

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.901.299>

Morphological and Cultural Characteristics of Different Collections of Medicinal White-Rot Bracket Fungi *Ganoderma* P. Karst

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ABSTRACT

Ganoderma is widely distributed mushroom with the longest record of medicinal use. Specimens of *Ganoderma* were collected from several locations in Navsari. These were identified on the basis of macroscopic and microscopic traits of fruiting bodies and biological properties of pure cultures. A total of seven *Ganoderma* isolates were collected and named based on genus, place and locality. These were GNN-1, GNN-2, GNN-3, GND-1, GND- 2, GNG-1 and GNG-2. The shape of basidiocarp varied from auriform to discoid, colour from light to dark brown; they were either sessile or stipitate with double walled ellipsoid brown coloured basidiospores and pore layers were white or brownish white coloured. The colour of mycelia varied from white to off white colour and density varied from cottony to velvety. Thick walled and elliptical to spherical shaped chlamydospores found in four isolates and the position was varied from terminal to intercalary. The maximum growth was recorded in Mushroom Complete Medium (MCM) media viz., 7.20cm, 6.67cm, 6.63cm and 5.97cm in GNN-2, GNG-1, GND-1 and GNN-1, respectively on fifth day of inoculation. Mycelial growth of GNN-2 was significantly more as compared to rest of the isolates which covered the petriplate earlier to all i.e. on sixth day.

Keywords

Ganoderma,
Medicinal
mushroom,
Morphology,
Cultural characters,
Basidiocarp

Article Info

Accepted:
20 December 2019
Available Online:
20 January 2020

Introduction

Ganoderma P. Krast is one of the most popular mushrooms in oriental medicine. It is found in tropical, sub-tropical, and temperate climates all around the world but prefers warmer climate on a large variety of trees such as deciduous and hardwood trees. The diverse climatic condition in India made the country a natural habitat for *Ganoderma*. This is

probably the first medicinal mushroom to gain importance in India. The genus *Ganoderma* is identified on the basis of their basidiocarp morphology, microscopic view of basidiospore and mycelium. *Ganoderma* are fairly large with a generally tough bracket like pileus (cap) that can reach more than 60 cm (24 inches) in diameter. Many species have a stipe (stalk) to support the pileus, some species are sessile and have a pileus attached

directly to the tree or dead stump or log. The pileus can be white, yellow, brown, red, or deep purple, often with a light-coloured border, though coloration patterns within species can vary depending on age and environmental conditions. They develop a bright, white, outer growth margin while growing. As growth continues, the margin may begin to change color and develop a tough skin that has a shiny lacquered appearance in many species. It is characterized by the presence of pores instead of the gills on the lower side of the fruiting body (Wang *et al.*, 2012, Kapoor and Sharma, 2014, Nithya *et al.*, 2014 and Rajesh *et al.*, 2014). Macro- and microscopic features of these *Ganoderma* basidiocarps are well investigated, but only a few data are available on their cultivation *in vitro* (Woo-Sik *et al.*, 2009, Badalyan *et al.*, 2015). Pure cultures of *Ganoderma* offer important taxonomic features and can be used in biotechnology and biomedicine (Balaes and Tanase, 2012, Badalyan *et al.*, 2015). In addition, some molecular analyzes for identification are only possible with the use of pure cultures – e.g. MALDI-TOF MS (Pristas *et al.*, 2017). Thus, we would like to contribute to knowledge of the mycelial characteristics of *Ganoderma* species along with the morphological characteristics. Thus, the aim of this study is to describe the macro- and microscopic characteristics of fruiting bodies as well as biological properties of pure cultures (color, shape, texture of the colonies) along with growth rate of mycelium of *Ganoderma* species.

