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Deployment of indigenous wild *Ganoderma lucidum* for better yield on different substrates

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***Ganoderma lucidum* is a medicinal mushroom growing on angiospermous trees that has been used in the Orient medicine for more than 2000 years. Since now studies have been almost entirely limited to laboratory scale and much more understanding on the growth morphology and mass production is necessary to develop economical large scale processes. In this study, domestication of wild isolate of *G. lucidum* was done, which was collected from Himalayan region of India. Effect of various kinds of sawdust and bran on its yield was also investigated in artificial cultivation. Four kinds of sawdust viz. *Mangifera indica*, *Jacaranda mimosifolia*, *Populus alba* and *Dalbergia Sissoo* along with Coir Pith were used as a substrate media in *G. lucidum* cultivation. Significant differences (P<0.01) were found among varieties of sawdust, and mixtures, both in yield and biological efficiency (BE). The highest yield and BE were obtained from Oak sawdust and its combination with wheat bran.**

Key words: *Ganoderma lucidum*, cultivation, sawdust, bran, substrate, yield.

INTRODUCTION

Ganoderma lucidum (Fr.) Karst (Polyporaceae) has been recognized as a medicinal mushroom for over 2000 years and its powerful effects have been documented in ancient scripts. Besides promoting longevity, it has unique properties of strengthening the immune system[1]. It is a popular remedy to treat conditions like chronic hepatitis, hypertension, cancer, low blood pressure, high blood pressure, diabetes, rheumatism, heart problems, paralysis, ulcers, arthritis, asthma, tiredness, hepatitis A, B, and C, sterility, psoriasis, mumps, epilepsy and alcoholism (Zang, 1999). It refreshes the body and mind, delays ageing and is also effective in reducing the blood glucose level of diabetic patients after two months of treatment. Tribes in India have also been using the extract of this fungus for the treatment of joint pain (Harsh et al., 1993). All parts of Reishi mushroom, such as spore, mycelium and basidiocarp are used for health purpose (Wasser, 2002).

The modern herbal industry and consumer demand genuine true-to-type *Ganoderma* which is mature and free from unwanted microbes and chemicals. The recent increase of interest in herbal medicines for various physiological disorders and recognition of many biological response modifiers (BRM) in *Ganoderma* products has attracted the attention of researchers globally. As a result, many countries have developed cultivation technology of this mushroom. Scientists have attempted artificial cultivation of Reishi first time in 1937 and mass production was achieved in 1971 by seeding the spawn in sawdust containing pots (Perumal, 2009). *G. lucidum* is

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most commonly cultivated in China, Taiwan, Japan, Korea, Malaysia and North America. In India, Directorate of Mushroom Research, Solan, has achieved success in cultivating this mushroom employing various combinations of sawdust and other nutrients supplements and environmental conditions such as light, pH, temperature, humidity under laboratory conditions (Rai, 2003).

Global production of *Ganoderma* was about 4900 to 5000 tonnes in 2002, out of which 3800 tonnes were produced in China (Lai et al., 2004). The fruit body of *G. lucidum* is sold in the market at Rs 600-700/kg (Perumal, 2009). Its trade is also growing fast in India due to import from other countries.

Though *Ganoderma* is available in the tropics, temperate and subtropical regions, huge requirements of fruiting body and their spores cannot be met from wild collections. To make *G. lucidum* easily available to the general public around the year its cultivation under *in vitro* conditions is of paramount importance. Therefore, in this work cultivation of wild isolate under *in vitro* conditions was tried along with determination of best kind of substrate for its cultivation.

MATERIALS AND METHODS

The strain of *G. lucidum* was collected from the forest of Dhualadhar range in Himalayan region of India and identified by using conventional description method and molecular techniques. Culture was prepared on malt extract agar (MEA) medium by tissue culture from the basidiocarp. The mycelium from MEA slant was used for spawn production on wheat grains. Experimental cultivation was attempted on four locally available sawdust based substrates and Coir Pith. All the substrates were used in various combinations. Sawdust from different broad leaf tree species (*Mangifera indica*, *Populus alba*, *Jacaranda mimosifolia*, and *Dalbergia Sissoo*) from sawmills and Coir Pith were collected and evaluated in various combinations with bran such as (10% wheat bran+ 90% sawdust) and (20% wheat bran + 80% sawdust). Apart from various combinations 100% sawdust of above mentioned trees and 100% wheat bran were also evaluated. All substrates were wetted to 65% moisture level. Calcium sulphate (gypsum) and Calcium carbonate (chalk powder) were added to get a pH of 5.0.

Then the mixed substrates (700 g dry wt.; 1000 g wet wt.) were filled in polypropylene bags and plugged with non-absorbent cotton after putting a plastic (PVC) ring at the neck. For each treatment, four replications were maintained. The substrates were sterilized at 121°C for 3 h. After cooling, sterilized bags were inoculated in the laminar flow cabinet and incubated at 28±2°C without light exposure. Spawn run period completed 12 days. When the mycelium had colonized on the substrate completely, the bags were shifted to cropping room at 30-32ºC, 85-90% relative humidity (RH) with a 10 h light exposure for the formation of fruiting bodies. The mouths of bags were opened and water was sprinkled twice in a day on the bags. Cap formation of *G. lucidum* initiated in 2 to 3 days, after opening the mouths of bags. Fruiting bodies were harvested when the caps become completely red and the white margin disappeared. Total yield (g kg^{-1}) was obtained from three flushes in a harvest period of 60 days. The biological efficiency (BE) percentage [fresh weight of harvested mushrooms/dry matter content of the substrate] × 100 was calculated. Experimental design was a Completely Randomized Block with four replicates. Each block was placed with 3 plastic bags containing totally 4 kg substrate. The data were analyzed by using the analysis of variance (ANOVA) and group means were compared by Duncan Multiple Range Test (DMR) using the MINITAB program. Correlation analyses were carried out to determine the relationships among yield and biological efficiency (BE) of the substrates.

