Comparative analysis of mycelial growth of yanagi matsutake mushroom (agrocybe aegerita, v. Brig.) Across various indigenous culture media

Cesar G. Della[1], Shielmyn Kate Junio-Manuel[2]

ABSTRACT

Media plays a critical role in the cultivation of mushrooms, serving as a method for storing viable cell lines of microorganisms. These cell lines can lead to significant advancements for the benefit of humanity. This study aimed to identify the optimal indigenous substrates and inoculum sizes that would promote faster mycelial growth in the preparation of culture media for the Yanagi Matsutake mushroom. The study assessed the impact of different indigenous culture media and inoculation sizes on the mycelial growth of Yanagi Matsutake, focusing on the number of days required for complete mycelial ramification.The research employed a laboratory experimentation approach using a Factorial in Randomized Complete Block Design (RCBD) to investigate the effects of culture media and inoculum size on the mycelial growth of Agrocybe aegerita. The data collected was analyzed using a repeated measures analysis of variance (ANOVA) to determine the effect of the different culture media and inoculation size in mycelial growth. It also compared these effects to determine the most cost-effective culture media and inoculum size using Return on Expenditure as a measurement tool. Additionally, the study sought to develop a learning module for cultivating the Yanagi Matsutake mushroom using various culture media and inoculation sizes. The findings indicate that the combination of specific indigenous culture media and inoculum sizes significantly affects the rate of mycelial growth and ramification of Yanagi Matsutake. Larger inoculum sizes (15-20 mm) generally resulted in faster mycelial growth and fewer days to full ramification, particularly with PDA and PSA media. The CGSA medium also showed a notable advantage for larger inoculum sizes (10 mm) compared to the smallest size of 5 mm. In conclusion, the choice of culture medium and inoculum size is essential for optimizing the mycelial growth of Yanagi Matsutake. This study provides valuable insights into the factors that influence the mycelial growth of Yanagi, emphasizing the importance of specific indigenous culture media and inoculum sizes in achieving optimal growth rates.

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Keywords: Yanagi Matsutake, mycelial growth, indigenous culture media, inoculum size, mycelial ramification, Potato Dextrose Agar (PDA), Rice Bran Sugar Agar (RBSA), Coconut Water Agar (CWA), Corn Grit Decoction Sugar Agar (CGDSA), Potato Dextrose Sugar Agar (PDSA), mushroom cultivation, sustainable agriculture.

Introduction

Mushroom cultivation, particularly of Yanagi Matsutake (Agrocybe aegerita), offers nutritional, health, and economic benefits, supporting sustainable livelihoods with minimal resources (Boa, 2004; Stamets, 2000). This prized edible mushroom is valued in Japan for its high market value (Pierson, 1958; Tippayawong & Chaichana, 2011). Optimizing Yanagi Matsutake cultivation requires suitable culture media and inoculum size.

Media composition plays a critical role in mushroom cultivation, influencing mycelial growth and spawn quality (Huerta et al., 2016). With the increasing costs of traditional media, exploring indigenous substrates could lead to cost-effective and sustainable production that aligns with Sustainable Development Goals (SDGs 1, 2, 3, and 12). Researching local materials for mushroom culture media preparation particularly for Yanagi mushroom, could lead to cost-effective methods.

This study focuses on identifying cost-effective methods using indigenous media and inoculum sizes, aligning with SDGs, addressing Goals 1: No Poverty, 2: Zero Hunger, 3: Good Health and Well-being, and 12: Responsible Consumption and Production. Moreover, this study examines and assesses various indigenous culture media, such as rice bran, corn grit, potato, and coconut water, along with different inoculum sizes (5 mm^2, 10 mm^2, 15 mm^2, and 20 mm^2), in order to optimize the mycelial growth of Yanagi Matsutake. The aim is to support more efficient cultivation practices. There has been limited research comparing indigenous media and inoculum sizes specifically for the mycelial growth of A. aegerita.

Methods

The research employed a laboratory experimentation approach using a Factorial in Randomized Complete Block Design (RCBD) to investigate the effects of culture media and inoculum size on the mycelial growth of Yanagi Matsutake (Agrocybe aegerita). The experiment involved two factors: culture media (with five levels - Potato Sugar Agar (PSA), Coconut Water Agar (CWA), Rice Bran Sugar Agar (RBSA), Corn grit decoction sugar agar (CGDSA), and Potato Dextrose Agar (PDA)) and inoculum size (with four levels - 5 mm^2, 10 mm^2, 15 mm^2, and 20 mm^2). This resulted in 20 treatment combinations, each replicated four times with 10 Petri dishes per experimental unit. The experimental method allowed the researcher to determine the relative effects of the treatments and infer conclusions about the optimal culture media and inoculum size for Yanagi mycelial growth. The inoculated petri dishes were incubated at 32^0C and observed mycelial growth. Further, the radial growth of mycelia were measured everyday. The data collected was analyzed using a repeated measures analysis of variance (ANOVA) to determine the effect of the different culture media and inoculation size in mycelial growth. It also compared these effects to determine the most cost-effective culture media and inoculum size using Return on Expenditure (ROE) as a measurement tool.

RESULTS

Characterizing the effect of the different indigenous culture media and inoculation size in the mycelial growth of Yanagi Matsutake in terms of the number of days to full mycelial ramification.

