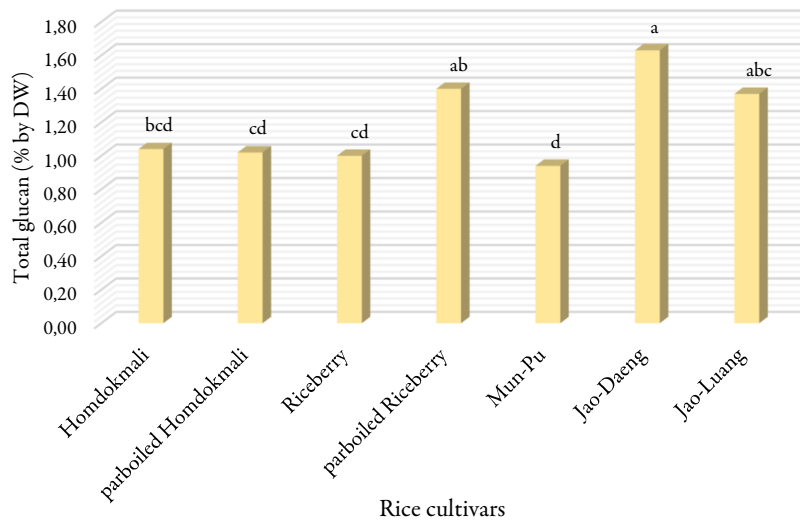
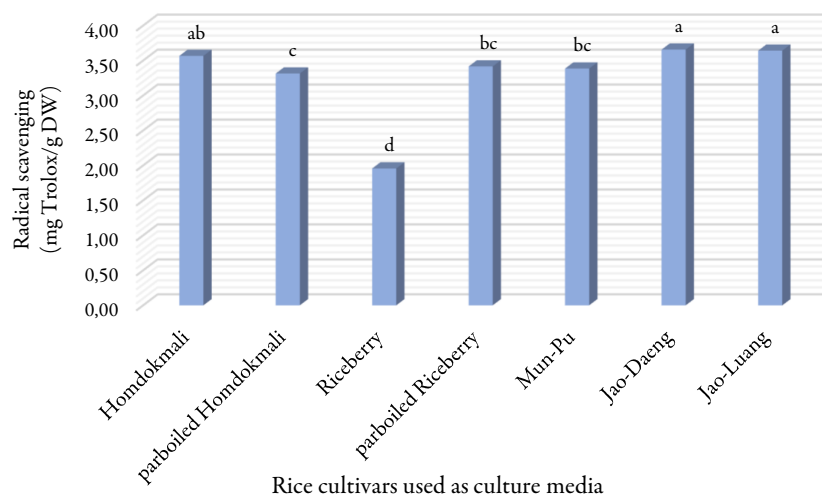


Figure 3. Total glucan content relative to dry weight obtained from fruiting bodies of mushrooms cultivated in various rice media expressed as means from triplicated experiments. Different letters indicate statistically significant differences based on LSD at $p \leq 0.05$.

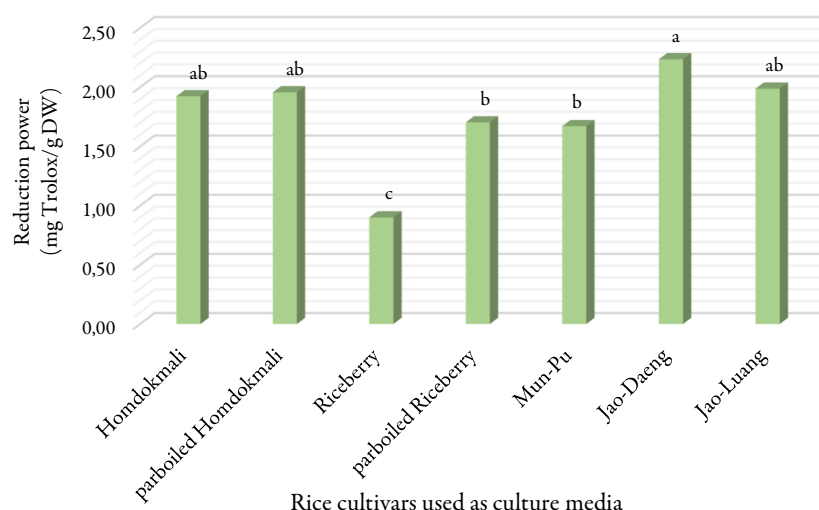
Effect of rice media on antioxidant activity

