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STUDIES ON DIFFERENT GROWTH PARAMETERS OF *Ganoderma lucidum*

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Abstract: *Ganoderma lucidum* (W. Curt: Fr.) P. Karst is one of the most popular mushrooms in oriental medicine. *Ganoderma Lucidum* is a species of the class Basidiomycetes, which belongs to the family Polyporaceae/Ganodermataceae of the order Aphyllophorales. Polysaccharides, peptidoglycans, and triterpenes are three major physiologically active constituents present in *G. lucidum* which are responsible for medicinal properties. The present investigation was conducted on its different growth parameters. The different isolates/strains were collected, cultured and identified on the basis of basidiocarp and basidiospore morphology. The cultures of different isolates were studied for their radial growth at different temperatures and pH. Malt extract medium, temperature range of 25-35˚C and acidic pH (5.0) were conducive for the mycelial growth of *G. lucidum*.
Keywords: *Ganoderma lucidum*, morphology, malt extract medium, temperature, pH.

Introduction

Ganoderma lucidum (*M.A Curtis: Fr.*) P. Karst is a species of the class Basidiomycetes, which belongs to the family Polyporaceae (or Ganodermataceae) of the order Aphyllophorales. It is known as "Ling Zhi" in china and "Reishi" or Mannentake in Japan means "Herb of Spiritual Potency" (Wagner *et al.* 2003).

Ganoderma Lucidum is commonly known as wood-decaying fungus; it causes white rot of a wide variety of trees and can thus be described as a phytopathogenic fungus. It is one of the most desired medicinal mushrooms and has been used for more than 2000 year. It is common in the tropical and warm temperate areas of India. *G. lucidum* is most commonly cultivated in China, Taiwan, Japan, Korea, Malaysia and North America.

Global production of *Ganoderma* was about 4900-5000 tonnes in 2002, out of which 3800 tonnes were produced in China (Lai *et al.* 2004). It is being cultivated artificially in over ten countries with an annual production of 4300 tonnes. World trade of this mushroom is around 2 billion dollar and in India, annual market for *Ganoderma* based nutriceuticals is estimated to be about Rs 120 crores (Anonymous 2002). USA is the biggest market for medicinal

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mushrooms and their products. The fruit body of *Ganoderma lucidum* is sold in the market @ Rs 600-700/Kg.

Ganoderma has a woody consistency and possesses chemicals which offer many health benefits. 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. It produces several metabolites such as polysaccharides, peptidoglycans and terpenoids, which are responsible for medicinal properties. Besides promoting longevity, *Ganoderma* has unique property of strengthening the immune system. *Ganoderma* products are available in various forms such as powders, tablets, capsules and syrups.

Ganoderma lucidum has been cultivated by using several different substrates and by maintaining growth parameters such as temperature, relative humidity, water content, air pH and light intensity (Chang and Miles, 2004). Hence, light and pH are the most important parameters for the mycelial growth of *Ganoderma lucidum.* The mycelial growth depends on some factors such as culture media, pH, temperature and nutrient elements. These factors greatly influenced the growth of fungi in both field and laboratory conditions. For this reason, it is very important to evaluate these factors for the optimum mycelial growth of *Ganoderma lucidum*. In this paper we studied the mycelial growth of *G. lucidum* on malt extract medium, at different temperature and pH under laboratory conditions.

Material and Methods

Collection, Identification and Maintenance

Different strains/isolates of *G. lucidum* were collected from the wild and also procured from DMR, Solan and IIHR, Banglore for undertaking the present study.

Different isolates of *Ganoderma lucidum* were identified on the basis of morphological and microscopic characteristics using standard description of the species. Isolation from the fresh specimens collected from wild was made following standard tissue culture technique. Young and fresh specimens were first washed with a jet of sterile water and then cut across the pileal region with the help of sterilized sharp blade and bits (2-3 mm) of tissue were taken using sterilized forceps and dipped in 0.1 per cent mercuric chloride solution for 5-10 seconds. These were repeatedly washed with sterile distilled water (5 washing) and placed on sterilized filter paper to remove excess moisture. The bits were then transferred on to Malt Extract Medium aseptically with the help of sterilized inoculating needle and were incubated

at 30± 1ºC for about 7-10 days for their growth. Stock cultures were maintained in the refrigerator for further studies. Culture was revived after a period of 10-15 days on fresh slants.

