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MORPHOLOGICAL CHARACTERIZATION OF GANODERMA SPECIES FROM MURREE HILLS OF PAKISTAN

Fakhar-ud-Din, Tariq Mukhtar

Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

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ABSTRACT

Proper identification of wild mushrooms is important to study their biodiversity and ecological role. The discovery of new medicinal mushroom species will lead to their exploitation as an industry. No research work has been done about medicinal mushrooms in Murree hills in the past. Keeping in view the importance of *Ganoderma* species, the present studies were carried out to determine the distribution and prevalence of *Ganoderma* species in Murree hills on different host plants. In all, eight species of *Ganoderma* were identified from Murree hills of Pakistan. *Cedrus deodara* and *Eucalyptus citriodora* were found to be the most susceptible hosts of *Ganoderma* species. The identified species were *Ganoderma praelongum*, *G. chalceum* (*Eucalyptus citriodora*), *G. applanatum* (*Abies pindro*), *G. curtisii*, *G. multicornum* (*Cedrus deodara*) *G. lipsiense* (*Acacia arabica*), *G. multiplicatum* (*Pinus logifolia*) and *G. boninense* (*Morus alba*). These identified species were non-mushroom and belonged to Phylum Basidiomycota. Variations were observed among different morphological and cultural properties. The color of the pileus surface was found light to dark brown and one species showed white to creamish color. The maximum length and thickness of pileus was found to be 32 cm and 10 cm respectively. Majority of *Ganoderma* specimens were lateral, central, stipitate, dimidiate and sessile. Among cultural characteristics, colonies of the *Ganoderma* species possessed brown color and some were white to creamish. Colonies were circular and smooth to slimy. Most of the species produced the hyphal system trimetic which had the generative and skeletal hyphae. Most spores were brown ellipsoid and oval shape. The maximum spore index was 2.5. Maximum tube size of 1 cm and minimum tube size 2 mm were recorded. Pale brown cystidia with thin walls were seen from the germinating cystidia.

Corresponding Author: Fakhar-ud-Din

Email: fakhar178@gmail.com

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INTRODUCTION

Ganoderma species belong to the kingdom Fungi, the division Basidiomycota, the class Homobasidiomycetes, the order Aphyllophorales, the family Poleporaceae

(Ganodermataceae) and the genus *Ganoderma*. Commonly known as a wood decaying fungus, it causes white rot of a wide variety of trees and can be described as a phytopathogenic fungus (Chang, 1999). *Ganoderma*

spp. are considered as medicinal fungi and over 250 *Ganoderma* spp. have been reported worldwide with most of them from the tropics. *Ganoderma* spp. are used in folk medicine to cure various diseases such as hypertension, hepatitis, hypercholesterolemia, gastric cancer and many others. Due to its ability to cure many different diseases it received different names like "Elixir of life", "Food of Gods", "Mushroom of the Universe" (Wasson, 1986).

Several strains of *Ganoderma* spp. are commercially cultivated for the preparation of health tablets or drinks, tonics and sedative drugs widely in Taiwan. Therefore, *Ganoderma* spp. have received a wide interest for studies ranging from identification, phylogenetic relationship to the content of natural products and their biological activities. *Ganoderma* has been known in Japan, China and other countries as a food and raw material for the development of drugs. It is a shelf like mushroom that grows on dead and dying trees, the fruiting body of *Ganoderma* is employed medicinally. Among the different colors and shapes of the fruiting bodies, the red variety is mostly used and commercially cultivated (Steyaert, 1980).

G. lucidum, a complex known in Chinese as Lingzhi, the word lingzhi, in Chinese, means "herb of spiritual potency" and has also been described as "mushroom of immortality" has been considered a symbol of good fortune and prosperity. Lingzhi may possess anti-tumor, immunomodulatory and immunotherapeutic activities, supported by studies on polysaccharides, terpenes, and other bioactive compounds isolated from fruiting bodies and mycelia of this fungus. It has also been found to inhibit platelet aggregation, and to lower blood pressure, cholesterol and blood sugar (Willard, 1990).

