DIVERSITY OF MACROFUNGI AND NUTRIENT ANALYSIS OF SELECTED WILD EDIBLE MUSHROOMS IN THULO BAN COMMUNITY FOREST OF ARJAM, MYAGDI DISTRICT

A Dissertation Submitted for the Partial Fulfillment of the Requirement for the Master's Degree in Science, Central Department of Botany, Tribhuvan University

Submitted By

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Symbol No: Bot. 529/074

T.U. Regd. No. : 5-2-53-387-2013

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May, 2022

DECLARATION

I, Shashi Shrestha, M.Sc student of botany declare that this dissertation entitled "**DIVERSITY OF MACROFUNGI AND NUTRIENT ANALYSIS OF SELECTED WILD EDIBLE MUSHROOMS IN THULO BAN COMMUNITY FOREST OF ARJAM, MYAGDI DISTRICT**" is a record of genuine work carried out by me under the supervision of **Dr. Sanjay Kumar Jha,** Associate Professor, Central Department of Botany. I further declare that the work reported in this research has not been previously submitted in any degree, in this or any other institute or University.

……………………

Shashi Shrestha

4 May, 2022

Date: 4 May, 2022

RECOMMENDATION

This is to certify that the dissertation work entitled **"DIVERSITY OF MACROFUNGI AND NUTRIENT ANALYSIS OF SELECTED WILD EDIBLE MUSHROOMS IN THULO BAN COMMUNITY FOREST OF ARJAM, MYAGDI DISTRICT"** submitted by **Ms. Shashi Shrestha** was completed under my supervision. To the best of my knowledge the work has not been submitted elsewhere for any academic purpose and hence is original work performed by the candidate. It is hereby recommended for the acceptance of this dissertation as a partial fulfillment of the requirement of Master's Degree in Botany at Tribhuvan University.

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LETTER OF APPROVAL

The M.Sc. Dissertation entitled **"DIVERSITY OF MACROFUNGI AND NUTRIENT ANALYSIS OF SELECTED WILD EDIBLE MUSHROOMS IN THULO BAN COMMUNITY FOREST OF ARJAM, MYAGDI DISTRICT"** submitted at the Central Department of Botany, Tribhuvan University by **Ms. Shashi Shrestha** for the partial fulfilment of her Masters of degree in Botany, has been accepted.

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ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my respected supervisor Dr. Sanjay Kumar Jha, Associate Professor of Central Department of Botany, Tribhuvan University for his persistent encouragement, support and guidance throughout the research work.

I am also thankful to Professor Dr. Ram Kailash Prasad Yadav, Head of Central Department of Botany, Tribhuvan University as well as other faculty members for their support.

I am equally thankful to my respected teacher Associated Prof. Dr. Hari Prasad Aryal, Associate Professor Dr. Lal Bahadur Thapa and Associate Professor Dr. Chandra Prasad Pokhrel of Applied Mycology and Advanced Plant Pathology Unit, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu for their suggestions and guidance,

I am indebted to my friends Punam Pun, Laxmi Thapa, Tulasa Chaudhary, Tej Lal Chaudhary, Sadikshya Thapa, Binod Malla, Binod Gautam and Muna Baral as well as my family members for their encouragement and moral support during the research work as well as in my ups and downs.

Last but not the least, I would like to express my heartfelt thanks equally to all people and institution that helped and reinforced me directly or indirectly during my research work.

Shashi Shrestha

ABSTRACT

Macrofungi are Ascomycetes and Basidiomycetes members that produce mature spore-bearing and morphologically distinct fruiting bodies. They grow either above ground (epigenous) or underground (hypogenous) in nature. They are key player conservation of forest ecosystems and biodiversity**.** The current study deals with the diversity of macrofungi in the subtropical mixed forest of Myagdi district. Survey was conducted from the end of June to the beginning of September 2020. Macrofungi were sampled using a systematic random procedure at altitudes ranging from 1250-1450 meters above sea level. The sampling was done by using a 10×10m quadrat in three transects at the distance of 100 m, and a total of 18 plots were made. Distance between each plot was approximately 50 m. A total of 70 macrofungal taxa were collected. Among them, 60 were identified at the species level, and 10 were identified at the generic level belonging to 26 different families and 12 orders. Russulaceae was found to be the most dominant family with 16 species whereas *Mycena* sp. was found to be much denser with value 11.88 % and *Cantharellus cibarius* was much frequent species containing 44.44 %. The Shannon diversity index was 3.49 and Simpson index was 0.95. The study analyze the nutrient content of three wild edible mushrooms namely *Cantharellus cibarius*, *Laccaria laccata*, and *Scleroderma cepa* commonly consume by the local people of Arjam, Myagdi district. In total, 13 parameters were analyzed such as ash, carbohydrate, fat, moisture, protein, calcium, magnesium, phosphorus, potassium, copper, iron, manganese and zinc. Test method used for ash was ignition whereas carbohydrate was done by calculation method, fat was done by soxhlet extraction, moisture was done by oven dry method, protein was done by kjeldahl digestion method similarly carbohydrate, calcium and magnesium was done by titration method and phosphorous were done by spectrophotometric method and iron, manganese, copper, zinc and potassium were done by AAS method. All macro and micro nutrient composition were determined on a dry weight basis. Ash, carbohydrate, fat, moisture, and protein ranges from 7.05-13.38%, 61.89-71.37%, 0.78-1.41%, 12.37-13.63% and 23.33-24.47% whereas calcium, magnesium, phosphorus and potassium ranges from 0.13-0.15, 0.09-0.11µg/g, 0.25-0.33 µg/g and 0.41-3.40 µg/g similarly copper, iron, manganese and zinc ranges from 2.40-30.94 µg/g, 0.08-0.20 µg/g, 12.29-16.06 µg/g and 45.70-77.34 µg/g.

Keywords: Macrofungi, Russulaceae, Diversity index

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
AOAC	Association of Official Analytical Chemists
Ca	Calcium
cm	Centimeter
Cu	Copper
DW	Dry Weight
ECM	Ectomycorrhizal
FAO	Food and Agriculture Organization
Fe	Iron
gm	Gram
K	Potassium
Mn	Manganese
Mg	Magnesium
mg	Milligram
µg	Microgram
µm	Micrometer
PPM	Parts Per Million
Sp.	Species
mm	Millimeter
a.s.l	Above sea level

CHAPTER I: INTRODUCTION

1.1 Background

Fungi are distinct group of organisms more closely related to animals than plants because they are heterotrophs. They are one of the most diverse groups of organisms on the planet and are important role in ecosystem processes. (Keizer, 1998; Seen- Irlet *et al*., 2008). They usually reside underground or under tree barks, and therefore their recognition or identification is possible only in term of fruiting mushroom. (lwabuchi *et al*., 1994).

Macrofungi are Ascomycetes and Basidiomycetes members that produce mature spore-bearing and morphologically distinct fruiting bodies that are visible to the naked eye and are generally ≥ 1 cm in size (Arnolds 1992; Redhead and Berch, 1997). They grow either epigenous or hypogenous in nature (Acharya, 2020). The best known example of macrofungi is the mushroom. The most familiar type of macrofungi is umbrella shaped while other species are in the form of gilled fungi, coral fungi, jelly fungi, bracket fungi, puffballs and bird's nest fungi (Jha and Tripathi, 2012).

Biodiversity, also known as biological diversity, refers to all living things on Earth or in a specific ecosystem. Mycodiversity is a branch of biodiversity that reflects the diversity of fungi (Pandey, 2008).

Macrofungi play key roles in the conservation of forest ecosystems and biodiversity (Hawksworth, 1991; Molina *et al.,* 2001). It is an important source of food for forest animals, and it also serves as a home for many soil insects and other small organisms that are part of the forest ecosystem (Teke *et al*., 2019). Several macrofungal species, such as *Trametes versiocolor*, have been identified as decomposers of organic persistent pollutants (Tran *et al.*, 2010; Tran *et al.*, 2013). Forestry managers and conservationists have realized that dead decaying wood, which is mostly facilitated by fungi, is an important source of biodiversity and an essential component of carbon and other nutrient recycling (Gates, 2009).

1.2 Structure of mushrooms

The mushroom is made up of two parts: an underground part (mycelium) and an above-ground, often edible part that also serves as the reproductive organ. Different mushrooms have distinctive shapes and forms. The obvious characteristics and importance in identifying mushrooms are variations in size, color, texture, and shape of the cap and stalk (Chang and Miles, 1989).

Mushrooms contain different part which is described below;

Pileus: Pileus refers to the upper part of mushrooms, which is shaped differently and protects the pores or gills. The pileus is available in a variety of colors, including white, brown, and yellow.

Gills, pores or spine: The spore-dispersing portion of the mushroom is found beneath the cap. Spores are mushroom seeds that are released into the air and land on a substrate to form new mushrooms. They can be seen by placing the mushroom cap on a piece of paper and covering it with a glass to maintain a high level of humidity. The color, shape, and manner in which the spores form aid in identifying the fungus (Boa, 2004). The spore producing tissue is called the hymenium (Etang *et al*., 2006). Underneath the cap we normally find gills but *Boletes* contain pores while other species even have spine. Attachment of gills to the mushroom stipe can be free, adnate, adnex, sinuate, decurrent.

Stipe: It is the axis that supports the mushroom top. The stems of some species may contain ring and volva. A ring is a narrow collar that wraps around the top of the stem. The volva is a bag-like structure that wraps around the stem's base.

Mycelium: Mycelium is a swarm of fine hair-like strands that grow outward and downward into the soil in search of nutrients.

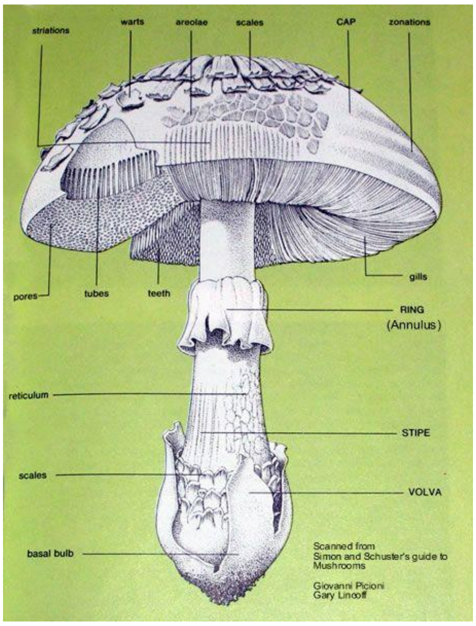

Figure 1: Structure of mushroom (Source: https://www.pinterest.com)

1.3 Habitat and Ecology

Mushrooms can be found in almost any habitat, including rotten logs, leaf litter and soil. Different terms have been given to signify their habitats. For example: species growing on grasslands are known as praticolous; on woodland – silvicolous; on wood, woody debris, trees, stumps, rotten or burnt wood - lignicolous; on dung-coprophilous; amongst moss - muscicolous; on leaf litter - humicolous and so on (Purkaryastha and Chandra, 1985). Fungi get their energy and many nutrients from the breakdown of plants and other organic matter (Hanson, 2008). Fungi are made up of fine threads known as hyphae that are hidden in the soil and join together to form a mycelium (Boa, 2004). When atmospheric conditions, particularly humidity, are favorable, the mycelium absorbs food nutrients while the hyphae form into mycelia, which form the fruit (sporocarp) structure on the surface (Okoro and Achuba, 2012). In terms of ecology, basidiomycetes can be saprophytic (growing on dead organic matter), parasitic (causing harm to other organisms), or mycorrhizal (growing in association with other organisms). Members of the genera *Amanita*, *Russula*, *Cortinarius*, *Boletes*, *Lacterius*, *Tricholoma*, *Inocybe*, and others are ectomycorrhizal, whereas *Mycena*, *Coprinus*, *Gymnopus*, *Collybia*, *Hygrocybe*, and others are saprotrophes.

1.4 Mushrooms diversity of Nepal

Due to wide variation of climatic condition, such as tropical, sub- tropical, temperate and alpine, Nepal is consider as the homeland for the mushrooms floral diversity (Aryal and Budhathoki , 2012; Poudel and Bajracharya, 2011). There are an estimated 1.5 million fungal species worldwide, but only 74,000-120,000 have been described (Garibay-Orijel *et al*., 2009). Approximately, 14,000 described species of the millions of fungi estimated to exist on the planet produced fruiting bodies large enough to be considered mushrooms. 7,000 of them are considered edible. So far, approximately 1,291 mushrooms species have been recorded from Nepal and 34 species of mushrooms have been described as endemic. Among them 159 species are edible (Devkota and Aryal, 2020). Because most of Nepal's mushroom biodiversity remains unexplored, it is essential to document the diversity, distribution, and abundance of these macrofungi in Nepal. Mycological exploration and investigation is carried out more in Central Nepal in comparison to eastern and western regions of Nepal (Adhikari, 1999, Adhikari, 2000; Adhakari and Bhattarai, 2014).

The distribution of macrofungal species is low during the hot and dry season, but abundant during the spring and autumn due to the humid climate (Sibounnavong *et al*., 2008).

1.5 Toxicity / poisoning of wild mushrooms

Mushroom poisoning refers to the adverse effects caused by the ingestion of toxic substances found in mushrooms. Although there are over 5,000 varieties of mushrooms in the world, only 100 are toxic (Chaudhary, *et al*., 2013). Many wild mushrooms are edible and have a delicious flavor, but some are deadly poisonous (Mehrotra and Aneja, 1990). Mushrooms growing on the ground in forest are more dangerous than mushrooms growing on living trees (McPartland *et al*., 1997). Mushroom poisoning is most common in the spring and autumn seasons, when cool, damp evenings encourage mushroom growth (Erguven *et al*., 2007). Eating of poisonous mushrooms can cause a variety of side effects, including allergic gastroenteritis, psychological relaxation, and fatal liver intoxication (Lin and Wang, 2004). The clinical manifestations of mushroom poisoning are primarily determined by the type of mushroom involved, the amount ingested, age (symptoms in children are more fatal than in adults due to their low body weight), geographic distribution (some of the dangerous species of mushrooms may be more asymptomatic in certain areas) and so on (Patowary, 2010). Undocumented poisoning is more prevalent in Nepal (Aryal, 2009).

1.6 Nutrient contents

Wild edible fungi have been collected and consumed by people for thousands of years. The archaeological record reveals edible species associated with people living 13,000 years ago in Chile (Rojas and Mansur, 1995), but it is in China, several hundred years before Christ's birth, that the eating of wild fungi is first reliably noted (Aaronson, 2000). Mushrooms had been consumed primarily for their distinct flavor, but due to extensive research into the chemical content of mushrooms, fungi can now be utilized as a meal to treat disease. They have a high protein content, carbohydrate, minerals, fibers, trace elements, and low/no calories and cholesterol (Agahar-Murugkar and Subbulakshmi, 2005; Wani *et al*., 2010). In general, the fruiting bodies of mushrooms contain approximately 56.8 percent carbohydrate, 25.0 percent protein, 5.7 percent fats, and 12.5 percent ash by dry weight (Demirbas, 2002; Mendil *et al*., 2004). According to some studies, the amino acid compositions of mushrooms are comparable to those of animal protein. (Ogundana and Fagade, 1982; Kalac, 2009). The protein found in wild edible mushrooms contains a high concentration of non-essential amino acids such as alanine, arginine, glycine, glutamic acid, aspartic acid, proline, and serine, which aid in tissue growth and repair, immune function, red blood cell formation, and hormone synthesis (Chan, 1981).

1.7 Research objectives

The general objective of the study is:

To find the diversity of macrofungi and nutrient analysis of selected wild edible mushrooms in Thulo Ban Community forest of Arjam, Myagdi district

Specific objectives are:

- To enumerate macrofungi found in study area
- To estimate the nutrient of wild edible mushrooms
- To assess patterns of species diversity and distribution along environmental variables

1.8 Rationale of the study

Macrofungi is not only important for maintaining forest ecosystems but also for their nutrient value. In the modern time, large number people across the world is suffering from obesity which has contributed to wide array of ailments leading to mortality and morbidity. Hence, there is a need of food that is low in calorie and fat. Mushrooms on the other hand are popular for their high nutritional value, protein content, low calories as well as of low fat containing. Even if they are gaining popularity for their above mentioned qualities they lack proper scientific research and validation. Sufficient research has not been done on various aspects of different mushrooms or macro fungi including nutrient composition, exploration, and phytochemical analysis and so on. It is well known that there are wide array of mushrooms containing high value of protein and low fat value; there are also mushrooms which contain highly toxic substances. Each and every year it is recorded that large number of people die by the consumption of wild mushrooms because of either lack of knowledge on edible mushrooms or lack of proper identification technique throughout the world including Nepal. In Nepal most of the region is backward in terms of exploration of mushrooms let alone differentiation between poisonous and non – poisonous mushrooms.

1.9 Hypothesis

- The diversity of macrofungi varies depending on the pH of the soil, amount of moisture in the soil, and the type of forest.
- Mushroom chemical composition varies across and among species.

1.10 Limitations

- Difficulty in identification and preservation due to immaturity or over maturity of mushroom species.
- Spore print of all collected mushroom were not been able taken as some mushroom were not fully matured.
- Only one season was selected for macrofungal diversity.
- Limited mushrooms were estimated for nutritional value.

CHAPTER II: LITERATURE REVIEW

2.1. Mycological investigation

The collections of macrofungi in Nepal started in 19th century with the works of Lloyd (1808) (with one *Ganoderma* species) and Berkeley (1838) (with one Polystictus species). J. D. Hooker (1848 – 1854) explored East Nepal in botanical study, and gathered numerous Nepalese fungi. The result of his gatherings was published by M.J. Berkeley (1854), who reported 44 higher fungi masked in "Indian Fungi" (In Hooker's Journal of Botany). His findings were 18 new species.

Adhakari, *et al*., (2005) studied the use of wild mushroom by ethnic group of Lumle and Kathmandu. They investigate that among 24 species of mushroom, 18 are used as culinary, 8 in medicine, and 3 for other purpose.

Devkota, (2008) recorded 44 macrofungi belonging to 25 families, and 37 genera from Taha, and Majphal VDCs of Dolpa district. Among the collected species 22 have with their culinary values, and 5 with medicinal values.

Aryal, (2009) gave glimpse into the mushroom poisoning problem in Nepal, and its mitigation on the basis of reported made by earlier authors.

Shrestha, (2011) reported 15 *Cordyceps* species recently classified as *Cordyceps* (5 spp.), Meta *Cordyceps* (1 sp.) and *Ophicordyceps* (9 spp.) from Nepal.

Aryal and Budathoki, (2012) reported 30 species of mushrooms (basidiomycetes) under 26 genera belonging to 18 families.

Aryal *et al.,* (2012) reported 21 species of higher fungi gathered from Peepaldanda Community Forest, Western Terai region of Nepal.

Jha and Tripathi, (2012) collected 50 species of macrofungi during survey among them 22 species were identified belonging to 14 families.

From the study area of India Tapwal *et al.,* (2013) recorded 30 macrofungal species belonging to 26 genera, and 17 families. They found 20 species of macrofungi edible out of which some had medicinal value. They also found 17 species were saprophyte, and 10 species were mycorrhizal.

A total of 84 species of macrofungi managed for <10 years, and 73 species in the forests managed for >10 years in Sal forest were recorded by Baral, *et al.,* (2015).

