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Development of low-cost cultivation protocol for *Ganoderma lucidum* (Curtis) P. Karst

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Abstract

Ganoderma lucidum commonly known as lingzhi mushroom, or reishi mushroom in some countries, is an edible mushroom known for its medicinal value. This study evaluated the optimum culture media, grain spawn and substrate formulation for the cultivation of *G. lucidum*. The use of different low-cost culture media, grains and substrate formulations in the preparation of pure cultures, grain spawn bags and fruiting bags of *G. lucidum* were tested. The largest mycelial diameter was observed in Potato Sucrose Agar (93.45mm) which was significantly higher among all the treatments used. It has very thick mycelial density. Cracked corn as spawning material had the shortest incubation period of 14 days, which showed significant difference compared to sorghum seeds and barley grains. The use of cracked corn also incurred the lowest cost and highest return of investment in grain spawn bag production. For fruiting bag production, substrate combination of 50% sawdust and 30% rice straw supplemented with 20% rice bran was the best formulation for fruiting bag production of *G. lucidum* which had the highest yield with a mean value of 91.30g and biological efficiency of 20.29%.

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Introduction

Mushrooms are considered ultimate healthy food and dietary supplements. They contain proteins, carbohydrates, minerals, vitamins, saturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids (Ho *et al.,* 2020). Thus, mushrooms can be used directly in the diet and promote health, taking advantage of both the additive and synergistic effects of all bioactive compounds present in it (Reis *et al.,* 2011).

The cultivation of edible mushrooms could become a way to augment farm income while making use of crop-based residues. The growth of a variety of mushrooms requires different type of substrates and availability of different type of materials. Substrates such as logs, wood sawdust, rice straw and hull, banana leaves, maize stalk, and various grasses can all support mushroom growth (Philippoussis, 2009). In some parts of the Philippines, these substrates may not be available or are available at relatively high prices. Thus, mushroom growers are continuously searching for alternative substrates that may be more readily available or cost effective, or that may provide higher yield and better mushroom quality (Royse D *et al.,* 2004).

Ganoderma lucidum (Curtis) P. Karst is a tropical, edible mushroom that is commonly known as "Reishi" in Japanese and "Lingzhi" in Chinese (Yang and Liau, 1998; Wagner *et al.,* 2003). World-wide, Lingzhi occupies a major source of medicine that has been used for more than 2000 years (Azizi *et al.,* 2012). Commercial *G. lucidum* products are available in various forms, such as powders, dietary supplements, and tea which are obtained from different parts of the mushroom, including mycelia, spores, and fruiting body (Wachtel-Galor *et al.,* 2011). To meet the gradually increasing demand for *G. lucidum* as a natural medicine, commercial cultivation of this mushroom has been initiated worldwide, especially in the tropical Asian countries (Chang and Buswell, 2008). As different members of the *Ganoderma* genus seek different conditions for growth and cultivation, and the traditional cultivation technique takes several months for fruiting body development, artificial cultivation of *G. lucidum* has been implemented using the available substrates such as grain, sawdust, wood logs and cork residues (Boh *et al.,* 2007). Several substrates have been investigated worldwide for the cultivation of *G. lucidum* (Tiwari *et al.,* 2004).

With the need to cultivate *G. lucidum* using low-cost inputs and locally available materials, this project envisioned to determine the best culture media, grain and substrate in terms of biological and cost efficiency. Discovering the best culture media, grain and substrate for *G. lucidum* may lead to the development of mushroom production technologies that may increase yield, and in effect further increase farmers' income.

Materials and methods

Source of Mushroom Strain

Immature fruiting bodies of *Ganoderma lucidum* were collected from a mushroom grower in Mapandan, Pangasinan. The mycelia of this mushroom were aseptically extracted following the tissue culture protocol in the laboratory. The strain was inoculated into potato sucrose agar (PSA) plates. The plates were covered with parafilm and were incubated at room temperature until full mycelial ramification.

Determination of best, low-cost culture media for G. lucidum pure culture production

The performance of the mycelial growth of *G. lucidum* was evaluated using different culture media. Table 1 shows the nine (9) treatments with five (5) replications.

Culture Media

The composition of each treatment is presented in Table 1. The decoctions were boiled until the gulaman bars and sugar added were totally dissolved. The media were then sterilized for 20 min at 121°C (15 psi). After sterilization, the culture media were aseptically pour plated in sterile petri plates.

Table 1. Composition of treatments used in *G. lucidum* pure culture trials.