Materials and Methods

Morphological characterization of *Ganoderma* isolates

Survey was carried out during June 2018 to September 2018 for collection of naturally growing *Ganoderma* at several locations of Navsari *viz.*, Navsari Agricultural University campus, Dandi and Gandevi. Collected

samples were named based on genus, place and locality. These are GNN-1, GNN-2 and GNN-3 were collected from Navsari Agricultural University campus, GND-1 and GND- 2 two were from Dandi and GNG-1 and GNG-2 two were from Gandevi. They were identified on the basis of macroscopic traits *viz.*, shape, size and colour of pileus, presence or absence of stipe, colour and length of stipe and microscopic traits *viz.*, size and shape of basidiospores.

Isolation of *Ganoderma* mycelium

The collected specimens were isolated using tissue culture technique (Kapoor and Sharma, 2014). Small pieces of basidiocarp (1-2 mm) were cut and surface sterilised with 0.1 per cent mercuric chloride solution for 10 to 20 seconds and plated on potato dextrose agar (PDA) and malt extract agar (MEA) media in Petri plates. Visible mycelia grown from the pieces of basidiocarp were subcultured onto fresh MEA media in Petri plates.

Diametric growth of different *Ganoderma* isolates on different media

Five different media *viz.*, Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Tryptone Glucose Extract Agar (TGEA), Yeast Malt Extract Agar (YMEA) and Mushroom Complete Medium (MCM) were taken to study the mycelial growth pattern of *Ganoderma*. Actively growing culture plates of *Ganoderma* mycelium was punched at the advancing edge with a cork borer. A 5mm diameter disc of the mycelium was then transferred into petri plates containing 20ml of each medium and placed at the centre of petri plates.

Statistical Analysis

The data was analysed using CRD and the difference among mean value was tested by

using critical differences (CD) values at 5% level of probability.

Results and Discussion

Morphological characterization of *Ganoderma* isolates

A total of seven *Ganoderma* isolates were collected from Navsari region during rainy season. Out of the seven isolates, three were collected from Navsari Agricultural University campus named as GNN-1, GNN-2 and GNN-3, two were procured from Dandi named as GND-1 and GND-2 and another two were from Gandevi named as GNG-1 and GNG-2. The isolates were named based on genus, place and locality. GNN-1, GNN-2 and GND-1 were found on dead stumps of unknown trees and two isolates GNN-3 and GNG-2 were on soil surface. While rest of the two isolates GND-1 and GNG-1 were found on the stem of Pink shower, *Cassia grandis*. The morphological characteristics varied among the basidiocarp collected within the same locality as well as between the three localities. The morphological features of seven *Ganoderma* isolates are listed in Table 1.

The size, shape (bud shape to discoid or auriform) and colour (white to golden brown or dark brown) of fruiting bodies of seven *Ganoderma* isolates varied with the growth stages. The basidiocarps were either sessile or stipitate. White or brownish white coloured pore layer was found beneath the pileus. Basidiospores were double walled ellipsoid brown in colour and the size varied from 8.7 x 5.3 to 10.7 x 7.1 µm. Reddish brown coloured spore print was obtained from isolate GNN-3. The present result supports the reports of Latiffah and Ho (2005), who studied the morphological characteristics of *Ganoderma* and identified the collected samples based on the morphological characters like habitat, shape, size and colour of pileus, size of stipe,

shape and size of basidiospore and pore structure. Smith and Sivasithamparam (2003), who studied the morphological characters of *Ganoderma* and observed that basidiocarps were sessile, dimidate, semicircular or irregular. It typically varies from 5-50cm wide and colour light brown to dark brown. Margins were white while growing, but same colour as crust or occasionally brownish black in older specimens. Pores were pale yellow or orange ageing to dark brown. Basidiospores were truncate to elliptic and 9.1-11.8 µm x 5.5-9.1 µm in size.

Cultural Characteristics

The cultural characteristics of seven *Ganoderma* isolates are listed in Table 2. The colour of mycelia varied from white to off white colour and density of mycelia varied from cottony to velvety. Isolates GNN-2, GNN-3, GND-1, GNG-1 and GNG-2 showed white whereas GNN-1 and GND-2 showed off white colour mycelia. Chlamydospores were thick walled, elliptical to spherical in shape and position varied from terminal to intercalary. Out of seven isolates, four isolates of *Ganoderma* (GNN-1, GNN-2, GND-1 and GNG-1) were selected on the basis of their fast growing nature on MEA for further study on the effect of five different media on the radial growth of mycelium on petri plates.

