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Performance of Reishi Mushroom (*Ganoderma lucidum* [Fr.] Karst) using Different Spawn Substrates

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ABSTRACT

Spawn production is an essential aspect in mushroom cultivation. This involves the induction of mushroom mycelial development in growing medium under controlled conditions. This study aimed to investigate the performance and economic profitability of Reishi Mushroom (*Ganoderma lucidum* [Fr.] Karst) cultivation in response to different spawn substrates. The study was laid out in Completely Randomized Design with six spawn grain substrates as treatments. Results revealed that the shortest time to full mycelial colonization among the different spawn grains at 50, 75 and 100% mycelial spawn run was in corn kernels. However, fruiting bags seeded with sorghum spawn grains significantly showed the earliest time (28.33 days) to full colonization and also produced the highest number of fruiting bodies. Moreover, fruiting bags seeded with sorghum grains produced the highest mushroom yield and consequently recorded the highest profit and return of investment. As per findings, sorghum grains as spawn substrate can be used for rapid mycelial growth, high yield and low cost of reishi mushroom spawn production. When not available, corn kernels or adlay grains may be used as substitute substrates for mushroom spawn production.

Keywords: *colonization, fruiting bags, grain spawn, reishi mushroom, substrates*

INTRODUCTION

Ganoderma lucidum (Leyss. Fr.) Karst is a basidiomycete that belongs to the Polyporaceae family, known as "reishi," "lingzhi" and "yeongji." It is a hard, bitter mushroom used to promote health and longevity in traditional Chinese medicine. Ganoderma is not just known as a nutritional supplement but also as a miraculous herb for its health-giving properties, as well as, an anti-aging natural food that helps prevent and reverse many diseases. China used this Ganoderma for medical treatment for over 4000 years and was crowned by ancient Chinese as "Miraculous King of Herbs". Cultivation of Ganoderma mushroom has been attempted for centuries with least success due to the environmental complexity and nutritional requirements to grow them. However, farmers can now grow them seasonally in the low-cost growing rooms and environmentally controlled cropping rooms commercially.

Spawn production is an essential aspect in mushroom cultivation. This involves the induction of mushroom mycelial development in supporting medium under controlled conditions. The supporting matrix is preferably composed of sterilized grains due to their biochemical and growth-enhancing properties compared to other substrates (Siddhant et al., 2013). However, limited information and studies have been reported on this aspect. Likewise, the quality and quantity of the spawn used in the cultivation of mushrooms could directly influence their quality and yield. Hence, this study was conducted to evaluate the performance of Reishi mushroom on different grains as spawn substrates. Specifically, it aimed to determine the most profitable and best medium for commercial cultivation and spawn production.

METHODOLOGY

The study was laid out in Completely Randomized Design (CRD) with six spawn grain substrates as treatments replicated three times. Ten fruiting bags were prepared per replication. The six treatments that served as growing media for mass production of mother spawn were: T1- sorghum grains, T2-adlay whole grains, T3-adlay grits, T4- rice grains, T5- corn kernels, and T6- cracked corn. Results were analyzed using the one-way Analysis of Variance (ANOVA). Differences among treatment means were tested using the Duncan's Multiple Range Test (DMRT).

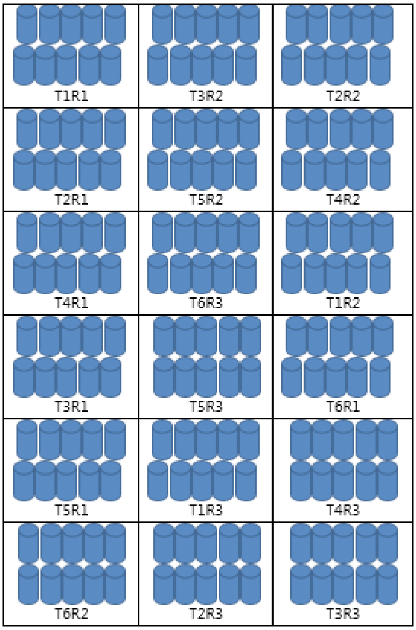

Figure 1. Lay-out of the experiment in CRD with three replications

The fresh reishi mushroom cap was acquired from a grower in Cagayan de Oro City. The cap was disinfected first with ethyl alcohol, cut into several pieces and placed in a dry blotting paper. Using a flamed needle or scalpel, the cap pieces were carefully transferred into the prepared plates of potato dextrose agar (PDA) equidistantly and incubated at room temperature.