Preparation of Inoculum

Starter cultures used in various experiments were obtained from the periphery of actively growing colonies on Malt Extract Medium. For cultural studies 5 mm discs of mycelial mat were cut with the help of a pre-sterilized cork borer. In the experiments on liquid media, the inoculum was prepared by thoroughly homogenizing the mycelial bits from slants in sterilized glass homogenizer. A uniform mycelial suspension (12-16 bits/low field of the compound microscope) was prepared and used throughout the studies. The cultures were incubated at 28±1˚C. Each treatment was replicated thrice.

Measurement of growth

Growth was measured by taking the average of linear growth of colony in three different directions. In liquid media study, mycelial mats were filtered through previously dried and weighed Whatman No. 42 filter paper. Filter papers along with mycelial discs were dried to constant weight in an electric oven at 50˚C, and weighed immediately in a digital electric balance. Average value of three replications in a treatment were worked out and used as quantitative measures for comparing the growth under different treatments.

Effect of temperature

Effect of different temperatures *viz*., 5˚C, 10˚C, 15˚C, 20˚C, 25˚C, 30˚C and 35˚C on the mycelial growth was studied in B.O.D incubators maintained at different temperatures. Each treatment was replicated three times. The data in terms of colony diameter, color and growth patterns were recorded after 7 days of incubation when the plate was completely colonized.

Effect of pH on growth

Different pH levels of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 were tested for the optimum growth of *Ganoderma lucidum*. The pH of the medium before and after sterilization was adjusted with the help of systronic pH meter by adding either N/10 NaOH or N/10 HCl. 25 ml of medium was poured into 100 ml Erlenmeyer flasks, plugged, sterilized and inoculated with 1 ml of standardized mycelial suspension. For each treatment, there were three replications and the flasks were incubated at 30±1˚C. At the end of growth period (10 days), the mycelium was filtered through previously dried and weighed filter paper (Whatman No. 42) with the help of suction pump. Malt Extract Medium was used as basal medium.

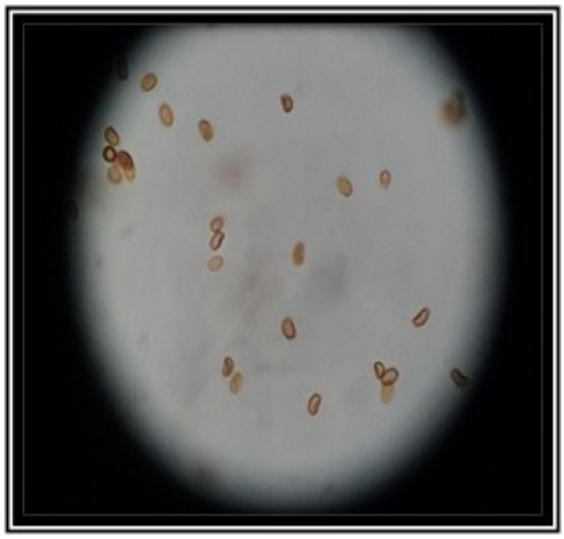
Result and Discussion

Collection

Two samples of *Ganoderma lucidum* were collected during July-August from stems of *Albizzia chinensis* (Osbeck) Merr., locally known as 'Oei' and from dead wood of angiospermous trees.(Plate1). Two cultures of *G. lucidum* were procured from DMR, Solan and two from IIHR, Banglore. (Table 1). *G. lucidum* has been reported to grow on wide range of host species. It grows near stumps of oak and broad leaved trees species in summer and autumn in the wild at a temperature of 25-30˚C. It may also grow saprophytically or parasitically on logs (Singh *et al*. 2007).

Out of five strains/isolates, four *viz.* GLI, PLP-2, OE-52 and OE-53 were selected as representative isolates for conducting preliminary cultural studies.