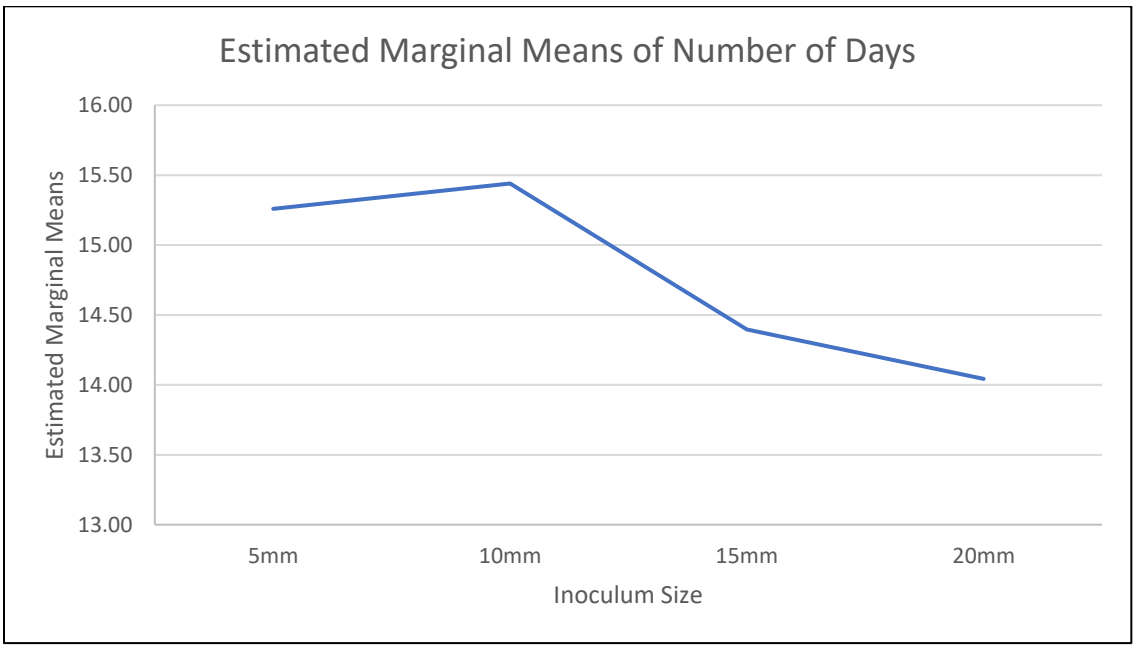
Table 1 Effect of the different indigenous culture media and inoculation size in the mycelial growth of Yanagi Matsutake in terms of the number of days to full mycelial ramification

Inoculum Size	Culture Media									
	PDA		PSA		CGSA		CWA		RBSA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5 mm^2	15.60	1.58	16.25	1.04	17.44	2.19	**13.50**	2.33	**13.50**	3.98
10 mm^2	16.43	0.98	17.00	1.07	**13.57**	1.51	16.20	3.29	14.00	0.00
15 mm^2	**12.22**	1.92	15.67	2.94	14.25	3.54	14.44	1.33	15.40	2.72
20 mm^2	12.40	1.35	**11.71**	2.14	14.50	2.98	15.60	3.41	16.00	2.67

The data provided shows that mycelial growth of Yanagi Matsutake in terms of the number of days to full mycelial ramification varies across different culture media mycelial growth of Yanagi Matsutake in terms of the number of days to full mycelial ramification.

PDA, known for supporting high mycelial growth rates, exhibited the fastest growth with a 15 mm^2 inoculum size. PSA showed a decrease in the number of days to full ramification with a 20 mm^2 inoculum size, suggesting its effectiveness for rapid growth when larger inocula are used. CGSA demonstrated optimal growth with a 10 mm^2 inoculum size, indicating its suitability for moderately sized inocula. Conversely, CWA and RBSA showed the best performance with a 5mm inoculum size, aligning with findings that smaller inocula can be effective in specific media.

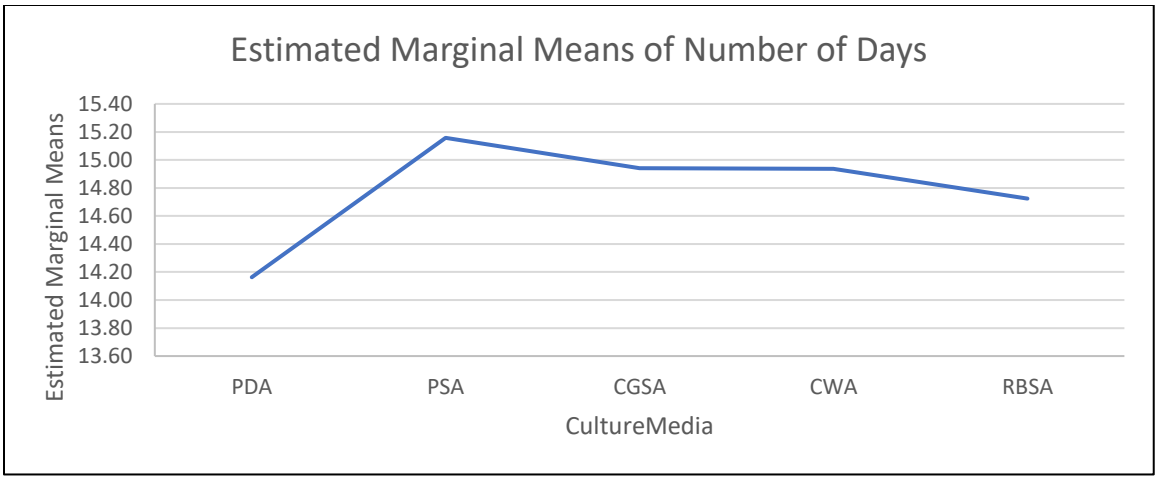
Comparing the effect of the different indigenous culture media and inoculation size on the mycelial growth of Yanagi Matsutake in terms of the number of days to full mycelial ramification.
Comparison of the effect of the different indigenous culture media on the mycelial growth of yanagi mushroom.