The six growing media for spawn production were washed separately with tap water and for at least 10 minutes. Washed grains were boiled until these expanded slightly. Boiled grains were drained and allowed to cool, then packed loosely filling about 2/3 of the bottles that were heat resistant. Each bottle that contained a spawn grain substrate was plugged with cotton and covered with aluminum foil. These were sterilized in an autoclave for about 1 hour at 15 psi, then allowed to cool. These were aseptically inoculated with pure cultures of *G. lucidum* were incubated until substrates were completely covered with the mycelia.

Sawdust and rice bran were used as primary components for the mushroom fruiting bags. Composted sawdust was piled into a heap not higher than 1.5 meters. This was mixed with 20% rice bran, 1% sugar and 1% lime. The mixture composed of about 78% sawdust was moistened and covered with canvass. The heap was mixed thoroughly and turned every three days. This was used for bagging after seven days.

Propylene plastic bags (6 x 12 x 0.02) filled with the mixture were used for growing the mushroom. Each bag was pressed down tightly, plugged with cotton at the opening, and tightly closed with a rubber band. These bags were sterilized for eight hours.

Inoculation of spawn in fruiting bags was done aseptically. Spawn was stirred with a sterile long flat end needle. Each sterilized fruiting bag was opened and added with 10 g of the specified spawn treatment. Inoculated bags were slightly tilted to equally distribute the spawns along its "shoulder area." These were placed on shelves and incubated at 28-35 0C in a closed dark room until each was fully covered with mycelia. After the complete spawn run (as indicated by white mycelia all over the bags), each fruiting bag was cut at the substrate level to completely expose the top side. Proper conditions for fruiting or pinning were provided. Fruiting bodies were harvested when the caps became completely red, and the white margins disappeared.

Several data were gathered. A number of fruiting bodies per bag were counted. Mushroom cap samples were collected per replication where five random caps per replication were measured for diameter. Total yield per replication per treatment was recorded from two flushes. The number of days to percent spawn running at 50, 75, and 100 on the six-grain spawn substrates were counted from inoculation of pure mushroom culture until the bottles of grain spawn were filled up. The appearance of heavy mycelial growth per bag was determined by counting the number of days from spawning to the date when complete mycelial growth was observed per fruiting bag. The number of days to fruiting bodies formation was counted from the time the fruiting bags were opened to the time of first harvest. The freshly harvested mushroom caps were collected from each treatment and each cap diameter was measured and the average of five (5) caps was computed. Number of fruiting bodies formation per bag per replication was counted. Freshly harvested mushroom per bag per replication were weighed. Mean of 10 bags per replication was computed to obtain the mean of three replications per treatment.

Return of Investment (ROI) was computed to compare and identify the most profitable treatment. ROI per treatment was computed using the formula: ROI = (Total Return –Total Cost)/Total Cost.

RESULTS AND DISCUSSION

Growth of Reishi mushroom (G. lucidum) as influenced by different spawn grain substrates

Table 1 shows that the number of days to 50%, 75% and 100% mycelial colonization differed significantly among substrates. Results show that corn kernels spawn development of reishi mushroom (G. lucidum) significantly exhibited the earliest time to attain 50%, 75% and 100% mycelial colonization (MC). However, it was comparable with other substrates. Corn kernels achieved the shortest time (3.67 days) to reach 50% MC. However, it did not differ from adlay whole grains, sorghum, and rice grains. Cracked corn was last at 6.67 days. For 75% MC, corn kernels were again the earliest (6.33 days), but this was comparable with rice grains, adlay whole grains, and sorghum grains. Cracked corn was last at 8.33 days but was comparable with adlay grits. For 100% MC, corn kernels consistently was earliest (9.67 days). However, it was comparable with sorghum grains, adlay whole grains, and adlay grits. Rice grains were last at 13.67 days but was comparable with cracked corn.