The antioxidant capacity of common split gill mushrooms cultivated with different rice cultivars was examined using two methods: DPPH and FRAP assays. The antioxidant activities of phenolic compounds can be influenced by the position and number of hydroxyl groups (Camargo *et al.*, 2019). The results showed that mushroom cultivated with *Oryza sativa* cv. 'Jao-Daeng' media exhibited the highest DPPH radical scavenging activity and FRAP reducing potential, at 3.66 and 2.24 mg Trolox/g DW, respectively (Figure 4). Antioxidants play a crucial role in protecting the body against oxidative stress and preventing cellular damage caused by free radicals. Therefore, cultivating mushrooms on specific rice media can enhance their antioxidant activity, increasing their potential as a natural source of antioxidants in the diet. Our findings regarding antioxidant activity align with research conducted by Ferrari *et al.* (2021), who investigated the effect of different substrates on the antioxidant properties of *Pleurotus ostreatus* and *Agaricus subrufescens* mushrooms. They found that mushrooms cultivated on specific substrates exhibited enhanced antioxidant activity compared to those grown

on conventional substrates. This suggests that manipulating cultivation media can positively influence the antioxidant potential of mushrooms, consistent with our findings.



(A)



(B)

Figure 4. Antioxidant activity of *Schizophyllum commune* Fr. extracts based on different rice media expressed as mean from triplicated experiments: (A) DPPH method and (B) FRAP method. Different letters indicate statistically significant differences based on LSD at $p \leq 0.05$.

Correlation analysis between different rice media and phytochemical properties of Schizophyllum commune Fr.

Principal component analysis (PCA) revealed variations in the effects of rice media on the physical and chemical properties of mushroom (Figure 5). The first two main components accounted for 93.73% of the overall variance, with 77.08% and 16.65% for PC1 and PC2, respectively. PC1 consisted of fresh weight of fruiting body, dry weight of fruiting body, yield, biological efficiency, α -glucan, DPPH, and FRAP. PC2 included dry weight of substrate, total glucan, and β -glucan.

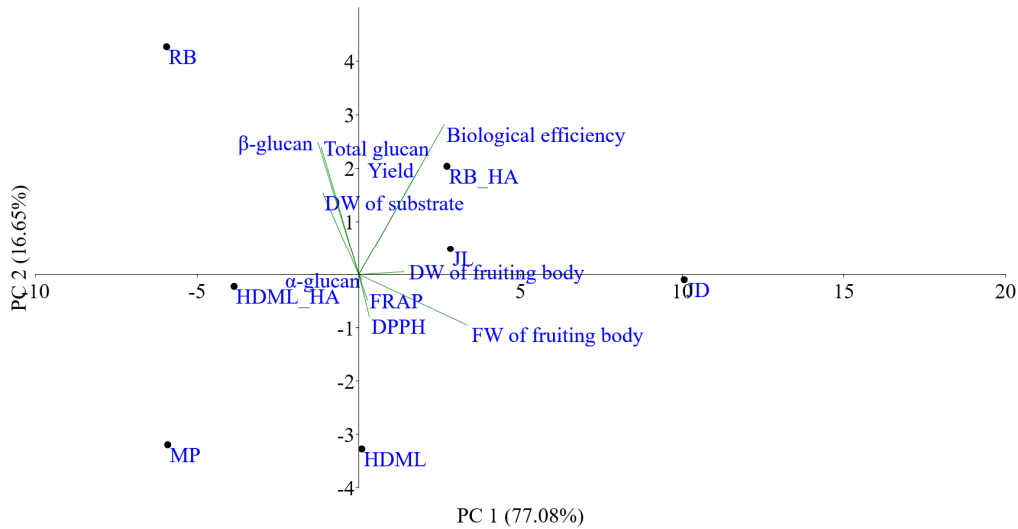


Figure 5. The relationships between phytochemical properties and different rice media

Pearson’s correlation analysis revealed relationships between phytochemical properties and antioxidant activity of mushrooms (Table 3). Yield positively correlated with biological efficiency, with a correlation coefficient of 1.000 ($p \leq 0.05$), while β -glucan was negatively correlated with FRAP, with a correlation coefficient of -0.958 ($p \leq 0.05$).