Ganoderma species are not classified as edible mushrooms, as the fruiting bodies are stipitate, dimidiate, or reniform and rarely sub orbicular. They are thick, corky, and tough and do not have the fleshy texture characteristics of true edible mushrooms such as common button mushroom. Several types of *Ganoderma* products are available in the market including, ground fruiting bodies or mycelium processed into capsule or tablet form or tea, *Ganoderma* beer and *Ganoderma* hair tonic (Jong and Birmingham, 1992).

Fungi from the family Ganodermataceae are classified as such as they have many tiny holes on the underside of fruiting bodies, which are pores that contain the reproductive spores. They have woody or leathery feel

and the presence of these pores are obvious characteristics that distinguish polypores from other common type of mushroom. They degrade the wood over time and produce a fruiting body (or conk) on the surface of wood. *Ganoderma* species are among those fungi that can thrive under hot and humid conditions and are usually found in subtropical and tropical region (Moncalvo and Ryvarden, 1998).

As early as 800 years ago in the Yuan Dynasty (1280-1368 A.D) *Ganoderma lucidum* has been represented in painting, carving, furniture, carpet design, jewelry, perfumes bottles and many more creative artworks. According to the two famous Chinese herbal medical books, *Shen Nong Ben Cao Jing* and *Ben Cao Gang Mu* by Li Shi-Zhen there were six known Lingzhi species in China at that time (Wasser and Weis, 1999). The *G. lucidum* complex is composed of species with annual fruiting bodies having a yellow to reddish laccate cuticle and an upper layer that is smooth and grooved. The surface is sometimes covered with brownish spore powder. Bracket fungi of the genus *Ganoderma* are known by mycologists for the crust-like upper surfaces of their fruit bodies, which in all species, such as *G. pfeifferi* and *G. resinaceum*, have a varnished appearance. Arborists and managers of plantation crops know them collectively as a cause of decay in a very wide range of tree species all over the world (Flood et al., 2000).

Proper identification of wild mushrooms is important to study their biodiversity and ecological role. The discovery of new medicinal mushroom species will lead to their exploitation as an industry. No research work has been done about medicinal mushrooms in Murree hills in the past instead of the fact that there is enormous amount of medicinal herbs and trees in those areas. Keeping in view the importance of *Ganoderma* species, following studies were carried out to determine the distribution and prevalence of *Ganoderma* species in Murree hills on different host plants. The *Ganoderma* species were also identified on the basis of morphological and cultural characteristics.

MATERIALS AND METHODS

Study areas: The studies on the distribution of *Ganoderma* spp. were conducted in five randomly selected union councils of Murree i.e. Ghora Gali, Charhan, Darya Gali, Sehr Bagla and Murree Urban. The morphological studies were carried out in the Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

Collection of specimens: A total of eight fresh fruiting bodies of *Ganoderma* specimens were collected from different host trees in the Murree hills from spring to monsoon (March to June, 2011). The specimens of *Ganoderma* were collected from living infected and dead trees of Ghora Gali, Charhan, Darya Gali, Sehr Bagla, and Murree Urban. The samples were collected on its vegetative stage and sorted accordingly. The location/site and substrate on which *Ganoderma* grew were recorded. Photographs of *Ganoderma* spp. were taken in their natural habitat to preserve their morphological characters. Specimens were cut out from the trunks of trees by sharp razor with care to avoid damage to the stipe and pileus, and soil was removed by washing with water. Field notes on the macro characters and habit were noted. The collected specimens were individually wrapped, brought to the laboratory and dried in an oven at a temperature of 35°C. Information about habit, habitat substratum colors and locality were recorded in the field. The specimens were labeled, placed in envelopes or air-dried. The polythene bags containing the samples were well labeled for easy identification. The identification was done with the help of available literature (Adaskaveg and Gilbertson, 1986; Bakshi, 1971; Gottlieb and Wright, 1999; Ryvardeen, 1995, 2000; Steyaert, 1972, 1980).

Morphological characters: Observations on different morphological characters i.e. pileus or cap size, pileus shape, margins, texture, attachment patterns, persistence and surface patterns, stipe: with or without stipe or directly attached to the trunk were recorded.