These results are best supported by Tinoco et al. (2001) who reported that substrates with larger surface area and pores support faster mycelium growth. Perhaps, such substrates have increased air flow and therefore, have higher O2 concentration. Oxygen has an important role in metabolism and is essential for respiration. The rate of respiration is associated with substrate O2 concentration (Mehravaran, 1993). Similarly, Shukla (2003) used different grains for spawn development of milky mushroom (Calocybe indica) and reported early spawn development on maize grains.

Table 1 summarizes the mean number of days to mycelial colonization on various grains spawn for production at 50%, 75% and the 100% mycelial spawn run (Fig. 2).

Table 1
Number of Days to 50%, 75% and 100% Mycelial Colonization of Various Grains for Spawn Production

Treatments	No. of Days to Mycelial Colonization		
	50%	75%	100%
Sorghum grains	4.0c	7.0bc	10.67bc
Adlay whole grains	4.33c	6.67bc	10.67bc
Adlay grits	5.33b	7.67ab	11.67bc
Rice grains	4.67bc	7.33bc	13.67a
Corn kernels	3.67c	6.33c	9.67c
Cracked corn	6.67a	8.33a	12.00ab
F-TEST	**	**	**
C.V (%)	11.03	7.30	9.25

Means in a column followed by the same letter are not significantly different at 5% level of probability (DMRT)
** highly significant

Figure2. Spawn Running Performance of Different Grains Spawn

Number of days to complete MC (on mushroom fruiting bags (Fig. 3) and number of days to fruiting body formation are presented in Table 2. ANOVA revealed highly significant differences among treatments on both parameters.

Fruiting bags seeded with sorghum grains had the shortest time to complete MC with mean of 28.33 days. However, this treatment did not differ from corn kernels and adlay grits. Complete MC was last in rice grains (34.33 days) but was comparable with adlay whole grains. Puri (2011) described that sorghum grains acted as a carrier for evenly distributing the mycelium and which also served as a nutritional supplement. Thus, it results in fast and uniform mycelial growth.

Figure 3. Fruiting bags completely colonized with mycelia of G. lucidum

Table 2
Growth Performance of Reishi Mushroom (G. lucidum) on Sawdust-rice Bran Mixture using Different Grain Spawns Substrates

Treatments	No. of Days to Complete Mycelial Colonization of Fruiting Bags	No. of Days to Fruiting Body Formation
Sorghum grains	28.33d	2.00c
Adlay whole grains	33.33ab	4.67a
Adlay grits	30.67bcd	2.33c
Rice grains	34.33a	4.33ab
Corn kernels	29.33cd	3.00bc
Cracked corn	31.33bc	3.33abc
F-TEST	**	**
C.V (%)	4.72	22.75

Means in a column followed by the same letter are not significantly different at 5% level of probability (DMRT)
** highly significant

The number of days to fruiting body formation ranged from 2.00 days (sorghum grains) to 4.67 days (adlay whole grains). Sorghum grains, however, were comparable with adlay grits, corn kernels and cracked corn. On the other hand, adlay whole grains were comparable with rice grains and (also) cracked corn for delayed formation of fruiting bodies.

Similar results were reported by Puri (2011) that Lentinula edodes colonized the substrates of sorghum and its combinations faster than the other substrates investigated. Thus, it resulted in faster and uniform mycelial growth.

Yield of Reishi Mushroom as Influenced by Different Spawn Grain Substrates

Table 3 and Figure 4 present the yield performance of *G. lucidum* in different treatments. ANOVA on number of fruiting bodies per bag showed highly significant differences among treatments. Fruiting bags seeded with sorghum grains spawn exhibited the highest mean number of fruiting bodies per bag (3.72). All the other treatments had less and were comparable. Table 3 presents that the cap diameter did not differ among treatments as per ANOVA, although the measurements ranged from 5.29 cm (rice grains) to 6.11 cm (adlay whole grains).