Diametric growth of different *Ganoderma* isolates on different media

The diametric growth patterns of four *Ganoderma* isolates were listed in Fig. 1.

Isolate GNN-1: It showed off white coloured mycelia with yellow pigmentation and less denser in the centre on petri plates containing PDA while uniformly scattered on rest four media and bead like structures formed on petri plates and scanty and thin mycelia layer formed on TGEA.

Fig.1 Mycelial growth of *Ganoderma* isolates on different media

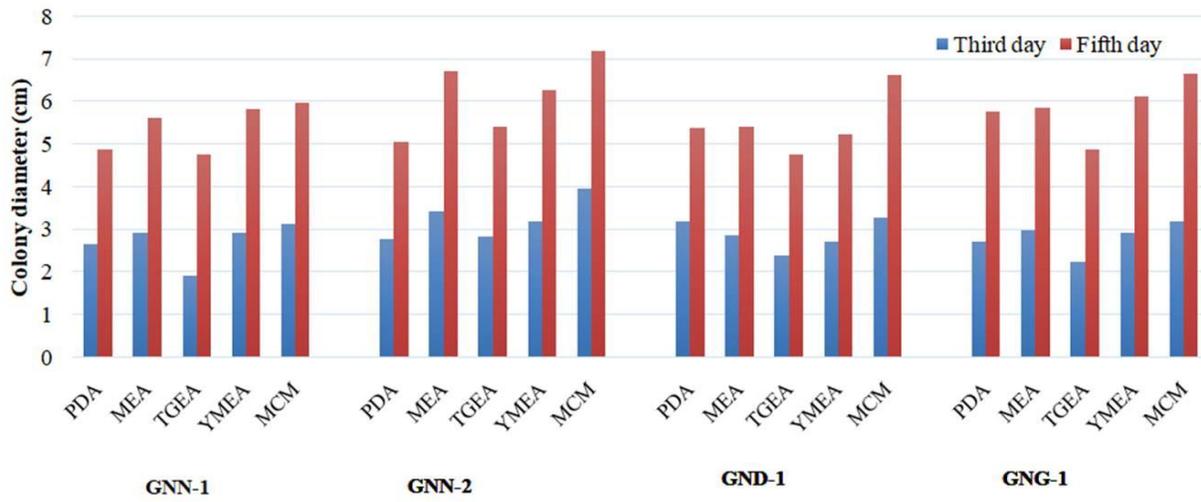
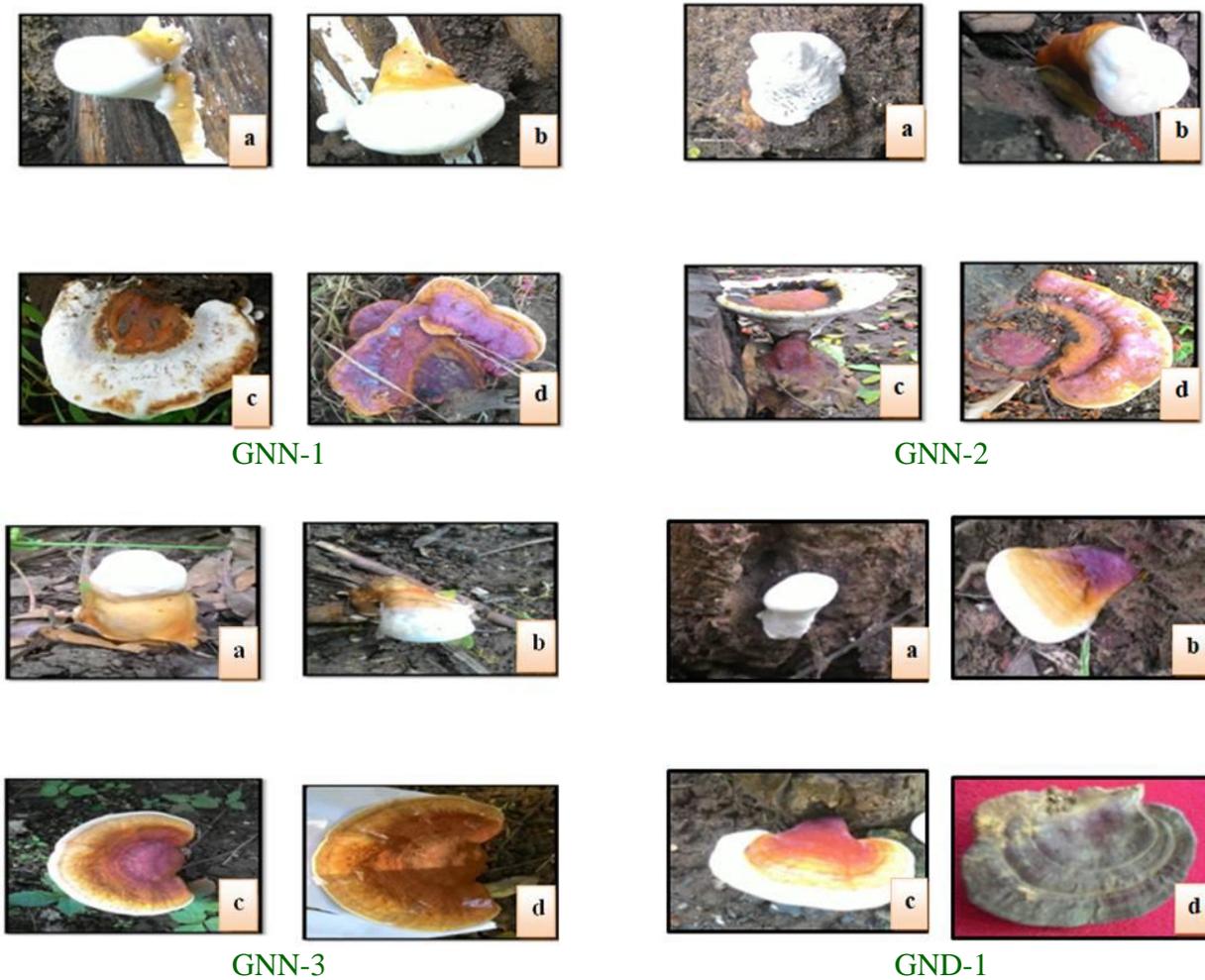
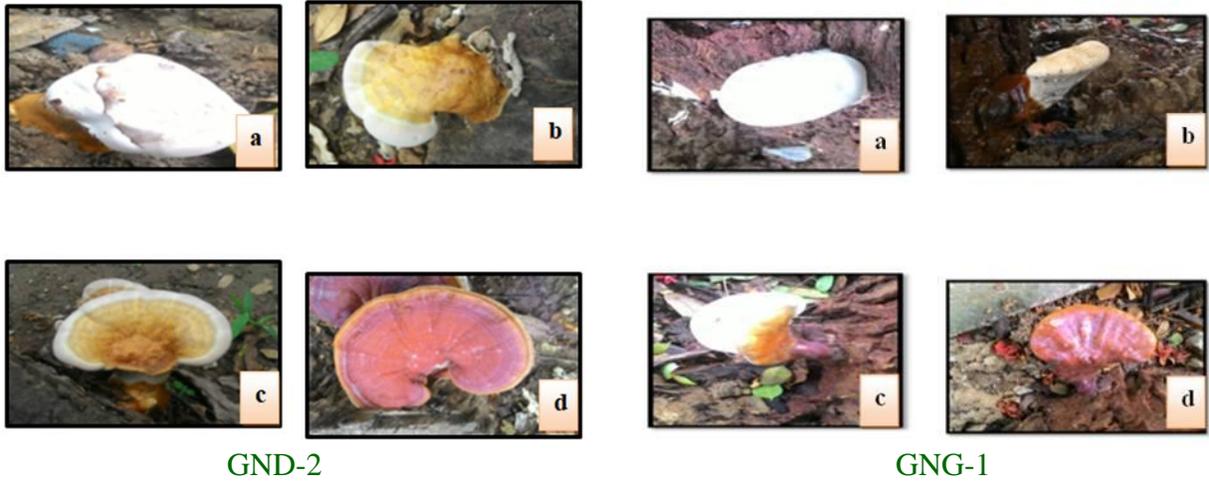