Collection of basidiospores: Spores were collected from the fruiting bodies in the field by suspending a square Perspex plate covered with clean paper, 1-2 cm from the surface of the pores. The entire fruit body and suspended plate was then covered with clean paper to minimize airborne contamination.

Preparation of media: Potato dextrose agar (PDA) was prepared by using starch 20 g, agar 20 g, dextrose 20 g and water to make volume of 1 liter. The medium was sterilized in autoclave at 15 psi for 20 minutes and transferred in the slants or Petri plates for isolation and multiplication of *Ganoderma* specimens.

Single spore isolations: Spores obtained from fruiting bodies were suspended in a drop of distilled water and streaked onto water agar soon after collection. Streaked plates were incubated at 30°C and after germination (1-4 days) single spores were transferred to PDA with the aid of a stereomicroscope at 50× under dark field

illumination. All the cultures were incubated at 30°C in the dark for 3-4 days.

Microscopic examination: Basidiospores obtained from spore prints of each specimen were examined under microscope by mounting in a drop of sterile water or Lactophenol drop. Measurement of basidiospores was carried out at 640× magnification using an eyepiece reticule calibrated with a stage micrometer. The length and width of basidiospores from each of the eight specimens were measured in this way. Spore index was calculated as:

$$\text{Spore index} = \frac{\text{Length}}{\text{Width}}$$

Preservation of specimens: The collected specimens were preserved in appropriate preservatives (formaldehyde or alcohol) in the refrigerator at 4°C until further processing. Usually 10% of formalin is used to preserve different specimens of *Ganoderma* spp. in bottles.

Transformation of culture: Fruiting bodies were cut into tiny pieces by using sharp razor and soaked in 0.5% sodium hypochlorite solution for 2 minutes, washed three times with sterilized water, dried and transferred to PDA Petri plates with minute amount of streptomycin sulphate, and were incubated at 30°C for seven days. Inoculums were taken from the slope part of the sample, transfer of material was done under aseptic conditions.

Isolation of *Ganoderma* mycelium: Isolation of mycelium was made from the basidiomata. Small pieces of basidiomata (4 mm × 4 mm × 5 mm) were cut and surface sterilized with 5% sodium hypochlorite for 3 minutes, 0.1% HgCl₂ for 1 minute, and 2% sodium hypochlorite for 2 minutes, blotted dry and plated on PDA with streptomycin sulphate in Petri dishes and were incubated at 30°C for seven days. Visible mycelia grown from the pieces of basidiomata were sub-cultured on fresh PDA medium in Petri plates.

Cultural study of *Ganoderma* spp.: Cultural characteristics such as hyphae, growth pattern, colony color, mycelial color, spore shape, basidia, basidiospore, cystidia and mycelium structure of the specimens were identified.

RESULTS AND DISCUSSION

Distribution of *Ganoderma* species: In all, eight species of *Ganoderma* were identified from the research areas. *Cedrus deodara* and *Eucalyptus citriodora* were found to be the most susceptible hosts of *Ganoderma* species (Table 1). *C. deodara* was found to be the most susceptible and showed high incidence of infection causing threat to the plantation. The species are well distributed except for

species *G. boninense*, *G. multiplicatum* and *G. lipsiense* which are restricted to specific locations. The overall counts of valid species described by various authors from Pakistan are *G. multiplicatum*, *G. applanatum*, *G. resinaceum*, *G. lucidum*, and *G. philipp*. Bilgrami et al. (1991) reported *G. annulare*, *G. adspermum* and *G.*

australe from India but all these species are the synonym of *G. austal* (Moncalvo and Ryvardeen, 1998). Murrill (1996) rejected the synonymy of *G. praelongum* with *G. resinaceum* and continued as separate species. Ryvardeen (2000) separated *G. resinaceum* as species with dimitic hyphal system, continuing the synonymy.