Acharya and Parmar, (2016) collected 42 species of basidiomycetous fungi from Bardia National Park and it's Buffer Zone area.

Aryal *et al*., (2016) discovered *Termitomyces palpensis* Aryal and *T. arghakhanchensis* Aryal in a subtropical deciduous forest at an altitude of 800-1500 m a.s.l. Its macro- and microscopic characteristics distinguish this taxon as a new species in science.

A total of 85 macrofungal taxa were recorded by Bhandari and Jha, (2017) among them 52 were identified up to species level, and 24 generic level and 9 taxa remain unidentified. In their finding they also found species richness increased with increased tree canopy cover, soil pH and soil moisture.

Prasad & Pokhrel, (2017) collected 38 wild mushroom specimens from Amrite community forest of Bhalwad, Banganga municipality. Out of the 38 species collected, 34 were identified to the generic level belonging to 16 families.

Acharya, (2020) recorded 31 fungal species, 5 species belonged to ascomycetes, and 26 species belong to basidiomycetes from Lumbini collaboration forest, Rupandehi district, Central Nepal.

Raut *et al*., (2020) described a new *Scleroderma* species from the Phulchoki Hill temperate oak forest in Nepal. The taxon's identity was also confirmed through phylogenetic analysis of data sets from the internal transcribed spacer region (ITS) of nuclear rDNA sequences, as well as scanning electron micrographs of its basidiospores.

Shrestha *et al*., (2021) explored the ecology and diversity of ectomycorrhiza in the Sunsari district of eastern Nepal's moist tropical forest. They discovered 18 ectomycorrhiza species belonging to 12 genera, and 7 families, with Russulaceae being the dominant family with 7 species.

2. 2. Nutrition composition of mushrooms

Caglarlrmak *et al*., (2002) investigated the nutrient profile of three wild edible mushrooms and discovered that wild edible mushrooms have a good nutritional value.

Agrahar-Murugkar and Subbulakshmi, (2004) investigated the nutrient content of seven wild edible mushrooms typically consumed in Meghalaya's Khasi hills, revealing that the wild mushrooms were high in protein and low in fat.

Giri and Rana, (2008) examined the nutritional values of 11 wild edible mushrooms in nine parameters, and found that the overall nutritional values were good.

Heleno, *et al*., (2009) documented nutritional composition of 10 Portuguese wild mushrooms, and revealed that the wild mushrooms were rich sources of protein, and carbohydrate however, low amounts of fat.

Ouzouni *et al*., (2009) did research on Nutritional value and metal content of ten wild edible mushrooms including *Cantharellus cibarius* and they found moisture content of mushrooms varied from 8.66 % to 17.43%, and dry matter varied from 21.57% to 34.77%.

For nutrient study, Johnsy *et al.*, (2011) gathered 10 wild edible mushrooms from various locations in India's Kanyakumari area, and concluded that wild mushrooms contain higher amounts of protein and carbohydrate and lower amounts of fat.

When Magrati *et al*., (2011) looked at the nutritional components of *Morchella conica*, they discovered that the carbohydrate value was the greatest, followed by protein, crude fiber, fat, ash, and moisture.

Jha and Tripathi, (2012) investigated seven nutritional parameters of three wild edible mushrooms obtained from four Kathmandu valley locations (Phulchowki, Chandragiri, Shivapuri, and Nagarjun).

Kumar *et al*., (2014) studied the nutritional composition of six species of *Russula* from Nagaland, India, and discovered that protein and carbohydrate content ranged from 28.12 to 42.86, and 49.33 to 55 %, respectively.

CHAPTER III: MATERIALS AND METHODS

3.1 Study Area

3.1.1 Physiography

The study was carried out in the Thulo Ban Community Forest of Arjam, Beni Municipality 1, Myagdi District, Gandaki Province, Nepal (Fig. 2). Geographically, it is located in between 28º19' 09.8" N Latitude and 83º34' 35.1" E Longitude. The forest is situated at an altitude 1450 meters above sea level. The study area has Subtropical climate. The total area of the forest is 114 ha. The distance from Arjam to Nepal's capital Kathmandu is approximately 185km.

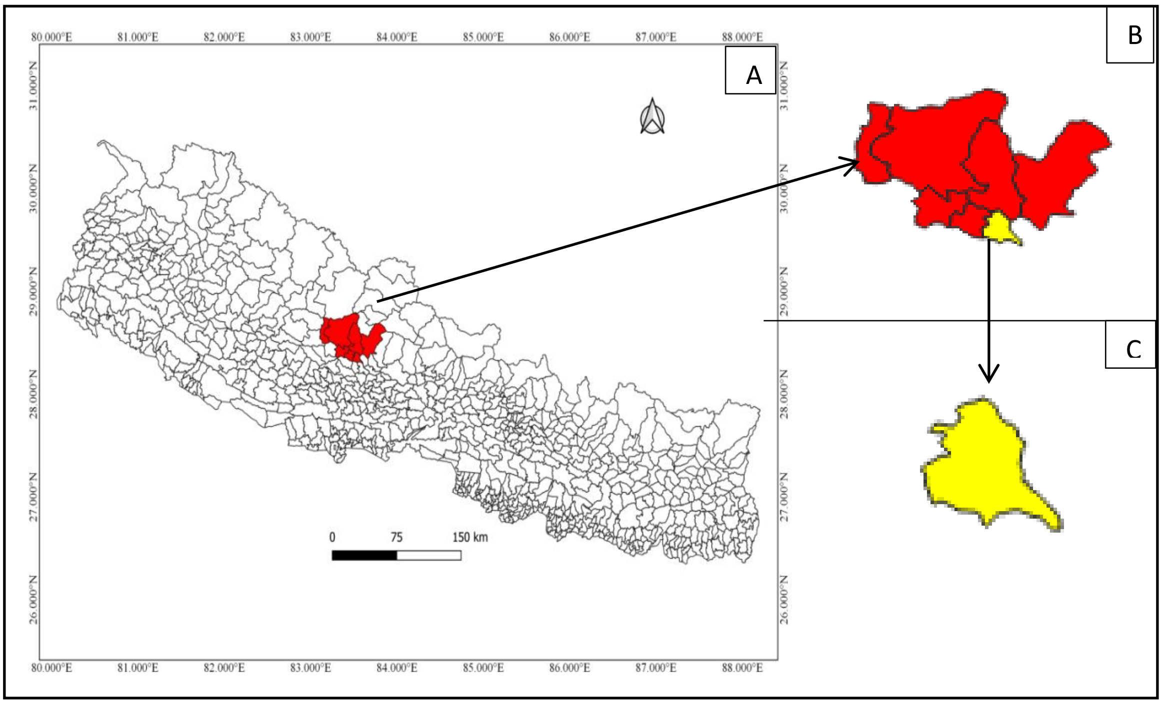

Figure 2: A) Map of Nepal showing study area B) Myagdi district C) Beni Municipality showing study site.

3.1.2 Climate

According to the data revealed by the Department of Hydrology and Meteorology, the maximum average temperature was recorded in the month of June (31.403 ˚C), and May, July, and August had nearly identical values to June (Fig. 3). Similarly, the minimum temperature was recorded in the month of January with a value of 6.277 ˚C whereas the maximum rainfall was recorded in July 548.83 mm and the minimum was recorded in November 0.991 mm.

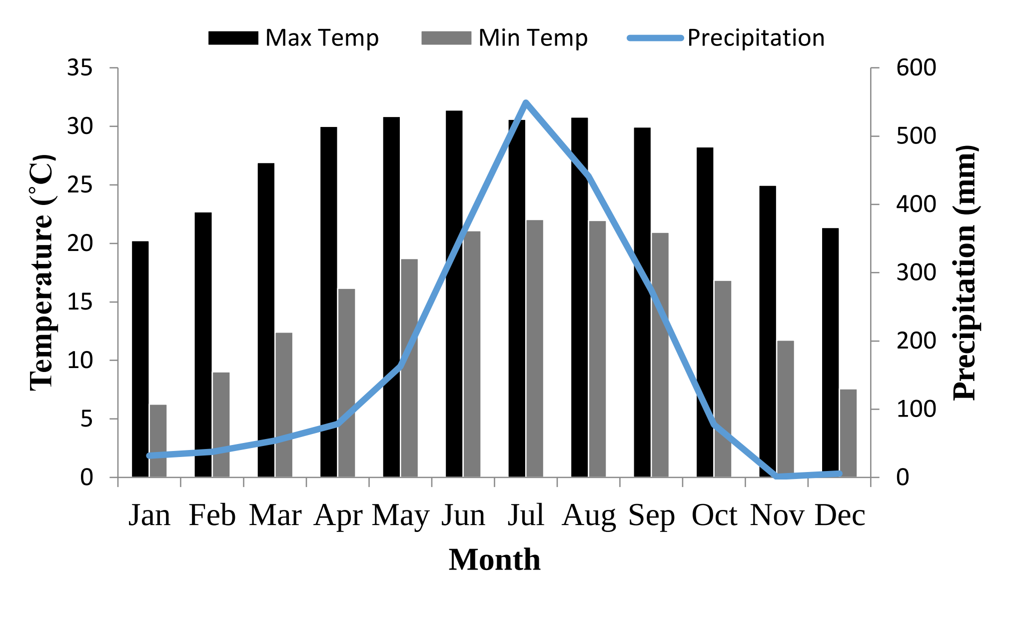

Figure 3: Average monthly precipitation and temperature of nearer station (Baglung), from 2012-2021 (Source: Department of Hydrology and Meteorology)

3.1.3 Vegetation

The study area consist of subtropical pine mixed forest dominated by tree species such as *Pinus roxburghii*, *Schima wallichii*, *Rhododendron arboreum*, *Egelhardia spicata* and *Lyonia ovalifolia*. The herbaceous plants found in that forest are *Chrysopogon* sp., *Andropogon* sp., *Cyanotis cristata* and so on.

3.2 Collection of Sample

Mushroom samples were gathered from the end of June to the beginning of September 2020. Macrofungi were sampled using a systematic random procedure at altitudes ranging from 1250- 1450 meters above sea level. The sampling was done by using a 10 m ×10 m quadrat in three transects at the distance of 100 m, and a total of 18 plots were made. Distance between each plot was approximately 50 m. (Baral *et al*., 2015). Before collecting, specimens were photographed in their natural habitat by using digital camera (Sony, DSC–W350) and their morphological characteristics such as size of fruiting body, cap color, cap surface, cap margin, scale, gill color, gill attachment, gill spacing, stipe length, width, color, shape, type of veil, annuls and volva, geographic location were noted (Srivastava and Bano, 2010). Date of collection, altitude, any change in color on cutting or bruising when fresh, growing solitary or in gregarious were also recorded.

The species found in soil were dug carefully with the help of a digger so that their bases were not damaged. Wood rotting mushrooms were removed from the bark of trees where they were attached. To prevent the intermixing of spores and external infections, the collected specimens were labeled, wrapped with aluminum foil, and placed in wax paper bags and brought to laboratory of Central Department of Botany for identification. From the middle of each plot, ecological factors such as tree canopy (percentage) were estimated visually.

3.3 Spore print

Mushrooms come in a variety of shape and size, including gilled mushrooms, boletus, puffballs, corals, hydnums, and bird nests, and their spore deposition varies as well. Also, there are different ways to take spores. For, taking spores of gills and pores bearing mushrooms stipe was cut off and placed the cap with the gills facing down on a piece of paper. Drop of water were put on the top of the cap to help release spores. And covered the cap with a bowl and left for 2-24 hours, depending on the humidity and freshness of the mushrooms. After removing the cap, paper containing spores were photographed and packed along with tag in zip- lock bag for microscopic view. The spore production in puffball entirely inside a ball and spores had taken when the outer covering exploded.

3.4 Preservation of mushrooms

Collected mushrooms were preserved using both dry and liquid methods. In liquid preservation, mushrooms were preserved in liquid using (25: 5: 70 mL of rectified alcohol + formalin + distilled water) (Hawksworth *et al.*, 1995). Air and sun drying were used for dry preservation. Only woody macrofungi were dried directly in the sun; fleshy fungi were air dried for 4–7 days at room temperature before being exposed to direct sunlight because rapid drying disfigures them.

3.5 Microscopic study

Obtained spore print was brought to the laboratory of Central Department of Botany for microscopic examination. First, the spore print papers were scratched with a needle and placed on a slide, stained with 1-2 drops of cotton blue and lactophenol, covered with a cover slip, and examined under a microscope to find the length and width of each species spore. In the case of small spores, immersion oil was used to magnify the spore.

3.6 Mushrooms identification

The preserved specimens were identified using various books and standard literatures (Phillips, 1981; Corner, 1970; Adhikari, 2000; Watling, 1973), consult with mushroom field guide as well as websites (http:/www.mycoweb.com; www.mushroomexpert.com). Macrofungi were also distinguished by spore print color and microscopic structure, i.e. spore shape and size, and morphological characteristics such as fruiting body size, cap color, cap surface, cap margin, scale, gill color, gill attachment, gill spacing, stipe length, width, color, shape, type of veil, annuls, and volva.

3.7 Evaluation Diversity index, Frequency and Density

The number of species in each sampling site was noted and those data used to calculate Diversity indices like the Shannon-Wiener diversity index (H ') and Simpson diversity index (D). Shannon-Wiener diversity index was calculated by following formula (Magurran, 2004).

$$H' = -\Sigma \, Pi \ln Pi$$

Where, H ' = Diversity index

Pi= ratio of individuals of species i divided by all individuals

n= number of species

N of all species

Σ= A Greek symbol that means "sum"

Similarly, Simpson Diversity Index was calculated by using following formula (Magurran, 2004).

Simpson Diversity Index (D) = $\frac{\Sigma n\ (n-1)}{N(N-1)}$

Where, D= Simpson's index

N= Total number of individuals of all species

n =Total number of organism of a particular species

The important quantitative analysis such as density and frequency of macrofungal species were determined as per (Daubenmire, 1959)

$$\text{Frequency \%: } \frac{\text{Number of quadrates with species}}{\text{Total number of quadrates taken}} \times 100\%$$

$$\text{Density (\%): } \frac{\text{Total number of individual of a species in all quadrates}}{\text{Area of one quadrate} \times \text{Total number of Quadrates}} \times 100\%$$

3.8. Soil sampling and analysis

To measure soil pH and moisture, soil samples were collected at a depth of 15 cm from four corners and the middle of each plot using a digger. These samples were well mixed before being placed in a Zipper bag containing approximately 200 g of soil for laboratory analysis.

3.8.1 Soil pH

pH was determined by using a pH meter (model-HM-1003) in a 1:2 ratio of the soil-water mixture. Before taking the measurement, the pH meter was calibrated with a buffer solution of known pH (pH 4 and pH 7). Following that, 50 mL of distilled water was poured into 25 g of soil. A magnetic stirrer was used to stir the mixture for up to 30 minutes before allowing it to settle for 5 minutes. The electrode was dipped into the mixture and the result (pH) value was recorded. Triplicate readings were taken from each soil sample.

3.8.2. Soil moisture

Moisture content was determined by using the formula by (Zobel *et al.*, 1987). For the calculation of the moisture content in the soil, clean and dry crucibles were taken. A 10 g fresh soil sample from each sample was heated in a hot air oven at 105˚C for 48 hours. Then the crucible was cooled thoroughly and weighted again.

$$\text{Moisture content (\%): } \frac{\text{Weight of fresh soil} - \text{weight of oven dried soil}}{\text{Weight of oven} - \text{dried soil}} \times 100\%$$

3.9 Nutrient analysis

3.9.1 Sample preparation

On the basis of most dominant & popularly known species of three wild edible mushrooms *Scleroderma cepa*, *Cantharellus cibarius,* and *Laccaria laccata* were taken for nutrient analysis. The collected mushrooms species were cleaned thoroughly to free them from mud, dried on blotting paper, sliced without division of pileus and stipe, air- dried, and powdered to about 1mm particle size and store at room temperature in polyethylene bottles until analysis (Mallikarjuna *et al*., 2013).

3.9.2 Determination of macronutrient

Nutrient content of three wild edible mushroom species were determined according to the Association of Official Analytical Chemists (AOAC, 2005).

Moisture - Moisture in mushroom sample was determined by oven dry method. In this method, 2 g sample was taken in a tarred oven dried crucible and placed into hot air oven for 110ºC, until it gives constant weight. Oven dried sample was cooled in desiccator and final weight was taken after proper cooling. The lost weight during the drying represents the moisture contents (%) calculated by following formula;

$$\text{Moisture contents (\%)} = \frac{\text{Loss in weight of sample}}{\text{weight of a sample taken for analysis}} \times 100\%$$

Ash – 1 g of air dried sample was taken qualitatively in a clean and dry porcelain crucible. The sample was ignited at 550ºC keeping inside the muffle furnace until it gives constant weight. After complete ignition, the crucible containing the ash was cooled in desiccator and the final weight of the crucible containing ash was taken. From the increased weight of the crucible, ash content of the sample was calculated. The ash contents (%) calculated by following formula;

$$\text{Ash contents (\%)} = \frac{\text{Weight of ash after incineration}}{\text{Weight of sample taken for ashing}} \times 100\%$$

Protein – Protein was determined by Kjeldal Digestion method. In this method, 3 gm powdered sample was mixed with about 10 grams digestion mixture (mixture of copper sulfate and sodium sulfate) in presence of 10 mL concentrated sulfuric acid. Unless the forth ceases, it was heated at low temperature and further heated at high temperature until the solution becomes transparent blue and white fumes comes. Then after digestion flask was cooled at room temperature for 20-30 min. Then digested sample was transferred into volumetric flask with the help of pipette and added distill water to make its volume and closed it.

For distillation, the apparatus was set in such a way that the cold water continuous flow through the unit. The distilled was then collect in a 4% boric acid (H_3BO_3) solution that absorb the liberated nitrogen content in a beaker. Nearly 200 mL beaker containing boric acid, was then titrated.

After completing distillation, distilled sample was titrated against the hydrochloric acid (HCL). By using the following formula total nitrogen content was calculated and the protein content was determined by multiplying with 6.25.

$$\text{Total Nitrogen (\%)} = \frac{14\times (V-V1)\times 100\times S}{W\times 1000} = X$$

Protein % = X × 6.25

Where;

14 = molecular weight of Nitrogen

V = Volume of standard acid used to neutralize the distillate

V1 = Volume of standard acid used to neutralize the blank

S = Normality of standard acid (strength)

X = Total nitrogen percent W = Weight of sample taken for digestion

6.25= Conversion factor

Fat - Fat in mushrooms sample was determined by Soxhlet Extraction method. In this method, 10 g of oven dry powdered sample was kept into thimble, weighted, noted the sample weight and placed cotton into the thimble in a way that covers the sample and folded. Dried round bottom flask was weighted and noted its weight. Then thimble along with sample placed into Soxhlet apparatus and extracted by petroleum spirit for 4-5 hours in Soxhlet apparatus. Extraction had been done for 7 hours. The solvent was evaporated in a tarred evaporating dish and weighted. From the increased weight of the dish, fat percentage of the sample was calculated by following formula;

$$\text{Fat (\%)} = \frac{M2-M1}{E}\times 100\%$$

M1= Initial weight (in g) of the dry empty round bottom flask

M2= Final weight (in g) of the dry empty round bottom flask

E= weight of the sample in grams

Carbohydrate - Carbohydrates calculated from the observed values of ash, fat and protein.