Legend: **PDA:** Potato Dextrose Agar **PSA:** Potato Sugar Agar
CGSA: Corn Grit Sugar Agar **CWA:** Coconut Water Agar **RBSA:** Rice Bran Sugar Agar

Figure 1. Estimated Marginal Means on the Number of Days to Full Mycelial Ramification Based on the Different Inoculum Sizes

Based on Figure 2, which provided estimated marginal means for the number of days to full mycelial ramification of Yanagi matsutake in different inoculation sizes, the estimated mean number of days for full mycelial ramification with a 5 mm^2 inoculation size is 15.2 mm^2, 10 mm^2 inoculation size is 15.44 mm^2, 15 mm^2 inoculation size is 14.40 mm^2 and 20 mm^2 inoculation size is 14.04 mm^2.
The estimated marginal means reveal a noticeable impact of the inoculation size on the mycelial ramification time. The observed differences in mycelial ramification time across the inoculation sizes suggest that the size of the inoculation significantly influences the speed of mycelial ramification. Specifically, the decreasing mean number of days with increasing inoculation size (from 15.44 for 10 mm^2 to 14.04 for 20 mm^2) indicates a trend towards faster mycelial ramification with larger inoculation sizes.



Legend: **PDA:** Potato Dextrose Agar **PSA:** Potato Sugar Agar
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Figure 2. Estimated Marginal Means based on the Number of Days to Full Mycelial Ramification Based on the Different Indigenous Culture Media

The estimated marginal means provide valuable insights into the average number of days required for full mycelial ramification across the different indigenous culture media tested. The data allows for a comparative analysis of the effectiveness of these media in promoting mycelial growth and ramification of Yanagi Matsutake. PDA has 14.16, PSA with 15.16, CGSA 14.94, CWA 14.94, and RBSA with 14.73. The data allows for a comparative analysis of the effectiveness of the different culture media in promoting mycelial ramification.
While the estimated marginal means vary slightly, ranging from 14.16 days for Potato Dextrose Agar (PDA) to 15.16 days for Potato Sugar Agar (PSA), these differences are not statistically significant based on the analysis. The estimated marginal means for Corn Grit Sugar Agar (CGSA), Corn Wheat Agar (CWA), and Rice Bran Sugar Agar (RBSA) fall within a narrow range of 14.73 to 14.94 days, further indicating the lack of a substantial impact of culture media on mycelial growth rate.

Comparing the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake mushroom

Table 3. Comparison of the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake

Factor	Level	Mean	SD	F	Sig	Partial Eta Squared
Inoculum Size	5 mm^2	15.26	2.85	3.215	0.025	0.06
	10 mm^2	15.44	2.27			
	15 mm^2	14.40	2.72			
	20 mm^2	14.04	3.02			

Legend: Mean: Average number of days to full mycelial ramification SD: Standard deviation
F: F-value indicating the significance of the factor
Sig: p-value indicating the significance level of the factor
Partial Eta Squared: Measure of effect size indicating the proportion of variance

The table above describes the comparison of the mean number of days to full mycelial ramification for each inoculum size. The table indicates the average effect of different inoculum sizes on the number of days to full mycelial ramification. The means and standard deviations for each inoculum size provide insights into the average mycelial growth rates and the variability of the data.

Comparing the Interaction Effect of the different indigenous culture media and inoculation size in the mycelial growth of Yanagi Matsutake in terms of number of days to full mycelial ramification

In Table 4, this table presents the mean mycelial growth rates for each combination of Culture Media and Inoculum Size. The mean and standard deviation values provide insights into the average mycelial growth rates and the variability of the data for each combination.

Table 4. Comparison of the Interaction Effect of the different indigenous culture media and inoculation size in the mycelial growth of Yanagi Matsutake in terms of number of days to full mycelial ramification

Factor	Culture Media	Inoculum Size	Mean	SD	F	Sig	Partial Eta Squared
Culture Media * Inoculum Size	PDA	5 mm^2	15.60	1.58	4.831	<0.001	0.277
		10 mm^2	16.43	0.98			
		15 mm^2	12.22	1.92			
		20 mm^2	12.40	1.35			
	PSA	5 mm^2	16.25	1.04			
		10 mm^2	17.00	1.07			
		15 mm^2	15.67	2.94			
		20 mm^2	11.71	2.14			
	CGSA	5 mm^2	17.44	2.19			
		10 mm^2	13.57	1.51			
		15 mm^2	14.25	3.54			
		20 mm^2	14.50	2.98			
	CWA	5 mm^2	13.50	2.33			
		10 mm^2	16.20	3.29			
		15 mm^2	14.44	1.33			
		20 mm^2	15.60	3.41			
	RBSA	5 mm^2	13.50	3.98			
		10 mm^2	14.00	0.00			
		15 mm^2	15.40	2.72			
		20 mm^2	16.00	2.67			

Legend: **PDA:** Potato Dextrose Agar **PSA:** Potato Sugar Agar
CGSA: Corn Grit Sugar Agar **CWA:** Coconut Water Agar **RBSA:** Rice Bran Sugar Agar

Based on the table, the fastest mycelial growth was observed in the PDA medium with a 15mm^2 or 20mm^2 inoculum size, taking only 12.22 and 12.40 days respectively to reach full ramification. This suggests that PDA is a suitable medium for promoting rapid mycelial growth of Yanagi Matsutake. The slowest mycelial growth was seen in the CGSA medium with a 5mm^2 inoculum size, taking 17.44 days on average to reach full ramification. This indicates that CGSA may not be an optimal medium for Yanagi Matsutake mycelial growth. Increasing the inoculum size generally decreased the number of days to full ramification, regardless of the culture medium used. This suggests that using a larger inoculum (e.g. 15-20 mm^2) can accelerate the mycelial growth of Yanagi Matsutake.