Table 1. Hosts of *Ganoderma* species in Murree.

| Species | Host tree | Collection site |
|-------------------------|------------------------------|-----------------|
| <i>G. praelongum</i> | <i>Eucalyptus citriodora</i> | Ghora Gali |
| <i>G. chaliceum</i> | <i>Eucalyptus citriodora</i> | Ghora Gali |
| <i>G. applanatum</i> | <i>Abies pindrow</i> | Charhan |
| <i>G. curtisii</i> | <i>Cedrus deodara</i> | Charhan |
| <i>G. lipsiense</i> | <i>Acacia arabica</i> | Darya Gali |
| <i>G. multiplicatum</i> | <i>Pinus logifolia</i> | Darya Gali |
| <i>G. multicornum</i> | <i>Cedrus deodara</i> | Sehr Bagla |
| <i>G. boninense</i> | <i>Morus alba</i> | Murree Urban |

The present study accepts the argument of Gottlieb and Wright (1999) since the specimens showed variations in their characters. *G. praelongum* was reported by Steyaert (1980) from India but was not confirmed due to insufficient material. Bakshi (1971) reported *G. ahmadii* from India; both these names continued and were accepted by Moncalvo and Ryvardeen (1998) but were not observed in the present study. *G. lucidum* was considered as invalid name by Moncalvo and Ryvardeen (1998) but latter Ryvardeen (2000) separated the same species under dimitic hyphal system. Similarly *G. perzonatum* was considered as synonym of *G. cupreum*, *G. chaliceum* and *G. purvulum* (Moncalvo and Ryvardeen, 1998), but latter these species (*G. chaliceum* and *G. perzonatum*) were separated under the dimitic hyphal system (Ryvardeen, 2000). *G. multicornum*, was described as new species by Ryvardeen (2000) with dimitic hyphal system and with only type locality. The present study reports a new locality for these species from Murree. Understanding the

taxonomic status of *Ganoderma* in Pakistan, it is confirmed that to date five valid species have been reported from Pakistan of which four species viz. *G. lucidum*, *G. applanatum*, *G. philippi*, *G. multiplicatum*, *G. resinaceum* were reported in the present study. Four species viz. *G. multicornum*, *G. chaliceum*, *G. perzonatum*, *G. lipsiense* were reported for the first time from Pakistan as shown in Table 1. Thus, there is an urgent need to examine all materials deposited in herbaria, under the genus *Ganoderma*. The work facilitates in understanding species diversity of *Ganoderma* from Murree, Pakistan.

Morphological characterization: Eight *Ganoderma* species were identified on the basis of morphological and cultural characteristics. The ecological data of *Ganoderma* species are given in Table 2. The identified species are non-mushroom and fall under Phylum Basidiomycota (Figures 1-8). Following morphological characteristics were considered for the identification of *Ganoderma* species.

Table 2. Ecological data of *Ganoderma* species.

| Species | Habitat | Growth type | Forest type |
|-------------------------|----------------|-------------|--------------|
| <i>G. praelongum</i> | Living tree | Solitary | Coniferous |
| <i>G. chaliceum</i> | Living tree | Colony | Coniferous |
| <i>G. applanatum</i> | Dead wood logs | Scattered | Broad leaves |
| <i>G. curtisii</i> | Dead wood logs | Scattered | Mixed |
| <i>G. lipsiense</i> | Wood logs | Colony | Mixed |
| <i>G. multiplicatum</i> | Dry tree | Solitary | Coniferous |
| <i>G. multicornum</i> | Dead trunk | Scattered | Mixed |
| <i>G. boninense</i> | Living tree | Colony | Coniferous |



Figure 1. *Ganoderma praelongum*



Figure 2. *Ganoderma chaliceum*.



Figure 3. *Ganoderma applanatum*.



Figure 4. *Ganoderma curtisii*.



Figure 5. *Ganoderma lipsiense*.



Figure 6. *Ganoderma multiplicatum*.



Figure 7. *Ganoderma multicornum*.



Figure 8. *Ganoderma boninense*.

Phenotypic evaluation: Morphological characteristics i.e. pileus surface color, pileus size, pileus shape, attachment patterns of pileus, stipe texture, surface patterns, persistence and margins of pileus were recorded for all the eight *Ganoderma* species and phenotypically diversities were assessed. The data have been summarized in (Table 3-10).