Carbohydrate (%) = 100- (Ash%+Fat%+Protein)

3.9.3 Determination of minerals

The mineral content such as Iron, Manganese, Copper, Zinc, Magnesium, Calcium and Potassium were determined through atomic absorption spectrophotometer (AAS). In this method, 5 g of mushroom sample was placed in a porcelain crucible and dried in a hot air oven set to 105°C for 3 hours. The samples were then ashed in a muffle furnace at 550˚C until the ash residue was white or grey. The obtained ash was dissolved in 5mL of a mixture of HNO3 and hydrochloric acid and slowly heated to dissolve the residue, and solution was transferred to a volumetric flask, and diluted to make 50 ml. Then, the sample containing element was determined by Atomic Absorption Spectrometry, by using Flame Atomic Absorption Spectrometer.

Phosphorous - Ash of the sample was extracted by 1:1 HCL, and distilled water then filtered through medium textured filter paper to get clear filtrate. Aliquot of sample was treated with Molybdovanadate Reagent to develop yellow color. Finally, the absorbance of the yellow color of the sample solution was measured by spectrophotometer at 400 nm. From the observed absorbance of the sample, concentration of phosphorous was calculated.

3.10 Statistical analysis

A regression analysis was performed to determine the impact of environmental variables on macrofungal species richness. To compare the mean value of nutrients between and within species, one way ANOVA and the non-parametric Kruskal Wallis test were done using SPSS. At the 5% probability level, significance was accepted. Analysis was carried out in triplicate to ensure reliability of the result.

CHAPTER IV: RESULTS

4.1 Macrofungal diversity

In total, 70 macrofungal taxa were collected. Among them, 60 were identified at the species level and 10 were identified at the generic level, of which 3 were ascomycota and 67 were basidiomycota. These fungi belonged to 26 different families and 12 orders (Fig. 4). The Russulaceae family (16 species) was found to be the most dominant family, followed by Amanitaceae (10 species), Boletaceae (9 species), Cortinariaceae (6 species) and so on whereas, families like Auriscalpiaceae (1 species) and Geastraceae (1 species) were the families containing the least species. Likewise, the Agaricales order was found to be the dominant order, containing 13 species, but the Geoglossales, Cantharellales, Xylariales, etc., contained only one species.

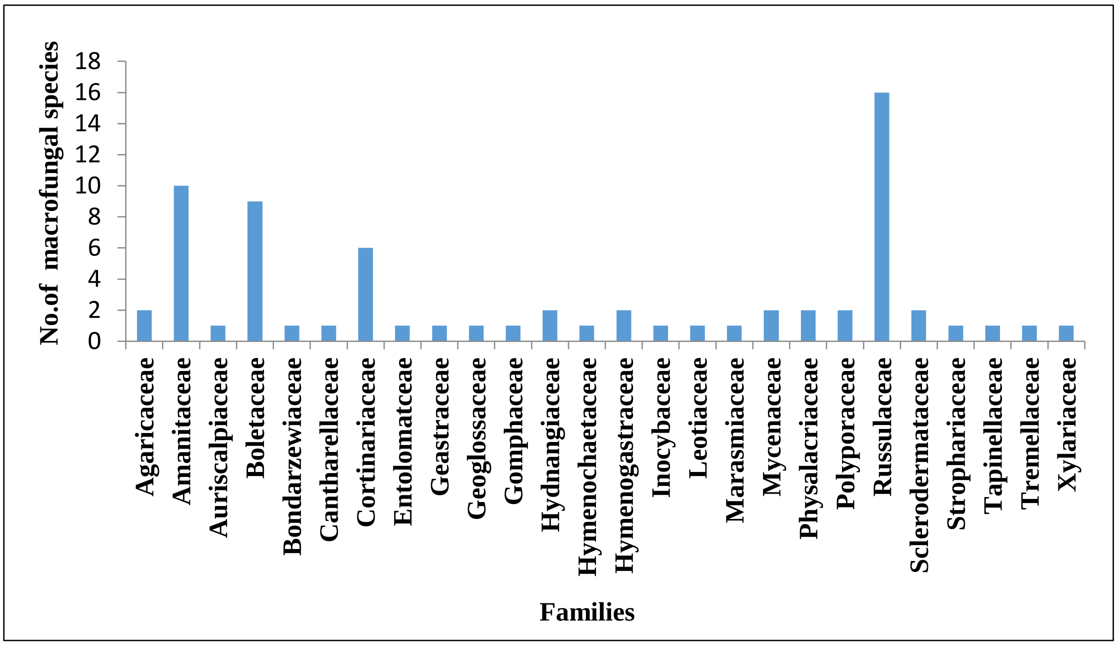

Figure: 4 Family wise distribution of macrofungi

During the survey mushrooms were found in different habitat (Fig: 5) i.e. on Cap of mushroom, fallen twig and branch, Pine cone, soil, stump and in tree trunk. Most of mushrooms were found in soil (82%) followed by stump (10%), tree trunk (3%) and fallen twig and branch (3%) while very few mushrooms were found in Pine cone, and cap of mushrooms, i.e. only (1%).

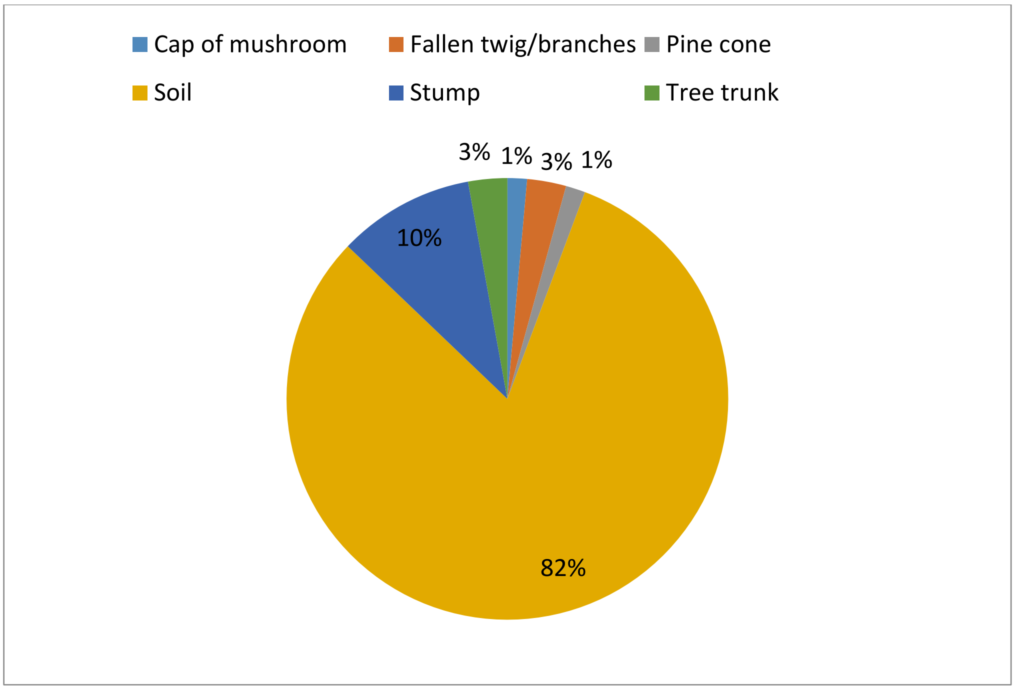

Figure: 5 Habitat wise distribution of mushroom

The majority of the mushrooms in the study were mycorrhizal (50), followed by saprophytic (17), and parasitic (3) (Fig. 6).

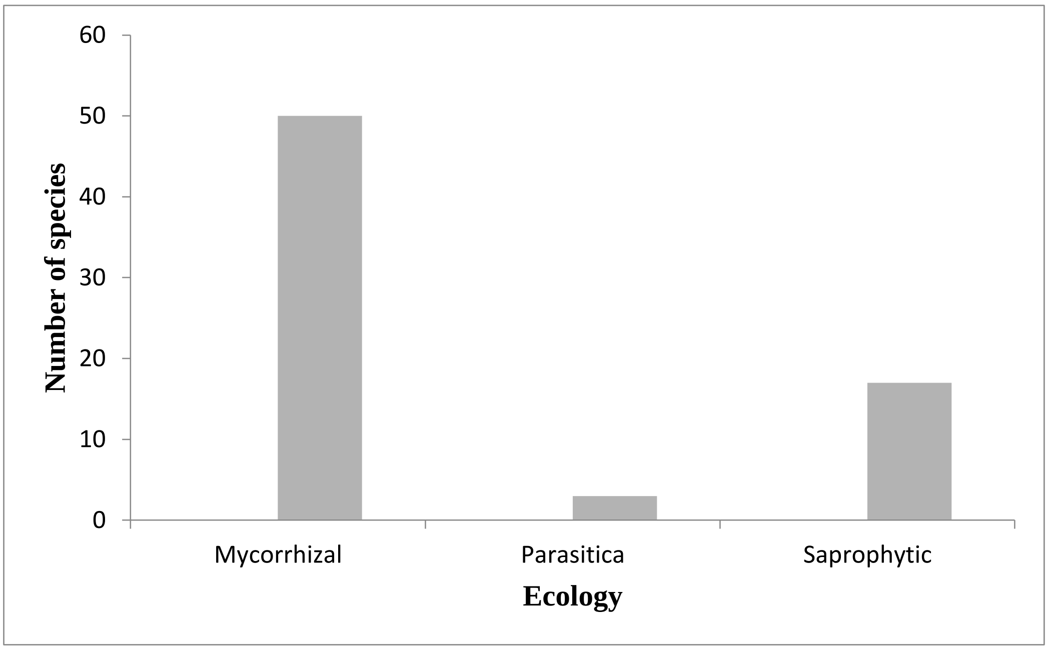

Figure 6: Ecology wise distribution of macrofungi

4.1.1 Diversity index, Frequency and Density

Among the collected species, *Mycena* sp. was found to be much denser (D =11.88 %) and *Cantharellus cibarius* was found to have much frequency (F=44.44 %) The

Shannon diversity index was 3.49 and Simpson index was 0.95 which indicates the very high species richness and dominance of the study site.

4.1.2 Detailed study of collected macrofungi

1. ***Amanita abrupta*** **Peck**

Family - Amanitaceae   Habitat - Soil, solitary or in small group

Pileus - Convex to flat, white, covered with conical white warts, 4-10 cm , **Gills -** white, crowded, close, free from the stem or slightly attached, **Stipe -** white, ring and volva, slender, 6.5-12 cm tall and 0.5-1 cm wide, solid, bulbous base, tapering towards the apex, , **Spore print -** White, **Spores -** 8-12 ×8-11 µm, subglobose or broadly ellipsoid, smooth.

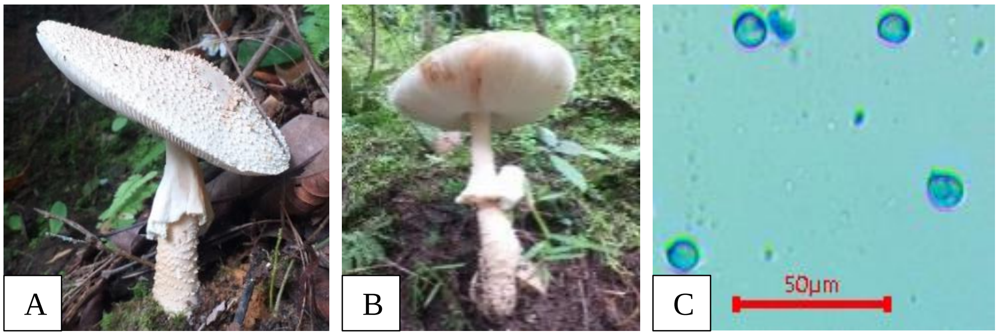

Photo plate 1: (A-B) *Amanita abrupta* in natural habitat (C) Spores of *A. abrupta*

2. ***Amanita caesarea*** **(Scop.) Pers.**

Family - Amanitaceae   Habitat - Soil

Pileus - Convex to flat, orange, smooth, up to 18 cm, **Gills -** yellow, free, crowded, **Stipe -** cylinder, yellowish white, 8-15 cm tall and 1-3 cm wide, ring and volva present, **Spore print -** white, **Spores -** ellipsoidal, 7-10 × 10-13 µm.

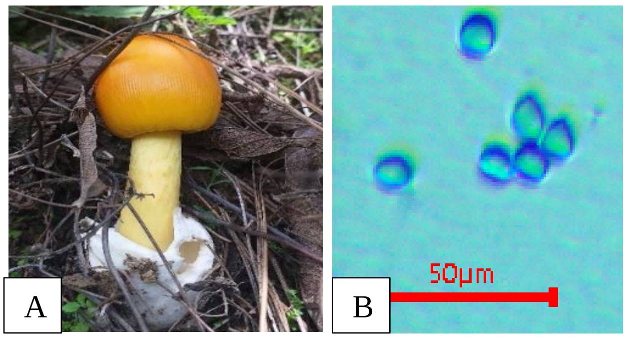

Photo plate 2: (A) *Amanita caesarea* in natural habitat (B) Spores of *A. caesarea*

3. ***Amanita fulva*** **Fr.**

Family - Amanitaceae Habitat - Soil growing alone

Pileus - Brown, convex or nearly flat, 5-8 cm in diameter, **Gill -** white, crowded, free from the stem, **Stipe -** pale brownish, 9-16 cm tall and 1-1.5 cm wide, no ring but volva present, slightly tapering towards apex, **Spore print -** white, **Spores -** globose to subglobose, smooth, 9.5-13.5 × 9.7-13 µm.

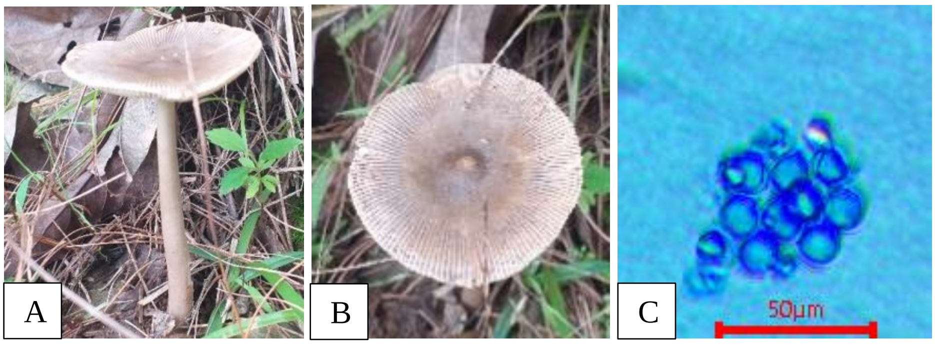

Photo plate 3: (A-B) *Amanita fulva* in natural habitat (C) Spores of *A. fulva*

4. ***Amanita multisquamosa*** **Peck**

Family - Amanitaceae Habitat - Soil

Pileus - Pale whitish, with a yellowish brown tan center, convex, margin is distinctly striate, 3.5 -10 cm, **Gill -** White, free from the stem, crowded, **Stipe -** white, annulated, the annulus funnel-shaped, volva white, 6-13 cm long and 0.5-1.5 thick, tapering to upward, hollow, not changing color when sliced, **Spore print -** white, **Spores -** subglobose to ellipsoid, smooth, inamyloid, 8-12 × 5-8 µm.

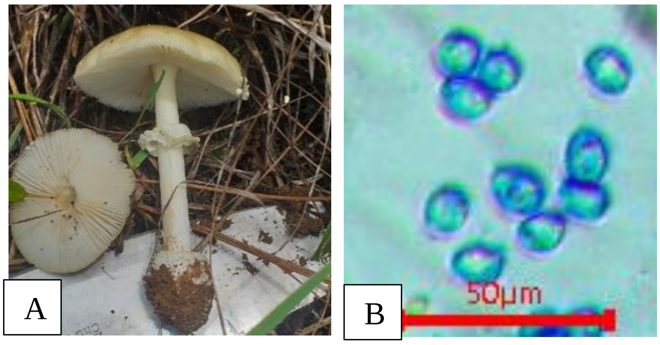

Photo plate 4: (A) *Amanita multisquamosa* in natural habitat (B) Spores of *A. multisquamosa*

5. *Amanita pantherina* (DC.) Krombh.

Family - Amanitaceae Habitat - Soil

Pileus - Convex, 4-18 cm wide, dark brown, whitish warts, **Gill -** white, free from the stem, crowded, **Stipe -** white, Ring and volva present, 5- 20 cm long and 2.5 cm thick, tapering to apex, **Spore print -** white, **Spores -** 8-12 × 7-9 µm, smooth, hyaline, globose to subglobose.

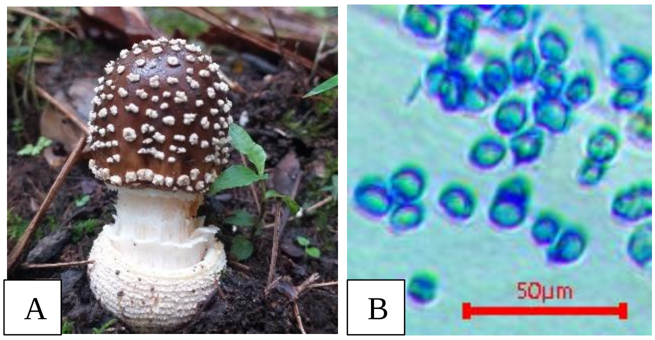

Photo plate 5: (A) *Amanita pantherina* in natural habitat (B) Spores of *A. pantherina*

6. *Amanita phalloides* (Vaill. ex Fr.) Link

Family - Amanitaceae Habitat - Soil

Pileus - Convex, yellowish green, 4.5-15 cm wide, **Gills -** crowded, white, free, **Stipe -**full when young and hollow with the maturity, bulbous at the base, annual ring present, 4.5-18 cm long and 1-3 cm, **Spore print -** white, **Spores -** ellipsoid to amayloid, 7-9 × 6-8 µm.

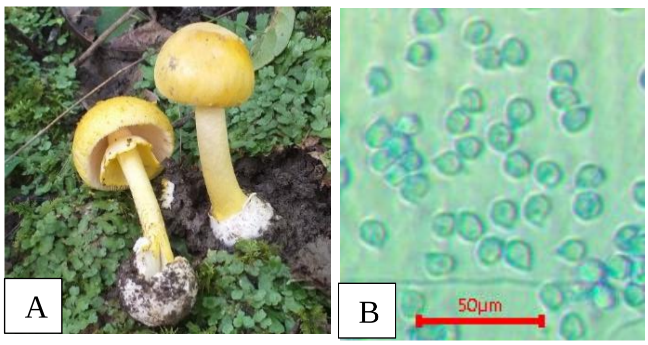

Photo plate 6: (A) *Amanita phalloides* in natural habitat (B) Spores of *A. phalloides*

7. *Amanita porphyria* Alb. & Schwein.

Family - Amanitaceae   Habitat - Soil

Pileus - Grey brown but darker in the center, flatten, 5-10 cm in diameter, **Gill -** crowded, whitish and free from stem, **Stipe -** 6-12 cm tall and 0.5-1.5 cm thick, ring gray in color, tapering towards the apex, grey brown, **Spore print -** white, **Spores -** 8.4-7.4 × 9-11 µm, globose to subglobose.