Treatment	Composition
T1 - Potato Dextrose Agar (PDA) - control	1 L distilled water, 39 grams PDA
T2 - Potato Sucrose Agar (PSA)	Potato decoction [1 L distilled water, 250 g peeled potatoes], 20 g shredded gulaman bars, 10 g white sugar
T3 - Sweet Potato Sucrose Agar (SPSA)	Sweet potato decoction [1 L distilled water, 250 g peeled sweet potatoes], 20 g shredded gulaman bars, 10 g white sugar
T4 - Taro Sucrose Agar (TSA)	Taro decoction [1 L distilled water, 250 g peeled taro], 20 g shredded gulaman bars, 10 g white sugar
T5 - Rice Wash Sucrose Agar (RWSA)	Rice wash decoction [1 L distilled water, 200 g rice], 20 g shredded gulaman bars, 10 g white sugar
T6 - Rice Bran Sucrose Agar (RBSA)	Rice bran decoction [1 L distilled water, 50 g rice bran], 20 g shredded gulaman bars, 10 g white sugar
T7 - Soybean Sucrose Agar (SBSA)	Soybean decoction [1 L distilled water, 200 g soybean], 20 g shredded gulaman bars, 10 g white sugar
T8 - Barley Sucrose Agar (BSA)	Barley decoction [1 L distilled water, 200 g barley], 20 g shredded gulaman bars, 10 g white sugar
T9 - Cracked Corn Sucrose Agar (CCSA)	Cracked corn decoction [1 L distilled water, 200 g cracked corn], 20 g shredded gulaman bars, 10 g white sugar

Inoculation and Incubation

Mycelial discs of 10 mm diameter from a seven (7) day old pure culture of the secondary mycelia of *G. lucidum* were inoculated centrally onto the plated culture media. Plates were sealed with parafilm and stored at room temperature (26°C – 34°C).

Data Gathering

The length of mycelia for each treatment was recorded by measuring the diameter of mycelial growth using a digital Vernier caliper after six (6) days of incubation. Mycelial density was also observed and noted.

Evaluation of Different Grains for the Spawn Production of G. lucidum

The performance of the mycelial growth of *G. lucidum* was assessed using different grains. Three (3) treatments were prepared in the grain spawn trial with five (5) replications each. The treatments were:

Treatment 1 - Barley (control);

Treatment 2 - Cracked corn; and

Treatment 3 - Sorghum seeds

Grain Spawn Preparation

Grains weighing 250 grams each were washed and were boiled for 10 minutes. The grains were then filtered using a strainer and were air dried on a cheese cloth for 20 minutes. After air drying, the grains were placed in 4x11 inches polypropylene bags. The bags were tied with rubber band and were then sterilized for 20 minutes at 15 psi. After sterilization, the bags were transferred into the inoculation room and were cooled down for one (1) hour.

Inoculation and Incubation

From the pure culture of *G. lucidum*, a mycelial disc measuring 10 mm in diameter was inoculated into each bag. Inoculated bags were then incubated at room temperature (26°C – 34°C).

Data Gathering

The incubation period and mycelial density upon full mycelial ramification of grain spawn bags were observed and recorded.

Assessment of Low-Cost Substrate Combination for G. lucidum Fruiting Bag Production

There were four (4) treatments in the substrate trial with 10 replications each.

The treatments were:

T1 - Sawdust (control);

T2 - Sawdust (70%) + Rice Straw (30%);

T3 - Sawdust (50%) + Rice Straw (40%) + Rice Bran (10%); and

T4 - Sawdust (50%) + Rice Straw (30%) + Rice Bran (20%)

Preparation of Fruiting Bags

Rice straws were soaked in water overnight to soften the material. After soaking, the rice straws were air-dried for three (3) hours to drain excess water. The rice straws were chopped into two (2) to three (3) inches length. The different substrates were then mixed according to the formulation of each treatment. A total of 750 g for each bag was loaded into 6x12 inches polypropylene bags. The bags were then inserted with PVC pipe and cotton plug, and were sterilized in an autoclave for 30 minutes at 15 psi and cooled overnight.

Inoculation, Incubation and Data Gathering

Forty (40) grams of spawn were inoculated in each bag. A total of 10 replications for each treatment were made. Inoculated bags were incubated in a dark room with temperature range of 18-20°C and monitored daily. The incubation period of fruiting bags under each treatment was observed and recorded.