Pileus surface color: Variations in pileus surface color were noted by observing whether the pileus surface color is light brown, dark brown, dull brown and creamish. Data pertaining to pileus surface color

(Table 3-10) showed that only *G. multicornum* and *G. boninense* showed white to creamish upper surface and all other specimens possessed light and dark brown upper surface. There was least diversity among these specimens phenotypically based on pileus surface color.

Pileus size: Data presented in Table 3-10 showed that, pileus size in the eight specimens varied greatly. Maximum length of pileus i.e. 32 cm and 10 cm thick was attained by *G. boninense* and minimum of 6 cm length and 1 cm thick was recorded by *G. curtisii*.

Table 3. Morphological characters of *Ganoderma praelongum*.

| Characteristics | Description |
|---------------------------------|--|
| A Pileus surface color | Upper surface glabrous, sulcate, laccate, bay to brownish brown spore appearing dusty. At mature stages dark brown with white edges, lower surface smooth and dark brown |
| B Pileus Size | 9 cm across, front length up to margins 6 cm and in the centre 3cm thick |
| C Pileus Shape | Orbicular dimidiata to flabelliform, rarely umbonate to stipitate |
| D Margins | Smooth, 6 to 7 mm thin, sterile, cream to ochraceous, acute to sulcate, rarely blunt |
| E Attachment patterns of pileus | Lateral |
| F Texture | Corky and flesh |
| G Surface patterns | 4 deep concentric zones |
| H Persistence | Perennial with annual growth |
| I Stipe | No stipe directly attached to the host tree |

Pileus shape and margins of pileus: Basidiomata examined generally conformed to the holotype but were variable in shape and habit. Data presented in Tables 3-10 showed that the majority of *Ganoderma* specimens were stipitate or dimidiata and generally had finely (concentrically) grooved surfaces with either acute, rounded or incurved margins only. Table

9 showed that pileus shape of *G. multicornum* was conical and sessile. There is great variation among the pileus margins. Data showed (Table 3-10), that majority of the specimens were waved, rounded and hard but the Table 3 and 9 showed that *G. praelongum* and *G. multicornum* were smooth and soft but the surface and margin of the pileus are rough.

Table 4. Morphological characters of *Ganoderma chalconeum*.

| Characteristics | Description |
|---------------------------------|---|
| A Pileus surface color | Upper surface reddish brown, highly sulcate, brown spore appearing dusty at mature stages dark brown with white |
| B Pileus Size | 8 cm across, breadth near margins was 2 cm and in the centre 2 cm was thick |
| C Pileus Shape | Laccate, acute to sulcate, rarely blunt |
| D Margins | Waved and somewhat smooth, 2 to 3 mm thick, with crust margin hard, acute, creamish yellow |
| E Attachment patterns of pileus | Central |
| F Texture | Leathery and flesh |
| G Surface patterns | Shallow waved with 1-3 concentric zones |
| H Persistence | Perennial with annual growth |
| I Stipe | Stipe length 7-8 cm |

Table 5. Morphological characters of *Ganoderma applanatum*.

| Characteristics | Description |
|---------------------------------|---|
| A Pileus surface color | Upper surface pale grey to dark brown, crustose with concentric zonation, covered with layer of chocolate, brown spore appearing dusty, below surface smooth and dark brown spore appearing dusty |
| B Pileus Size | 9 cm across, breadth near margins was 3 cm and in the centre 5 cm was thick |
| C Pileus Shape | Woody to corky, applanate or shelf like |
| D Margins | Waved, 6 to 7 mm thick, sterile, rounded, turning brown on drying |
| E Attachment patterns of pileus | Sessile |
| F Texture | Leathery and flesh |
| G Surface patterns | 6-7 deep concentric zones |
| H Persistence | Perennial with annual growth |
| I Stipe | Directly attached to the trunk |

Table 6. Morphological characters of *Ganoderma curtisii*.