Table 3. Correlation coefficients among phytochemical properties of common split gill mushroom from different rice media

Correlated traits	DW of fruiting body	Yield	DW of substrate	BE	Total glucan	α -glucan	β -glucan	DPPH	FRAP
FW of fruiting body	0.986*	0.795	-0.858	0.795	-0.762	0.028	-0.670	0.669	0.715
DW of fruiting body		0.882	-0.773	0.882	-0.675	0.100	-0.627	0.589	0.671
Yield			-0.457	1.000*	-0.344	0.156	-0.365	0.230	0.371
DW of substrate				-0.457	0.918	0.197	0.707	-0.684	-0.670
BE					-0.344	0.156	-0.365	0.230	0.371
Total glucan						-0.081	0.900	-0.848	-0.844
α -glucan							-0.508	0.301	0.521
β -glucan								-0.865	-0.958*
DPPH									0.939

DW – dry weight; FW – fresh weight; BE – biological efficiency

* = significantly different at $p \leq 0.05$ level

A correlation between glucan content and antioxidant capacity was observed. The β -glucan content exhibited a strong correlation with two standard antioxidant assays, namely DPPH and FRAP reagents, with correlation coefficients of 0.865 and 0.958, respectively, as reported by Brindzová *et al.* (2008). Furthermore, the total glucan content exhibited a higher correlation with β -glucan content than with α -glucan content. This is because the β -glucan content was determined by subtracting the α -glucan content from the total glucan content (Ćirić *et al.*, 2020). Notably, β -glucan can be extracted from various fungi and has a positive impact on immunomodulation for both human and animal health (Menkovska and Ibrahim, 2022).

Overall, these findings highlight the importance of rice media selection in mushroom cultivation for optimizing yield, improving biological efficiency, and enhancing nutritional and medicinal properties. By manipulating the composition of the media, mushroom cultivators can customize the growth conditions to meet specific objectives, such as increasing yield, enhancing glucan content, or boosting antioxidant activity.

Moreover, the results contribute to our understanding of the relationship between cultivation conditions and the biochemical composition of mushrooms, providing valuable insights for the cultivation industry and researchers in the field of mushroom cultivation and functional food production. This research provides a foundation for future studies aimed at improving cultivation practices and developing functional foods with enhanced health benefits.

Conclusions

The choice of rice medium significantly shapes the cultivation outcomes of common split gill mushrooms, influencing critical factors such as yield, biological efficiency, glucan content, and antioxidant activity. Notably, *Oryza sativa* cv. 'Jao-Daeng' media emerged as remarkably effective, demonstrating superior performance across multiple parameters. These findings underscore the substantial potential for optimizing mushroom production and enhancing their nutritional and medicinal properties through the strategic selection of cultivation media. The observed correlations between glucan content and antioxidant capacity yield valuable insights into the sophisticated dynamics of mushroom development. Furthermore, when considering all parameters, especially the comparison of total glucan content to the dry weight obtained from fruiting bodies of mushrooms cultivated in different rice media, it was observed that the fruiting bodies cultivated from media containing *Oryza sativa* cv. 'Jao-Daeng' exhibited the highest total glucan content when compared to dry weight. Therefore, it is suggested that the rice media from *Oryza sativa* cv. 'Jao-Daeng' is optimal for mushroom cultivation.

This study collectively highlights the paramount importance of rice media selection in achieving optimal mushroom cultivation, leading to heightened yield, enhanced biological efficiency, and enriched nutritional and medicinal qualities. The outcomes contribute significantly to the body of knowledge in mushroom cultivation and functional food production, establishing a groundwork for further exploration into tailoring growth conditions to specific objectives. The implications of these results extend to the development of sustainable cultivation practices, positioning mushrooms as valuable functional food ingredients. Subsequent research in this domain is poised to deepen our understanding and propel the field of mushroom cultivation towards enhanced productivity and nutritional excellence.

Authors' Contributions

Conceptualization: HC, AA, SH, PP; Data curation: HC, AA, SH, PP; Formal analysis: HC, AA, PP; Funding acquisition: HC; Investigation: HC, SH, PP; Methodology: HC, SB, PP; Project administration: HC, PP; Resources: SB; Writing - original draft: HC, PP; Writing - review and editing: PP, HC. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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