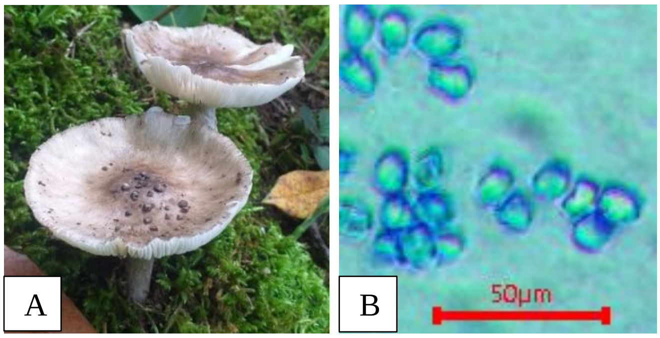

Photo plate 7: (A) *Amanita porphyria* in natural habitat (B) Spores of *A. porphyria*

8. *Amanita rubescens* Pers.

Family - Amanitaceae   Habitat - Soil, scattered in small group

Pileus - Convex at early stage while flattened at maturity, 4-20 cm in diameter, **Gill -** White, crowded, when handled the gills blush into dull red, **Stipe -** 6-15 cm tall and 1.5 cm wide, hollow, **Spore print -** white, **Spores -** ellipsoidal to ovoid, smooth,11-15 × 4-6 µm.

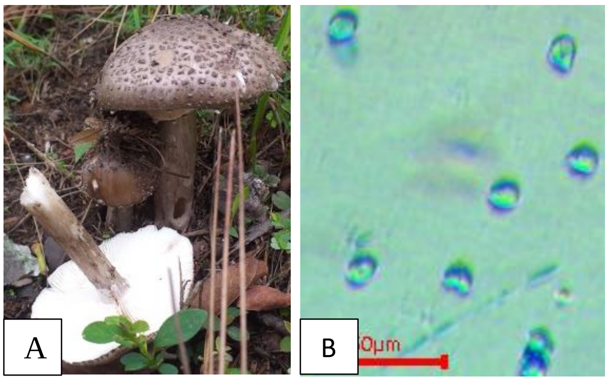

Photo plate 8: (A) *Amanita rubescens* in natural habitat (B) Spores of *A. rubescens*

9. *Amanita spissacea* S. Imai

Family - Amanitaceae Habitat - Soil

Pileus - Convex, grayish brown, brown scale present, 3-8 cm in diameter, **Gill -** Free, creamy white, **Stipe -** 4-12 ×0.5-1 cm, hollow, grayish brown, tapering towards apex volva bulbous, annulus present, **Spore print -** white, **Spores -** hyaline, smooth, elliptical, 6-11× 5-10 µm.

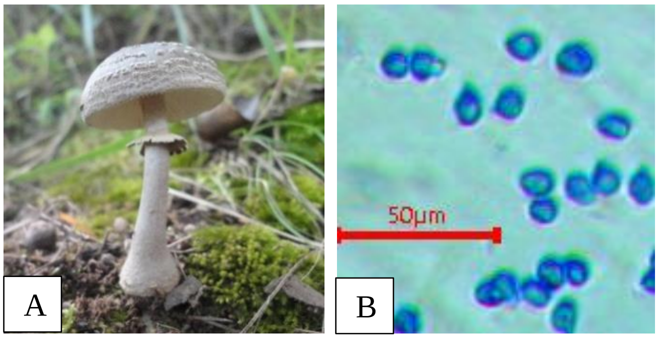

Photo plate 9: (A) *Amanita spissacea* in natural habitat (B) Spores of *A. spissacea*

10. *Amanita vaginata* (Bull.) Lam.

Family - Amanitaceae Habitat - Soil

Pileus - Gray,convex with a central bump, 3-10 cm in diameter, **Gills -** white, free, crowded, does not change color when bruising or injure, **Stipe -** lack of ring, volva present, 8-20 cm long and 1-2 cm thick, slightly tapering to apex, the stem surface is covered with a fine scales especially near the top, **Spore print -** white, **Spores -** subglobose, smooth, 13-16 µm in diameter.

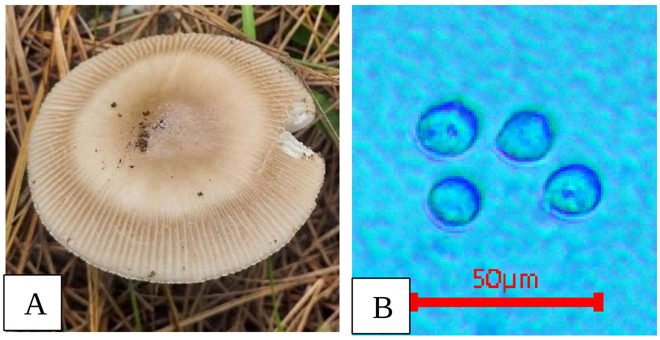

Photo plate 10: (A) *Amanita vaginata* in natural habitat (B) Spores of *A. vaginata*

11. *Auriscalpium vulgare* Gray

Family - Auriscalpiaceae Habitat - Pine cone

Pileus - Dark brown, spoon shape, hairy, 2.1 cm in diameter, **Teeth -** White, crowded, **Stipe -** Dark brown, hairy, 2.3 cm long and 0.3 cm width.

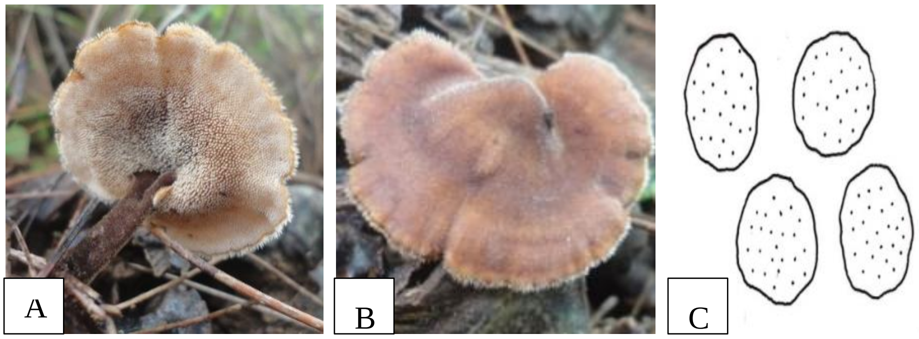

Photo plate 11: (A-B) *Auriscalpium vulgare* in natural habitat

(C) Illustrated spores of *A. vulgare*

12. *Boletus edulis* Bull.

Family - Boletaceae Habitat - Soil

Pileus - Flat, wrinkled and somewhat cracked with age, yellowish – brown, 5-20 cm, **Pores -** yellow when young and olive green when aged, **Stipe -** 5-15 cm long and 4-7 cm wide, sturdy, pale in color, rounded, **Spore print -** brown, **Spores -** 14.8-19.9× 5-7 µm, smooth, spindle-shaped.

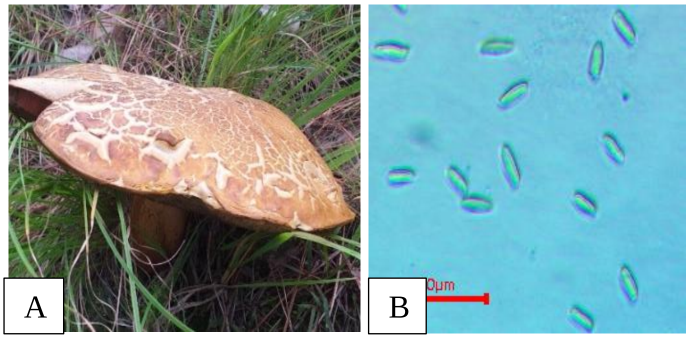

Photo plate 12: (A) *Boletus edulis* in natural habitat (B) Spores of *B. edulis*

13. *Boletus eximius* Peck

Family - Boletaceae   Habitat - Soil

Pileus - Brown, 5 cm, convex, **Pores -** brown**, Stipe -** 5-10 cm long and 1-3 cm wide**, Spore print -** brown, **Spores -** 10.9-14.1× 8.1-8.4 µm, smooth.

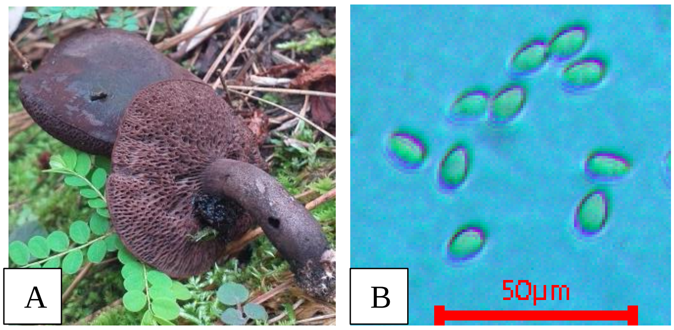

Photo plate 13: (A) *Boletus eximius* in natural habitat (B) Spores of *B. eximius*

14. *Cantharellus cibarius* Fr.

Family - Cantharellaceae   Habitat - Soil

Pileus - Orange yellow, funnel - shaped, wavy irregular margin, smooth, convex, up to 10 cm in diameter, **Gill -** orange yellow, fake gills or the wrinkle veins on the underside of the cap, the veins are very thick and decurrent, **Stipe -** orange yellow, smooth, 3-10 cm long and 2 cm in width, **Spore print -** pale yellow**, Spores -** ellipsoidal, smooth, inamyloid, hyaline, 7-11× 5-7 µm.

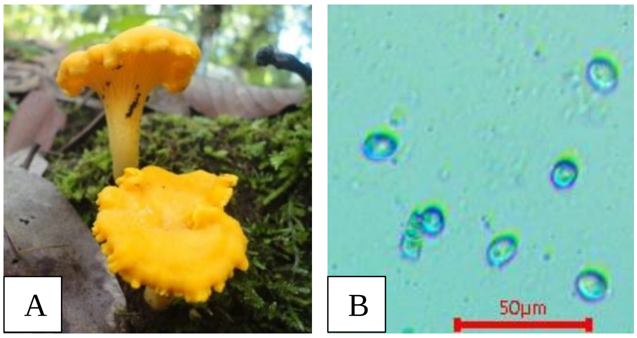

Photo plate 14: (A) *Cantharellus cibarius* in natural habitat (B) Spores of *C. cibarius*

15. *Coltricia cinnamomea* (Jacq.) Murrill

Family - Hymenochaetaceae Habitat - Soil

Pileus - Brown, 1-5 cm, flat, with concentric band, margin wavy, **Pores -** brown, angular, not bruising, **Stipe -** brown, 1-5 cm long and 1 cm wide**,** velvety, **Spore print -** brown, **Spore -** 6-8.5 x 3-5 µm.

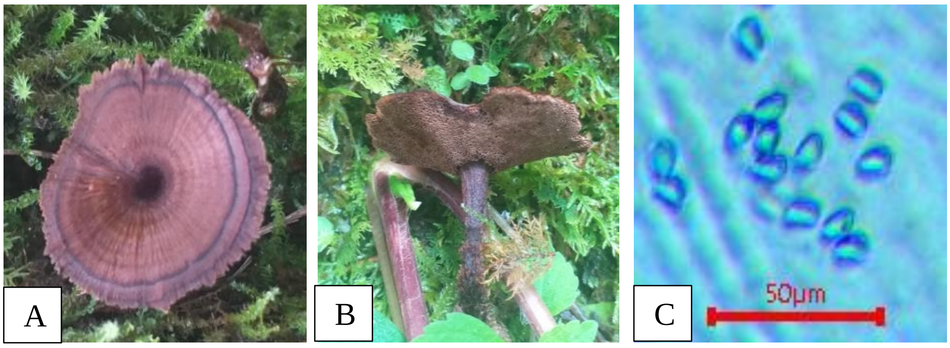

Photo plate 15: (A-B) *Coltricia cinnamomea* in natural habitat (C) Spores of *C. cinnamomea*

16. *Cortinarius laniger* Fr.

Family - Cortinariaceae Habitat - Soil

Pileus - Light brown, dome- shaped with whitish veil remnants along the edge, **Gills -** cinnamon brown, **Stipe -** bulbous, white to brownish, **Spore print -** brown, **Spore -** 10.1-13.4 x 7.4-7.8, µm, ellipsoid, slightly almond shaped.

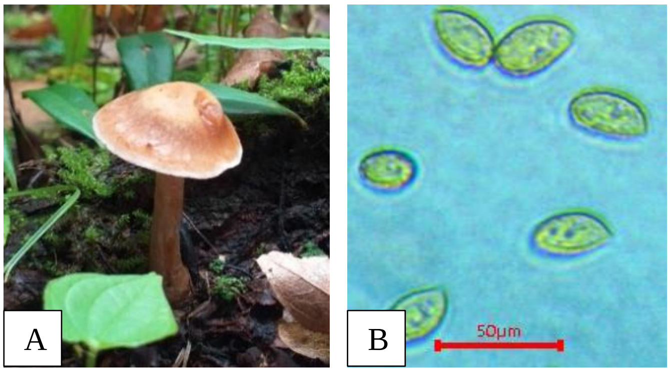

Photo plate 16: (A) *Cortinarius laniger* in natural habitat (B) Spores of *C. laniger*

17. ***Cortinarius purpurascens* Fr.**

Family **-** Cortinariaceae Habitat **-** Soil, rocky area, grow in group

Pileus - Dirty ochraceous brown, convex, wavy margin, 3-10 cm in width, **Gill -** violet brown, thin, crowded, when young gilled are covered by a spider web like structure called a cortina, **Stipe -** violaceous, swollen towards the base, solid, **Spore print -** brown, **Spores -** ellipsoid, slightly almond shaped, verrucos, 8-10 × 4-6 µm.

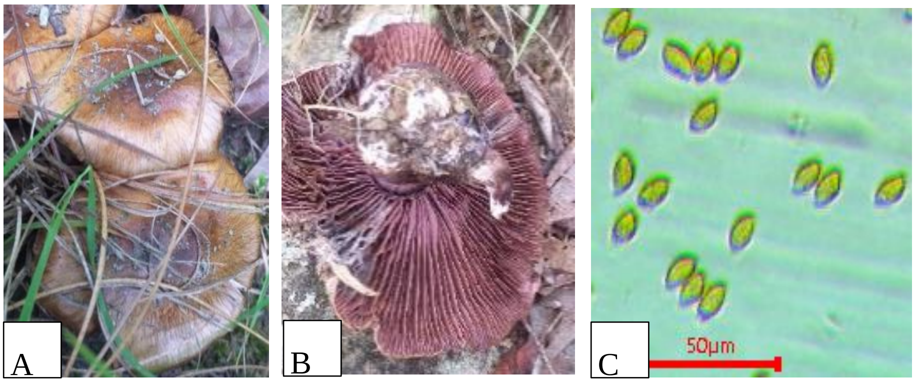

Photo plate 17: (A-B) *Cortinarius purpurascens* in natural habitat (C) Spore of *C. purpurascens*

18. ***Cortinarius rubellus* Cooke**

Family **-** Cortinariaceae Habitat **-** Soil

Pileus - Convex to flatten, dry, brown in color cover with fine, fibrous scales, 3-8 cm, **Gills -** deep brown, adnate, **Stipe -** 4-11 cm tall and 0.5-1.5 cm thick, color same as cap, fibrous, **Spore print -** Yellow, **Spores -** ellipsoidal to sub globose, 7-10 × 9-12 µm, surface rough.

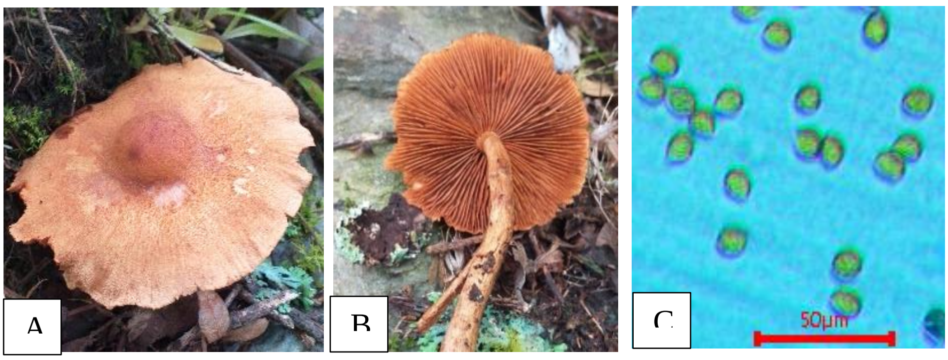

Photo plate 18: (A-B) *Cortinarius rubellus* in natural habitat (C) Spores of *C. rubellus*

19. *Cortinarius callisteus* (Fr.) Fr.

Family **-** Cortinariaceae   Habitat **-** Soil

Pileus - convex, 5.1 cm, bell shaped, smooth, reddish yellowish brown, **Gill -** rusty brown, attached to the stem, close, **Stipe -** 7.7 cm long and 0.9 cm thick, whitish to pale brownish, basal mycelial whitish, **Spore print -** rusty brown**, Spores -** ellipsoid, warty, 9-14 ×6.6-8.5 µm.

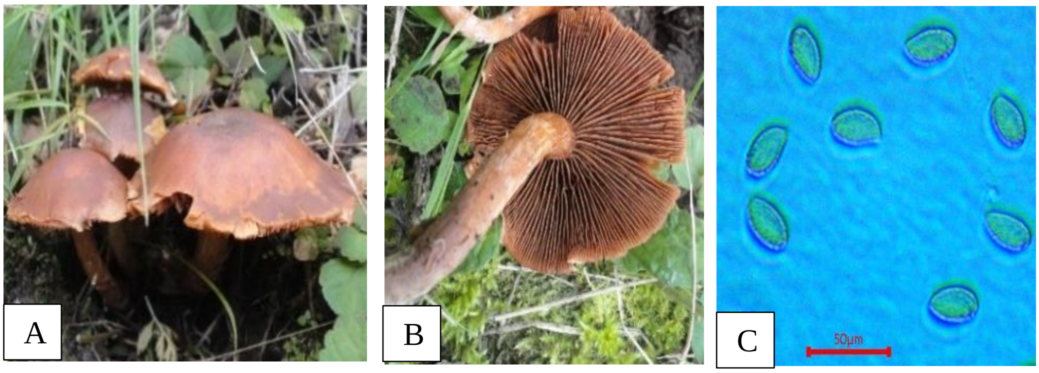

Photo plate 19: (A-B) *Cortinarius callisteus* in natural habitat (C) Spores of *C. callisteus*

20. *Cortinarius* sp.

Family **-** Cortinariaceae   Habitat **-** Soil

Pileus - Dark brown, 4-10 cm, **Gill -** Dark brown, attached to stem, distant, **Stipe -** light brown, 2 -7 cm tall and 0.8 – 2 cm thick, **Spore print -** Rusty brown**, Spore -** 13.1-15.4 ×6.6- 8.5 µm, almond-shaped, warted.

