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Locally Available Beans as an Alternative Low-Cost Nutrient Source for Yanagi (*Agrocybe aegerita*) Culture

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Locally Available Beans as an Alternative Low-Cost Nutrient Source for Yanagi (*Agrocybe aegerita*) Mushroom Culture in the Philippines

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Abstract

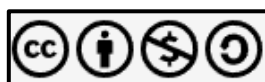
Mushroom farming provides economic opportunities, but in the Philippines, the high cost of commercial culture media hinders its development. This study aimed to determine whether locally available beans could serve as a cheaper nutrient source for cultivating *Agrocybe aegerita* in Tabuk City. In this study, researchers measured the mycelial diameter of *A. aegerita* on five media: Water Agar (T⁻), Potato Dextrose Agar (T⁺), and black bean (T₁), mung bean (T₂), and white bean (T₃) agar. A One-Way ANOVA followed by Tukey's HSD test was applied at the 5% significance level. The species was identified on the basis of its appearance, and the media were prepared by boiling the beans to obtain a broth. The results indicated that PDA (T⁺) gave the greatest mycelial diameter (49.63 ± 13.28 mm), followed by black bean (31.87 ± 0.517 mm), mung bean (29.09 ± 0.522 mm), and white bean (27.19 ± 0.522 mm) agars. All the legume-based media outperformed the negative control. Higher humidity also slightly inhibited mycelial growth. Although PDA remained the most effective, agars made from black, mung, and white beans are viable alternatives. The study suggests that further research should be carried out to improve methods of media preparation and to promote affordable mushroom production in rural and low-resource areas.

Keywords: *Agrocybe aegerita*, culture media, edible mushroom, legume-based media, low-cost

Introduction

Because of its nutritional, medicinal, and economic value, mushroom farming is attracting increasing attention (Thakur, 2020). In the Philippines, the mushroom industry is expanding but remains limited, which in turn hinders broader use and market development (Ballesteros et al., 2021). At present, local production accounts for only about 5% of the possible mushroom yield (Chang et al., 2014). The cultivation of mushrooms is highly dependent on temperature, humidity, and light, and the process involves a great deal of time (Rukhiran et al., 2023). Yanagi (*A. aegerita*), also called the Pioppino mushroom, is a saprotrophic basidiomycete known for having funnel-shaped fruiting bodies (Gupta et al., 2018). As the cap matures, it changes from a silky light ochre to a darker chestnut brown, and the gills go from pale cream to light brown as the spores develop.

In the warm-temperate forests of Europe and Asia, *A. aegerita* is found on decaying hardwoods, particularly on poplar and willow, and functions as a white-rot fungus by breaking down lignin and cellulose. Because of recent advances in cultivation techniques, the fungus is now grown commercially in Europe, North America, Asia, Australia, and Chile (Gupta et al., 2018).



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The fruiting body of *A. aegerita* has a dietary fiber content of about 25 to 30% on a dry weight basis and contains little fat. It is also a good source of minerals including potassium, phosphorus, iron, and zinc. The methanolic extract of this mushroom shows strong antioxidant activity, and its polysaccharides have immunomodulatory and anti-aging effects (Falandysz et al., 2024). Most of the research into mushroom mycelial growth has been directed towards improving the nutrient sources and the culture media. Potato dextrose agar (PDA) is commonly used and promotes the growth of a number of mushroom species (Dulay et al., 2021a; Dulay et al., 2021b), although alternative substrates can result in different growth rates and yields (Kalaw et al., 2021; Dulay & Damaso Jr., 2020). The high price of commercial media such as PDA is, however, a problem, particularly for small-scale growers in rural areas (Santos et al., 2021; Gamit et al., 2023).

For this reason, there is increasing interest in using locally available and inexpensive nutrient sources such as mung beans, white beans, and black beans. These legumes are common in rural areas like Kalinga in the Philippines and are rich in proteins, amino acids, and other nutrients that are beneficial for mycelial growth (Syamsia et al., 2021). The present study compares bean-based substrates with PDA with the aim of identifying a cheap, local nutrient medium which can reduce spawn production costs in Kalinga.