Harvesting and Data Gathering

Fully ramified fruiting bags were hanged inside a growing house using rope and every four (4) layers was secured with wire. Bags were watered three (3) times a day for three (3) months. Mature fruiting bodies with a maturity period of 25 days from primordial formation (Royse *et al*., 1990) were harvested from three (3) flushes of each treatment. Harvested fruits were weighed and the yield from all flushes was recorded. The size of pileus and stipe was measured using a digital Vernier caliper. The biological efficiency was computed using the formula developed by (Chang *et al.* 1981).

$$\text{Biological Efficiency (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Statistical Analysis

The experiments were laid out in Complete Randomized Design. The data gathered were analyzed using Statistical Tool for Agricultural Research (STAR). Analysis of Variance (ANOVA) was performed. Comparison among means was computed using Tukey's Multiple Comparison Test.

Cost-Benefit Analysis

Cost-benefit analysis from each treatment in each stage of production - pure culture, grain spawn, and fruiting bag production was done by computing the cost of production and comparing it to the computed return of investment (ROI).

Results

Determination of best, low-cost culture media for G. lucidum pure culture production

The mycelial growth performance and density of *G. lucidum* on nine (9) different culture media is shown in Table 2. The largest mycelial diameter was observed in PSA with a mean of 93.45 mm which is significantly higher among all the treatments. It also exhibited very thick mycelial density as seen in Fig. 1. On the other hand, PDA recorded the smallest mycelial diameter with a mean of 71.68 mm.

Table 2. Mycelial diameter and density of *G. lucidum* on various nutritional culture media after six (6) days of incubation.

Culture Media	Mycelial diameter (mm)	Mycelial density
T1 - Potato Dextrose Agar	71.68[g]	++++
T2 - Potato Sucrose Agar	93.45[a]	++++
T3 - Sweet Potato Sucrose Agar	83.72[d]	+++
T4 – Taro Sucrose Agar	81.83[e]	+++
T5 – Rice Wash Sucrose Agar	84.97[d]	++
T6 – Rice Bran Sucrose Agar	76.53[f]	++++
T7 – Soybean Sucrose Agar	89.86[b]	+++
T8 – Barley Sucrose Agar	88.00[c]	++
T9 - Cracked Corn Sucrose Agar	87.96[c]	+++

*Note: Means with the same letter are not significantly different at 5% level using Tukey's Multiple Comparison Test.

**Note: Mycelial density were evaluated as (+) very thin, (++) thin, (+++) thick, (++++) very thick and (-) no growth (De Leon *et al*., 2017).

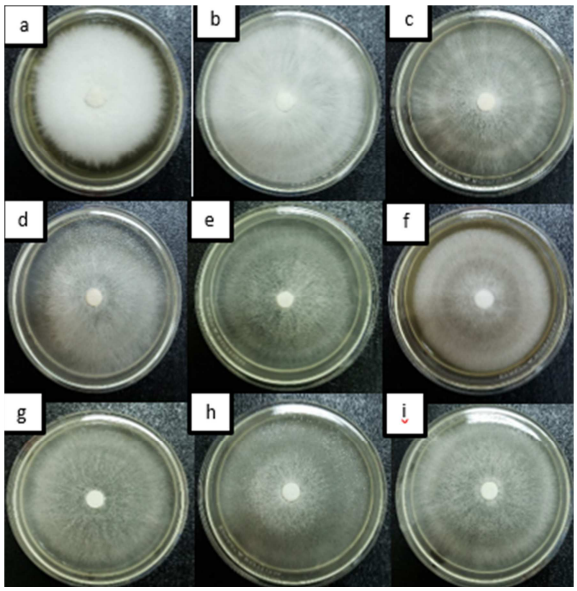

Fig. 1. Mycelial growth of *G. lucidum* on different culture media: a) PDA, b) PSA, c) SPSA, d) TSA, e) RWSA, f) RBSA, g) SBSA, h) BSA and i) CCSA after six (6) days of incubation.

Evaluation of Different Grains for the Spawn Production of G. lucidum

Incubation period to reach full ramification of *G. lucidum* on various grain spawn materials is shown in Table 3. *G. lucidum* had the shortest incubation period in cracked corn with 14 days, which showed significant difference compared to *G. lucidum* in sorghum seeds (20 days) and barley grains (31 days). It also had very thick mycelial density in cracked corn as seen in Fig. 2.

Table 3. Incubation period to reach full ramification and mycelial density of *G. lucidum* on various grain spawn materials.