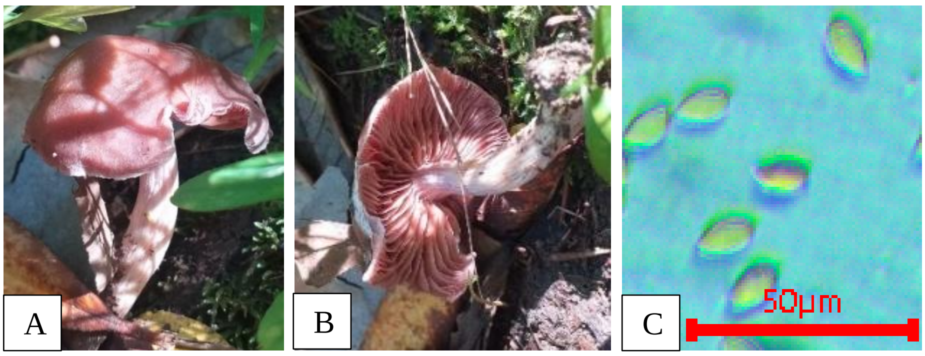

Photo plate 20: (A-B) *Cortinarius* sp. in natural habitat (C) Spores of *Cortinarius* sp**.**

21. *Cortenarius* sp.

Family - Cortinariaceae Habitat - Soil

Pileus - Yellowish brown,1.5-8 cm, convex, bell shaped, dry **Gill -** attached to the stem, close, crowded, attached to stem, **Stipe -** 2-7 cm long and 1-2 cm thick, **Spore print** -brown, **Spore -** 6-8×64-6 µm, almond-shaped, warted.

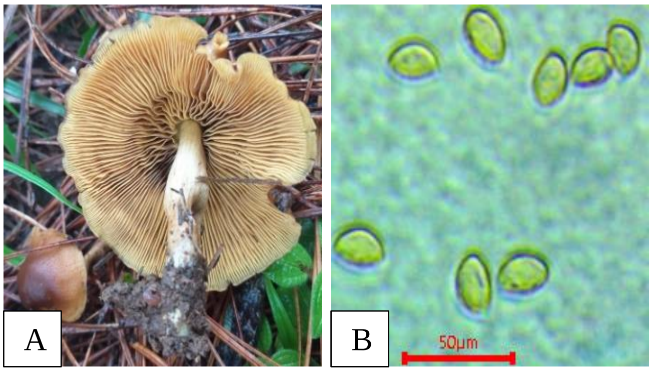

Photo plate 21: (A) *Cortinarius* sp. in natural habitat (B) Spores of *Cortinarius* sp**.**

22. *Cyathus olla* (Batsch) Pers.

Family - Agaricaceae Habitat - Cluster or scattered on soil

Nest - Base narrow, tapering upward, 0. 9-1.2 cm tall and 0.7-1.5 cm wide, brownish hairs on outer surface and smooth inside, eggs, rounded to somewhat flattened, silvery in color, **Spores -** ovate to ellipsoid, smooth, hyaline, 10-13 µm in length and 5-8 µm wide.

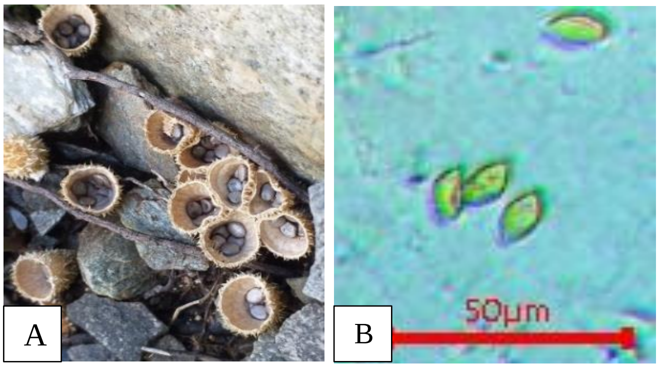

Photo plate 22: (A) *Cyathus olla* in natural habitat (B) Spores of *C. olla*

23. *Entoloma* sp.

Family - Entolomataceae Habitat - Soil

Pileus - White, convex, fibrous, brownish at the center, **Gills -** attach to the stem, distant or slightly close, **Stipe -** white, finely hairy, hollow, base enlarge, **Spore print** -yellowish pink, **Spores -** angular, 9-11.3 x 6-8 µm.

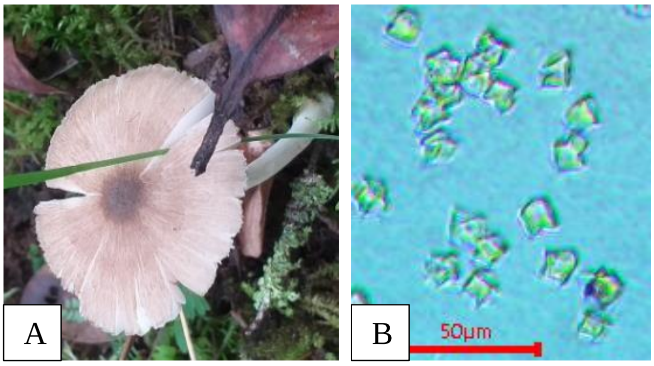

Photo plate 23: (A) *Entoloma* sp. in natural habitat (B) Spores of *Entoloma* sp.

24. *Favolus* sp.

Family - Polyporaceae Habitat - Decaying wood of *Schima wallichii*

Pileus - white nearly transparent, glabrous, 5.5 cm wide, **Pores -** angular to elongated, white to creamy, **Stipe -** short, Cylindrical, whit 0.7 cm long and 0.6 cm wide, **Spore print -** yellowish white, **Spores -** smooth, 6-8 × 3-4 µm.

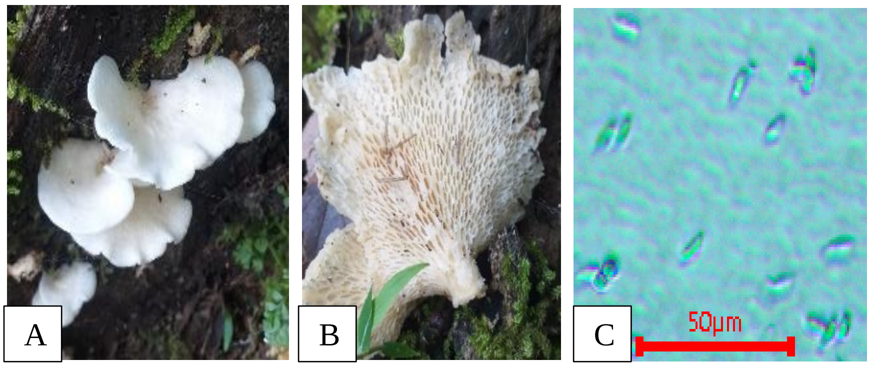

Photo plate 24: (A-B) *Favolus* sp. in natural habitat (C) Spores of *Favolus* sp.

25. *Geastrum* sp.

Family **-** Geastraceae Habitat **-** Soil near leaf litter

Fruiting body - dirty white to pale brown in color and composed of 2 layers. A thick outer layer has 7-9 lobes and Star shape where inner layer globular in shape with a circular pore at the top petal curl up around the spore sac, **Spores -** round, spiny warts, 11.0-15.3 µm.

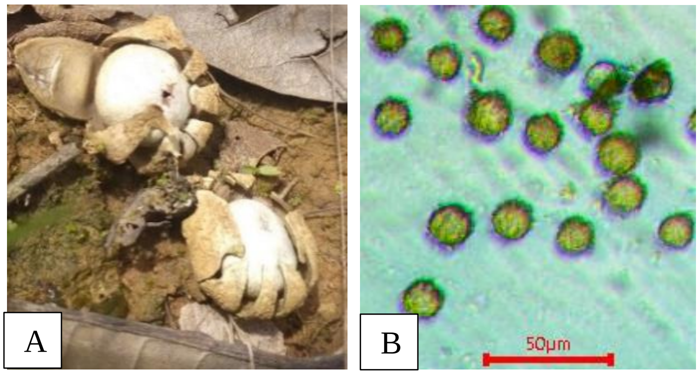

Photo plate 25: (A) *Geastrum* sp. in natural habitat (B) Spores of *Geastrum* sp.

26. *Glutinoglossum glutinosum* (Pers.) Hustad, A.N. Mill., Dentinger & P.F. Cannon

Family **-** Geoglossaceae Habitat **-** Soil in moss

Fruiting body - Club-shaped, smooth, nearly black with a well-defined head that is clearly separate from the stem ranging from 1.5-5 cm in height, **Head -** black, ellipsoidal to cylindrical, 0.7 cm height, **Stipe -** dark gray to brown, glutinous, **Spores -** pale brown to brown, cylindrical, clavate, slightly curved, septate, mostly three septate through occasionally seven-septate at maturity, one celled conidia inside the saci.

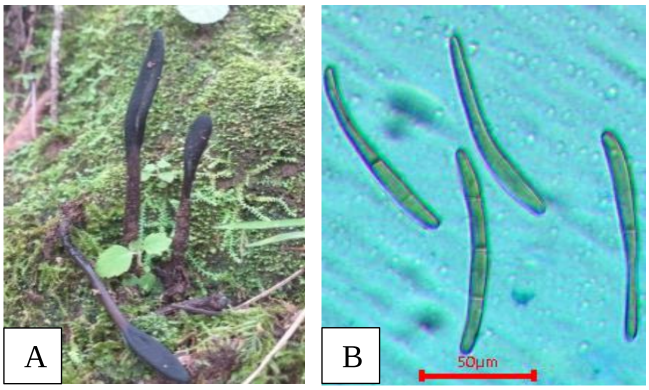

Photo plate 26: (A) *Glutinoglossum glutinosum* in natural habitat (B) Spores of *G. glutinosum*

27. *Hebeloma sinapizans* (Paulet) Gillet

Family - Hymenogastraceae   Habitat - Soil, Growing gregariously

Pileus - convex, sticky, white, margin slightly wavy, 4.7-15.5 cm, **Gill -** brown, crowded, attached to the stem, **Stipe -** 3-12 cm long and 1-3 cm wide, swollen base, white to brown, no ring, scales present near the apex, **Spore print -** brown, **Spores -** 13-16× 6-9 µm, elliptical.

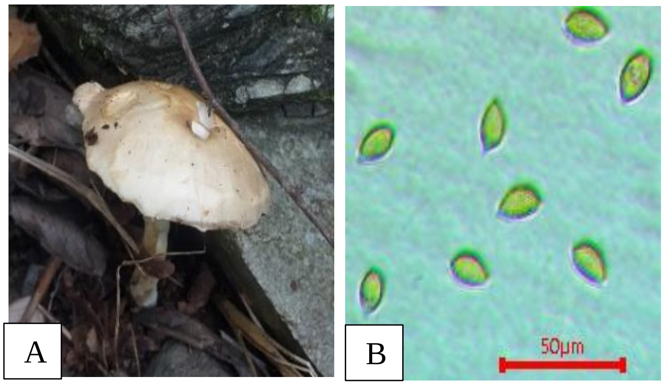

Photo plate 27: (A) *Hebeloma sinapizans* in natural habitat (B) Spores of *H. sinapizans*

28. *Heterobasidion annosum* (Fr.) Bref.

Family - Bondarzewiaaceae   Habitat - Stump of *Pinus roxburghii*

Pileus - Kidney shaped or irregular, whitish around the margins and dark brown on upper surface, **Pore -** present, **Stipe -** absent, **Spore print -** white

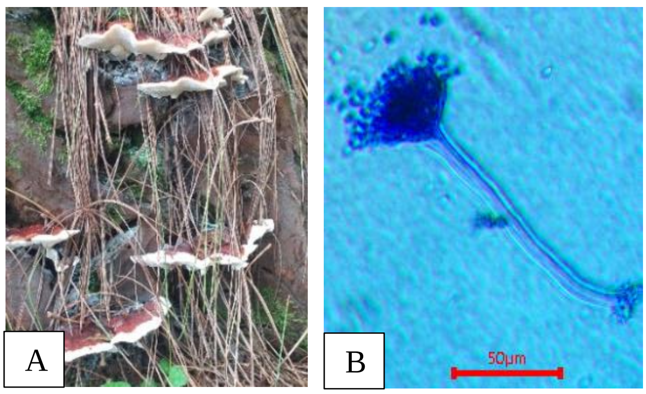

Photo plate 28: (A) *Heterobasidion annosum* in natural habitat (B) Spores of *H. annosum*

29. *Hortiboletus rubellus* (Krombh.) Simonini, Vizzini & Gelardi

Family - Boletaceae Habitat - Soil, growing alone, scattered, or gregariously

Pileus - Reddish, cracked, white band around the cap margin, 2.5-6.2 cm in diameter, **Pores -** yellow, stain blue when cut or bruised, **Stipe -** reddish, 3-5.5 cm long and 0.4-0.9 cm thick, **Spore print -** yellowish brown, **Spores -** smooth, subfusoid, 13.9-17.7 × 5.0-8.8 µm.

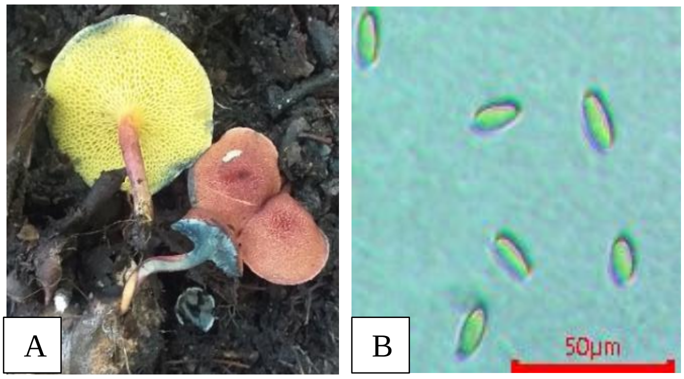

Photo plate 29: (A) *Hortiboletus rubellus* in natural habitat (B) Spores of *H. rubellus*

30. *Hypholoma fasciculare* (Huds.) P. Kumm.

Scientific Name - *Hypoloma fasciculare*

Family - Strophariaceae Habitat - Saprotrophic on *Lyonia ovalifolia*

Pileus - Convex, ranges from1.9-5 cm in diameter, smooth, light orange in color with darker center, **Gills -** adnate, crowded, initially yellow but later become greenish yellow and blackish spores develop on the yellow flesh therefore spotted blackish, **Stipe -** 2.8-10 cm tall and 0.3 cm thick, more or less equal, light yellow, **Spore print -** purple brown, **Spores -** ellipsoid, smooth, 5-9 × 3-6 µm.

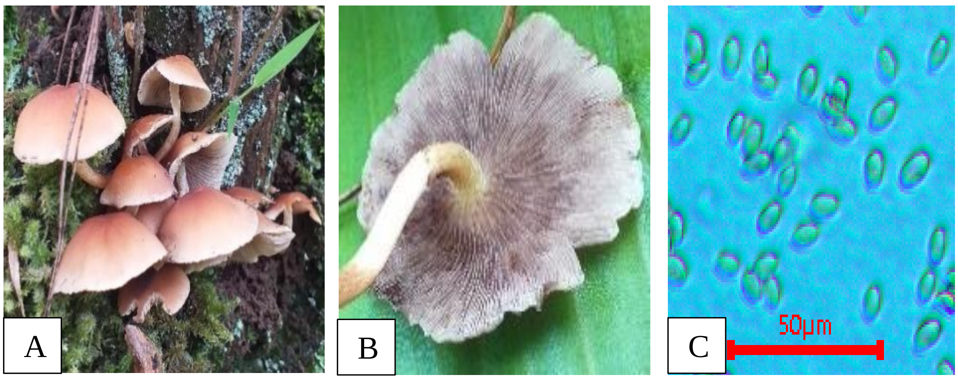

Photo plate 30: (A-B) *Hypholoma fasciculare* in natural habitat (B) Spores of *H. fasciculare*

31. *Inocybe* sp.

Family - Inocybaceae Habitat - Soil

Pileus - Light brown, 1-3 cm in diameter, conical, fibrous, **Gills -** moderately spaced, adnexed gill, pale brown, **Stipe -** 2-6 cm tall and 0.3 cm thick, pale brown, fibrous, **Spore print -** dark brown, Spores - irregularly stellate, 11.1-12.5 × 7-9 µm.

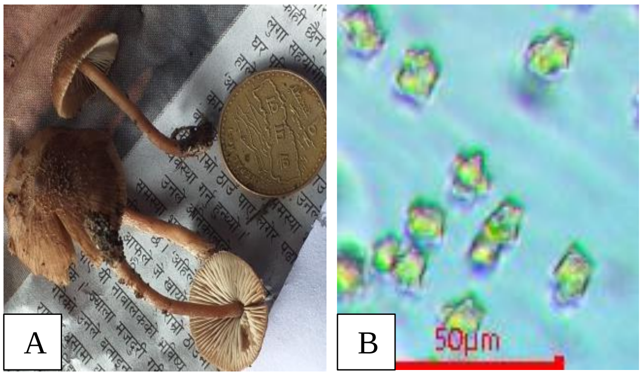

Photo plate 31: (A) *Inocybe* sp. (B) Spores of *Inocybe* sp.

32. *Laccaria amethystina* Cooke

Family - Hydnangiaceae Habitat - Soil, rocky area

Pileus - Deep purple, initially convex or depressed and become almost flat at maturity, finely hairy-scaly, 1-7 cm in diameter, **Gill -** attached to the stem, distant, dark purple, **Stipe -** Purplish, fibrous, hollow, 1.5-12 cm in tall and 0.5-1.5 cm in diameter, **Spore print -** white, **Spores -** spiny, hyaline, subglobose or broadly elliptical or 10-11.16 × 9-10 µm.

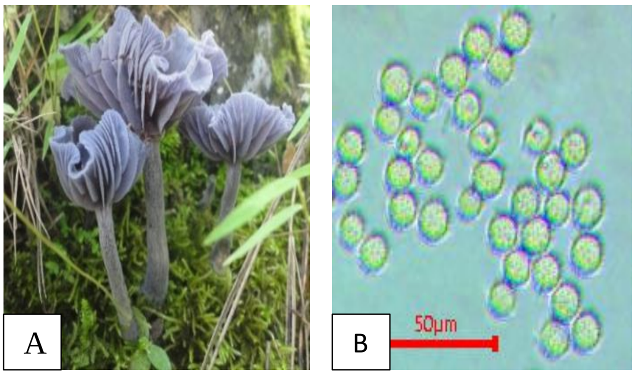

Photo plate 32: (A) *Laccaria amethystina* in natural habitat (B) Spores of *L. amethystina*

33. *Laccaria laccata* (Scop.) Cooke

Family - Hydnangiaceae Habitat - Soil cover by litters

Pileus - Pinkish brown, convex in very young stage but flat with age, 2-7 cm in diameter, **Gills -** decurrent, widely spaced, pinkish brown, **Stipe -** hollow, hairy towards the base, no ring, 3-9 cm long and 0.4-1 cm wide, Pinkish brown, **Spore print -** white, **Spores -** ellipsoidal to round with warty ornamentation, hyaline, 7-10 μm diameter.