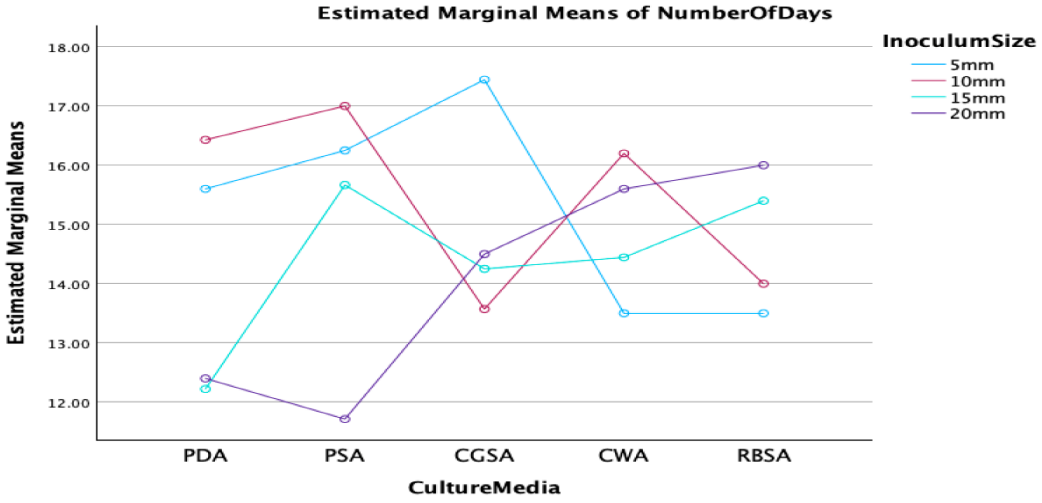
Pairwise Comparison of the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake

Table 5. Pairwise Comparison of the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake

Group I	Group J	Mean Difference (I-J)	Sig
5 mm^2	10 mm^2	-0.294	0.945
	15 mm^2	0.9111	0.302
	20 mm^2	1.0667	0.163
10 mm^2	15 mm^2	1.2051	0.119

	20 mm^2	1.3607	0.055
15 mm^2	20 mm^2	0.1556	0.991

The mean difference between the mycelial growth of Yanagi Matsutake at 5mm^2 and 20mm^2 inoculum sizes is 1.0667, indicating a significant increase in mycelial growth at 20mm^2 compared to 5mm. The associated significance level of 0.163 suggests that this difference is approaching statistical significance. Additionally, the mean difference between the mycelial growth of Yanagi Matsutake at 10mm^2 and 20mm^2 inoculum sizes is 1.3607, indicating a significant increase in mycelial growth at 20 mm^2 compared to 10mm. The associated significance level of 0.055 suggests that this difference is approaching statistical significance. All the other mean difference between the mycelial growth of Yanagi Matsutake suggests that the difference is not statistically significant.



Legend: **PDA:** Potato Dextrose Agar **PSA:** Potato Sugar Agar
CGSA: Corn Grit Sugar Agar **CWA:** Coconut Water Agar **RBSA:** Rice Bran Sugar Agar

Figure 6. Estimated Marginal Means on the Number of Days to Full Mycelial Ramification Based on the Different Indigenous Culture Media and Inoculum Sizes

PDA exhibited the fastest mycelial growth when paired with a 15 mm^2 inoculation size.PSA demonstrated the fastest mycelial growth when combined with a 20 mm^2 inoculation size. CGSA resulted in the fastest mycelial growth when used with a 10 mm^2 inoculation size. CWA and RBSA showed the fastest mycelial growth when paired with a 5 mm^2 inoculation size.

These findings suggest that the combination of specific indigenous culture media with particular inoculum sizes can significantly influence the rate of mycelial growth of Yanagi Matsutake. The study by Sharma and Pandey observed that different culture media significantly influenced the vegetative growth, colony morphology, and sporulation of various fungi. This supports the idea that the choice of culture medium, along with inoculum size, plays a crucial role in determining the growth rate and characteristics of fungal mycelium.

The provided data in Table 6 presents the pairwise comparison of the interaction effect of different indigenous culture media and inoculation sizes in the mycelial growth of Yanagi Matsutake in terms of the number of days to full mycelial ramification.

For PDA medium, the 5 mm^2 inoculum size had a significantly longer time to full mycelial ramification compared to the 15mm^2 (mean difference = 3.378, p=0.017) and 20mm^2 (mean difference = 3.200, p=0.022) inoculum sizes. The 10mm inoculum size also had a significantly longer time to full ramification compared to the 15mm^2 (mean difference = 4.206, p=0.005) and 20mm^2 (mean difference = 4.029, p=0.006) inoculum sizes.

