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Cultivation of *Ganoderma Lucidum* (Curt.fr.) P.karst.

Microbiology

KEYWORDS : *Ganoderma lucidum,* substrate, mother spawn, paddystraw

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ABSTRACT

Ganoderma lucidum (reishi mushroom, Ling Zhi) is a medicinal mushroom that has been used in the Orient for more than 2000 years. It has been an economically important species, particularly in the Far East countries (China, Japan, Korea, etc.), for over 4000 years. It is widely grown on a commercial scale and is commonly purchased for its medicinal and spiritual properties. Therefore, present investigations were undertaken to use locally available sawdust, wheat bran, sorghum and sorghum grains in combination with chalk powder and gypsum for selection of an ideal material for Ganoderma lucidum spawn production. G.lucidum had maximum and faster linear growth rate having sorghum and its combinations. It colonized sorghum substrate rapidly without any contamination and the spawn prepared with sorghum had maximum yield and biological efficiency. The results showed that the parameters which are used for the preparation of mother spawn, and fruit body development.

INTRODUCTION

Ganoderma lucidum (Curt. Fr.) Karst, is a basidiomycete belonging to the Polyporaceae, also known as Ling zhi in China and Reishi in Japan. While many wood utilising gourmet and medicinal mushrooms have traditionally been cultivated on hardwood logs outdoors in the natural environment an alternative, more intensive and regulated cultivation technique has been developed in several Asian laboratories over the last 2-3 decades. The success of this new approach largely reflects the major increase in world production of wood utilizing mushrooms. It has been widely used as a traditional medicine in the Orient for more than 2000 years. The first mention of this mushroom dates from the period of the first emperor of China, Shih-huang of the China Dynasty (221–227 B.C.) (Stamets 1993). Even today the basidiocarp (fruiting body) of *G. lucidum* is a popular remedy to treat conditions like hepatopathy, chronic hepatitis, nephritis, hypertension, hyperlipemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcer, arteriosclerosis, leukopenia, diabetes, anorexia and cancer. (Stamets 1993, Mizuno 1995) *G. lucidum* produces several metabolites with biological activity, such as polysaccharides and terpenoids, which might be responsible for some of these medicinal properties. Polysaccharide fractions containing (1-3) β D-glucans, branched mainly at the C-6 position, demonstrate high antitumor activity (Sone 1985). Terpenoids are cytotoxic to hepatoma cells, they inhibit the response of platelets to various aggregating agonists (*5*) and inhibit eukaryotic DNA polymerase and HIV type 1 reverse transcriptase (Mizushina *et al*., 1999). Although (1-3) β D-glucans and a group of terpenoids known as ganoderic acids are found both in basidiocarp and cultured mycelium, most of the commercial *G. lucidum* products derive from its basidiocarp. There has been recent interest in producing these biologically active substances by fermentation techniques, prompted by the fact that *G. lucidum* is rarely found in nature and traditional basidiocarp production in logs or sawdust takes months.

MATERIALS & METHODS

STRAIN

Fruiting bodies of *G.lucidum* was collected from Thanjavur district, Tamil Nadu. Tissue pieces of Ganoderma fructification were surface sterilized with tap water and 0.1 % mercuric chloride solution for 2 min rinsed in distilled water. Fruiting bodies of *G.lucidum* isolates were done onto potato dextrose agar (PDA) medium. After inoculation the fungal colonies cultures were purified using pure culture technique and stock culture was maintained in PDA slants for further studies.

CULTURE PLATES

G.lucidum isolates were replicated on potato dextrose agar medium for mother spawn preparation.

SUBSTRATES

Different substrates were used for cultivation of mushrooms. In the case of *G.lucidum* sorghum grains, wheat bran (*Triticum duram*) was used for mother spawn and paddy straw and saw dust for cultivation process.

MOTHER SPAWN PREPARATION (Smita Puri, 2011)

Disease- free sorghum grains are used as substrate for growing the spawn materials. Bag system was adopted for cultivation. 65% moisture level was adopted for substrate and maintained by adding appropriate quantity of water. The substrate includes sorghum grains, wheat bran, saw dust mixed with 1% $CaCo_3$, 1% $CaSo_4$, 1% sucrose. The water content was approximately 67%. For 500g dry substrate (containing 400g sorgum grains, 90g wheat bran), 1 litre of distilled water containing sucrose and $CaCo_3$ was added. (5g each). The purpose of mixing calcium carbonate is to remove the excess moisture present in the cooked grains, to neutralize the pH of the grains and to avoid caking of grains after sterilization. At first sorghum grains were soaked in clean water to remove chaffy and damaged grains. Then shadow dry was takenout. At 50% moisture level calcium carbonate ($CaCO_3$) was mixed thoroughly with the cooked, dried grains containing sterilized saw dust. These mixture was filled only 3/4 the capacity, in 2 kg capacity polypropylene bags. The necks of the bags were plugged with non-absorbent cotton and sterilized at 22 lbs pressure. After cooling inoculation was done with PDA plate containing fungal cultures. Then the paltes were incubated at room temperature for 27 days.