A. Fruiting body



B. Basidiospores

C. Culture in petriplates

D. Culture in slants

Plate 1: Morphological and microscopic characteristics of *G. lucidum*

Morphology

Different isolates collections were studied in details and identified following standard description of the species as under:

Fruit bodies are usually large, stipitate, dimidiate, rarely suborbicular, reddish brown, lateral and upper surfaces coated with hard shiny substance resembling with sealing wax. Pileus 2.0-5.0 cm broad, pileal surface rough, reddish brown in colour. Stipe 1.5-4.5 cm long and 0.5-2.0 cm thick. Pileus surface often appeared varnished. Basidiospores brown, ovate, with a rounded base and truncate to narrowly rounded apex; spore surface slightly too strongly dimpled; wall composed of several layers. Outermost wall connected to the inner wall by inter-wall pillars; basidiospores 10-12 x 6.5-8.0 µm in size.

All the isolates were found to be typical of *G. lucidum*. The basidiocarp and basidiospores morphology has also been studied by Pegler and Young (1973) and Adoskaveg and Gilbertson (1986). Furtado (1965) reported that the varnished appearance of the basidiocarp of many polypores is due to an amorphous substance secreted by the hyphae and the characteristic shiny cover is seen particularly in isolates of *Ganoderma*.

Raising of pure culture

Pure culture of *G. lucidum* was raised on Malt Extract Medium following tissue culture method. Malt extract medium has also been reported to support good growth of *G. lucidum* by earlier workers also (Biley *et al*. 2000 and Curvetto *et al*. 2002). Potato Dextrose Agar has also been also found to be good medium but it takes slightly more time (Biley *et al.* 2000).

Effect of Temperature

Temperature is one of the important factors for the growth of fungi. Mycelial growth pattern of four strains/isolates of *G. lucidum* was recorded at six different temperatures *viz.* 10˚C, 15˚C, 20˚C, 25˚C, 30˚C and 35˚C for 7 days on Malt Extract Medium when the plate was completely colonized. The data recorded is presented in Table 2.

The data (Table 2) indicate that a temperature range of 25-35˚C is optimum for most of the *G. lucidum* isolates/strains except PLP-2 which preferred a temperature range of 25-30˚ C, respectively for its optimum mycelial growth. The CRD analysis of data at 5 per cent level of significance showed that strain OE-53 exhibited maximum mycelial growth at temperature 30˚C among all the strains at different temperature levels (Plate 2).

The optimum temperature of 25-30˚C has been reported by Song *et al.* (2007). Similarly, a temperature of 30-35˚C has also been found suitable for growth of *G. lucidum* (Rai 2003 and Veena and Pandey 2006). Earlier findings are comparable to the temperature requirement for

vegetative growth of the present isolate/strains. The wide range of variation in temperature requirement can be attributed to ecological diversity of *G. lucidum.*