Grain Spawn	Incubation period (no. of days)	Mycelial density
T1 - Barley	31[a]	+
T2 - Cracked corn	14[c]	++++
T3 - Sorghum	20[b]	++

*Note: Means with the same letter are not significantly different at 5% level of significant difference using Tukey's Multiple Comparison Test.

**Note: Mycelial density were evaluated as (+) very thin, (++) thin, (+++) thick, (++++) very thick and (-) no growth (De Leon *et al.*, 2017)

Fig. 2. Grain spawn bags of *G. lucidum* on different medium: T_1 - Barley grains, T_2 - Cracked corn and T_3 - Sorghum seeds after three (3) weeks of incubation.

Assessment of Low-Cost Substrate Combination for G. lucidum Fruiting Bag Production

Incubation Period

Table 4 shows the incubation period of *G. lucidum* on various substrate combinations. Fruiting bags with 100% sawdust had the fastest spawn run of 18 days, followed by the substrate combination of 70% sawdust and 30% rice straw which fully ramified after 20 days, while the substrate supplemented with 10% and 20% rice bran recorded the longest spawn run of 23 days.

Table 4. Incubation period of *G. lucidum* on various substrate combinations.

Substrate	Incubation period (no. of days)
100% Sawdust	18[c]
70% Sawdust + 30% Rice Straw	20[b]
50% Sawdust + 40% Rice Straw + 10% Rice Bran	23[a]
50% Sawdust + 30% Rice Straw + 20% Rice Bran	23[a]

*Note: Means with the same letter are not significantly different at 5% level of significant difference using Tukey's Multiple Comparison Test.

Size of pileus

The diameter of pileus of *G. lucidum* grown on various substrate combinations is presented in Table 5. *G. lucidum* cultivated in substrate supplemented with 20% rice bran had the largest pileus diameter with a mean of 69.41 mm significantly different among all treatments, while the smallest pileus diameter was recorded in 10% rice bran supplementation with a mean of 47.15

mm which had no significant difference to the pileus diameter observed in 100% SD and substrate combination of 70% RS and 30% SD.

Table 6. Mean size of pileus of *G. lucidum* on various substrate combinations.

Substrate	Size of pileus (mm)
T1 - Sawdust	48.41[b]
T2 - 70% Sawdust + 30% Rice Straw	47.63[b]
T3 - 50% Sawdust + 40% Rice Straw + 10% Rice Bran	47.15[b]
T4 - 50% Sawdust + 30% Rice Straw + 20% Rice Bran	69.41[a]

*Note: Means with the same letter are not significantly different at 5% level of significant difference using Tukey's Multiple Comparison Test.

Size of stipe

The length of stipe of *G. lucidum* grown on various substrate combinations is presented in Table 6. The longest stipe was recorded on substrate supplemented with 20% rice bran with a mean value of 14.78 mm. However, statistical analysis revealed no significant difference in the size of stipe among the different treatments.

Table 6. Mean length of stipe of *G. lucidum* on various substrate combinations.

Substrate	Length of stipe (mm)
T1 - Sawdust	12.08[a]
T2 - 70% Sawdust + 30% Rice Straw	13.67[a]
T3 - 50% Sawdust + 40% Rice Straw + 10% Rice Bran	12.48[a]
T4 - 50% Sawdust + 30% Rice Straw + 20% Rice Bran	14.78[a]

*Note: Means with the same letter are not significantly different at 5% level of significant difference using Tukey's Multiple Comparison Test.

Weight of fruiting bodies

In this study, weight of fruiting bodies of *G. lucidum* on various substrate formulations is presented in Table 7. The highest mean weight of fruiting bodies was observed in substrate supplemented with 20% RB with 91.30 g followed by substrate supplemented with 10% RB with a mean weight of 78.20 g, while the lowest mean weight was recorded in 100% SD with mean weight of 63.30 g.

Table 7. Mean weight of fruiting bodies of *G. lucidum* on various substrate combinations.

Substrate	Weight of fruiting bodies (g)
T1 - Sawdust	63.30[c]
T2 - 70% Sawdust + 30% Rice Straw	69.90[bc]
T3 - 50% Sawdust + 40% Rice Straw + 10% Rice Bran	78.20[ab]
T4 - 50% Sawdust + 30% Rice Straw + 20% Rice Bran	91.30[a]

*Note: Means with the same letter are not significantly different at 5% level of significant difference using Tukey's Multiple Comparison Test.