| Characteristics | Description |
|---------------------------------|---|
| A Pileus surface color | Upper surface dull brown, milky coffee near the margin, concentric sulcate, below surface smooth and dark brown spore appearing dusty |
| B Pileus Size | 6 cm across, front length up to margins was 3 cm and in the centre 1 cm was thick |
| C Pileus Shape | Woody to corky, applanate or plane, shelf like |
| D Margins | Margin thin lobate, sterile, brown at old age 1 mm thick |
| E Attachment patterns of pileus | Lateral |
| F Texture | Soft and flesh |
| G Surface patterns | Smooth |
| H Persistence | Perennial with annual growth |
| I Stipe | Stipe length 4-5 cm, 6 mm thick |

Attachment and surface patterns of pileus: Data pertaining to attachment and surface patterns of pileus showed significant differences among the specimens. The pileus of the normal fruit body is laterally attached to the stipe, but eccentric and central. Majority of the specimens were lateral but Table 4 and 5 showed central and sessile. The result were in agreement with those of Schwab

(1988). The surface of the pileus was rough, waved and had many narrow concentric zones but the Table 6, 7 and 9 showed the smooth.

Texture, persistence and stipe: Data presented in Tables 3-10 showed that there were great similarities among the *Ganoderma* specimens. Most specimens' texture was corky and flesh. All the specimens were perennial with annual

growth. There was less difference among the stipe attachment of all the specimens which were directly attached to the trunk except Table 4 and 6. The maximum stipe length of 7-8 cm was observed in case of *G. chaliceum*. Although variations existed in the morphological characteristics of basidiomata from the five union councils of Murree, the overall morphology of the basidiomata falls Table 7. Morphological characters of *Ganoderma lipsiense*.

within the characteristics of *G. boninense* based on Steyaert (1972) description. Variations in basidioma characteristics within a species have been reported for several species of *Ganoderma* and the variations may be caused by different environmental conditions and developmental stages of the basidiomata (Adaskaveg and Gilbertson, 1989; Ho and Nawawi, 1985; Zhao, 1989).

| Characteristics | Description |
|---------------------------------|--|
| A Pileus surface color | Upper Surface slightly zonate, pulverulent glabrous, tuberous, crust, rigid, up to 1 mm thick, reddish grey or cinnamon. Lower surface smooth and dark brown |
| B Pileus Size | 7 cm across, front length 5 cm. margins was 3 cm and in the centre 4 cm was thick |
| C Pileus Shap | Orbicular dimidiate to flabelliform, rarely umbonate to stipitate |
| D Margins | Margin hard, obtuse, slightly thick and lobate, cinnamon to grayish white or slightly yellowish |
| E Attachment patterns of pileus | Lateral |
| F Texture | Corky and flesh |
| G Surface patterns | Smooth |
| H Persistence | Perennial with annual growth |
| I Stipe | Directly attached to the trunk |

Table 8. Morphological characters of *Ganoderma multiplicatum*.

| Characteristics | Description |
|---------------------------------|--|
| A Pileus surface color | Upper surface concentrically sulcate, brown of chestnut, lower surface smooth and dark brown |
| B Pileus Size | 12 cm across, front length up to margins was 9 cm and in the centre 1 cm was thick |
| C Pileus Shap | Pileate, stipitate, dimidiate |
| D Margins | Waved, 8 to 9 mm thin, sterile, brown to ochraceous, acute to sulcate, rarely blunt |
| E Attachment patterns of pileus | Lateral |
| F Texture | Corky and flesh |
| G Surface patterns | Shallow waved |
| H Persistence | Perennial with annual growth |
| I Stipe | Directly attached to the trunk |

Table 9. Morphological characters of *Ganoderma multicornum*.

| Characteristics | Description |
|---------------------------------|--|
| A Pileus surface color | Upper Surface slightly creamish white, below surface light brown, sulcate and zonate |
| B Pileus Size | 15 cm across, front length up to margins was 8 cm and in the centre 10 cm was thick |
| C Pileus Shap | Conical, sessile |
| D Margins | Smooth, regular, soft, obtuse, creamish white sterile, 2 to 2.5 cm thick |
| E Attachment patterns of pileus | Lateral |
| F Texture | Corky and brittle |
| G Surface patterns | Smooth |
| H Persistence | Perennial with annual growth |
| I Stipe | Directly attached to the trunk |

