

Short Communication

CULTURING AND SPAWNING STRATEGIES FOR CULTIVATION OF GANODERMA LUCIDUM (CURTIS) P. KARST

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ABSTRACT

Objective: To access the vegetative growth of *Ganoderma lucidum* on ten solid media and four liquid media. Spawn development on different grains viz., wheat, barley, maize, soybean and pearl millet was also assessed for lab scale artificial cultivation of this mushroom.

Methods: Culture was raised on Potato Dextrose Medium and growth parameters were assessed on different solid and liquid media at 25±2 °C. Various grains were analyzed for best spawn development of the mushroom mycelium. Oak sawdust and wheat bran mixture was used as a substrate for cultivation of the mushroom.

Results: Malt Agar Extract medium and Glucose Asparagine Solution supported the best mycelial growth among the solid and liquid media screened for the purpose. Barley grains were the best for the spawn development. Maximum yield was observed on the Oak sawdust and the wheat bran (4:1 w/w) mixture used as a substrate.

Conclusion: The study revealed that this pharmacologically important mushroom can be grown under lab conditions, and more yield of the mushroom can be obtained using barley based spawn.

Keywords: Cultivation, Fruiting bodies, *Ganoderma lucidum*, Spawn, Substrates.

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Ganoderma lucidum (Curtis) P. Karst. is a species of basidiomycetes which belongs to Polyporaceae (or Ganodermaceae) of Aphyllophorales. Its fruiting body is called "Reishi" in Japanese and "Lingzhi" in Chinese [1, 2]. *Ganoderma lucidum* is one of the most famous traditional Chinese medicinal herbs and used as a healthy food and medicine in the Far East for more than 2000 y [3]. This mushroom contains various chemical substances, including more than 119 different triterpenes and several types of polysaccharides [4]. A successful artificial cultivation of *G. lucidum* has been reported on most broadleaf hardwood trees and commonly used species include oak, pecan, elder, choke, cherry and plum [5, 6]. Moreover, *Ganoderma* species can also be cultivated on the sawdust which may originate from different kinds of trees [7, 8]. Artificial cultivation of *G. lucidum* is still in its early stages in India as compared to China, Japan, Taiwan, Korea, and North America, where it is being cultivated on the large commercial scale. Few studies have been carried out on the feasibility of cultivation of *G. lucidum* in India [9-11]. The scope of the market for *Ganoderma* based products is enormous in national and international markets both but the paucity of knowledge on its cultivation practices is the main constraints.

The aim of this paper is to collect knowledge on mycelial growth using various media and also different attempts regarding spawn production on various substrates were made to find out the best substrate for spawn production and cultivation.

The materials used in the study were the fruiting bodies of *G. lucidum* collected from Glen forest of District Shimla (Himachal Pradesh) in North-West India. It was taxonomically identified following Lincoff [12] and the specimen has been deposited in the Herbarium of Biosciences Department, Himachal Pradesh University, Shimla. The pure culture was raised on the Potato Dextrose Agar medium by tissue culture method.

Both solid as well as liquid media were evaluated for the vegetative growth of *G. lucidum*. The composition of each media used was based on Tuite [13]. Ten solid media, used for evaluation were Yeastal Potato Dextrose Agar (YPDA), Potato Dextrose Agar (PDA), Coriander Extract (CE), Maize Grain Extract (MGE), Wheat Grain Extract (WGE), Malt Yeast Agar Extract (MYAE), Pridham Yeast Malt

Dextrose Medium (PYMD), Czapek Dox Agar (CDA), Malt Agar Extract (MAE) and Dimmick Agar Extract (DAE). Four liquid media, Glucose Asparagine Solution (GAS), Czapek's Solution (CS), Dimmicks Solution (DS) and Asthana and Hawker's Solution (AHS) were used during experimentation for making a comparative observation on vegetative growth of *G. lucidum*.