Biological efficiency

As presented in Table 8, treatment supplemented with 20% RB produced the highest biological efficiency of 20.29% comparable with treatment supplemented with 10% RB, while 100% SD got the lowest value of 14.07%.

Table 8. Mean weight of fruiting bodies of *G. lucidum* on various substrate combinations.

Substrate	Biological efficiency (%)
T1 - Sawdust	14.07[c]
T2 - 70% Sawdust + 30% Rice Straw	15.53[bc]
T3 - 50% Sawdust + 40% Rice Straw + 10% Rice Bran	17.38[ab]
T4 - 50% Sawdust + 30% Rice Straw + 20% Rice Bran	20.29[a]

*Note: Means with the same letter are not significantly different at 5% level of significant difference using Tukey's Multiple Comparison Test.

Cost-benefit Analysis

The cost to produce, net profit, and return of investment (ROI) were computed. In Table 9, it is shown that the highest ROI can be attained by using cracked corn sucrose agar in the pure culture production of *G. lucidum.* Table 10 shows that the ROI in using different grains do not differ substantially. In fruiting bag production (Table 11), it can be observed that the highest ROI can be obtained in 70% SD + 30% RS since rice straw is free and no other supplements with cost were added.

Table 9. Cost and return of the different culture media used as treatments.

Culture Media	Cost to produce 1 flat bottle of pure culture (Php)	Net profit per bottle (Php)	Return of Investment % (ROI)
T1 - Potato Dextrose Agar	40.44	459.56	889.12
T2 - Potato Sucrose Agar	9.85	490.15	3,960.91
T3 - Sweet Potato Sucrose Agar	8.85	491.15	4,419.77
T4 – Taro Sucrose Agar	9.35	490.65	4,178.07
T5 – Rice Wash Sucrose Agar	8.75	491.25	4,471.43
T6 – Rice Bran Sucrose Agar	8.25	491.75	4,748.48
T7 – Soybean Sucrose Agar	8.65	491.35	4,524.28
T8 – Barley Sucrose Agar	8.45	491.55	4,633.73
T9 - Cracked Corn Sucrose Agar	8.35	491.65	4,690.42

*Note: One (1) flat bottle of pure culture of *G. lucidum* costs Php 500.00

Table 10. Cost and return of different grain spawn for *G. lucidum.*

Grain Spawn	Cost per bag containing 300 g (Php)	Net profit per spawn bag	Return of Investment % (ROI)
T1- Barley grains	35.0	115.0	328.57
T2- Cracked corn	33.5	116.5	347.76
T3- Sorghum seeds	36.5	113.5	310.96

*Note: One (1) spawn bag of *G. lucidum* containing 300 g costs Php 150.00.

Table 11. Cost and return of different substrate combinations for *G. lucidum.*

Substrate Combination	Cost per bag containing 750 g (Php)	Net profit per bag (Php)	Return of Investment % (ROI)
T1- Sawdust	16.8	33.2	197.62
T2- 70% Sawdust + 30% Rice Straw	16.2	33.8	208.65
T3- 50% Sawdust + 40% Rice Straw + 10% Rice Bran	18.0	32.0	177.78
T4- 50% Sawdust + 30% Rice Straw + 20% Rice Bran	20.4	29.6	145.10

*Note: One (1) fruiting bag of *G. lucidum* containing 750 g costs Php 50.00.

Discussion

The study focused on determining the best culture media, grain and substrate in terms of biological and cost efficiency for the cultivation of *G. lucidum* for increased yield and income.

A culture medium is an enriched material often derived from plant-based sources that can promote and sustain the mycelial growth of the desired mushroom to be artificially cultivated (Reyes *et al.,* 2009). Results obtained in the study imply that the use of PSA in culturing *G. lucidum* results to luxuriant mycelial growth.

The same results were observed by Landingin *et al.* (2020) wherein PSA, used in *Cyclocybe cylindracea*, was among the culture media with the highest mean mycelial growth rate at 9.00 mm/day and very thick mycelial density. In addition, Dulay and Garcia (2017) also observed the highest mycelial growth rate of Philippine strain of *Lentinus strigosus* in PSA. The dense growth of fungal mycelia in PSA can be attributed to the nutritional content of potato such as fructose, sucrose, amylase, protein, vitamins and minerals (Borlingame *et al.,* 2009; Kalaw *et al.,* 2016).