For PSA medium, the 5mm inoculum size had a significantly longer time to full ramification compared to the 20mm^2 inoculum size (mean difference = 4.536, p=0.002). The 10 mm^2 inoculum size also had a significantly longer time to full ramification compared to the 20mm^2 inoculum size (mean difference = 5.286, p<0.001). The 15 mm^2 inoculum size had a significantly longer time to full ramification compared to the 20mm inoculum size (mean difference = 3.952, p=0.024).

Pairwise Comparison of the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake

Table 6 Pairwise Comparison of the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake

Dependent Variable	Group I (Inoculum Size)	Group J (Inoculum Size)	Mean Difference (I-J)	Sig,
PDA	5 mm^2	10mm	-0.829	1.000
		15mm	3.378*	0.017
		20mm	3.200*	0.022
	10 mm^2	15mm	4.206*	0.005
		20mm	4.029*	0.006
	15 mm^2	20mm	-0.178	1.000
PSA	5 mm^2	10mm	-0.750	1.000
		15mm	0.583	1.000
		20mm	4.536*	0.002
	10 mm^2	15mm	1.333	1.000
		20mm	5.286*	<0.001
	15 mm^2	20mm	3.952*	0.024
CGSA	5 mm^2	10mm	3.873*	0.011
		15mm	3.194*	0.045
		20mm	2.944	0.081
	10 mm^2	15mm	-0.679	1.000
		20mm	-0.929	1.000
	15 mm^2	20mm	-0.250	1.000
CWA	5 mm^2	10mm	-2.700	0.122
		15mm	-0.944	1.000
		20mm	-2.100	0.420
	10 mm^2	15mm	1.756	0.704
		20mm	0.600	0.704
	15 mm^2	20mm	-1.156	1.000
RBSA	5 mm^2	10mm	-0.500	1.000
		15mm	-1.900	0.492
		20mm	-2.000	0.136
	10 mm^2	15mm	-1.400	1.000
		20mm	-2.000	0.579
	15 mm^2	20mm	-0.600	1.000

Legend: **PDA:** Potato Dextrose Agar **PSA:** Potato Sugar Agar
CGSA: Corn Grit Sugar Agar **CWA:** Coconut Water Agar **RBSA:** Rice Bran Sugar Agar

For CGSA medium, the 5mm inoculum size had a significantly longer time to full ramification compared to the 10 mm^2 (mean difference = 3.873, p=0.011) and 15 mm^2 (mean difference = 3.194, p=0.045) inoculum sizes.
The other pairwise comparisons within the CWA and RBSA media did not show any statistically significant differences. The results indicate that larger inoculum sizes (15-20 mm^2) generally led to faster mycelial growth and shorter number of days to full ramification, particularly for the PDA and PSA media. The CGSA medium also showed a significant advantage for larger inoculum sizes over the smallest 5mm size. These findings highlight the importance of optimizing both culture medium and inoculum size to maximize the mycelial growth of Yanagi Matsutake.

Determining the most cost-effective culture media and inoculum size using Return on Expenditure (ROE) as a tool

Table 7. Return on Expenditure using the Different Culture Media

Culture Media	Total Expenses	Total Revenue	ROE (In Percentage)
PDA	346	9900	2761.27

PSA	115	9900	8508.70
CGSA	87	9900	11279.31
CWA	85	9900	11547.06
RBSA	87	9900	11279.31

Legend: **PDA:** Potato Dextrose Agar **PSA:** Potato Sugar Agar
CGSA: Corn Grit Sugar Agar **CWA:** Coconut Water Agar **RBSA:** Rice Bran Sugar Agar

Based on the ROE percentages, the culture media CWA and RBSA are considered the most cost-effective, with ROE percentages of 11547.06% and 11279.31% respectively. These culture media demonstrate the highest efficiency in generating revenue relative to the total expenses incurred. The high ROE percentages for CWA and RBSA could be attributed to the use of locally available or inexpensive ingredients, such as potato, coconut water, corn, and rice bran. These indigenous culture media may require fewer resources or have lower production costs compared to commercially available media like PDA.

Table 8. Return on Expenditure Considering Contamination Rate using the Different Culture Media

Culture Media	**Total Expenses**	**Total Revenue**	**Contamination Rate**	**ROE (In Percentage)**
PDA	346	9900	7.50	2546.68
PSA	115	9900	27.50	6141.30
CGSA	87	9900	20.00	9003.45
CWA	85	9900	7.31	10694.49
RBSA	87	9900	9.52	10196.00

Legend: **PDA:** Potato Dextrose Agar **PSA:** Potato Sugar Agar
CGSA: Corn Grit Sugar Agar **CWA:** Coconut Water Agar **RBSA:** Rice Bran Sugar Agar

Based on the provided data and considering the contamination rate, the culture media CWA is considered the most cost-effective, with an ROE (Return on Expenditure) percentage of 10694.49%. This indicates that CWA is the most efficient in generating revenue relative to the total expenses, even when factoring in the contamination rate.

Among the listed culture media, CWA stands out as the best culture media considering the contamination rate and cost-effectiveness. It demonstrates the highest efficiency in generating revenue relative to the total expenses, even with a relatively low contamination rate.

Analysis/Discussion

Characterizing the effect of the different indigenous culture media and inoculation size in the mycelial growth of Yanagi Matsutake in terms of the number of days to full mycelial ramification

In Table 1, the data suggests a critical role of inoculum size in influencing mycelial growth. Smaller sizes (5 mm^2 and 10 mm^2) generally resulted in faster growth rates in CGSA, CWA, and RBSA, indicating that these media are well-suited to smaller inocula. In contrast, larger sizes (15 mm^2 and 20 mm^2) were more effective in PDA and PSA, with these media showing enhanced growth rates with larger inocula.