To measure the growth rate of mycelium in various solid media, the diameters of mycelial colonies were measured in cm scale and the average growth of mycelium was calculated. The mycelial growth rate in various liquid media was calculated by harvesting mycelial mat from each flask, washed and dried at 65 °C for 24 h. The dry weight of mycelium was recorded for two subsequent days and an average was taken as the actual weight.

Different types of grain substrates were evaluated to see their effect on spawn development of *G. lucidum*. Spawn was prepared using cereal grains (maize, barley, wheat, pearl millet and soybean) as substrates. The grains were washed, boiled and filled in polypropylene bags plugged with non-absorbent cotton, and sterilized in an autoclave. Thereafter, these bags were inoculated with equal sized mycelial bit of pure culture, and incubated at 25±2 °C and observations were recorded when the mycelium covered the entire grains in every treatment. Three replications were kept in each treatment.

The cultivation experiment was done using oak sawdust and wheat bran mixture (4:1 w/w). The substrate media were mixed homogeneously. The pH was stabilized by gypsum and CaCO₃ at 5.5-6.5 [14]. After inoculating with the barley and pearl millet based spawn the bags were kept in the dark at 25 °C for spawn run with 80-85% relative humidity. After few days of spawning, small buttons appear, at this stage, polyethene top is cut at the level of the substrate totally exposing the top side. Proper conditions for fruiting were provided in the cropping chamber. Relative humidity was maintained at 95 % and temperature of the chamber was kept around 28-30 °C with a 10 h light exposure. The cap formation of *G. lucidum* initiated in 2-3 d. Fruiting bodies were harvested when the caps become completely red and the white margins disappeared [15]. Fresh weight and dry weight of mushrooms harvested in each flush were recorded. The mean colony diameter of the mycelium

(±SD) in different solid media is graphically represented in fig. 1. The mean colony diameter of the mycelium was maximum on Malt Agar Extract (8.1±0.06 cm), followed by Maize Grain Extract, Pridham Yeast Malt Dextrose Medium, Yeastal Potato Dextrose Agar, Potato Dextrose Agar Medium, Coriander Extract, Malt Yeast Agar Extract, Wheat Grain Extract, Dimmick Agar Extract and Czapek Dox Agar Medium respectively. The average dry weight of the mycelium on Glucose Asparagine Solution (145.60±2.64 mg) was maximum, followed by Czapek's Solution, Dimmick Solution and Asthana and Hawker's Solution respectively (fig. 2).

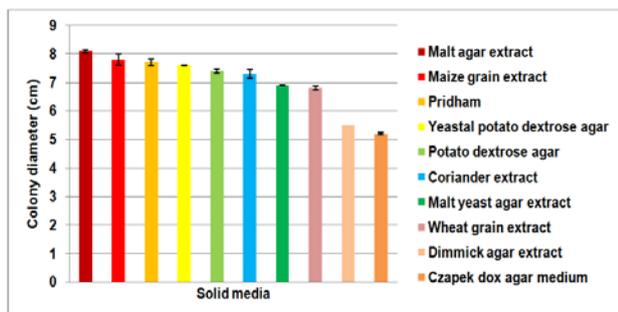


Fig. 1: Average colony diameter of *G. lucidum* on different solid media

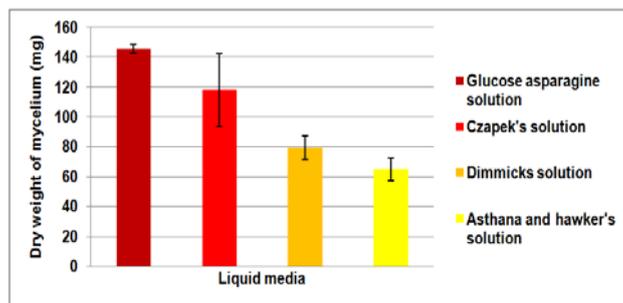


Fig. 2: Average weight of mycelium of *G. lucidum* in different liquid media

Among the five grain substrates used for spawn production, minimum (8 d) period was recorded in barley grains where grains were completely covered by the mycelium and tightly held, followed by pearl millet, wheat and maize. However, soybean grains took maximum (20 d) with poor mycelia growth and grains were not fully covered (table 1, fig. 3).