Materials and Methods

A Completely Randomized Design (CRD) was employed in this study to examine the mycelial growth of *A. aegerita* on various legume-based media. The five treatments included Water Agar (T-), Potato Dextrose Agar (T+), and three legume-based agars—those made from black beans (T₁), mung beans (T₂), and white beans (T₃). For each treatment, there were 10 replicates (n = 10 plates per treatment).

The black beans (*Phaseolus vulgaris*), mung beans (*Vigna radiata*), and white beans (*Phaseolus lunatus*) were purchased at the Bulanao Public Market in Tabuk City. They were sorted, rinsed, and then 100 g of each type was boiled in 1000 mL of distilled water for 60 minutes (Al-Asmari et al., 2015). The resulting liquid was filtered, made up to 1000 mL, and 26 g of agar powder was added before being dissolved by heating and stirring. The pH was determined and brought to 6.0 using 1 M HCl. The media were sterilized in an autoclave at 121 °C and 15 psi for 20 minutes, then cooled and their pH checked before being poured into sterile Petri plates within a biosafety cabinet.

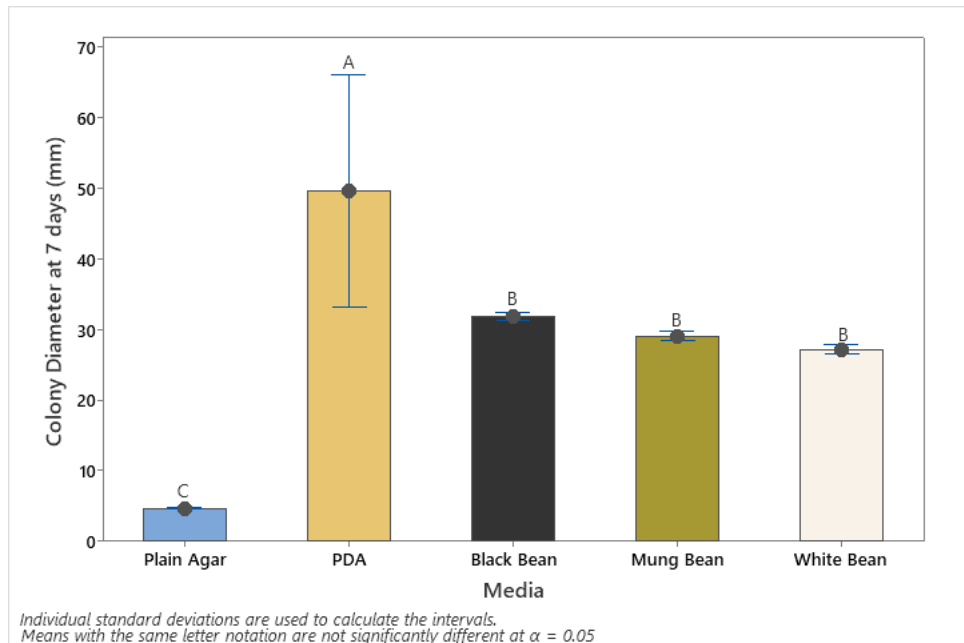
The plates were allowed to solidify before inoculation. The grain spawn of *A. aegerita* was supplied by “Mycosphere®, Muñoz, Nueva Ecija”. A single grain was placed in the center of each plate. The plates were randomly positioned on the shelves of the incubator in order to prevent any positional effects. The temperature and humidity were recorded with a digital hygrometer and thermometer. Each day, the perpendicular diameters were recorded to measure mycelial growth over a period of 7 days. The data were analyzed using one-way ANOVA and Tukey’s HSD test ($\alpha = 0.05$) in Microsoft Excel (v2018). A regression analysis was carried out to investigate the effects of media type, temperature, and humidity on mycelial growth.

Results and Discussion

Colony Diameter of Yanagi (*A. aegerita*) on Different Legume-based Culture Media