Fig.2 Different growth stages of *Ganoderma* isolates





GND-2

GNG-1



GNG-2

a. Primordia formation, b. Elongation of primordial, c. Flattening and thickening of cap, d. Maturation of cap

Fig.3 Pore layer and basidiospores

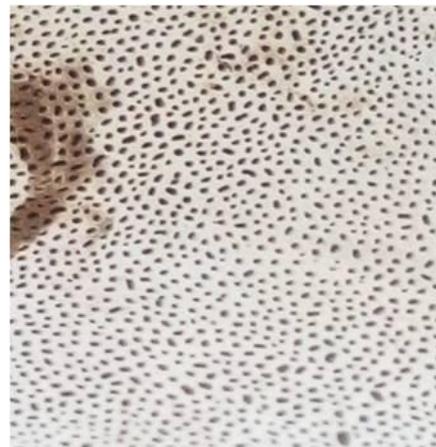
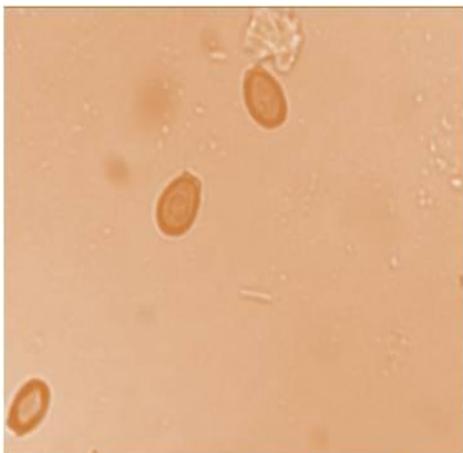