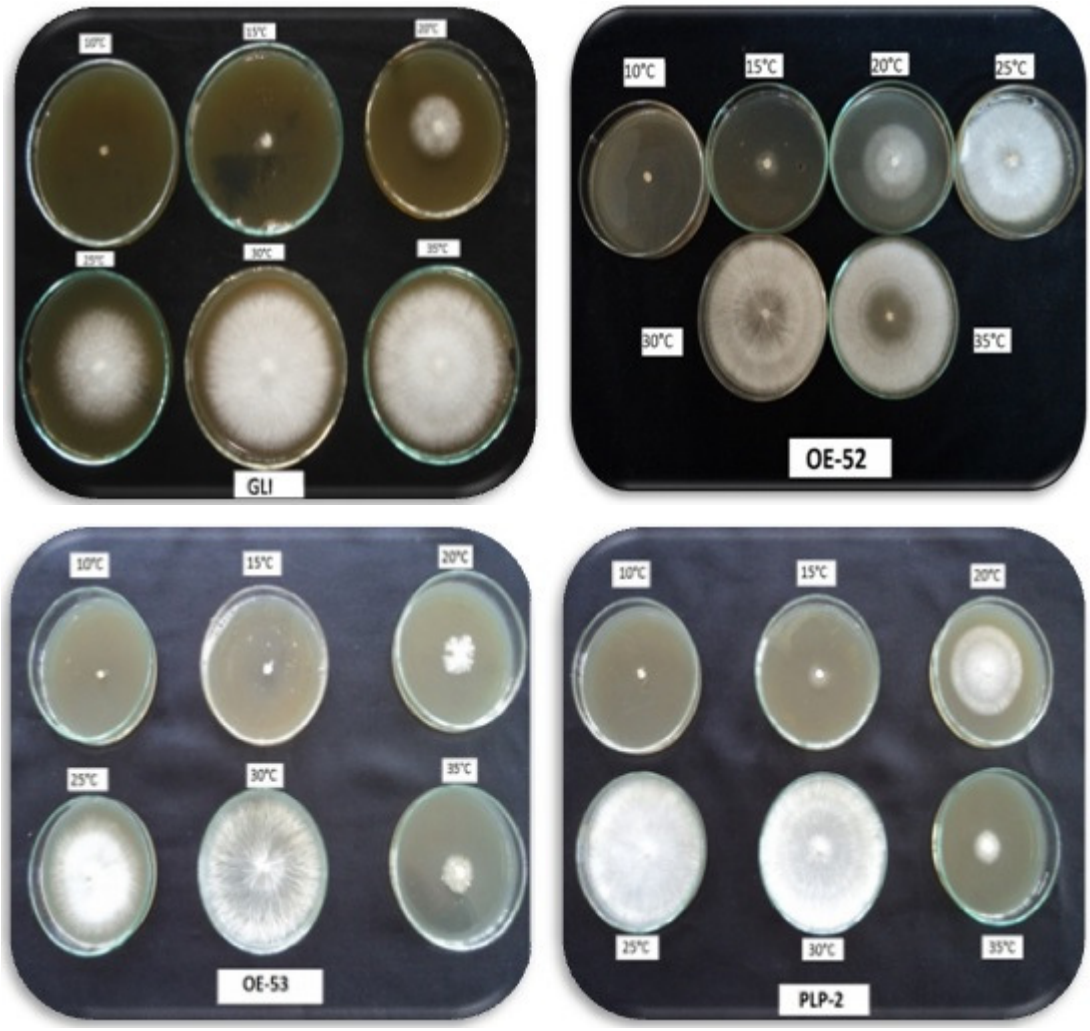


Plate 2: Effect of different temperature on mycelial growth of various isolates/strains of *Ganoderma lucidum*

Effect of pH

pH is also an important factor for the growth of fungi. An experiment was, therefore, conducted to find out the optimum pH for the growth of OE-53 strain of *G. lucidum* on Malt Extract Broth Medium. Different pH levels ranging from 3.0 to 12.0 were tested with three replications each. The data were recorded in terms of average dry mycelial weight after 10 days of incubation at 30˚C and presented in Table 3. The CRD analysis of data at 5% level of significance showed that pH 5.0 is best for the growth of mycelium and on the alkaline side, there was a sudden drop. However, the test fungus could grow over a wide range of pH between 3.0 to 11.0.

Both lower and higher levels of pH showed adverse effect on the mycelial growth (Plate 3). Rai (2003) have reported mycelial growth of the *G. lucidum* at acidic pH. Veena and Pandey (2006) reported pH range of 4.0- 6.5 to be the best for growth of *G. lucidum*.