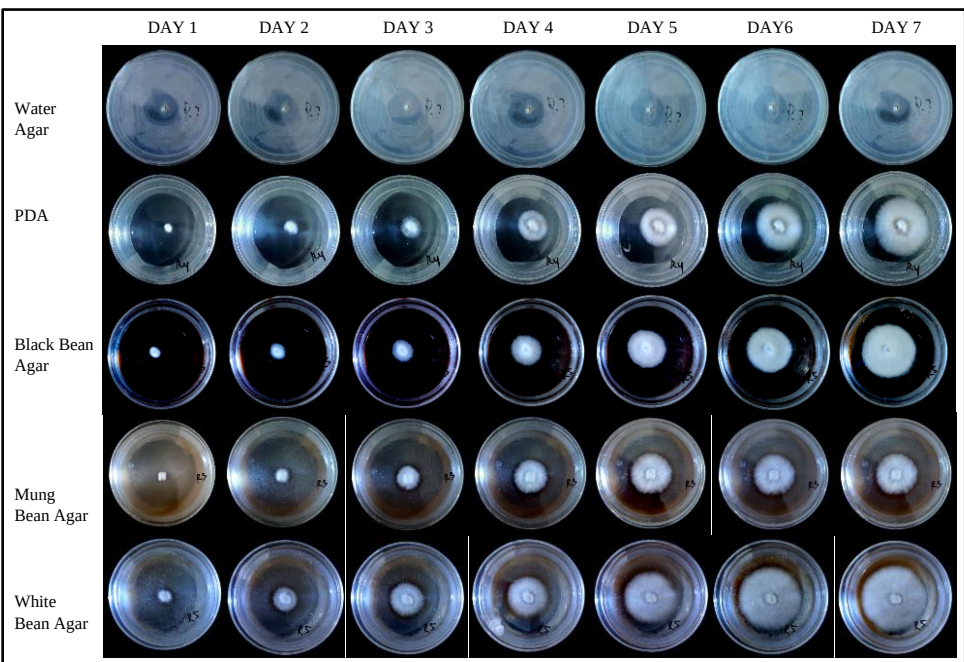
There was a significant difference in the diameter of the *A. aegerita* colonies among the various media (see Figure 1). Growth on Water Agar was the smallest (4.72 mm), whereas that on PDA was the greatest (49.63 ± 13.28 mm). The bean-based media also exhibited good growth: black bean (31.87 ± 0.517 mm), mung bean (29.09 ± 0.522 mm), and white bean (27.19 ± 0.522 mm).

Figure 1
Colony Diameter of Yanagi (A. aegerita) on Different Culture Media



Statistical analysis proved that the type of nutrient had a significant effect on mycelial growth ($p < 0.000$). The Tukey’s HSD test indicated that legume-based media promoted mycelial growth, although PDA still produced the highest values (see Figure 2). Legume-based media supply fungi with important nutrients such as vitamins, minerals and organic compounds, all of which aid mycelial growth (Bhatia et al., 2015). The proteins, carbohydrates, and amino acids contained in legumes provide a favorable environment for fungal growth.

Figure 2
A. aegerita Mycelial Cultures in Legume-Based Agar, Water Agar and Potato Dextrose Agar (PDA)



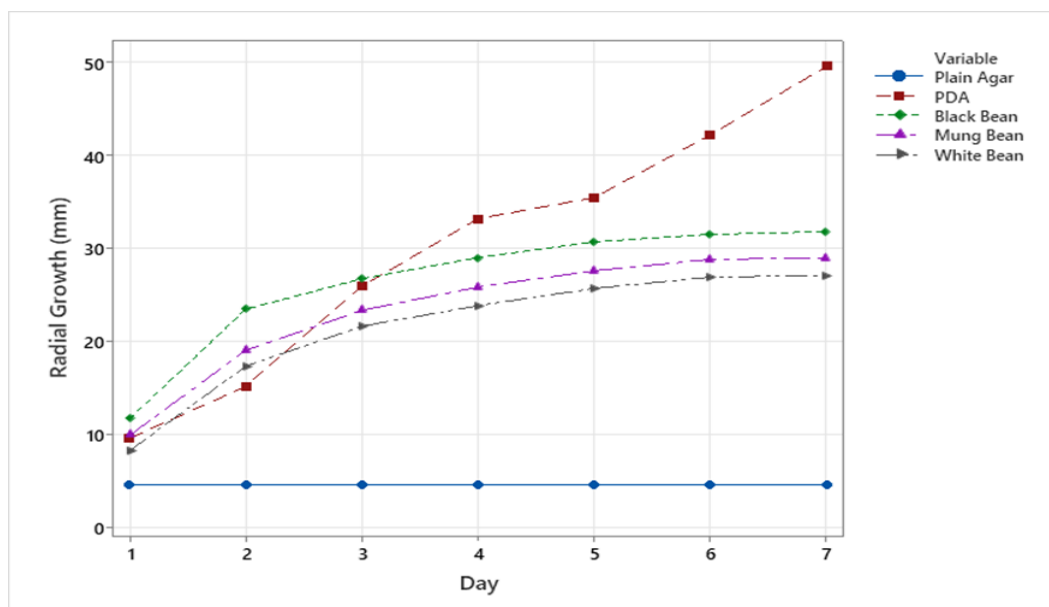
In addition to carbon and nitrogen, growth-promoting hormones that influence fungal growth may be supplied by media based on legumes. Cytokinins and various other substances are found in black beans, mung beans, and white beans (Hammerton et al., 1996; Fujimoto et al., 1998). The beans also contribute to stress tolerance via jasmonic acid and abscisic acid (Adolfsson et al., 2017). Whether or not these hormones function as growth regulators in mushroom culture media will depend on their concentrations.