Table 10. Morphological characters of *Ganoderma boninense*.

| Characteristics | Description |
|---------------------------------|--|
| A Pileus surface color | Upper Surface slightly zonate, pulverulent glabrous, white to creamish, lower surface smooth and light brown |
| B Pileus Size | 32 cm across, front length up to margin 15 cm, and in the centre 10 cm was thick |
| C Pileus Shape | Stipitate or dimidiate and generally had finely (concentrically) grooved surfaces |
| D Margins | Rounded or incurved margins hard, thick and lobate, cinnamon |
| E Attachment patterns of pileus | Lateral |
| F Texture | Corky and flesh |
| G Surface patterns | Smooth and somewhat 3 waved |
| H Persistence | Perennial with annual growth |
| I Stipe | Directly attached to the trunk |

Cultural characterization: Due to the variability of *Ganoderma* species on the hosts, it is evident that identification must be supported with cultural data. Cultural studies were therefore, conducted to investigate the colony color, hyphal system, mycelial color, cutis, tube size, context layer, spore index, spore shape and cystidia shape. The results as found in this study are as follows.

Colony color and hyphal system: Colonies of the *Ganoderma* species grew very slowly. Table 11-14 showed that the majority of the specimens possessed brown color and some white to creamish. Colonies were circular, smooth to slimy, finely radiating with regular margins. There was no production of conidiomata on the host material. Shape tends to be variable, but the size, color and the ornamentation of the hyphal system were consistent and there were less difference found, most species produced the hyphal system trimetic which had

the generative and skeletal hyphae as shown in Table 11-14. Mycelium appeared 'stringy' in growth. There was some production of conidiomata on the host material.

Spore production: Basidiomata maturity were marked by spore production when the fruiting body reached a constant size. Spores were strongly ejected from the pore layer and fell all over the basidiomata surface. The spore deposits were light brown and powder form, they were also found on the upper surface of the basidiomata, giving it a velvety golden appearance. Spores discharge were light in the first three days, becoming very heavy from the forth. Most deposited spores were ellipsoid and oval shape. The maximum spore index of 2.5 was recorded for *G. multiplicatum* and minimum spore index of 1.66 was found in *G. chaliceum*. There were great similarities among the spore print color of all *Ganoderma* specimens as shown in Table 11-14.

Table 11. Cultural Characteristics of *G. praelongum* and *G. chaliceum*.

| Characters | <i>G. praelongum</i> | <i>G. chaliceum</i> |
|-------------------|----------------------|---------------------|
| Colony color | White to pale yellow | Light brown |
| Hyphal system | Trimitic | Dimitic |
| Mycelial color | Yellowish brown | Brown |
| Cutis | Diverticulate | Claviform |
| Generative hyphae | 3.3-4.1 μ m | 3.5 μ m |
| Skeletal hyphae | 5 μ m | 7.5 μ m |
| Binding hyphae | 6.6 μ m | 7.5 μ m |
| Tube size | 2 mm | 13 mm |
| Context layer | 4 mm | 15 mm |
| Spore index | 1.77 | 1.60 |
| Spore shape | Ellipsoid | Ovoid |
| Cystidia shape | Gloeocystidia | Macrocystidia |

Tube size, cutis shape and context layer evaluation: Data pertaining to tube size (Table 11-14) showed differences

among specimens. Maximum tube size was 1 cm and minimum tube size was 2 mm. Most cutis shape was

diverticulate but some showed the claviform. The maximum context layer of 30 mm and minimum of 4 mm were recorded.

Cystidia shape evaluation: Fungus produced other yeast-like characteristics. Cystidia were produced by

budding directly from hyphae. Pale brown cystidia with thin walls were seen from the germinating cystidia or directly from the hyphae. There were great variations among cystidial shapes as shown in Table 11-14.