On sawdust and wheat bran mixture as a substrate while using pearl millet and barley as a spawn source, the spawn run was completed in 23.3 d and 23.8 d respectively. Pinheads were developed in 32.8 and 32.0 d respectively. The highest yield was obtained on the substrate having barley as spawn source (65.24 g/kg) (table 2).

Table 1: Evaluation of different grain substrates for spawn development of *G. lucidum*

Grain substrate	Spawn development (days)*	Mycelial characters
Wheat	10	White cottony compact mycelia growth, all grains were completely colonised by the mycelium and tightly held with each other
Pearl Millet	9	White cottony compact mycelia growth, all grains were completely colonised by the mycelium and tightly held with each other
Barley	8	White cottony compact mycelial growth, all grains were completely colonised by the mycelium and tightly held with each other
Maize	15	Mycelial growth was poor, and grains were not fully covered
Soybean	20	Very poor mycelial growth and grains were not fully covered

* Average of three replications

Table 2: Effect of cereal grains on spawn run and yield of *G. lucidum* on Oak sawdust

Spawn source	Spawn run (days)**	Pinhead stage (days)**	Yield (g/kg)
Pearl millet	23.3	32.8	59.72
Barley	23.8	32.0	65.24

**Average of three replications



Fig. 3: Spawn growth on various grain substrates. A. Wheat, B. Pearl millet, C. Barley, D. Maize, E. Soybean

On comparing the previous work on other mushrooms, Potato Dextrose Agar and Yeast Malt Agar found the best medium for *Ganoderma applanatum* [16]. Malt extract medium was found best for radial growth of *Lentinus cladopus* [17].

Maximum growth of *L. squarrosulus* was observed on yeast glucose medium [18] and malt broth was found best for *L. cladopus* [17]. In our results, maximum growth of mycelium in Glucose Asparagine Solution could be attributed to asparagine, which as a nitrogen source can enhance the growth of mycelium in culture whereas other synthetic media do not contain asparagine, hence show poor growth of mycelium.

Cereal grains and millets are generally used as spawn substrates. They act as a reservoir of carbohydrates; which offer sufficient nutrition for mycelia growth and provide the vehicle for eventual distribution of mushroom inoculants [19]. Barley grains were best for the spawn development and took only 8 d for spawn development as compared to other substrates.

Days required for complete colonization of the substrate was minimum with pearl millet. After seeding the substrate, pearl millet grain spawn allows a quick spreading of mycelium from a small propagation centre because, small grains provide more points of inoculum per gram of spawn [19, 20]. The present findings are very close to the results obtained by earlier workers [21-25]. Oak

sawdust and wheat bran combination was used in the present study and yield of the mushroom was also in conformity with some other authors who reported hardwood sawdust as a preferred substrate for the commercial production [14, 15].