Daily Average Radial Growth of Yanagi (*A. aegerita*) on Different Media

Over a period of 7 days, the study monitored mycelial growth. From Day 1 to Day 3, the Black Bean Agar had the greatest diameter of all the legume-based media. The fastest growth occurred between Day 1 and Day 2, after which the rate of growth decreased right through to Day 7. This kind of pattern is usual in fungal cultures since the nutrients are consumed and waste products accumulate.

Figure 3

Daily Growth Trend Of Yanagi (A. aegerita) Mycelial Culture Over a 7-Day Incubation Under Various Legume-Based Media



The reduced growth rate in the legume-based media could be because there are fewer nutrients available or because waste products have built up. This is normal in fungal growth, as it typically involves a lag phase followed by rapid growth and then a stationary phase (Jain et al., 2017). Although legumes supply essential minerals and protein, the characteristics of these legumes differ according to species (Kebede, 2021; Semba et al., 2021; Wani et al., 2016). Zhang and Elser (2017) found that fungi grow best under carbon-rich conditions, with an ideal C: N ratio of about 15–20:1. Unless the substrate is altered, these features may restrict growth. The mean radial growth of *A. aegerita* on PDA (49.63 ± 13.28 mm after 7 days) is in agreement with findings in other studies. Muthu & Shanmugasundaram (2015) reported approximately 80 mm on PDA and 88 mm on Malt Extract Agar after 15 days. *Agrocybe cylindracea* reached about 84 mm on PDA after 10 days at 25 °C and pH 6 (Alam et al., 2010). *A. cylindracea* grown on PDA and Potato Dextrose Broth produced robust mycelia and proteins that are important for growth (Pothiratana et al., 2020).

These results indicate that PDA is suitable for *Agrocybe* species. As no previous studies have examined *A. aegerita* on legume-based agar, this research is the first to demonstrate that black bean,

mung bean, and white bean media can support good mycelial growth, even if at a slower rate than that on PDA. Other studies have also found good growth in fungi such as *Trichoderma harzianum*, *Trichophyton rubrum*, and *Candida albicans* on legume-based media (Platon et al., 2025; Widyanur et al., 2023; Condrillon et al., 2024). Because of limited resources, this study did not carry out a detailed analysis of the individual properties of each legume substrate. Future research should include biochemical profiling in order to better understand how legumes affect fungal growth.

Effects of Humidity and Temperature on Mycelial Growth of Yanagi (*A. aegerita*)

The impact of humidity and temperature was found to be important for the growth of the mycelium on media based on legumes. For a period of more than seven days, both temperature and humidity were recorded, the average values being $29 \pm 0.8^{\circ}\text{C}$ and $68.3 \pm 2.2\%$ RH (see Table 1).

Table 1
*Temperature and Humidity of the External Environment During the 7 Days Growing Period of Yanagi (*A. aegerita*) Mycelial Cultures*

	Day after inoculation							Mean (n=7)
	1	2	3	4	5	6	7	
Temperature ($^{\circ}\text{C}$)	30.9	29	28.6	28.4	28.5	29	28.6	29 ± 0.80
Humidity (%)	64	66	70	69	70	69	70	68.29 ± 2.18