RESULTS AND DISCUSSION

Sawdust is the most preferred main ingredient used in substrate mixtures for *G. lucidum* cultivation. From the initial studies, it was found that *G. lucidum* took significantly less time for spawn run on Coir pith (100%) as compared to other combinations. Coir pith supplemented with 10 and 20% wheat bran showed quick and complete spawn run (10-12 days) among all the substrate combinations (Figure 1). In case of *D. Sissoo* and its different combinations with wheat bran, incomplete and patchy colonization with thick white rope like mycelial strands was observed. Likewise, Coir Pith it also failed to develop fruiting primordia. Failure of *D. Sissoo* as a substrate medium for *G. lucidum* was also reported in earlier studies (Kapoor, 2011).

Similar results have also been reported stating sawdust as the best substrate for cultivation of *G. lucidum* (Perumal, 2009; Dadwal and Jamaluddin, 2004). Chen (1999) reported Rice bran as an essential ingredient for the cultivation of *G. lucidum* and suggested that addition of WB to the substrate might be taking care of thiamine requirement due to which mycelial growth and reproduction of *G. lucidum* is enhanced (Chen, 1999).

No primordial formation took place, in case of 100% *M. indica*, *P. alba*, Coir Pith and *D. Sissoo*. Combinations of Coir Pith and shesham with wheat bran too failed to form fruiting bodies, thus suggesting them as unsuitable substrate for *G. lucidum*. This might be due to lack of carbohydrate source in case of treatments having 100% sawdust. Reasons for failure of *D. Sissoo* and Coir Pith combinations are yet to be determined.

The results revealed that all the substrates showed significant difference to one another in terms of yield. Among all the different substrates screened for yield performance of *G. lucidum*, *J. mimosifolia* sawdust based combinations were superior in terms of yield (156.0 g kg^{-1}) and biological efficiency (22.33) (Table 1). *J. mimosifolia* sawdust supplemented with 20% WB gave significantly higher yield of 195.0 g kg^{-1} and biological efficiency (27.9%) among all the substrates evaluated and minimum yield (49.4 g kg^{-1}) was obtained with poplar combined with 20%. Among *M. indica* based substrates, *M. indica* sawdust supplemented with 10% WB gave maximum yield with all isolates. Higher yield in local isolates of *G. lucidum* with increase in rate of supplementation was recorded[7]. In the present studies similar trend of increase in yield with supplementation was observed.

Figure 1. Effect of different substrate combinations on *Ganoderma lucidum* (GL18) on different substrate combinations.

Table 1. Yield potential of *Ganoderma lucidum* isolates/strain on various substrate combinations*.

Substrate combinations	Yield	Mean	B.E. (%)	Mean
100% *Mangifera indica* sawdust	88.2^{c}		12.6^{b}	
90% *Mangifera indica* sawdust + 10% WB**	176.5^{b}	138.83^{b}	25.2^{a}	19.83^{b}
80% *Mangifera indica* sawdust + 20% WB	151.8^{b}		21.7^{a}	
100% *Jacaranda mimosifolia* sawdust	99.0^{c}		14.2^{b}	
90% *Jacaranda mimosifolia* sawdust + 10% WB	174.0^{b}	156.00^{a}	24.9^{a}	22.33^{a}
80% *Jacaranda mimosifolia* sawdust+ 20% WB	195.0^{a}		27.9^{a}	
100% *Dalbergia sissoo* sawdust	57.8^{d}		8.3^{c}	
90% *Dalbergia sissoo* sawdust + 10% WB	91.7^{c}	66.3^{d}	13.1^{b}	9.50^{c}
80% *Dalbergia sissoo* sawdust + 20% WB	49.4^{d}		7.1^{c}	

*Means with different letters within a column are significantly different (P<0.05). **WB, Wheat Bran.

These findings are in conformity with some authors who reported hardwood sawdust have been preferred for the commercial production (Chen, 1999) although *Ganoderma* can be cultivated on the sawdust which may originate from different kinds of trees (Wasser, 2005; Olei, 2003; Erkel, 2009).

Kapoor (2011) observed that strain OE53 gave maximum biological efficiency of 17.4% on mango sawdust supplemented with 10% wheat bran (Kapoor, 2011). Rai (2003) reported biological efficiency of *G. lucidum* between 10 to 15% (Rai, 2003). Biological efficiency of 15 to 17% was reported on wheat straw supplemented with 5% rice bran (Mishra and Singh, 2006). The findings of present studies showed significantly higher biological efficiency (27.9%) of isolate GL18 with supplementation of 20% WB on *J. mimosifolia* sawdust. These results suggest that the yield and BE were effected not only by the sawdust types but also by their combinations. Very little information about the combinations of various kinds of sawdust and wheat bran in the cultivation of *G. lucidum* is available. Most of studies have focused on submerged media in obtaining mycelial biomass. Sawdust of *D. Sissoo* and Coir Pith were found to be most unsuitable material for cultivation of *G. lucidum.*

Conclusions

The effects of various kinds of sawdust and their combination with wheat bran, on the yield of *G. lucidum* were investigated in this study. As described above, yield and biological efficiency of *G. lucidum* varied widely, depending on the kind of sawdust and their combinations. Therefore, it is important to use the proper combination of substrate formulations for the commercial production of *G. lucidum.*

Conflict of Interest

The authors have not declared any conflict of interest.

Abbreviations: BE, Biological efficiency; **BRM,** biological response modifiers; **MEA**, malt extract agar; **WB,** wheat bran; **SD,** saw dust.

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