Based on the investigations, mushroom growers are advised to maintain the pure culture of *G. lucidum* on Malt Agar Extract Medium and develop spawn on barley grains. Cultivation of *G. lucidum* with enormous pharmacological value is highly recommended in India. The authors express their sincere thanks to the Chairman, Department of Biosciences, HPU, Shimla for providing laboratory facilities. Financial support from UGC, (F.17-40/08 SA-1), New Delhi is also gratefully acknowledged.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Yang FC, Liau CB. Effect of cultivating conditions on the mycelial growth of *Ganoderma lucidum* in submerged flask cultures. *Bioprocess Eng* 1998;19:233-6.
2. Wagner R, Mitchell DA, Sasaki GL, Amazonas MALA, Berovic M. Current techniques for the cultivation of *Ganoderma lucidum* for the production of biomass, ganoderic acid and polysaccharides. *Food Technol Biotechnol* 2003;41:371-82.
3. Fang QH, Zhong JJ. Effect of initial pH on the production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. *Process Biochem* 2002;37:769-74.
4. Hsieh C, Yang F. Reusing soy residue for the solid-state fermentation of *Ganoderma lucidum*. *Bioresour Technol* 2004;91:105-9.
5. Chen KL, Chao DM. Ling Zhi (*Ganoderma* species). In: KT Hsu. Ed. *Chinese Medicinal Mycology*. United Press of Beijing Medical University and Chinese United Medical University, Beijing, China; 1997. p. 496-517.
6. Chen AW. Cultivation of the medicinal mushroom *Ganoderma lucidum* (Curt. Fr.) P Karst. (Reishi) in North America. *Int J Med Mushrooms* 1999;1:263-82.
7. Olei P. *Mushroom Cultivations*. Leyden, the Netherlands, Backhuys Publishers; 2003. p. 429.
8. Wasser SP. Reishi or Ling zhi (*Ganoderma lucidum*). *Encyclopaedia of Dietary Supplements*; 2005. p. 603-22.
9. Rai RD. Successful cultivation of medicinal mushroom *Ganoderma lucidum* (Reishi). *Mushroom Res* 2003;12:87-91.
10. Veena SS, Pandey M. Evaluation of the locally available substrate for the cultivation of indigenous *Ganoderma lucidum* isolate. *J Mycol Plant Pathol* 2006;36:434-8.
11. Nithya M, Ambikapathy V, Panneerselvam A. Cultivation of *Ganoderma lucidum* (Curt. Fr.) P. Karst. *Int J Sci Res* 2014;3:39-41.
12. Lincoff G. *Guide to Mushrooms*. A Fireside Book, Published by Simon and Schuster, New York; 1981. p. 512.
13. Tuite J. *Plant pathological methods, Fungi and Bacteria*. Burgess Publishing Company. Minn. USA; 1969.
14. Smith JE, Rowan NJ, Sullivan R. Medicinal mushroom: A rapid developing area of biotechnology for cancer therapy and other bioactivities. *Biotechnol Lett* 2002;24:1839-45.
15. Royse DJ. *Specialty mushrooms*. ASHS Press: Arlington VA; 1996. p. 464-75.
16. Woo-Sik J, Yun-Ju C, Doo-Hyun C, So-Deuk P, Young-Bok Y, Soon-Ja S. Culture conditions for the mycelial growth of *Ganoderma applanatum*. *Mycobiology* 2009;37:94-102.
17. Atri NS, Lata. Studies for culturing and cultivation of *Lentinus cladopus* Lev. *Mycosphere* 2013;4:675-82.
18. Atri NS, Upadhyay RC, Singh R, Saini MK. Effect of culture media and physical factors on vegetative growth of *Lentinus squarrosulus* (Mont.) Singer. *Punjab Academy Sci* 2007;4:1-2.
19. Siddhant, Yadav S, Singh CS. Spawn and spawning strategies for cultivation of *Pleurotus eous* (Berkeley) Saccardo. *Int J Pharm Chem Sci* 2013;2:1494-500.
20. Bahl N. *Handbook on Mushrooms*. Oxford and IBH Publishing Co. New Delhi; 1984.
21. Mathew AV, Mathai G, Suharban M. Performance evaluation of five species of *Pleurotus* (Oyster mushroom) in Kerala. *Mushroom Res* 1996;5:9-12.
22. Hafeez ZMK, Shawani M, Shakeel, Hafiz-ul-Allah. Effects of temperature on the linear colony and spawn growth of *Pleurotus florida* (Strain Yelova-1). *Balochistan J Agric Sci* 2000;1:48-51.
23. Sharma BB. Effect of different substrates grains/straws on spawn growth and yield of pink oyster mushroom *Pleurotus djamor* Boedijn. *J Mycol Plant Pathol* 2003;33:265-8.
24. Sagir A, Yildiz A. Growth of the mycelium of *Pleurotus* sp. on different grains and determination of their competition with some contaminant fungi. *Acta Aliment* 2004;33:249-57.
25. Asghar R, Tariq M, Rehman T. Propagation of *Pleurotus sajor-caju* (oyster mushroom) through tissue culture. *Pak J Bot* 2007;39:1383-6.