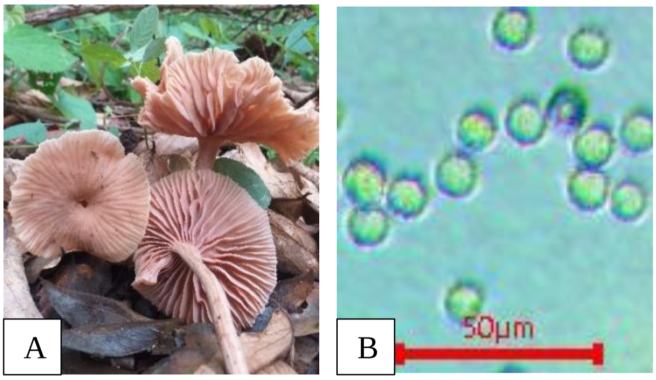

Photo plate 33: (A) *Laccaria laccata* in natural habitat (B) Spores of *L. laccata*

34. *Lactarius cinereobrunneus* D. Stubbe & Verbeken

Family - Russulaceae Habitat - Soil

Pileus - Gray brown, latex white at first but becoming pinkish reddish some time later, convex, smooth, surface dry, margin wavy, dark greyish brown in the central, 3-5 cm, **Gills -** creamy, subdistantes, **Stipe -** grey brown, approximately narrower towards the base, surface dry, 2.5-5 cm long and 0.5-1 cm wide. 12.1-14.1 × 10.4-11.9 μm.

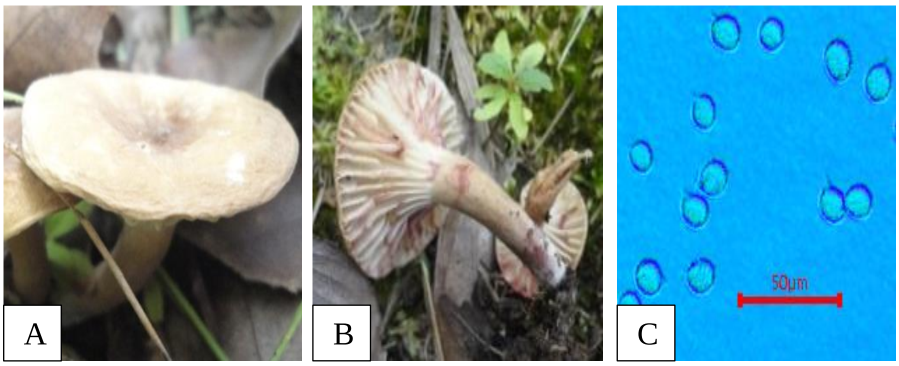

Photo plate 34: (A-B) *Lactarius cinereobrunneus* in natural habitat (C) Spores of *L. cinereobrunneus*

35. *Lactarius deterrimus* Gröger

Family **-** Russulaceae Habitat **-** Soil, solitory

Pileus - Flat, slightly sticky, light orange, bruised area turn into greenish, 7-10 cm, **Gill -** decurrent, crowded, light orange, developing green stain, red milk produce when cut later change into green, **Stipe -** Orange, hollow, 3 cm long and 1.5 cm wide, **Spore print -** yellow, **Spores -** subglobose to ellipsoidal, warts, 7.6-11.2× 9.0-9.9 µm.

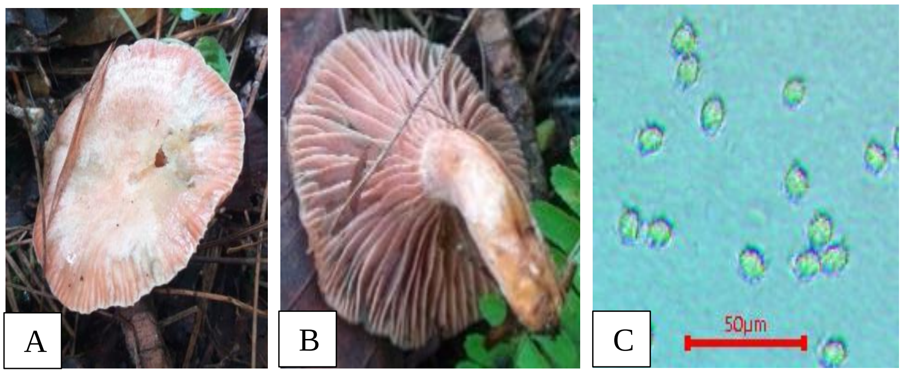

Photo plate 35: (A-B) *Lactarius deterrimus* in natural habitat (C) Spores of *L. deterrimus*

36. *Lactarius piperatus* (L.) Pers.

Family **-** Russulaceae Habitat **-** Soil

Pileus - Creamy- white, smooth, dry, broadly convex, funnel shaped, glabrous, 4-15 cm in width, **Gill -** white, attached to the stem, narrow, very crowded, forking frequently, **Stipe -** White, bare, smooth, solid, cylindrical, sometimes tapering towards the base, 2-8 cm tall and 1-2.5 cm thick, **Milk -** copious, white, unchanging after exposure, **Spore print -** white **, Spores -** warted and reticulate, broadly ellipsoid, 5-10× 5-8 µm.

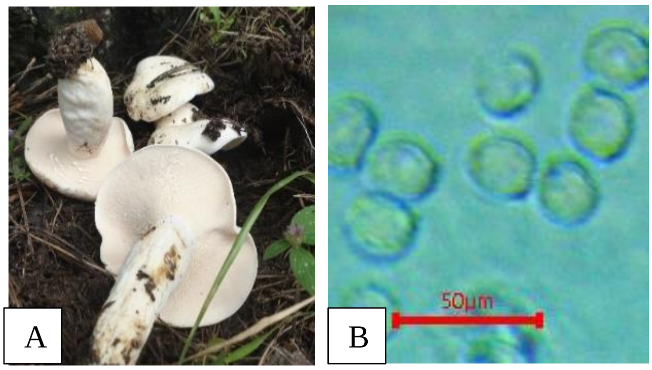

Photo plate 36: (A) *Lactarius piperatus* in natural habitat (B) Spores of *L. piperatus*

37. *Lactifluus volemus* (Fr.) Kuntze

Family - Russulaceae Habitat - Soil

Pileus - Convex, brownish orange, 5.5-10 cm wide, **Gills -** decurrent, pale golden yellow, closely spaced, latex exudes when gills are damaged, **Stipe-** 5-11 cm long and 0.9-1.5 cm wide, color paler than cap, **Spore print -** white, **Spores -** Spherical, hyaline, 8-11× 8-10 µm.

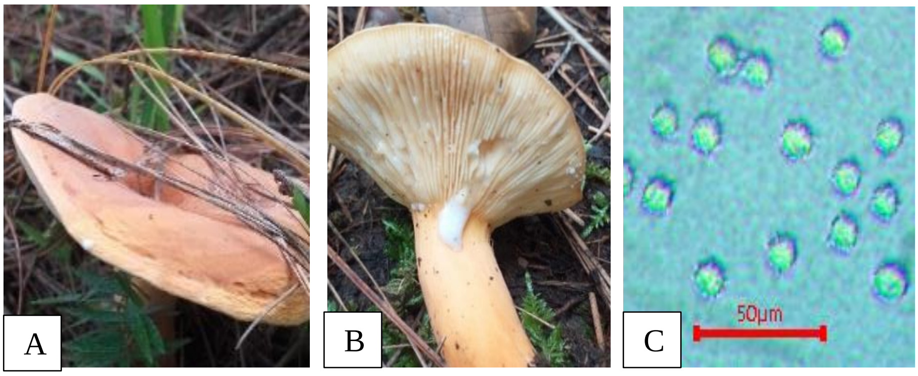

Photo plate 37: (A-B) *Lactifluus volemus* in natural habitat (C) Spores of *L. volemus*

38. *Leotia lubrica* (Scop.) Pers.

Family - Leotiaceae Habitat - Soil

Head - Olive-green, surface smooth, sticky and slightly wrinkled, convex, head has irregular lobes ad undulations, margin inrolled, 1-4 cm, **Stalk -** paler than head, smooth, sticky, surface is cover with very small greenish color granules, filled with gelatinous material, 2-8 cm long and 0.5- 1 cm wide, **Spore print -** white, **Spores -** 21-27×5-9 µm, smooth, ellipsoid to subfusiform, curved, septate.

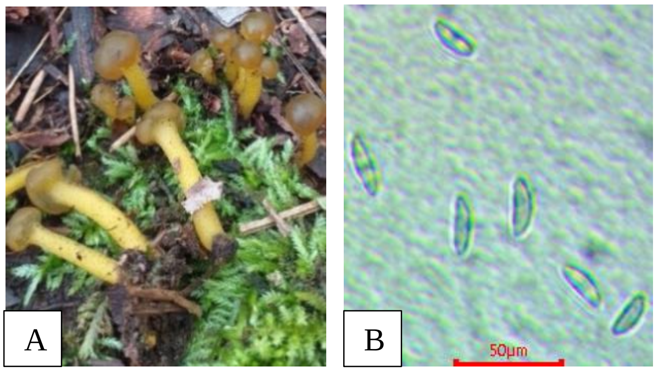

Photo plate 38: (A) *Leotia lubrica* in natural habitat (B) Spores of *L. lubrica*

39. *Lycoperdon molle* Pers.

Family - Agaricaceae Habitat - Solitary

Fruiting body - 2-7 cm tall and 2-5 cm broad, well developed pseudo stipe, greyish brown covered with short spines, **Spore deposition -** brown, **Spores -** 4-5 µm, small, round to subglobose, warted.

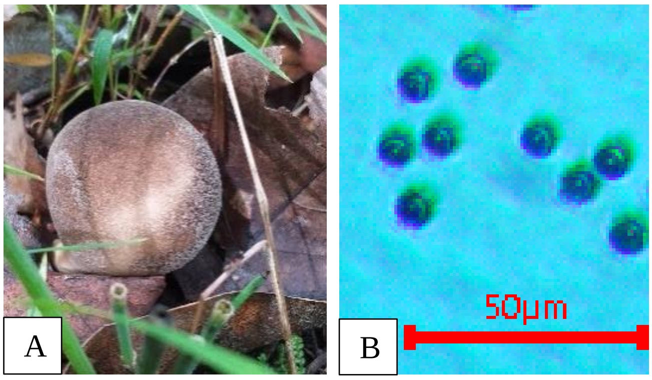

Photo plate: (A) *Lycoperdon molle* in natural habitat (B) Spores of *L. molle*

40. *Marasmius* sp.

Family - Marasmiceae Habitat - Fallen twig

Pileus - White, 0.7 cm, convex, wrinkled at the margin, **Stipe -** black, 1.8 cm length and 0.1 cm width, no stem ring, **gills -** narrow, very distant, **Spore print -** white, **Spores -** ellipsoidal, smooth, 6.2×6.4 µm.

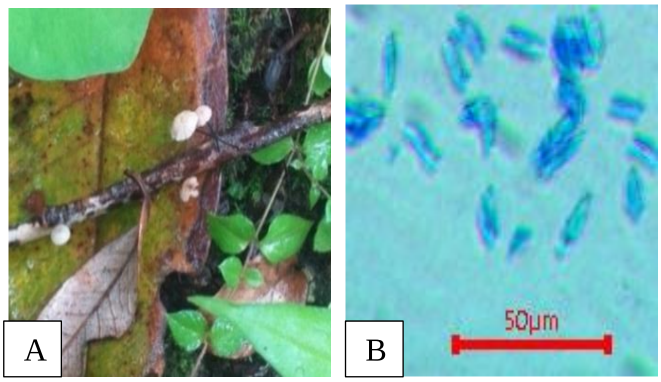

Photo plate 40: (A) *Marasmius* sp. in natural habitat (B) Spores of *Marasmius* sp.

41. *Microporus xanthopus* (Fr.) Kuntze

Family **-** Polyporaceae Habitat **-** Fallen branches of conifer

Pileus - Funnel-shaped, concentrically zoned in various shades of brown, and have a margin, up to 3-7.5 cm in diameter, **Pores -** white, pores are very tiny, **Stipe -** cylindrical, pale yellow, 0.5 cm long and 0.6 cm wide, **Spore print** – white, **Spores -** hyaline, 2.5-3.9 × 7-12 µm.

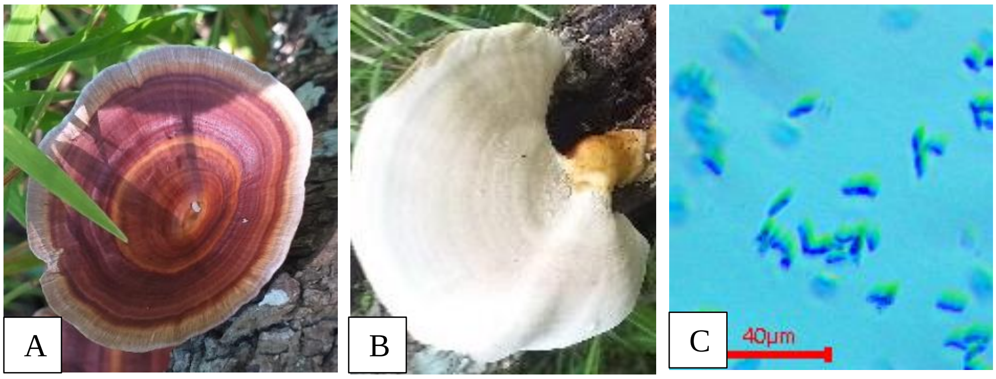

Photo plate 40: (A-B) *Microporus xanthopus* in natural habitat (C) Spores of *M. xanthopus*

42. *Mycena* sp.

Family **-** Mycenaceae Habitat **-** Saprobic on decaying wood of *Schima wallichii*

Pileus - Reddish brown, conical to bell shaped, 1.5-2 cm in diameter, **Gill -** adnate **Stipe -** reddish brown, hollow 5 cm tall, **spore print -** white, **Spores** - ellipsoid, smooth, 8-10 × 7.7-9.7 µm.

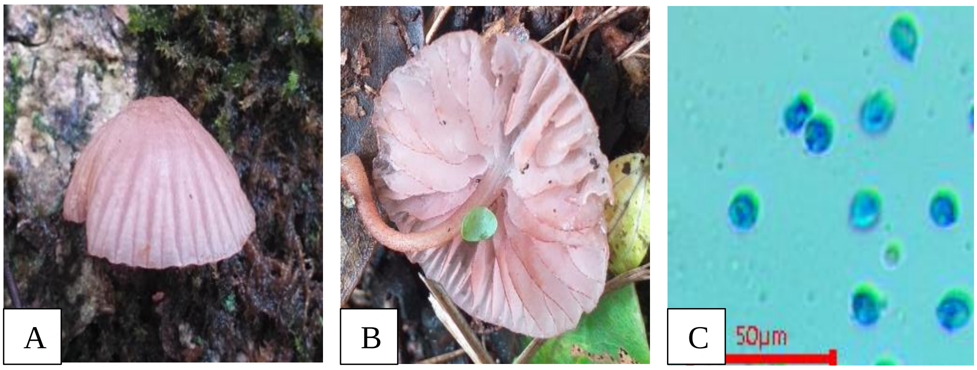

Photo plate 40: (A-B) *Mycena* sp. in natural habitat (C) Spores of *Mycena* sp

43. *Mycena* sp.

Family - Mycenaceae   Habitat - Saprobic on decaying wood of Schima wallichii

Pileus - Pale whitish brown, slightly darker at center, convex, bell shaped, 1-7 cm in diameter, **Gill -** attached to the stem, nearly distant, whitish to pale brown, **Stipe -** pale whitish brown, hollow, 3-10 cm long and 1 cm thick, **Spore print -** white, **Spores -** ellipsoid**,** 8-11 × 7-9 µm.

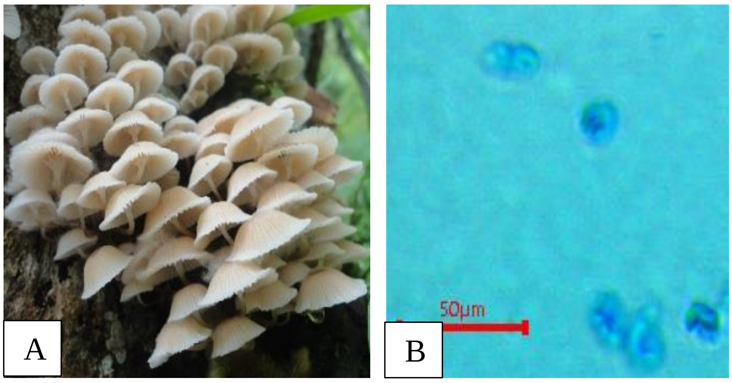

Photo plate 43: (A) *Mycena* sp in natural habitat (B) Spores of *Mycena* sp.

44. *Nyctalis agaricoides* (Fr.) Bon & Courtec.

Family - Lyophylloideae   Habitat - On rotting *Russula nigricans*

Pileus - First whitish but later covered with a thick powdery chocolate brown layer, 1-3 cm, **Gills -** grey-brown, **Stipe -** white, no ring, cylindrical, **Spore print -** white, **Spores -** smooth, ellipsoidal, 4-5 × 3-4 µm, spore shaped.

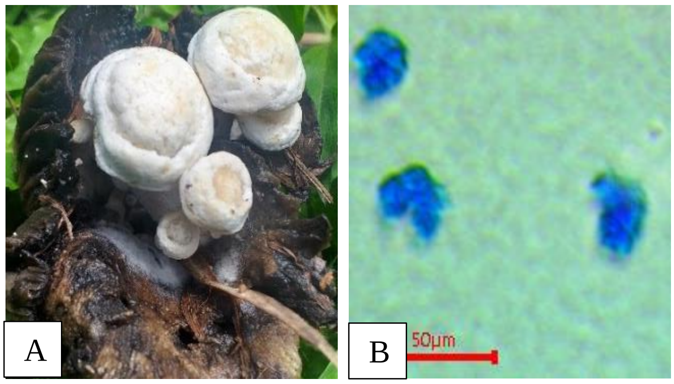

Photo plate 44: (A) *Nyctalis agaricoides* in natural habitat (B) Spores of *N. agaricoides*

45. *Oudemansiella* sp. (Jungh.) Höhn.

Family - Physalacriaceae Habitat - living tree of Schima wallichii, forming small group

Pileus - 6.5 cm wide, plano-convex, glutinous, yellowish brown, **Gill -** white, lamellulae of two orders, **Stipe -** central, 2 cm tall and 1 cm wide, curved, tapering towards the apex, sub bulbous, **Spore print -** white**, Spores -** 29.9-34.8 × 30.3-32.8 µm, smooth, hyaline, globose to subglobose.

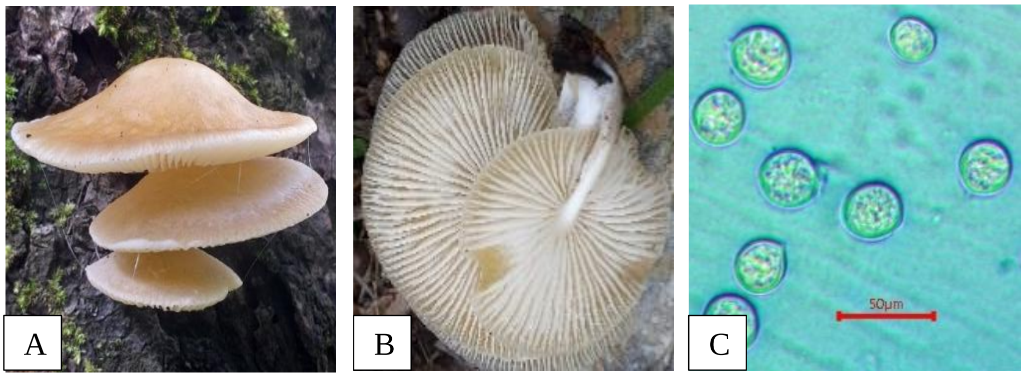

Photo plate 45: (A-B) *Oudemansiella* sp. in natural habitat (C) Spores of *Oudemansiella* sp.