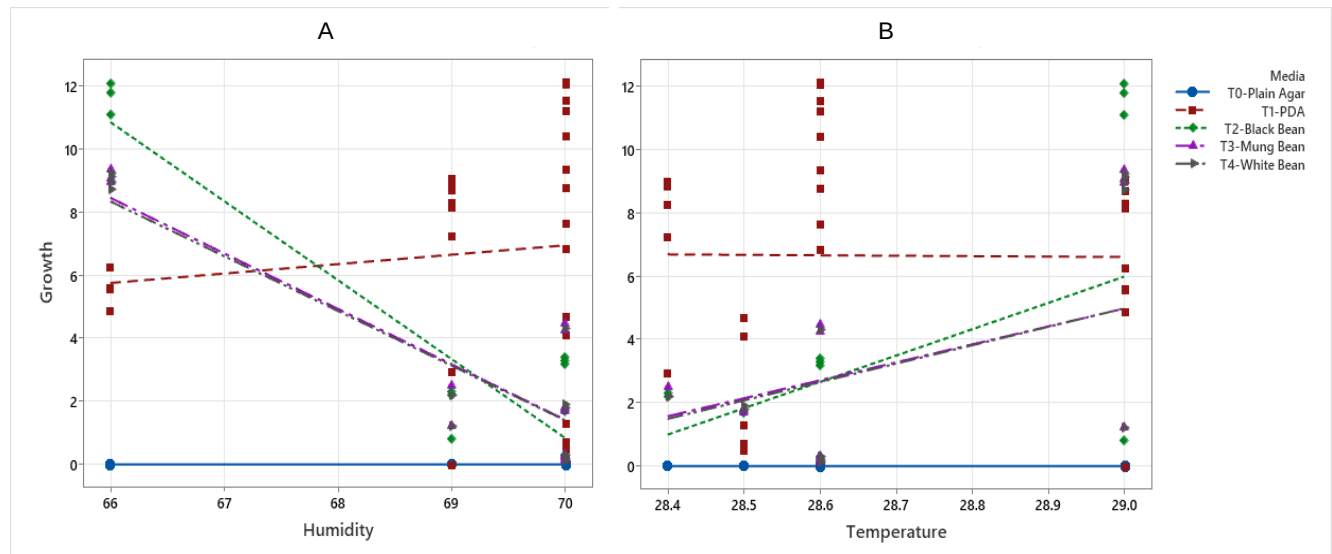
The impact of humidity and temperature was found to be important for the growth of the mycelium on media based on legumes. For a period of more than seven days, both temperature and humidity were recorded, the average values being $29 \pm 0.8^{\circ}\text{C}$ and $68.3 \pm 2.2\%$ RH (see Table 1). *A. aegerita* grows most efficiently at a temperature between 28 and 30°C and under conditions of high humidity (Miles & Chang, 2004), although other studies have indicated that good growth can also be achieved at temperatures between 24 and 28°C (Zhan et al., 2021). The temperatures that were favorable in this study probably corresponded to the mushroom's natural habitat (Philippoussis et al., 2001; Phonemany et al., 2025).

The humidity level observed (ranging from 64 to 70%) was suitable for mycelial growth, spore germination, and colonization of the substrate (Bellettini et al., 2019; Zak & Wildman, 2004). Interestingly, lower humidity within this range was associated with greater mycelial growth. These figures are lower than the optimal 80–90% required by many fungi, including *A. aegerita* (Islam et al., 2016). Voutetaki & Mpalaskas (2024) pointed out that maintaining the correct level of humidity prevents the medium from drying out and promotes growth. Low humidity can reduce moisture, affect enzymes, and limit the availability of nutrients (Ene et al., 2015). This indicates that *A. aegerita* might prefer moderate humidity, or that the combination of substrate and moisture influences its growth (Talley et al., 2002). Higher humidity can also lead to an increase in volatile organic compounds, which may inhibit mycelial growth (Lyu et al., 2020).

These factors may account for the negative relationship between humidity and mycelial growth observed in this study. Keeping the humidity slightly lower could improve aeration, stability, and activity, thus resulting in better and faster colonization on legume-based media. The connection between humidity, temperature, and mycelial growth is illustrated in Figure 4 and Figure 5.