Table 3
Yield Performance of Reishi Mushroom (G. lucidum) Mushroom as Influenced by Different Spawn Grains Substrates

Spawn Grain Substrates	No. of Fruiting Bodies/Bag	Cap Diameter (cm)	Yield (g/bag)
Sorghum grains	3.72a	5.53	38.72a
Adlay whole grains	2.36b	6.11	28.47b
Adlay grits	2.48b	5.65	33.18ab
Rice grains	2.99b	5.29	31.76ab
Corn kernels	2.67b	5.62	33.61ab
Cracked corn	2.25b	6.08	26.33b
F-TEST	**	ns	**
C.V (%)	14.04	7.32	11.95

Means in a column followed by the same letter are not significantly different at 5% level of probability (DMRT)
** highly significant
ns non-significant

Figure 4. Fruiting Bodies of G. lucidum with Sorghum Grains Spawn; (a) Young Fruiting Bodies at 5 Days after Emergence, and (b) Mature Fruiting Bodies

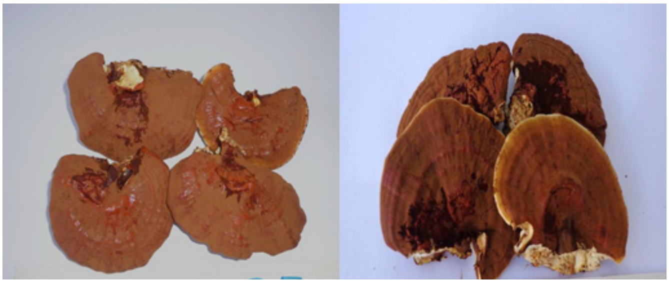

Figure 4. Fresh Samples of Reishi Mushroom Caps (G. lucidum)

Table 3 present the mean yield of Reishi mushroom per treatment that was obtained and recorded from two flushes or harvests. ANOVA showed highly significant differences in mushroom yield (g/bag) among treatments. Therefore, yield was influenced by different grain substrates. Mean yield was highest in the sorghum grains treatment (38.72 g/bag) and was least in cracked corn (26.33 g/bag). However, each was comparable with at least two other treatments.

Previous reports (Puri, 2011) cited that sorghum grains acted or served as an excellent medium for an even distribution of mycelium, as well as, as source of nutritional supplement resulting in rapid and uniform mycelial growth. Further, Puri (2011) also reported that yield of Shiitake mushroom was higher in sorghum spawn than in poplar sawdust. He further identified sorghum as an ideal material under Indian conditions for production of Shiitake spawn. Similar findings were reported by Kumla et al. (2013) that sorghum grains as substrate were best for spawn production utilized for Thai oyster mushroom cultivation.

Table 4 presents the cost and return analysis on producing *G. lucidum* using six spawn grains substrates. ROI computation showed profitability of mushroom production in all treatments. However, sorghum grains were most profitable (ROI of 293.10%) whereas, the least was cracked corn (167.99%). The second most profitable were corn kernels, adlay grits, rice grains, and adlay whole grains. Mushroom growth performance and yield were best in sorghum grains hence, the highest ROI.

Table 4
Cost and Return Analysis of Mushroom Yield of G. lucidum using Different Spawn Grain Substrates

Spawn Grain Substrates	Mushroom Yield (g/bag)	Gross [1/] Income (PhP/bag)	Treatment Cost[2/] (g/bag)	Net Profit (PhP/bag)	ROI (%)
Sorghum	38.72	46.46	11.82	34.64	293.10
Adlay whole grains	28.47	34.16	11.95	22.21	185.89
Adlay grits	33.18	39.82	11.85	27.97	236.00
Rice grains	31.76	38.11	11.78	26.33	223.53
Corn kernels	33.61	40.33	11.74	28.59	243.54
Cracked corn	26.33	31.60	11.79	19.81	167.99

[1/] Price of mushroom at PhP 120.00/kg (PhP 0.120/grams)
[2/] Expenses incurred in different treatments per bag

CONCLUSION

On the basis of the results, sorghum grains as spawn substrate can be used for rapid mycelial growth, high yield and low cost for reishi mushroom spawn production. In the absence of sorghum grains, corn kernels or adlay grains may be used as substitute substrates for mushroom spawn production.

RECOMMENDATION

Based on the data gathered, mushroom growers for Ganoderma are advised to develop their spawn on sorghum grains, though in the absence of sorghum grains, corn kernels, and adlay grits may be used as good substitute substrate for mushroom spawn production.

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