46. *Oudemansiella radicata* (Relhan) Singer

Family - Physalacriaceae Habitat - Soil near stump of tree or attached to roots or buried wood

Pileus - Yellowish brown, Sticky when moist, becomes wrinkled with age, convex, 5-12 cm, **Gill -** white, adnexed , thick, broad, distant, **Stipe -** dark chocolate brown, bare, 10-20 cm long and 0.5-1 cm in diameter, deeply rooting, no, ring, **Spore print -** white, **Spores -** ellipsoidal to lemon - shaped , hyaline, smooth, non-amyloid, 12-17 × 9-14 µm, with a germ pore.

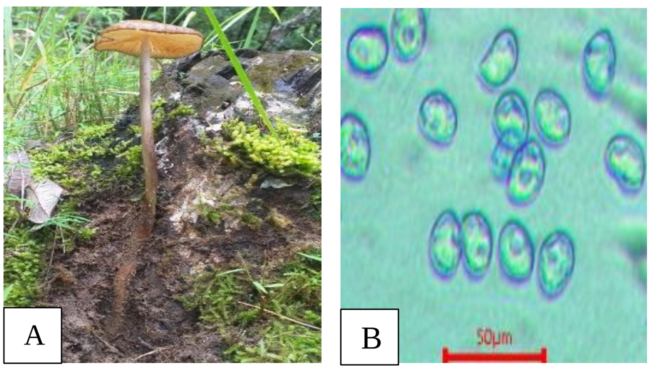

Photo plate 46: (A) *Oudemansiella radicata* in natural habitat (B) Spores of *O.*

47. ***Phellinus tremulae*** **(Bondartsev) Bondartsev & P.N. Borisov**

Family **-** Hymenochaetaceae Habitat **-** Stump of *Rhododendron arboretum*

Pileus - Convex, 5-20 cm, brown to blackish**, Stipe -**2-3 cm**, Pore -** yellowish brown

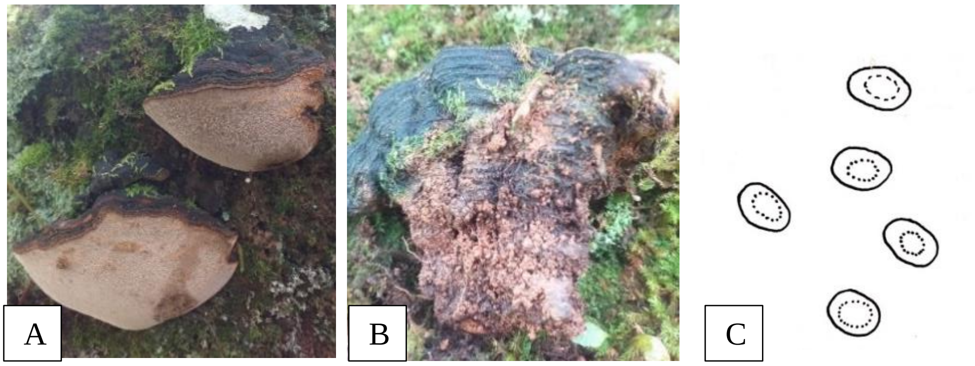

Photo plate 47: (A-B) *Phellinus tremulae* in natural habitat (C) Spores of *P. tremulae*

48. ***Pulveroboletus ravenelii*** **(Berk. & M.A. Curtis) Murrill**

Family **-** Boletaceae Habitat **-** Soil

Pileus - Yellowish, convex or flat, surface is dry and small scale present,1-10 cm in wide, **Pore -** bright yellow, angular to almost circular, it stains greenish blue then grayish brown after injury, **Stipe -** Yellow, solid, covered with minute hairs, 4.5-14 cm long and 0.6-1 cm thick, a cottony and powdery partial veil remains as a ring on the stipe, **Spore print -** olive brown, **Spores -** Elliptical to oval, smooth, subfusoid, 8-12.5 × 4.5-6.5 µm.

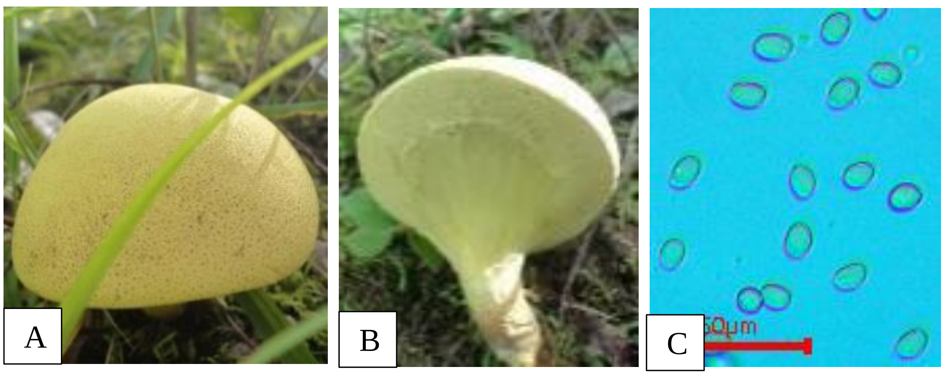

Photo plate 48: (A-B) *Pulveroboletus ravenelii* in natural habitat (C) Spores of *P. ravenelii*

49. *Phylloporus rhodoxanthus* (Schwein.) Bres.

Family **-** Boletaceae Habitat **-** Soil, scattered in small group

Pileus - Olive brown, velvety, initially convex later planer, surface dry, 3-8 cm diameter, **Gill -** yellow, subdistant, decurrent, bruise green, **Stipe -** yellow, flushed with red in an irregular pattern, tapering towards base, 4-8 cm long and 0.5-1.5 cm wide, , **Spore print -** yellow-brown, **Spores -** smooth, cylindrical, nonamyloid, 11-15 × 4-6 µm.

Photo plate 49: (A-B) *Phylloporus rhodoxanthus* in natural habitat (C) Spores of *P. rhodoxanthus*

50. *Ramaria stricta* (Pers.) Quél.

Family **-** Gomphaceae Habitat **-** Soil

Fruit body - Pale creamy, 4 to 14 cm tall, base short or nearly absent, **Spore print -** Yellow, **Spores -** ellipsoid, surface minutely warted, 6.5-9.5 × 4-6 µm.

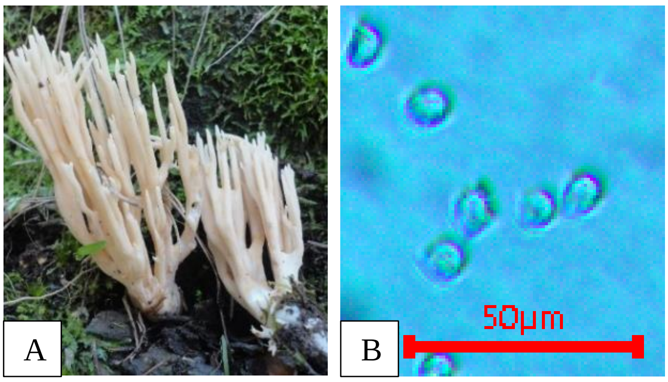

Photo plate 50: (A) *Ramaria stricta* in natural habitat (B) Spores of *R. stricta*

51. ***Retiboletus nigerrimus*** **(R. Heim) Manfr. Binder & Bresinsky**

Family - Boletaceae   Habitat - Soil

Pileus - Black,10.5 cm width, smooth, **Gill -** white to black, angular pore, brownish when injure, **Stipe -** black, 6.7 cm long and 1.6 cm width, reticulate on the upper side of stalk, solid, **Spore print -** black, Spores - 10-14 × 4-6 µm.

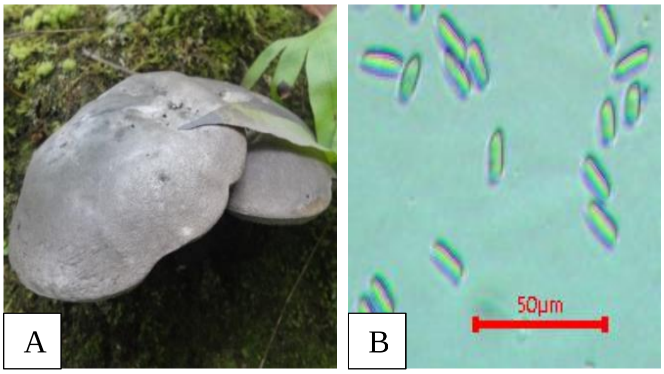

Photo plate 51: (A) *Retiboletus nigerrimus* in natural habitat (B) Spores of *R. nigerrimus*

52. ***Russula compacta*** **Frost**

Family - Russulaceae   Habitat - Moss, solitary or occurring in small group

Pileus - Orangish yellow, 3-15 cm, convex at early stage but later becoming flattened, upper surface smooth when young however cracked with maturity, **Gill -** white, staining brown when bruised, close, **Stipe -** white, 2-7 cm long and 1-2.5 cm in diameter, **Spore print -** white, **Spores -** 7-10 × 6-9 µm, elliptical, warts.

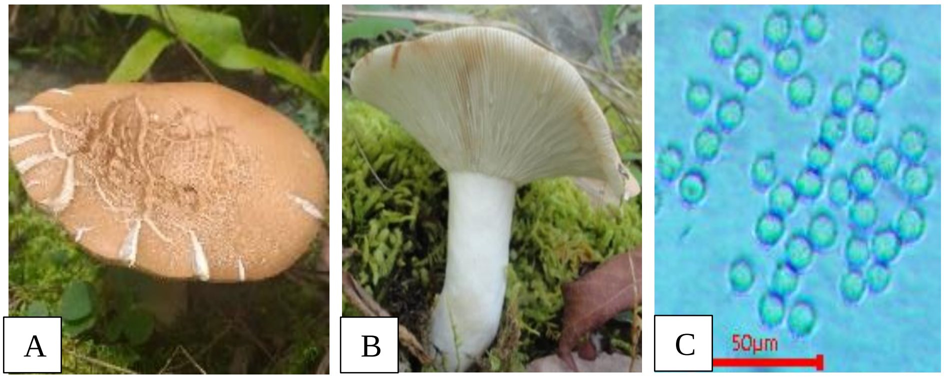

Photo plate 52: (A-B) *Russula compacta* in natural habitat (C) Spores of *R. compacta*

53. *Russula cyanoxantha* (Schaeff.) Fr.

Family - Russulaceae Habitat - Moss, scattered to gregarious

Pileus - Violet, convex at first and later flattened, dry or slightly moist, 5-18 cm in diameter, flexible and do not break, **Gill -** white, close or nearly distant, almost decurrent, crowded, **Stipe -** white, 4-12 cm tall and 1.1-5 cm wide, **Spore print -** white, **Spores -** ellipsoidal, 9.7-11.1×7.3-9.8 µm.

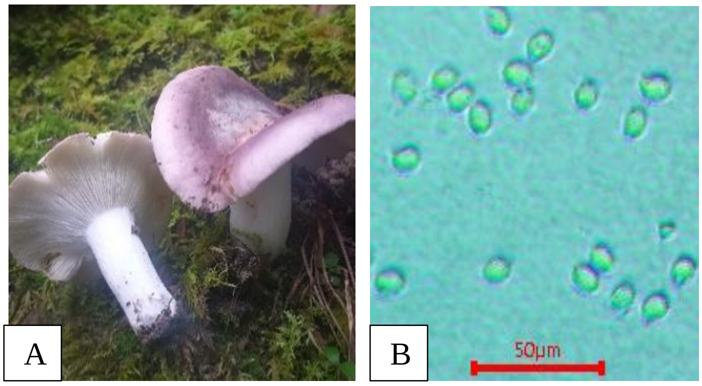

Photo plate 53: (A) *Russula cyanoxantha* in natural habitat (B) Spores of *R. cyanoxantha*

54. *Russula densifolia* Secr. ex Gillet

Family - Russulaceae Habitat - Soil, growing alone, scattered or gregariously

Pileus - Cap color initially white and turn into gray and then black, convex at first then become flattened with age, bruising slowly reddish, up to 15 cm in diameter, **Gills -** initially creamy white then turn into black adnate to slightly decurrent, crowded, bruising slowly reddish, **Stipe -** 1.5-8.0 cm tall and 1-3 cm thick, solid, white in color before turning into grayish brown, slightly scaly, bruising slowly reddish, **Spore print -** white, **Spores -** oval to elliptical, hyaline, 8.1-9.3 × 7.6-8.8 µm.

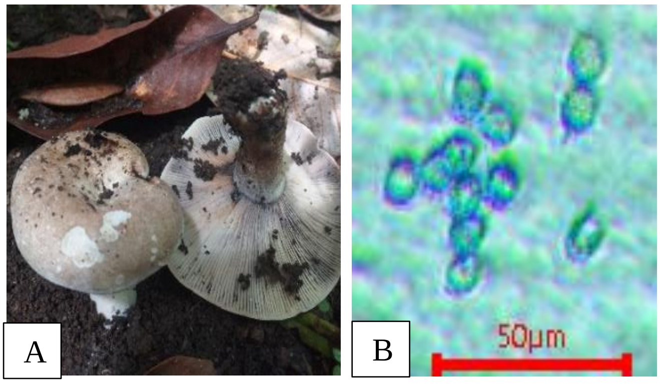

Photo plate 54: (A) *Russula densifolia* in natural habitat (B) Spores of *R. densifolia*

55. *Russula earlei* Peck

Family - Russulaceae   Habitat - Soil

Pileus - Dirty orangish yellow, convex, waxy to the touch, surface often cracking up with age, not peeling easily, 3-11 cm, **Gill -** dull yellow, attached to the stem, distant, **Stipe -** whitish to dull yellow, dry, waxy, water soaked appearance, 2-7 cm long and 2.5 cm thick, **Spore print -** white, **Spores -** warts, 3.5-6.5× 5-8.5 µm.

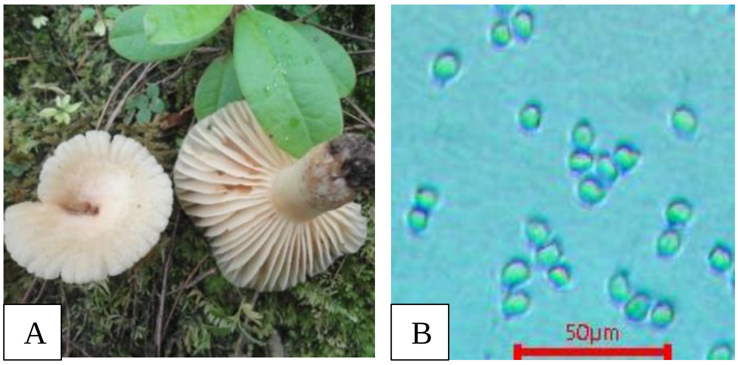

Photo plate 63: (A) *Russula earlei* in natural habitat (B) Spores of *R. earlei*

56. *Russula emetica* (Schaeff.) Pers.

Family - Russulaceae   Habitat - Soil

Pileus - Red, convex to flat, 3-8 cm wide, **Gills -** pale cream, crowded, adnate to adnexed or free, **Stipe -** white, cylindrical, the base slightly swollen, 4.6-10.6 cm long and 0.7-2.5 cm wide, **Spore print -** Yellowish white, **Spores -** elliptical to egg shaped, warts and spines present.

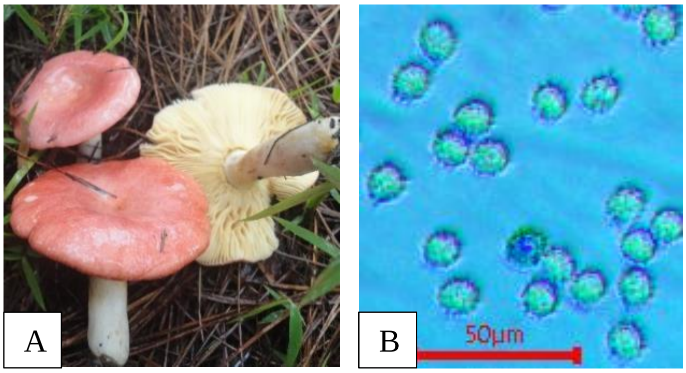

Photo plate 56: (A) *Russula emetica* in natural habitat (B) Spores of *R. emetica*

57. *Russula fellea* (Fr.) Fr.

Family - Russulaceae Habitat - Soil

Pileus - Honey yellow, convex, smooth, furrow margin, margin not lined or with very short line, 5-10 cm in width, **Gill -** crowded, adnexed, does not change color on cutting, **Stipe -** color as cap, tapering towards the cap, **Spore print** - white, **Spores -** ellipsoidal, warted, 8.1-10.4 × 8-9.07 µm.

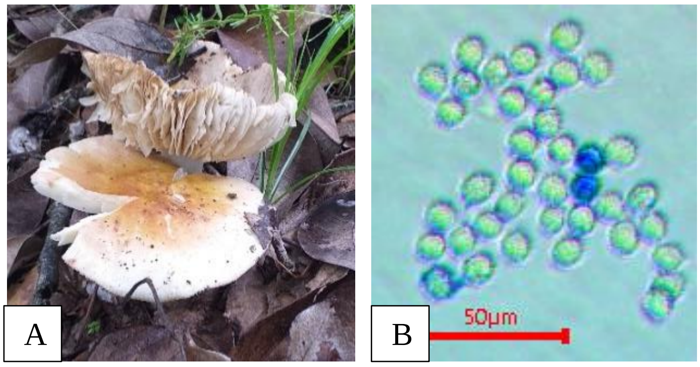

Photo plate 57: (A) *Russula fellea* in natural habitat (B) Spores of *R. fellea*

58. *Russula nigricans* Fr.

Family - Russulaceae Habitat - Gregarious or solitary on the ground

Pileus - Dirty white when young, but swiftly turns into brown, and then black on aging, convex, umbilicate, 5-20 cm in diameter, **Gill -** white, sub-distant to distant, broad, alternating long and short, adnate, when bruised turn red then grey and finally black, **Stipe -** white, too blackens with age, smooth, straight, no stem ring, stout, changing to reddish then black when bruised, solid, 5 – 8 cm long and 2 – 5 cm wide, **Spore print -** white, **Spores -** warty, ellipsoid, hyaline, subglobose, 7-9 × 6-8 µm.

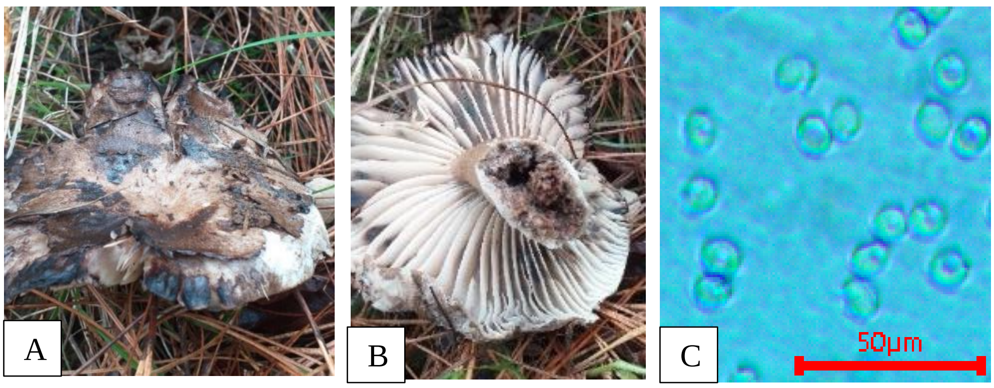

Photo plate 58: (A-B) *Russula nigricans* in natural habitat (C) Spores of *R. nigricans*

59. *Russula rosea* Pers.

