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Artificial Cultivation of *Ganoderma lucidum* (Reishi Medicinal Mushroom) Using Different Sawdusts as Substrates

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Abstract: *Ganoderma lucidum* is a kind of medicinal mushroom possessing anti-tumor, anti-inflammatory, immune-modulatory, antioxidant and other biological traits which render it to be used as medicinal herbs to combat against variety of diseases. Present study was designed to implement a suitable method for artificial cultivation of *G. lucidum* in polypropylene bags with variety of cheap and readily available substrates. Sawdusts of five woods (*Swietenia mahagoni*, *Dipterocarpus turbinatus*, *Tectona grandis*, *Gmelina arborea* and *Michelia champaca*) were used as substrates and each was supplemented with calcium carbonate ($CaCO_3$) and either rice or wheat bran for cultivation. *T. grandis*, *G. arborea* and *M. champaca* were not found to provoke the further extension of mycelial growth and hence the growth was stunted. On the contrary, *S. mahagoni* and *D. turbinatus* were noticed to impart comparatively good yield with biological efficiency. Wheat bran was found to be more efficient as supplement than rice bran. However, *S. mahagoni* supplemented with wheat bran provided the best yield of mushroom among the substrates which took 6 days, 33 days and 60 days for the mycelial growth, primordial formation and harvesting, consecutively with the subsequent yields of 235.2 g/kg and biological efficiency of 7.6%.

Keywords: *Ganoderma Lucidum*, Reishi, Lingzhi, Medicinal Mushroom, Cultivation, Sawdust

1. Introduction

Ganoderma lucidum (Fr.) Karst is a member of fungal group Basidiomycetes which belongs to Polyporaceae (Ganodermaceae) of Aphyllophorales. Its fruiting body is named as "Reishi" in Japanese and "Lingzhi" in Chinese [1, 2]. World-wide Reishi occupies a major source of medicine that has been used for more than 2000 years [3-5]. Commercial *G. lucidum* products are available in various forms, such as powders, dietary supplements, and tea which are farmed from different parts of the mushroom, including mycelia, spores, and fruit body [4]. *G. lucidum* has been used in Traditional Chinese Medicine (TCM) as a remedy to treat more than 20 different illnesses which include migraine and headache, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis hemorrhoids, hyper-cholesterolaemia, nephritis, dysmenorrhoea, constipation, lupus erythematosis, hepatitis, leucopoenia, cardiovascular problems and cancer [3, 6-8]. Besides, reishi or lingzhi also attribute some health benefits which principally include the control of blood glucose levels, modulation of the immune system, hepato-protection, and bacteriostasis [4]. Recent studies on lingzhi have demonstrated numerous biological activities amongst this type of mushroom, including anti-tumor, anti-inflammatory, hepato-protective, anti-microbial, hypotensive, anti-diabetic and hypolipodemic effects [3, 9, 10].

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 [4, 11]. 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 [3, 11-13]. Several substrates have been investigated worldwide for the cultivation of *G. lucidum* till date [3, 14-18].

Usually in Bangladesh the summer season is considered as the best time for the cultivation of *mushroom* [19, 20]. Different environmental factors, oxygen level, and calcium ion concentration, etc. are also important for the cultivation [21, 22]. Bangladesh Council of Scientific and Industrial Research (BCSIR) investigated the efficacy of sawdust supplemented with rice or wheat bran as substrate, and found the 9:1 ratio of sawdust and rice bran/wheat bran to be effective for the cultivation of *G. lucidum* with elevated production, even in the large scale. Although extensively used in several Asian and tropical countries, unfortunately its application as medicine in Bangladesh is still in scarce. Commercial-based cultivation of such mushroom in this country is thus critical to confer its extended medicinal use as it could be a suitable alternative to synthetic drugs with less adverse effects. Along these lines, the present study assessed the best cultivation media for achieving high yield, biological efficiency, and growth (mycelial, primordial and fruiting body) rate of *G. lucidum* using different sawdusts (*Swietenia mahagoni, Dipterocarpus turbinatus, Tectona grandis, Gmelina arborea,* and *Mechelia champaca*), and the supplements including rice bran, wheat bran and calcium carbonate ($CaCO_3$) for implementing its large scale cultivation in Bangladesh.

2. Materials and Methods

2.1. Setting and Sampling

Experiments relating to the present study were carried out in the laboratory of Plant Pathology Section, Biological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh within the time frame of May 2014 to July 2014. The five types of sawdusts (Table 1) were collected randomly from different saw mills of Dhaka city, and the supplements (rice bran, wheat bran and $CaCO_3$) were collected randomly too from rice mills and other shops. The sample spawn of *G. lucidum* was collected from National Mushroom Development and Extension Center, Savar, Bangladesh.