CULTIVATION (Biswass 2006 and Tewari 2007)

Mushrooms can be cultivated through a variety of methods. Some methods are extremely simple and demand little or no technical expertise. On the other hand, cultivations which require aspects of sterile handling technology are much more technically demanding (Chang and Miles, 1989). All mushroom growing techniques require the correct combination of humidity, temperature, substrate (growth medium) and inoculum (spawn or starter culture). Wild harvests, outdoor log inoculation and indoor trays all provide these elements.

Paddy straw which is to be used should be uncrumpled, not very leafy, not more than one year old and preferably should be hand threshed. It should be stored at a proper place so that it does not get wet during rain. Paddy straw is made into bundles. The bundles are soaked in water for 18 to 24 hours. Soaking can be done in small tanks and the bundles should be completely immersed in water. The bundles are taken out and excess water drained off. The bundles are arranged such that they make one layer on which small bits of spawn are placed 3 to 4 inches inside the margin, leaving a space of 5 to 5.5 cm from each other.

A small quantity of sterilized saw dust was sprinkled over the spawn bits. Each layer was spawned in the same way. Finally the last layer was spawned allover and covered with a thin layer of loose straw and the bed was pressed down. The bed was covered with a polythene sheet. The bags were kept in the crop room at relative humidity of 80-85 % and 25°C temperature in the dark for complete spawn run. Watering depends upon the humidity of the air. Mostly no watering is required for the first 3 or 4 days. After the spawn run, slitting was done and relative humidity of 80-90 % was maintained by sprinkling water. Small buttons start appearing 7 to 10 days after spawning. At that time the polythene sheet should be removed from the bed. They remain in the button stage for 4 to 5 days and then grow into full size.

Picking is done by gently twisting the fruiting bodies. Different methods are used for cultivation by different workers. Spawning the different layers of the bed is an influencing factor in straw mushroom production. Spacing between the spawn bits also influences the yield. Smaller spacing (5 cm) between small spawn bits (10 mm) and larger spacing (10 cm) between large spawn bits (100 mm) gives better yield.

RESULTS & DISCUSSION

SUBSTRATE FORMULATION AND CULTIVATION OF GANODERMA BASIDIOCARPS

Formulation of the supplemented paddystraw, sorghum grains, sawdust substrate and the process of producing Ganoderma fruiting bodies were first tested on a laboratory scale with positive results. High humidity and proper air circulation are crucial for the formation of pileated fruiting bodies. The *G. lucidum* mycelium had a good development on the paddystraw, which was firstcolonized about 25 days. Primordia appeared in 26, 27 and 28 days, respectively.

Linear growth was significantly low in wheat and teak sawdust combinations. Even though, wheat is a very common material used for spawn production (Rai, 2003, Dadwal and Jamaluddin, 2004), the linear growth was not promising on wheat grains. Mushrooms were harvested when the caps were full sized at 30 days. Clearly a supplement to the paddystraw is needed to obtain optimal *G. lucidum* production. These findings confirms with some authors who reported that molasses has stimulative growth effect and, the highest mycelial growth was found on the bag with paddystraw addition (Paterrson-Beedle *et.al*, 2000; Hsieh *et al*., 2005).

Structure of *Ganoderma lucidum*

Dorsal **Ventral**

Culture Plate and Slant of *G. lucidum*

Mother Spawn of *G. lucidum*

Ist Day **25th Day**

Cultivated bags of *G. lucidum*

Cultivation Room **Mycelial formation**

Cultivation Bag **Primordia formation**

CONCLUSION

Increase in population is creating an alarming situation in the food problem in India. Malnutrition in terms of "protein" deficiency is becoming major health hazard in the developing countries. It is unfortunate to realise that mushrooms rooms did not receive universal acceptance over the years since a number of naturally growing mushrooms are poisonous. Today the concept has changed because the cultivated species of mushrooms are totally safe for human consumption. Mushroom farming is becoming successful because of its very low inputs. In India, mushroom growing can be highly rewarding because of a variable climate. The technology can be profitably considered in areas where land is a limiting factor and agricultural residues are abundantly available able.

G. lucidum, the most famous species in this group is a legendary mushroom in China with a long fascinating history dating back over two thousand years. Not only it is a sparkling beautiful woody mushroom, but 33 more importantly, *G. lucidum* is

known as the mushroom of immortality and is the number one medicinal mushroom in China. The cultivation of *G.lucidum* is an outstanding tedious process of a biotechnological enterprise that challenges the combined skills of industrial and biological technologies. Many wood utilising gourmet and medicinal mushrooms have traditionally been cultivated on hardwood logs outdoors in the natural environment an alternative, more intensive and regulated cultivation technique has been developed in several Asian laboratories over the last 2-3 decades. The success of this new approach largely reflects the major increase in world production of wood utilizing mushrooms.

Detailed descriptions of the many growing techniques can be found in the Royse *et al*. (1985), Przbylowicz and Donoghue (1989) and Kozak and Krowczy (1999).

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