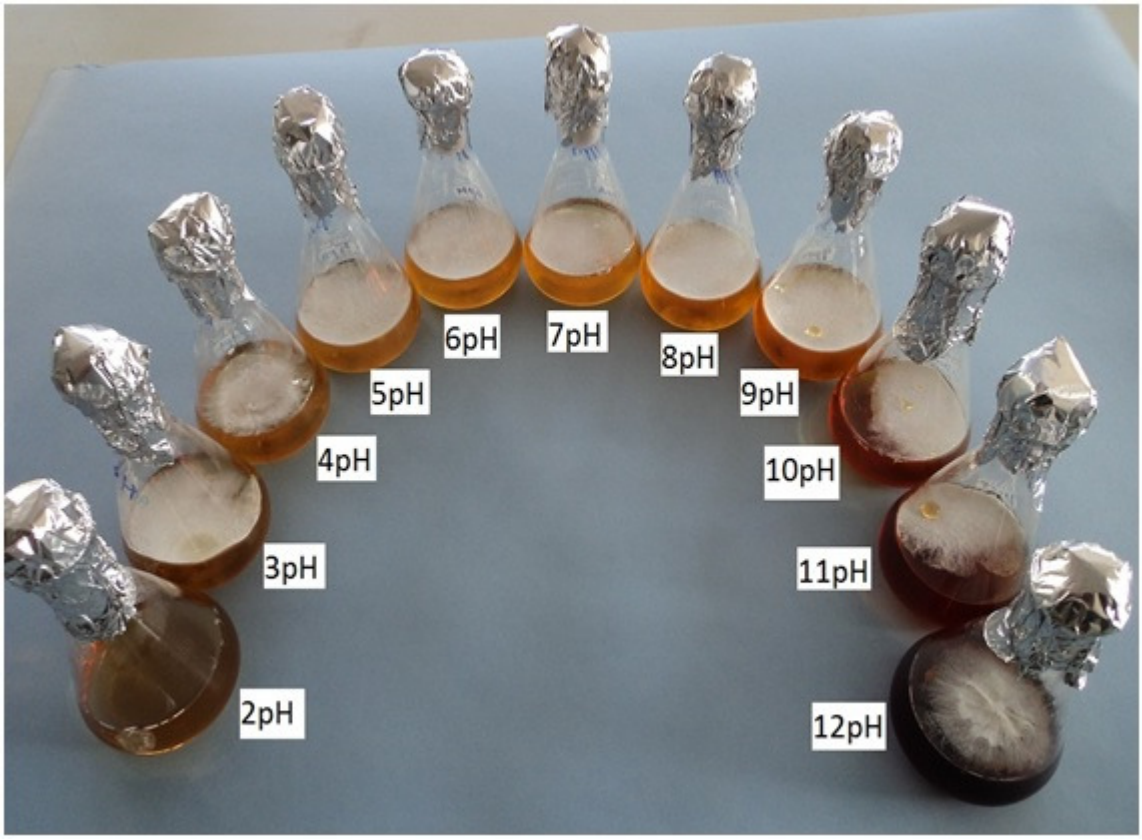


Plate 3: Effect of different hydrogen ion concentrations on the mycelial growth of strain OE-53 of *Ganoderma lucidum*

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Table 1: Procurement of different strains/isolates of *Ganoderma lucidum*

S. No.	Strains/ Isolates	Host/Habitat	Location/Source
1	GLI	On angiospermous wood	IIHR,Bangalore
2	PLP-1	Tree trunk of *Albizzia chinensis*	Palampur
3	PLP-2	-do-	Palampur
4	OE-52	-	DMR, Solan
5	OE-53	-	DMR, Solan

Table 2: Effect of temperature on the mycelial growth of various isolates/strains of *Ganoderma lucidum*

S.No.	Isolates/ Strains	Mean Diameter in (mm)*						Growth Type at 30˚C
		10˚C	15˚C	20˚C	25˚C	30˚C	35˚ C	
1	GLI	10.6	24.3	66.3	67.3	77.3	79.3	Growth slow, strandy, white
2	OE-52	3 .3	15.6	47.3	82.0	83.6	84.0	Growth fast, strandy, white
3	OE-53	0.0	3.0	21.3	83.0	85.0	36.6	Growth fast, strandy, white
4	PLP-2	0 .0	14.3	55.3	83.0	84.0	23.3	Growth fast, strandy, white
	CD (5%)	0.07	0.09	0.01	0.05	0.08	0.18	

***Data recorded after 7 days of Inoculation**
***Average of three replication**

Table 3: Effect of different hydrogen ion concentrations on the mycelial growth of a strain OE-53 of *G. lucidum*

pH	Average mycelial dry weight (mg)*	Mycelial characteristics
2	50.00	Very poor growth, very thin, incomplete mycelial mat
3	83.33	Mat incomplete, strandy, cottony, whitish growth, growth poor
4	96.66	Mat incomplete, strandy, cottony, whitish growth, growth poor

5	173.33	Mat complete, strandy, cottony, growth very good
6	153.33	Mat complete, strandy, cottony, growth very good
7	133.33	Mat incomplete, thick, strandy, cottony, whitish growth, growth good
8	126.66	Mat incomplete, thick, strandy, cottony, whitish growth, growth good
9	106.66	Mat incomplete, thin, strandy, cottony, whitish growth, growth poor
10	86.66	Mat incomplete, thin, strandy, cottony, whitish growth, growth poor
11	66.66	Mat incomplete, thin, strandy, cottony, whitish growth, growth poor
12	40.00	Mat incomplete, thin, strandy, cottony, whitish growth, growth very poor
CD (5%)	12.00	

***Average of three replication**