Fig.4 Mycelial growth of *Ganoderma* isolates

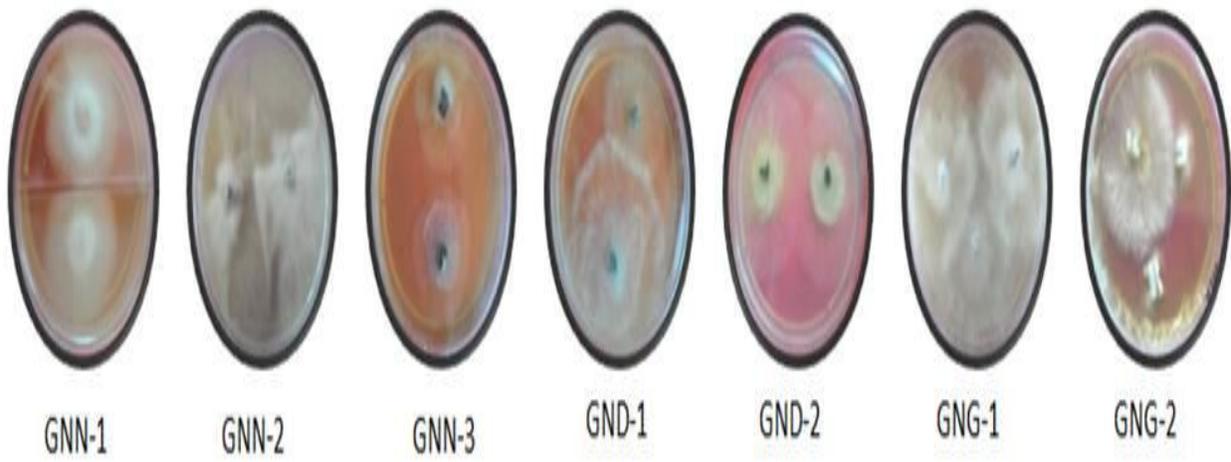


Plate.4 Chlamydospores

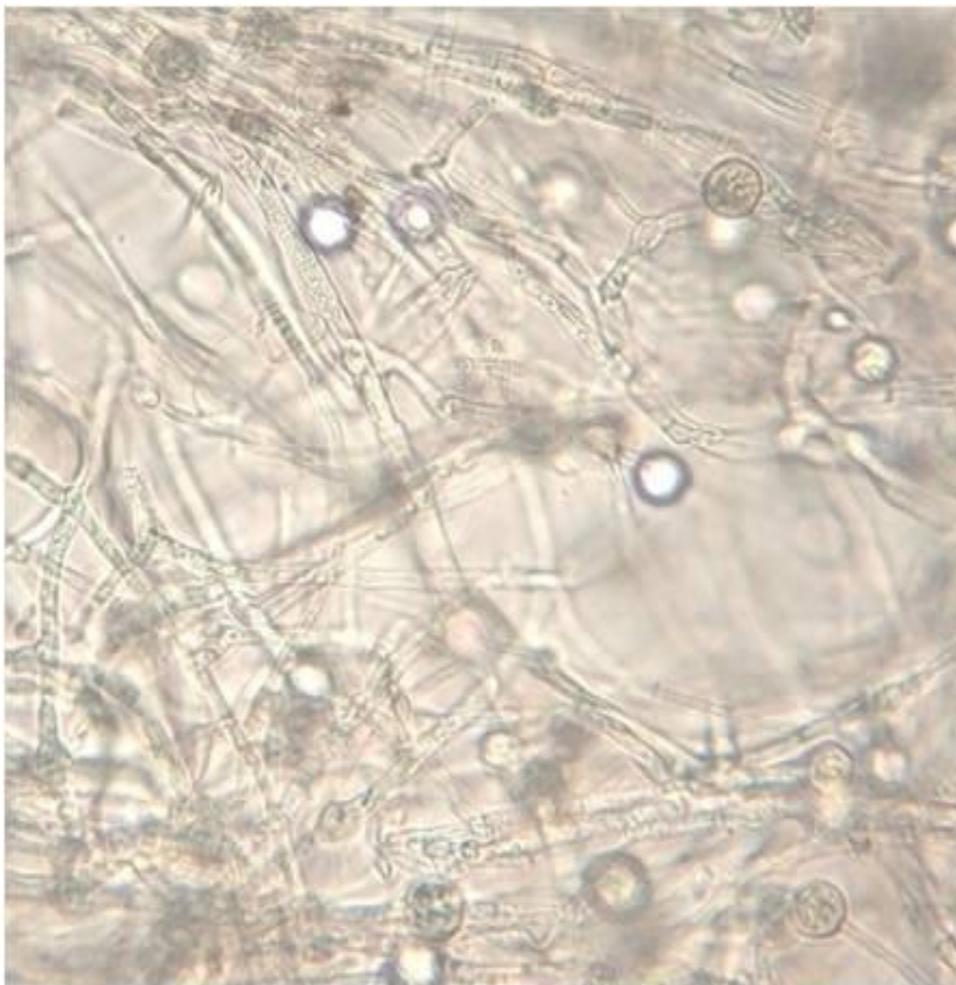


Table.1 Morphological description of *Ganoderma* isolates

Sr. No.	<i>Ganoderma</i> isolates	Pileus				Stipe		Basidiospore		
		Texture	Colour	Shape	Diameter (cm)	Colour	Size (cm)	Shape	Colour	Size (µm)
1	GNN-1	Non-laccate	Dark brown with light brown margin	Auriform	14.2 x 8.4	Absent		Ellipsoid	Brown	9.2-9.8 x 5.6-6.3
2	GNN-2	Non-laccate	Light brown	Discoid	7.5 x 5.8	Dark brown	5.3 x 0.75-1.2	Ellipsoid	Brown	9.3-10.7 x 6.5-7.1
3	GNN-3	Laccate	Light brown	Auriform	9.4 x 5.2	Dark brown	4.3 x 0.6-0.8	Ellipsoid	Brown	9.1-10.21 x 5.7-7.3
4	GND-1	Laccate	Dark brown	Auriform	4.5 x 3.8	Dark brown	2.2 x 1.3	Ellipsoid	Brown	8.7-10.2 x 5.3 -6.5
5	GND-2	Laccate	Dark brown	Auriform	6.5 x 4.8	Dark brown	4.2 x 1.3	Ellipsoid	Brown	8.11-10.21 x 5.6-6.2
6	GNG-1	Laccate	Brown	Auriform	4.7 x 3.5	Brown	2.2 x 1.0-1.3	Ellipsoid	Brown	8.7-10.12 x 5.7-6.3
7	GNG-2	Laccate	Reddish brown with whitish margins	Discoid	22 x 18	Black	12 x 1.5-1.62	Ellipsoid	Brown	9.1-9.28 x 4.87-5.18

Table.2 Cultural characteristics of *Ganoderma* isolates on Malt Extract agar (MEA)

Sr. No.	<i>Ganoderma</i> isolates	Mycelia			Chlamydospore	
		Colour	Density	Type	Shape	Position
1	GNN-1	Off white	Low	Velvety	Elliptical to spherical, thick walled	Terminal or intercalary
2	GNN-2	White	High	Cottony	Elliptical to spherical, thick walled	Terminal or intercalary
3	GNN-3	White	Low	Cottony	Absent	
4	GND-1	White	High	Cottony	Elliptical to spherical, thick walled	Terminal or intercalary
5	GND-2	Off white	Low	Velvety	Absent	
6	GNG-1	White	High	Cottony	Elliptical to spherical, thick walled	Terminal or intercalary
7	GNG-2	White	Low	Cottony	Absent	

Isolate GNN-2: Cotton like texture was observed on all five media with the white colour colonies. On PDA, colonies formed thinner at the center where as on TGEA denser colonies formed at the centre. On MEA, YMEA and MCM, colonies were continuous and dense in the center with dense radial margins.