Figure 4

Relationship of (A) Humidity and (B) Temperature on Mycelial Growth of Yanagi (A. aegerita)

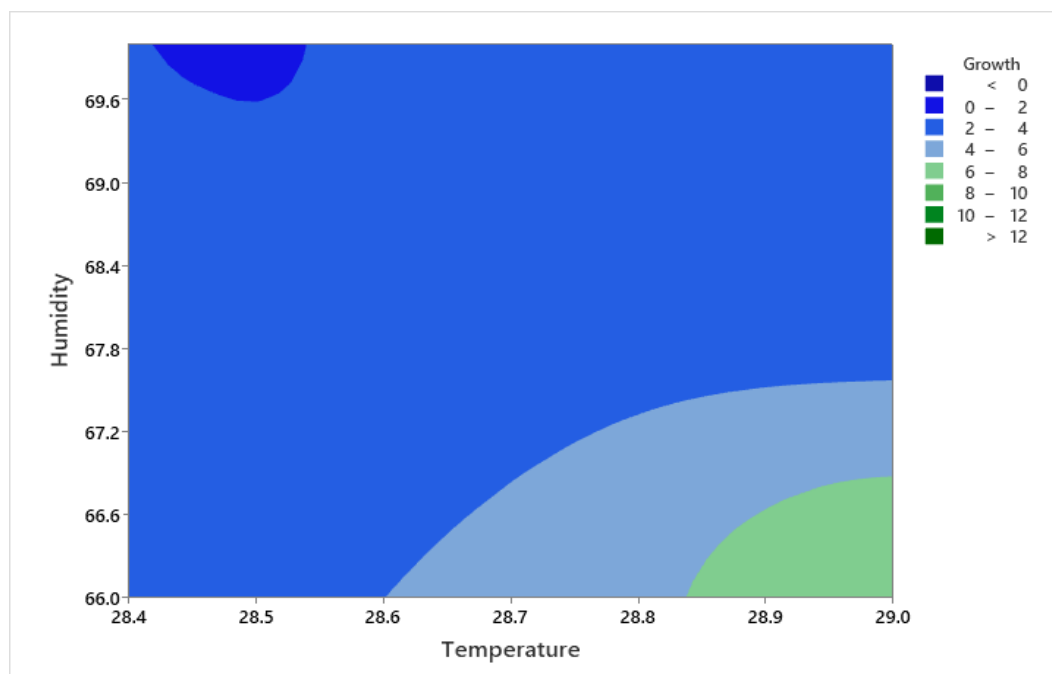


Contour map of the mycelial growth of fungi as affected by the interaction of humidity and temperature is visualized Figure 5.

Regression analysis indicated that although higher temperatures appeared to promote growth, there was no significant correlation, so temperature was not included in the final model. The analysis revealed that for each one-unit increase in humidity, the growth rate decreased by 1.141 mm/day ($R^2 = 52.35\%$). The regression equations for each type of medium are given below.

Figure 5

Contour Plot of Mycelial Growth affected by Humidity and Temperature



Regression Equation

Growth in Water Agar	=	78.8 - 1.141 Humidity
Growth in PDA	=	85.4 - 1.141 Humidity
Growth in Black Bean Media	=	82.1 - 1.141 Humidity
Growth in Mung Bean Media	=	81.9 - 1.141 Humidity
Growth in White Bean Media	=	81.9 - 1.141 Humidity

This study shows that PDA remains the best medium for *A. aegerita*, although media based on legumes are also suitable alternatives. Environmental conditions, particularly humidity, have a great influence on mycelial growth, since higher humidity is associated with slower growth. A small decrease in humidity can have an effect on the fungus's growth.

Conclusion

In conclusion, the study found that *A. aegerita* grows well on media based on legumes, with black bean agar producing the most favorable results of all the options. Although PDA resulted in the highest growth, legume-based media—particularly black, white, and mung bean agar—also performed well. The fact that growth is slower on legume-based media does not diminish their value since these media are cheaper and easier to obtain than PDA. It therefore follows that legume-based media can serve as practical and affordable alternatives to commercial media and thus support local mushroom production in rural areas.

Recommendations

It is recommended that further research be carried out in order to standardize legume-based media by improving the methods of preparation, the concentration, and the pH. It has also been suggested that longer trials and tests should be conducted regarding fruiting performance in order to confirm whether legume-based media are appropriate for both spawn and fruiting-body production. Furthermore, including other local legumes and agricultural by-products in future studies could help to promote sustainable and low-cost mushroom farming in rural areas such as Kalinga.

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