Furthermore, the variability of mycelial growth, as indicated by standard deviations, provides insights into the consistency of growth across different media and inoculum sizes. For instance, PDA showed a decrease in standard deviation with increasing inoculum size, from 1.58 for the 5 mm^2 size to 1.35 for the 20 mm^2 size, suggesting that larger inocula may lead to more consistent growth in this medium.

The standard deviations varied significantly across different culture media, highlighting the impact of medium choice on growth consistency. RBSA, for example, exhibited high variability, with standard deviations ranging from 0.00 to 3.98, indicating inconsistent growth patterns compared to other media.

Moreover, the interaction between culture medium and inoculum size significantly influenced the variability in mycelial growth. Notably, the 10 mm^2 inoculum size on RBSA had a very low standard deviation of 0.00, while the 5 mm^2 size on the same medium showed a much higher standard deviation of 3.98.

Comparing the effect of the different indigenous culture media and inoculation size on the mycelial growth of Yanagi Matsutake in terms of the number of days to full mycelial ramification.

In table 2, comparison of the effect of the different indigenous culture media on the mycelial growth of Yanagi mushroom. The F-value and significance (Sig) indicate the results of the statistical analysis. The F-value is 0.82, and

the significance is 0.514. The F-value and significance (Sig) are used to determine if there are statistically significant differences in the mean number of days to full mycelial ramification among the different culture media. In this case, the p-value is high (>0.05), indicating no statistically significant difference in mycelial growth rates among culture media.
Given the non-significant difference (p = 0.514), it suggests the choice of culture media might not drastically affect mycelial growth. The small effect size (Partial Eta Squared = 0.021) supports this, implying only 2.1% of growth variation is attributed to culture media.. PSA has slightly higher mean growth (15.24 mm), but since it's not significant, it's likely due to chance. This could guide cost-effective choices, like opting for cheaper media (e.g., CWA or RBSA) without compromising growth.

Comparing the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake mushroom
In Table 3, the statistical analysis, as indicated by the F-value, significance (Sig), and partial eta squared, suggests that there may be a significant difference in the number of days to full mycelial ramification based on the inoculum size. The F-value of 3.215 and the significance level of 0.025 indicate that the differences in the mean mycelial growth rates among the inoculum sizes are statistically significant. The partial eta squared value of 0.06 suggests a moderate effect size.

Comparing the Interaction Effect of the different indigenous culture media and inoculation size in the mycelial growth of Yanagi Matsutake in terms of number of days to full mycelial ramification
The results presented in the table 4, demonstrate that the interaction effect between the different indigenous culture media and inoculation size had a significant impact on the mycelial growth of Yanagi Matsutake, as measured by the number of days to full mycelial ramification. The interaction effect was statistically significant, with a p-value less than 0.001. This indicates that the combination of culture medium and inoculum size had a strong influence on the mycelial growth rates of Yanagi Matsutake.
The partial Eta squared value, which measures the proportion of the total variance in the dependent variable (days to full ramification) that is attributable to the interaction effect, was 0.277. This suggests that 27.7% of the variation in mycelial growth rates can be explained by the interaction between culture media and inoculum size. The partial Eta squared value of 0.277 is considered a large effect size, meaning the interaction effect had a substantial impact on the mycelial growth of Yanagi Matsutake. This highlights the importance of optimizing both the culture medium and inoculum size to achieve the fastest mycelial growth rates for this mushroom species.
The results presented in the table demonstrate that the interaction effect between the different indigenous culture media and inoculation size had a significant impact on the mycelial growth of Yanagi Matsutake, as measured by the number of days to full mycelial ramification. The interaction effect was statistically significant, with a p-value less than 0.001. This indicates that the combination of culture medium and inoculum size had a strong influence on the mycelial growth rates of Yanagi Matsutake.

Pairwise Comparison of the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake
In Table 5, the partial Eta squared value, which measures the proportion of the total variance in the dependent variable (days to full ramification) that is attributable to the interaction effect, was 0.277. This suggests that 27.7% of the variation in mycelial growth rates can be explained by the interaction between culture media and inoculum size. The partial Eta squared value of 0.277 is considered a large effect size, meaning the interaction effect had a substantial impact on the mycelial growth of Yanagi Matsutake. This highlights the importance of optimizing both the culture medium and inoculum size to achieve the fastest mycelial growth rates for this mushroom species.

Pairwise Comparison of the effect of the different Inoculum Sizes on the mycelial growth of Yanagi Matsutake
The pairwise comparison data in Table 6 indicates that the combination of specific indigenous culture media with particular inoculum sizes can significantly influence the rate of mycelial growth of Yanagi Matsutake. PDA, PSA, and CGSA exhibit significant differences in mycelial growth across different inoculum sizes, with specific combinations leading to faster mycelial ramification.

Determining the most cost-effective culture media and inoculum size using Return on Expenditure (ROE) as a tool
CWA and RBSA emerged as the most cost-effective culture media, boasting high ROE percentages of 11547.06% and 11279.31%, respectively. Their efficiency stems from using locally sourced, inexpensive ingredients like potato, coconut water, corn, and rice bran, likely reducing production costs compared to commercial media like PDA. This highlights the potential of indigenous materials in optimizing production costs.