Isolate GND-1: On TGEA, colonies grew radially and formed thinner zones i.e. scanty mycelial growth on petri plates whereas on rest four media colonies were continuous and formed denser zones. Colonies on all the five media had cotton like texture with white colour.

Isolate GNG-1: Colonies had cotton like texture with white colour and grew radially and formed thinner zones at center with fine rays at the margins on all the five media. Thinner zones were observed in the case of TGEA whereas less or almost nil in the case of MCM and MEA.

Among the media tested, the maximum growth was recorded in Mushroom Complete Medium (MCM) viz., 7.20cm, 6.67cm, 6.63cm and 5.97cm in GNN-2, GNG-1, GND-1 and GNN-1, respectively on fifth day of inoculation. So, it can be concluded from the present investigation that MCM was the best for the mycelia growth of all isolates.

Out of all four isolates GNN-2 mycelia growth was significantly more which covered the petri plate earlier to all i.e. after five days of inoculation while rest isolates covered the petri plates after six days of inoculation on five different media and 7.20cm on MCM on fifth day of inoculation. The present result supports the reports of Jayasinghe *et al.*, (2008) who isolated eight different strains of *Ganoderma* using potato dextrose agar (PDA) and screened the optimal mycelia growth on ten different solid culture media. The trend of

growing of *Ganoderma* on MCM and YMEA was similar with this result. Badalyan *et al.*, (2015) isolated the four isolates on PDA and MEA media and found the different isolates formed phenotypically different colonies on MEA and PDA media.

Acknowledgements

We are thankful to Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari for providing the facility to conduct the study.

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How to cite this article:

Anjali Suansia and Priya John. 2020. Morphological and Cultural Characteristics of Different Collections of Medicinal White-Rot Bracket Fungi *Ganoderma* P. Karst. *Int.J.Curr.Microbiol.App.Sci*. 9(01): 2636-2644. doi: <https://doi.org/10.20546/ijcmas.2020.901.299>