Any nutritious substance inoculated with mycelia is called spawn. It is used to transfer mycelia onto a substrate for the mass production of mushroom. Based on the results, cracked corn is the most efficient spawn material for rapid multiplication of *G. lucidum.* The efficient growth of *G. lucidum* on cracked corn could be attributed to its high carbohydrate, fatty acid, and protein that could stimulate fruiting as observed by Magday, *et al.* (2017) and Awi-waadu and Stanley (2010) also found out that large surface area and aperture of substrates results to faster mycelial growth as supported also by Tinoco *et al*. (2001). Meanwhile, the thin and slow mycelial growth observed in barley seeds could be attributed to the protective covering of the barley grain. Dulay*, et al*. (2012) explained that the hull serves as the barrier that limits the penetration of the fungal mycelia needed to acquire the nutritious part of the barley.

Sawdust is the main ingredient used in substrate mixtures for the artificial cultivation of *G. lucidum*. The difference in the number of days of spawn run of *G. lucidum* observed could be due to the supplementations used in the cultivation which might affected the mycelial ramification. However, active mycelial growth was still observed in substrate supplemented with 20% rice bran showing primordial formation on the 13th day of incubation though fruiting bags were not yet fully ramified (Fig. 3). Gurung, *et al.* (2012) in their report on the cultivation of *G. lucidum* in various kinds of sawdust and supplement showed that supplementation had a positive role in mycelial growth of mushroom.

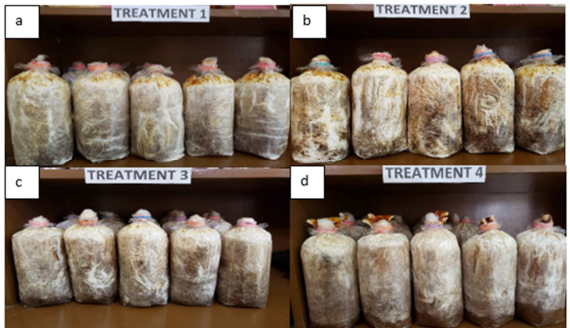

Fig. 3. Fruiting bags of *G. lucidum* on different substrates: a) T1 – 100% Sawdust, b) T2 – 70% Sawdust + 30% Rice Straw, c) T3 – 50% Sawdust + 40% Rice Straw + 10% Rice Bran and d) T4 – 50% Sawdust + 30% Rice Straw + 20% Rice Bran.

The pileus is the technical name for the cap, or cap-like part of a mushroom that has a spore-bearing surface. This result indicated that supplementation affects the size of the pileus of the mushroom as it produced larger pileus diameter compared to un-supplemented cultivation as reported by Dulay *et al.*, (2012). Meanwhile, a *stipe* is the stem or stalk-like feature supporting the cap. This indicates that the amount of supplementation does not affect the stipe of the mushroom.

Adesina, *et al.* (2011) reported that cultivation of *L. squarrosulus* on bark and leaves of fruit trees supplemented with rice bran exhibited best result. The result of this study proved that high yield of *G. lucidum* is dependent on the ratio of supplements used. Biological efficiency is a measure of how efficient a substrate is converted into yield. This result could be attributed to the amount of rice bran present in the substrate. Moonmoon *et al.* (2011) reported that rice bran increased productivity of mushrooms due to its components such as carbohydrates, amino acids, and mineral elements. Based on the result of this study, supplementation is important in *G. lucidum* cultivation since it can increase the biological efficiency of un-supplemented substrate. Rice bran as an industrial by-product that is economically cheaper than wheat bran and more readily available is recommended as a supplement with sawdust to cultivate *G. lucidum*.

It is important to know the cost-benefit analysis of each treatment used in each stage of the mushroom cultivation process - pure culture, grain spawn bag, and fruiting bag production. This will help mushroom growers understand the costs and benefits of an intervention that would help in deciding which culture media, grain, and substrate combination to use in the cultivation of *G. lucidum.* Based on the cost and return analyses conducted, it proves that cultivation of *G. lucidum* has potential for mass production because of high percentage of return of investment. Lingzhi mushroom production can contribute to mushroom growers as another variety of mushroom that is beneficial to grow in terms of business purposes.

Conclusion

G. lucidum can be cultured in locally available agro–industrial residues and may be useful for its large-scale production with minimal cost. It can be concluded that Potato Sucrose Agar was the best culture medium for mycelial growth and cracked corn was the best grain for spawn production of *G. lucidum*. For fruiting bag production, substrate combination of 50% sawdust and 30% rice straw supplemented with

20% rice bran was the best formulation for *G. lucidum* in terms of yield and biological efficiency.

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