Table 1. *Plant source of sawdust for substrate base.*

Local name	English name	Scientific name	Family
Mahogani	Mahogani	*Swietenia mahagoni*	Meliaceae
Gerjan	Gerjan	*Dipterocarpus turbinatus*	Dipterocarpaceae
Segun	Teak	*Tectona grandis*	Verbenaceae
Gamar	Beechwood	*Gmelina arborea*	Lamiaceae
Chapa	Teak chambul	*Michelia champaca*	Verbenaceae

2.2. Isolation and Obtaining Pure Culture of G. lucidum

To obtain the pure culture of *G. lucidum*, Potato Dextrose Agar (PDA) culture or tissue culture method was used. A small piece of tissue was collected from the fruiting body of *G. lucidum* mushroom and placed on the sterilized PDA medium under aseptic conditions. The inoculated medium was incubated at 25 ^{o}C for 7-10 days for sufficient mycelia growth which was identified on the basis of basidiocarp and basidiospore morphology. Subsequent sub-culturing was then performed to obtain the pure culture of *G. lucidum* mycelium on PDA which was further added to the substrate for fruiting body generation [3, 23].

2.3. Substrate Preparation

A total of 10 kg of substrate mixture (65% wet weight and 35% dry weight) was prepared for each category of the 5 sawdusts. Therefore, Moisture level of the mixture was maintained at 65% (6.5 L) [3, 20, 23]. To achieve 3.5 kg dry weight of substrate (35% of the mixture), and sawdust and supplements ratio of 9:1, 3.1 kg (90%) of sawdust was mixed with 0.3 kg (8%) rice bran or wheat bran and 0.1 kg (2%) of $CaCO_3$.

2.4. Spawn Preparation

A total of ten spawn bags for each of the substrate mixture were prepared. Each of the ten polypropylene bags (18×25 cm) were filled with 400 g of the above prepared mixture and packed tightly. The neck of the bag was prepared by using heat resistant PVC (Polyvinyl chloride) tube. A hole of about 2/3 deep of the volume was made at the center of polypropylene bags with sharp end stick for space to put inoculum. The neck was plugged with cotton and covered with a sterile brown paper and tied with a rubber band. The packets were sterilized on an autoclave for one hour at 120 ^{o}C under 1.5 kg/cm^2 pressure. Following sterilization, the packets were transferred into a clean aseptic chamber and were allowed to cool for 24 hours [3, 19, 20, 23].

2.5. Cultivation

A piece of pure culture medium containing mycelium (6-7 days old) of *G. lucidum* mushroom was placed aseptically in the hole of center of the polypropylene bags and again plugged with cotton wool in a laminar flow chamber. During incubation, the inoculated packets were kept in almost dark at about 25 ^{o}C and transferred to the culture room at 25-32 ^{o}C, together with 85-95% relative humidity and 250-350 lux light [3]. Water was sprayed 4-5 times per day and proper aeration was maintained in culture house to facilitate the development of the fruiting bodies [3, 23]. Different parameters such as days required to mycelial growth, primordial initiation, fruit body maturation, and harvesting were recorded regularly. Number of fruiting bodies and dry yield of mushroom (g/packet) were also calculated. The biological efficiency was determined for each packet using the formula [3, 19, 23, 24]:

Total biological yield (g)

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of fruit bodies (g)}}{\text{Total dry substrate used (g)}} \times 100$$

3. Results and Discussion

Due to the increased rate of resistance and several adverse effects for toxicity of the synthetic drugs, natural products like herbs have attracted lots of interest to be a suitable drug alternatives or sources for new drug discoveries in recent years [8, 24-37]. *G. lucidum* has already proved its competency as medicinally active mushrooms with a wide range of therapeutic and biological effects [3-5, 7, 9, 23]. To meet the huge demand of such mushroom for medicinal purposes, artificial cultivation using different substrates have been employed so far round the globe [3, 4, 11-13, 20, 23]. However, in context to economical ease of cultivation, no significant effort has been initiated to develop a suitable and cheap cultivation method in Bangladesh till date. Present study thus endeavored to establish an effective cultivation technique using sawdusts along with rice bran or wheat bran.

The primordial formation period for *S. mahagoni* with rice bran and wheat bran was in 35 days and 33 days, respectively, which were 40 days and 36 days, respectively in case of *D. turbinatus*. The average first harvest days of fruit bodies of *G. lucidum* for *S. mahagoni* with both supplements (rice bran & wheat bran) were 71 days and 60 days, respectively, which were 90 days and 66 days in case of *D. tarbinatus*. In the study conducted by Karma and Bhatta (2013), it was noted to be up to 92 days [8]. After obtaining the first harvest of *G. lucidum*, the second third and fourth harvest can be obtained from the same sample within each 18 to 20 days. *G. arborea*, *T. grandi*s and *M. champaca* could not lead to the harvesting of *G. lucidum* because of the retarded mycelial growth. Thus, those sawdust samples could not be suggested to consider as substrate for the cultivation of mushroom of interest.