Return on Expenditure Considering Contamination Rate using the Different Culture Media

CWA is the most cost-effective culture media, boasting an ROE of 10694.49% even after factoring in contamination rates. Its efficiency in generating revenue relative to expenses makes it stand out as the best option, leveraging its low contamination rate and cost-effectiveness.

Conclusions

In view of the foregoing findings, the following are the conclusions:

The combination of specific indigenous culture media and inoculum sizes significantly influences the rate of mycelial growth and ramification of Yanagi Matsutake.

Larger inoculum sizes (15-20 mm^2) generally led to faster mycelial growth and shorter number of days to full ramification, particularly for the PDA and PSA media.

The CGSA medium also showed a significant advantage for larger inoculum sizes (10 mm^2) over the smallest 5 mm^2 size.

The choice of culture medium and inoculum size plays a crucial role in optimizing the mycelial growth of Yanagi Matsutake.

Recommendations

Based on the conclusions of the study, the following are the recommendations:

For optimal mycelial growth and ramification of Yanagi Matsutake, it is recommended to use PDA or PSA media in combination with larger inoculum sizes of 15mm or 20mm, respectively.

If using CGSA medium, an inoculum size of 10mm is recommended for faster mycelial growth compared to smaller or larger sizes.

Further research should be conducted to investigate the underlying mechanisms and factors influencing the observed interactions between culture media, inoculum size, and mycelial growth in Yanagi Matsutake.

Optimization of culture conditions, including media formulation and inoculum size, should be considered for efficient cultivation and commercial production of Yanagi Matsutake and other valuable mushroom species.

Bibliography

1. Boa ER. Wild edible fungi: a global overview of their use and importance to people. In: Food & Agriculture Organization; 2004. (No. 17).
2. Buswell J. A, Tomlinson B, Benzie I. F. F. Lingzhi. (2004). polyphorous fungus. In: Herbal and Traditional Medicine: Molecular Aspects of Health. New York: Marcel Dekker Inc. pp. 179-228. [3] Russell R, and Paterson M. Ganoderma, a therapeutic fungal biofactory. Phytochemistry. (2006) 67:1985-2001. [4] Ferreira ICFR, Vaz JA,
3. Dulay RMR, Batangan JN, Kalaw SP, De Leon AM, Cabrera EC, Kimura K, Eguchi F, Reyes RG. Records of wild mushrooms in the Philippines: A review. J App Biol Biotech. 2023;11(2):11-32. https://doi.org/10.7324/JABB.2023.110202
4. Dulay, Rich & Ray, Karen & Hou, Ching. (2015). Optimization of liquid culture conditions of Philippine wild edible mushrooms As a potential source of bioactive lipids. Biocatalysis and Agricultural Biotechnology. 4. 10.1016/j.bcab.2015.04.003.
5. Engola APO, Eilu G, Kabasa JD, Kisovi L, Munishi PKT, Olila D. The ecology of edible indigenous mushrooms of the Lake Victoria basin (Uganda). Res J Biol Sci. 2007;2(1):62–8.
6. Evangelista FR, Chairez I, Sierra S, Leal Lara H, Martínez-González CR, Garín Aguilar ME, Valencia Del Toro G. A novel coconut-malt extract medium increases the growth rate of morels in pure culture. AMB Express. 2021 Dec 15;11(1):167. doi: 10.1186/s13568-021-01325-2. PMID: 34910284; PMCID: PMC867497.
7. Gougouli M, Kalantzi K, Beletsiotis E, Koutsoumanis KP. Development and application of predictive models for fungal growth as tools to improve quality control in yogurt production. Food Microbiol. 2011 Dec;28(8):1453-62. doi: 10.1016/j.fm.2011.07.006. Epub 2011 Jul 20. PMID: 21925028.
8. Griggs D, Stafford-Smith M, Gaffney O, Rockström J, Öhman MC, Shyamsundar P, Steffen W, Glaser G, Kanie N, Noble I. Policy: Sustainable Development Goals for people and planet. Nature. 2013;495(7441):30
9. Heneghan MN, Burns C, Costa AM, Burton KS, Challen M, Bailey AM, Foster GD. Functional analysis of Agaricus bisporus serine proteinase 1 reveals roles Wendiro et al. Journal of Ethnobiology and Ethnomedicine (2019) 15:64 Page 13 of 15 in utilization of humic-rich substrates and adaptation to the leaf-litter ecological niche. Environ Microbiol. 2016;18(12):4687–96
10. Hoa HT, Wang CL. The Effects of Temperature and Nutritional Conditions on Mycelium Growth of Two Oyster Mushrooms (Pleurotus ostreatus and Pleurotus cystidiosus). Mycobiology. 2015 Mar;43(1):14-23. doi: 10.5941/MYCO.2015.43.1.14. Epub 2015 Mar 31. PMID: 25892910; PMCID: PMC4397375.