Table 12. Cultural Characteristics of the *G. applanatum* and *G. curtisii*.

| Characters | <i>G. applanatum</i> | <i>G. curtisii</i> |
|-------------------|--------------------------|--------------------|
| Colony color | Light grey | Yellowish brown |
| Hyphal system | Trimitic | Trimitic |
| Mycelial color | Pale yellow | Yellowish brown |
| Cutis | Trichodermis | Claviform |
| Generative hyphae | 3.3 -4.1 μm | 2.5 μm |
| Skeletal hyphae | 5.8 to 6.6 μm | 5 μm |
| Binding hyphae | 7.5 μm | 3.3 μm |
| Tube size | 8 mm | 10 mm |
| Context layer | 6 mm | 10 mm |
| Spore index | 2.16 | 1.87 |
| Spore shape | Oval | Obpyriform |
| Cystidia shape | Leptocystidia | Echinidia |

Table 13. Cultural Characteristics of *G. lipsiense* and *G. multiplicatum*.

| Characters | <i>G. lipsiense</i> | <i>G. multiplicatum</i> |
|-------------------|---------------------|-------------------------|
| Colony color | Creamish white | Brown |
| Hyphal system | Trimitic | Dimitic |
| Mycelial color | Brown | Yellowish |
| Cutis | Trichodermis | Diverticulate |
| Generative hyphae | 3.3 μm | 3.8 μm |
| Skeletal hyphae | 5 μm | 5.8-7.5 μm |
| Binding hyphae | 5 μm | 3.4 μm |
| Tube size | 13 mm | 3 mm |
| Context layer | 30 mm | 5 mm |
| Spore index | 1.91 | 2.5 |
| Spore shape | Ellipsoid | Ellipsoid |
| Cystidia shape | Macrocystidia | Chrysocystidia |

Table 14: Cultural Characteristics of *G. multicornum* and *G. boninense*.

| Characters | <i>G. multicornum</i> | <i>G. boninense</i> |
|-------------------|-----------------------|---------------------|
| Colony color | Brown | White |
| Hyphal system | Dimitic | Dimitic |
| Mycelial color | Light brown | Pale Orange |
| Cutis | Diverticulate | Diverticulate |
| Generative hyphae | 3-5 μm | 4.1 μm |
| Skeletal hyphae | 15 μm | 5-6.6 μm |
| Binding hyphae | 5 μm | 3.4 μm |
| Tube size | 5 mm | 1 cm |
| Context layer | 13 mm | 5 mm |
| Spore index | 2 | 1.82 |
| Spore shape | Obviate | Reniform |
| Cystidia shape | Leptocystidia | Macrocystidia |

Basidiospore dimensions of *Ganoderma* species: The eight species of *Ganoderma* also showed variations in basidiospore dimensions i.e. spore color, spore length, spore shape and spore index as shown in Table 15. This finding may hold further repercussions for the

identification and publication of other *Ganoderma* species. Without cultures, it appears difficult to accurately identify a *Ganoderma* spp. or distinguish whether variation is caused by environmental factors or is due to collections representing different taxa.

Table 15. Basidiospore dimensions of *Ganoderma* species.

| Species | Spore color | Spore Length | Spore shape | Spore index |
|-------------------------|---------------|--------------|-------------|-------------|
| <i>G. praelongum</i> | Reddish brown | 8.0 × 4.5 | Ellipsoid | 1.77 |
| <i>G. chaliceum</i> | Reddish brown | 7.4 × 4.6 | Ovoid | 1.60 |
| <i>G. applanatum</i> | Pale yellow | 7.8 × 3.6 | Oval | 2.16 |
| <i>G. curtisii</i> | Brown | 9.0 × 4.8 | Opyriform | 1.87 |
| <i>G. lipsiense</i> | Brown | 9.2 × 4.3 | Ellipsoid | 1.91 |
| <i>G. multiplicatum</i> | Brown | 8.5 × 3.4 | Ellipsoid | 2.5 |
| <i>G. multicornum</i> | Brown | 6.8 × 3.4 | Obviate | 2 |
| <i>G. boninense</i> | Reddish brown | 9.5 × 5.2 | Reniform | 1.82 |

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