Table 2. *Comparison of growth (mycelial and primordial) using different sawdust samples supplemented with rice and wheat brans.*

Sawdust	Supplements	Growth (cm)						
		3 days	6 days	9 days	12 days	15 days	18 days	19 days
Swietenia mahagoni	Rice bran	3.5 cm	10.5 cm	14 cm	Primordial initiation	-	-	
	Wheat bran	12 cm	14 cm	Primordial initiation	-	-	-	
Dipterocarpur turbinatus	Rice bran	1.2 cm	2.4 cm	4.9 cm	8.7 cm	11.2 cm	13.5 cm	Primordial initiation
	Wheat bran	2.4 cm	5.8 cm	7.2 cm	10.5 cm	12.5 cm	13 cm	Primordial initiation
Tectona grandis	Rice bran	-	-	-	-	1.2 cm	Stunted	
	Wheat bran	-	-	-	1.4 cm	2.2 cm	Stunted	
Gmelina arborea	Rice bran	-	-	-	-	2.1 cm	Stunted	
	Wheat bran	-	-	-	-	3.1 cm	Stunted	
Michelia champaca	Rice bran	-	-	-	1.2 cm	Stunted		
	Wheat bran	-	-	-	1.2 cm	Stunted		

Average data for each of the substrate has been shown.

3.1. Comparison of Growth with Different Types of Sawdust

An equal colonization period was observed with both supplements (rice bran and wheat bran) in case of D. turbiratus, T. grandis and M. chmpaca which were 18 days, 15 days and 12 days, consecutively. In case of G. arborea, colonization period with rice bran and wheat bran was 13 days and 15 days, respectively, which was 9 days and 6 days for *S. mahagoni*. But it was observed that, only *S. mahagoni* and *D. tarbinatus* allowed primordial initiation from their mycelial growth, while other three sawdusts (*T. grandis*, *M. chmpaca* and *G. arborea*) could not extend the mycelium growth to primordial formation and the growth was stunted (Table 2). Growth attained for *S. mahagoni* up to 14 cm in rice bran and wheat bran before primordial initiation after 9 days and 6 days, respectively.

For *D. turbiratus*, the final mycelial growth was found to be 13.5 cm with rice bran, and 13 cm with wheat bran before primordial initiation after 18 days (Table 2). Recently, Karma and Bhatt (2013) in India noticed primordial initiation after 35 days using sawdust as substrate supplemented with rice and wheat brans, maize flour, and bagasse [8].

3.2. Effect of Different Types of Sawdust on Yield and Biological Efficiency

The feasibly of any of the sawdust samples used in this study was determined through measuring the biological yield of *G. lucidum* and biological efficiency of the substrate. Cultivation of reishi mushroom could only be achieved using *S. mahagoni* and *D. turbinatus*. The yield of 235.2 g/kg with biological efficiency of 7.6% and yield of 210.9 g/kg with biological efficiency of 6.8% were attained for *S. mahagoni* and *D. turbinatus*, respectively supplemented with wheat bran (Table 3). The yield was 132.9 g/kg with biological efficiency of 4.3%, and 110.4 g/kg with biological efficiency of 3.6% for *S. mahagoni* and *D. turbinatus*, respectively when rice bran was used as substrate (Table 3).

Karma and Bhatt (2013) found the yield of 570 g/100 kg of the sawdust used [8]. Azizi et al. (2012) reported the yield of 102.58 g/kg with biological efficiency of 12.89% using hornbeam saw dust supplemented with 5% malt extract and 10% wheat bran [3]. Erkel (2009) also found the highest yield (63.66 g/kg) and biological efficiency (18.63%) using oak sawdust where he used wheat bran as supplement [5]. The

finding of present study in agreement with previous studies [3, 5, 8, 18, 38], and further suggested that wheat bran would be suitable supplement for mycelial growth when sawdust being used as substrate.

Table 3. *Effect of different type of supplement and control on yield and biological efficiency of Ganodarma lucidum.*

Sawdust	Supplement	Yield (g/kg)	Biological Efficiency (%)
Swietenia mahagoni	Rice bran	132.9	4.3
	Wheat bran	235.2	7.6
Dipterocarpur turbinatus	Rice bran	110.4	3.6
	Wheat bran	210.9	6.8
Tectona grandis	Rice bran	0	0
	Wheat bran	0	0
Gmelina arborea	Rice bran	0	0
	Wheat bran	0	0
Michelia champaca	Rice bran	0	0
	Wheat bran	0	0

Average data for each of the substrates has been shown

4. Conclusion

The effect of various kinds of sawdust and supplements on the yield of *G. lucidum* was investigated in this study. As described above yield of *G. lucidum* varied widely depending on the kind of sawdust and supplements. Therefore it is important to use the proper substrate for the commercial production of *G. lucidum*. *S. mahagoni* sawdust with wheat bran showed highest biological efficiency with better yield among all treatments. However, marginal difference between rice bran & wheat bran supplement was observed. As rice bran and wheat bran are the industrial by-products and are economically cheaper than other supplements like gram flour, corn flour etc., these could have better applicability in low income countries. The present study recommended wheat bran to be used preferably with *S. mahagoni* for the commercial production of *G. lucidum*. However, rice bran could be used as an alternative supplement.

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