11. Hornby JM, Jacobitz-Kizzier SM, McNeel DJ, Jensen EC, Treves DS, Nickerson KW. Inoculum size effect in dimorphic fungi: extracellular control of yeast-mycelium dimorphism in Ceratocystis ulmi. Appl Environ Microbiol. 2004 Mar;70(3):1356-9. doi: 10.1128/AEM.70.3.1356-1359.2004. PMID: 15006753; PMCID: PMC368305.
12. Houlton BZ, Morford SL. A new synthesis for terrestrial nitrogen inputs. SOIL Discussions. 2014;1:497–540 https://doi.org/10.1016/j.bcab.2017.07.001.ISSN 1878-8181
13. Huerta A. I., Molina T. J., Garnica R. Ma. G. and Yahuaca J.B. (2016). Total Polyphenols and Antioxidant Activity of Ganoderma curtissii extracts. Journal of Medicinal Plants Studies 4(4): 136 – 141.
14. Kuypers MM, Marchant HK, Kartal B. The microbial nitrogen-cycling network. Nat Rev Microbiol. 2018;16(5):263
15. La Notte A, D'Amato D, Mäkinen H, Paracchini ML, Liquete C, Egoh B, Geneletti D, Crossman ND. Ecosystem services classification: a systems ecology perspective of the cascade framework. Ecol Indicators. 2017;74:392– 402. 42.
16. Leiva F, Saenz-Díez JC, Martínez E, Jiménez E, Blanco J. Environmental impact of mushroom compost production. J Sci Food Agriculture. 2016; 96(12):3983–90
17. Liu SR, Zhang WR, Chen AP, Kuang YB. Investigating the Effect of Tissue Size on Mycelial Growth of Seven Mushroom Species by Using a Novel Device for Precise Tissue Isolation. Indian J Microbiol. 2016 Dec;56(4):516-521. doi: 10.1007/s12088-016-0615-3. Epub 2016 Aug 16. PMID: 27784952; PMCID: PMC5061704.
18. Martins A. The numbers behind mushroom biodiversity. In: ICFR F, Morales PG, Barros L, editors. Wild plants, mushrooms, and nuts: functional food properties and applications. Chichester: Wiley; 2016. p. 15
19. Meyrath, J. Influence of the size of inoculum on various growth phases inAspergillus oryzae . Antonie van Leeuwenhoek 29, 57–78 (1963). https://doi.org/10.1007/BF02046039
20. Moonmoon M, Shelly NJ, Khan MA, Uddin MN, Hossain K, Tania M, Ahmed S. Effects of different levels of wheat bran, rice bran, and maize powder supplementation with sawdust on the production of shiitake mushroom (Lentinus edodes (Berk.) Singer). Saudi J Biol Sci. 2011 Oct;18(4):323-8. doi: 10.1016/j.sjbs.2010.12.008. Epub 2010 Dec 25. PMID: 23961143; PMCID: PMC3730944.
21. Obua J, Banana AY, Turyahabwe N. Attitudes of local communities towards forest management practices in Uganda: the case of Budongo Forest Reserve. Commonwealth For Rev. 1998;77(2):113–8.
22. Opige M, Kateyo E, Kabasa JD, Olila D. Indigenous knowledge and usage of indigenous edible and medicinal mushrooms among the Teso people of Eastern Uganda. J Food Technol. 2006;4(4):325–30. Pages 161-165
23. Pandey, R. R., & Sharma, G. (2010). Influence of culture media and environmental factors on mycelial growth and sporulation of Lasiodiplodia theobromae (Pat.) Griffon and Maubl. Journal of Yeast and Fungal Research, 1(8), 157-164. Retrieved from https://academicjournals.org/article/article1379517754_Pandey%2520and%2520Sharma.pdf
24. Peng, Jin-Torng & Lee, Chien-Ming & Tsai, Yin-Fung. (2000). Effect of Rice Bran on the Production of Different King Oyster Mushroom Strains during Bottle Cultivation. Journal of Agricultural Research of China. 49. 60-67.
25. Rich Milton R. Dulay, Arthur Grisha C. Rivera, Mycelial growth and fruiting body production of Philippine (CLSU) strain of Polyporus grammocephalus (BIL7749)
26. Rosales-López C, Vargas-López A, Monge-Artavia M, Rojas-Chaves M. Evaluation of Conditions to Improve Biomass Production by Submerged Culture of Ganoderma sp. Microorganisms. 2022 Jul 12;10(7):1404. doi: 10.3390/microorganisms10071404. PMID: 35889123; PMCID: PMC9322093.
27. Soriano, Emily, The Mushroom Industry in Central Luzon: Towards Finetuning R&D and Extension for Industry Revitalization (October 10, 2017). Available at
28. SSRN: https://ssrn.com/abstract=4132835 or http://dx.doi.org/10.2139/ssrn.4132835
29. Tudses, Nootjaree. (2016). Isolation and Mycelial Growth of Mushrooms on Different Yam-based Culture Media. Journal of Applied Biology and Biotechnology. 033-036. 10.7324/JABB.2016.40505
30. Vasconcelos MH, and Martins A. (2010). Compounds from wild mushrooms with antitumor potential. Anti-cancer Agents in Medicinal Chemistry. 10:424-436. [5] Nasim G, Malik S. M., Bajwa., Afzal, M and Mian, W. (2001). Effect of Three Culture Media on Mycelial Growth of Oyster and Chinese Mushroom. Asian Network for Scientific Information. Online Journal of Biological Sciences 1 (2): 1133
31. Wachtel-Galor S, Katende AB, Segawa P, Birnie A, Holding C, Tengnäs B. Wild food plants and mushrooms of Uganda. Regional Land Management Unit. Stockholm: Swedish International Development Cooperation Agency; 1999.
32. Wendiro, D., Wacoo, A.P. & Wise, G. Identifying indigenous practices for cultivation of wild saprophytic mushrooms: responding to the need for sustainable utilization of natural resources. J Ethnobiology Ethnomedicine 15, 64 (2019). https://doi.org/10.1186/s13002-019-0342-z