Family - Russulaceae Habitat - Soil

Pileus - Convex to flat, light red, 5.5 cm, **Gills -** white, crowded **Stipe -** white, bare, 5- 10 cm tall and 2-3 cm thick, **Spore print -** ochre, **Spores -** 10-12 × 8-10 µm, warts, hyaline, ovoid.

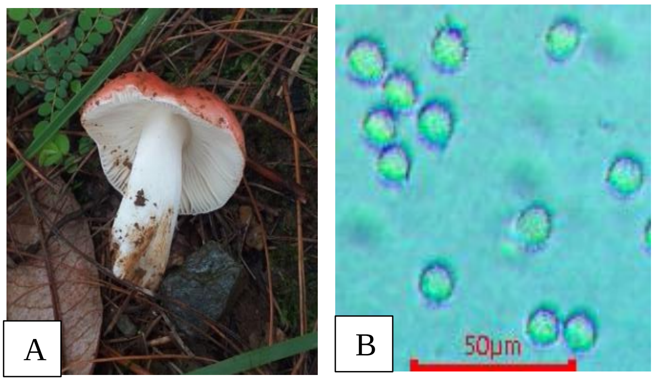

Photo plate 59: (A) *Russula rosea* in natural habitat (B) Spores of *R. rosea*

60. *Russula sanguinea* Fr.

Family - Russulaceae Habitat - Soil

Pileus - Convex to flat sticky when wet, bloody red, the cap skin peels at the margin only, Up to10 cm, **Gills -** adnate to slightly decurrent, close, white, **Stipe -** white, flushed red over a base of stipe, 4-10 cm tall and 1.5-3 cm thick, **Spore print -** creamy, **Spores -** 7.2-9.8 × 6.1-8 µm, warts, hyaline, ovoid.

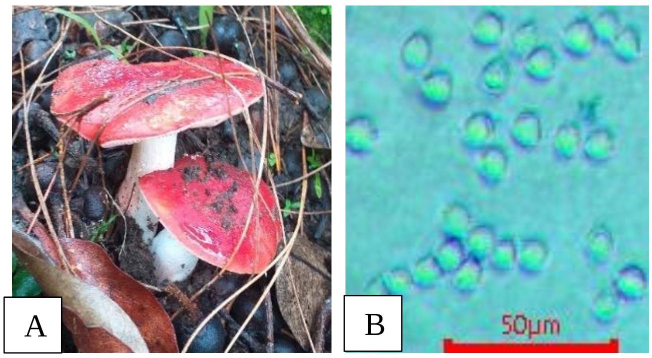

Photo plate 60: (A) *Russula sanguinea* in natural habitat (B) Spores of *R. sanguinea*

61. *Russula ochricompacta* Bills & O.K. Mill.

Family - Russulaceae   Habitat - Soil in moss

Pileus - White, concentric zonation, 5 cm, **Gill -** golden yellow**,** dichotomously forked, crowded, **Stipe -** white, 1.5 cm long and 1 cm width, volva absent, **Spore print -** orange, **Spores -** elliptical to ellipsoid with warts, hyaline, 4-5 × 6-7 µm.

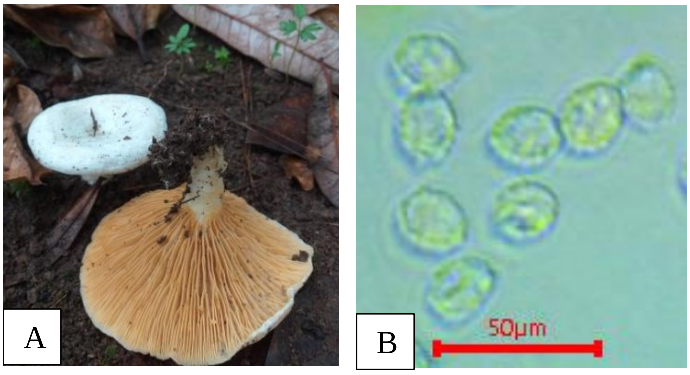

Photo plate 61: (A) *Russula ochricompacta* in natural habitat (B) Spores of *R. ochricompacta*

62. *Russula virescens* (Schaeff.) Fr.

Family - Russulaceae   Habitat - Soil in moss

Pileus - Grass green and cracked towards the edge, white background, up to 15 cm, convex initially and later flattened with age, **Gill -** creamy white, crowded, adnexed and brittle when handled, **Stipe -** white, 6 cm long and 3.2 cm wide, sometimes tapering at base, **Spore print** – white, **Spores -** elliptical to ellipsoid with warts, hyaline, 6-9 × 5-7 µm.

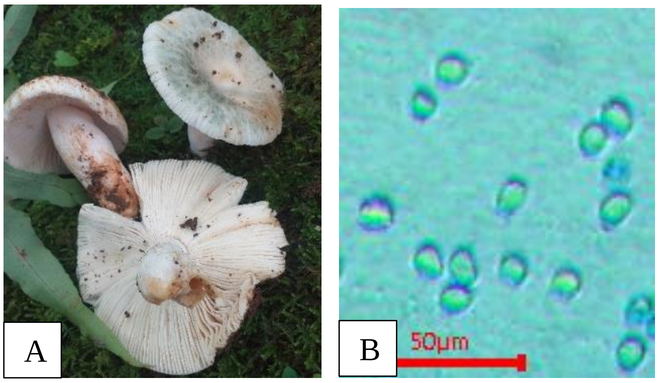

Photo plate 62: (A) *Russula virescens* in natural habitat (B) Spores of *R. virescens*

63. *Scleroderma cepa* Pers.

Family - Sclerodermataceae Habitat - Soil

Fruiting body - Yellow-brown with markings resembling scales, harder texture, **Spores -** Globose to slightly flattened, brown, 9-12 × 10-14 µm in diameter, conical warts at border.

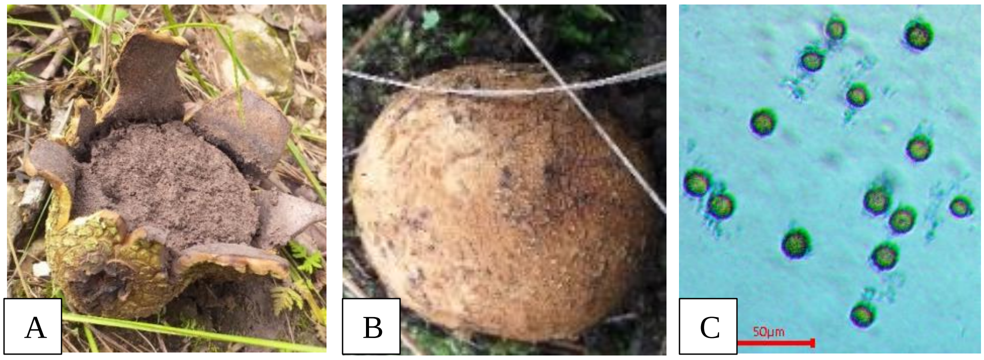

Photo plate 63: (A-B) *Scleroderma cepa* in natural habitat (C) Spores of *S. cepa*

64. *Scleroderma verrucosum* (Bull.) Pers.

Family - Sclerodermataceae Habitat - soil

Fruit body - Surface is covered with dark brown scale, 4-8 cm tall, thick stem like base, from the base white mycelial cord emanate, **Spore mass -** dark brown, **Spores -** spherical, covered with minute spines, 8-12 µm diameter.

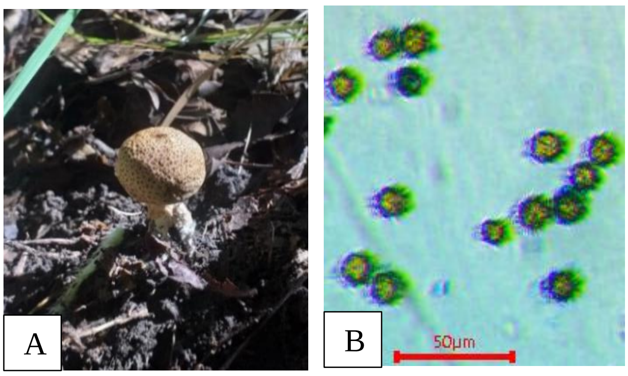

Photo plate 64: (A) *Scleroderma verrucosum* in natural habitat (B) Spores of *S. verrucosum*

65. *Strobilomyces strobilaceus* (Scop.) Berk.

Family **-** Boletaceae Habitat **-** Moss, found solitary, not only in deciduous but also found in coniferous forest

Pileus - Convex, Covered with soft dark grey to black pyramidal scales, 3.1-12 cm wide, **Pore -** hexagonal, white, turn black when bruised. flesh white but when exposed to air becoming pink, orange red and slowly black, **Stipe -** ring, scaly, up to 14 cm tall and 1-2 cm in diameter, **Spore print -** blackish brown, **Spores -** ellipsoidal, smooth, 8-12 × 7 -10 µm.

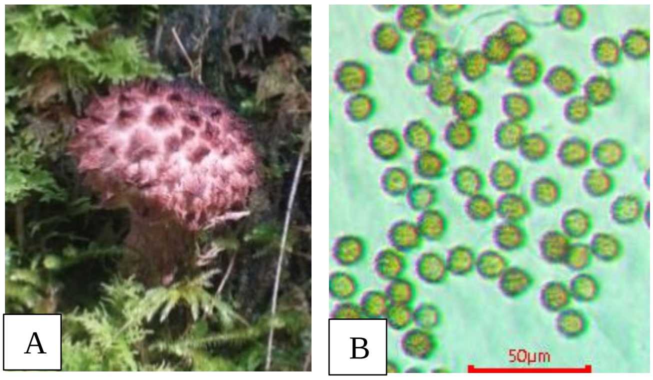

Photo plate 65: (A) *Strobilomyces strobilaceus* in natural habitat (B) Spores of *S. strobilaceus*

66. *Suillus granulatus* (L.) Roussel

Family **-** Suillaceae Habitat **-** Soil

Pileus - Yellowish at first, becoming orangish brown, flat of convex, sticky, smooth, 5 -15 cm, **Stipe -** pale yellow, without a ring, brownish glandular dots on the upper side of the stem, hollow, 4-8 cm long, 1-2 cm thick, **Pores -** small and exude pale milk droplets when young, **Spore print -** brown, Spores **-** 7-9×2.5-3.5 µm, Smooth, subfusoid.

Photo plate 66: (A-B) *Suillus granulatus* in natural habitat (C) Spores of *S. granulatus*

67. *Tapinella panuoides* (Fr.) E.-J. Gilbert

Family **-** Tapinellaceae Habitat **-** Overlapping clusters on pinus stumps

Pileus - Orangish brown, fan or oyster shaped with an eccentric attachment, plano convex, dry, finely velvety, 2.5-10 cm wide, **Gill -** bright yellowish, decurrent, crowded, radiating from point of attachment, frequently forked, with cross veins, **Stipe -** absent, or present only as a small lateral extension, **Spore print -** yellowish brown, **Spores -** smooth, ellipsoid, hyaline, 5-6.5× 3-4.5 µm.

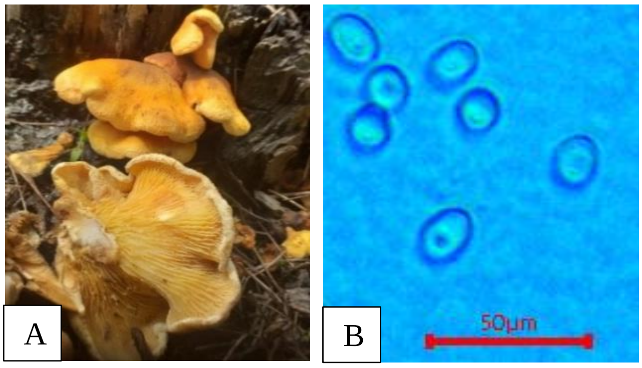

Photo plate 67: (A) *Tapinella panuoides* in natural habitat (B) Spores of *T. panuoides*

68. *Tremella mesenterica* Retz.

Family **-** Tremellaceae Habitat **-** Tree stump, cluster on about to decaying wood

Fruiting body - Orange, jelly like, up to 7 cm in diameter, lack of stipe.

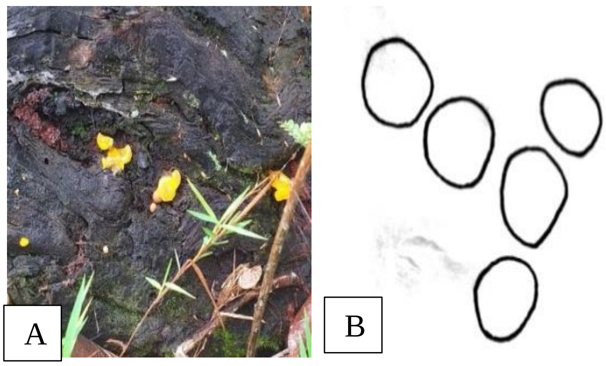

Photo plate 68: (A) *Tremella mesenterica* in natural habitat (B) Spores of *T. mesenterica*

69. *Tylopilus felleus* (Bull.) P. Karst.

Family - Boletaceae Habitat - Soil

Pileus - Brown, smooth, convex to flat, 12.5 cm in diameter, **Stipe -** light brown, ring absent, dark brown net like pattern present on stem, 7-10 cm tall and 3 cm in wide, **Pores -** white initially and pinkish with age, bruise carmine or brownish, **Spore print -** buff, **Spores -** subfusiform, 11-15 × 4-5 µm.

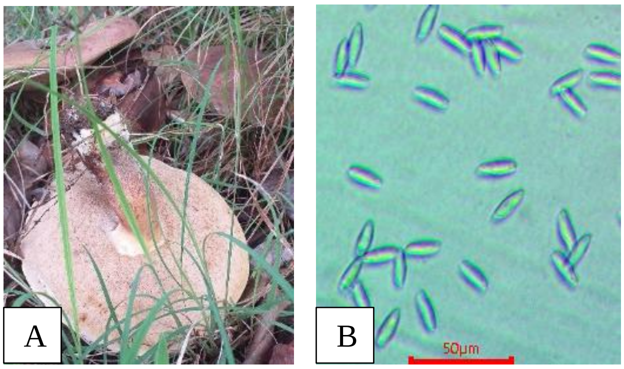

Photo plate 69: (A) *Tylopilus felleus* in natural habitat (B) Spores of *T. felleus*

70. *Xylaria polymorpha* (Pers.) Grev.

Family - Xylariaceae Habitat - Decaying wood of *Rhododendron arboreum*

Fruit bodies - Erect, lightly black to grey in color, producing white conidia, 2-8 cm tall.

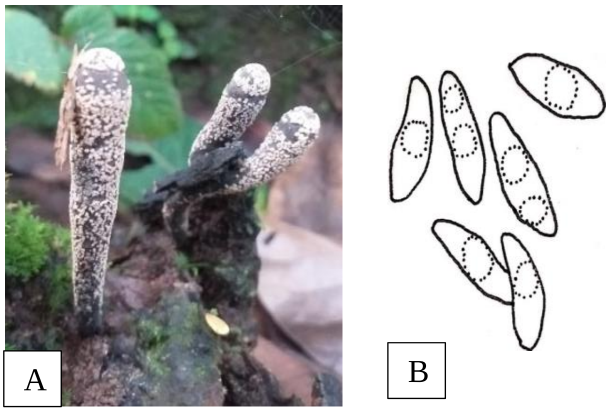

Photo plate 70: (A) *Xylaria polymorpha* in natural habitat (B) Spores of *X. polymorpha*

4.2 Relationships between macrofungal species richness and environmental variables

Study found a strong relationship between environmental variables such as Soil pH, soil moisture and tree canopy cover with macrofungal species richness. In the investigation, soil moisture, tree canopy coverage ranged from 30-61.2% and 5-70%. Similarly, soil pH ranged from 5-6.1. These environmental variables had the positive relation ($P>0.05$) with macrofungal species richness. Among them tree canopy cover showed stronger (R^2 = 0.494; P = 0.001) relationship with macrofungal species richness as comparison to soil pH (R^2 = 0.301; P = 0.018) and soil moisture (R^2 = 0346; P = 0.010). The relationship between macrofungal species and environmental variables has been showed in the below.

A. Soil moisture

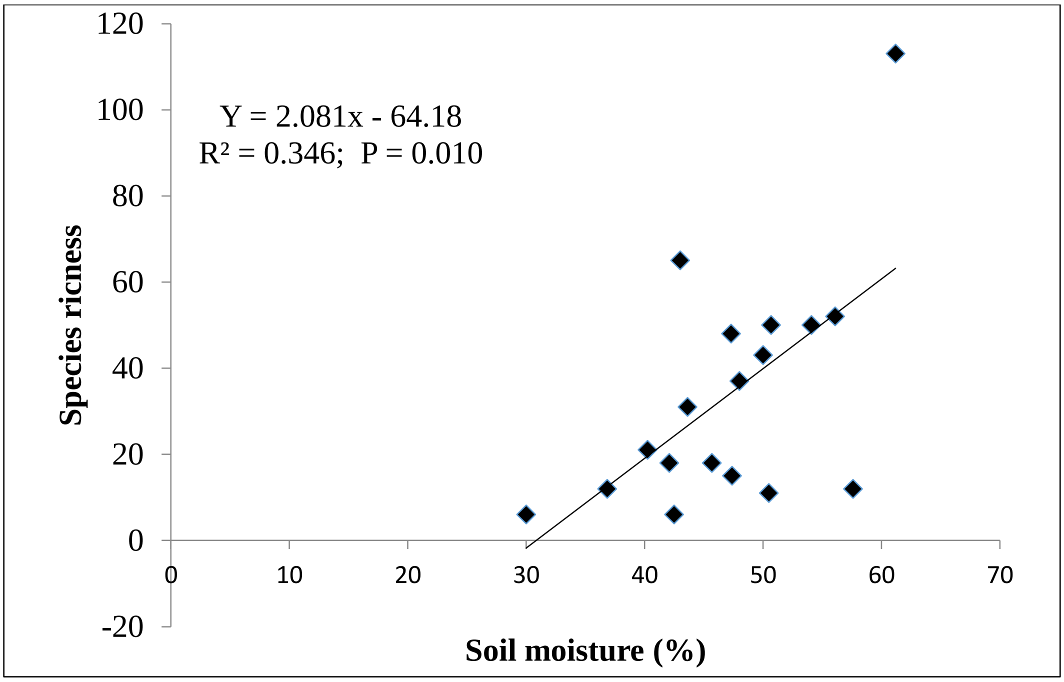

Figure 7: Relationships between macrofungal species richness and soil moisture

B. Tree canopy cover

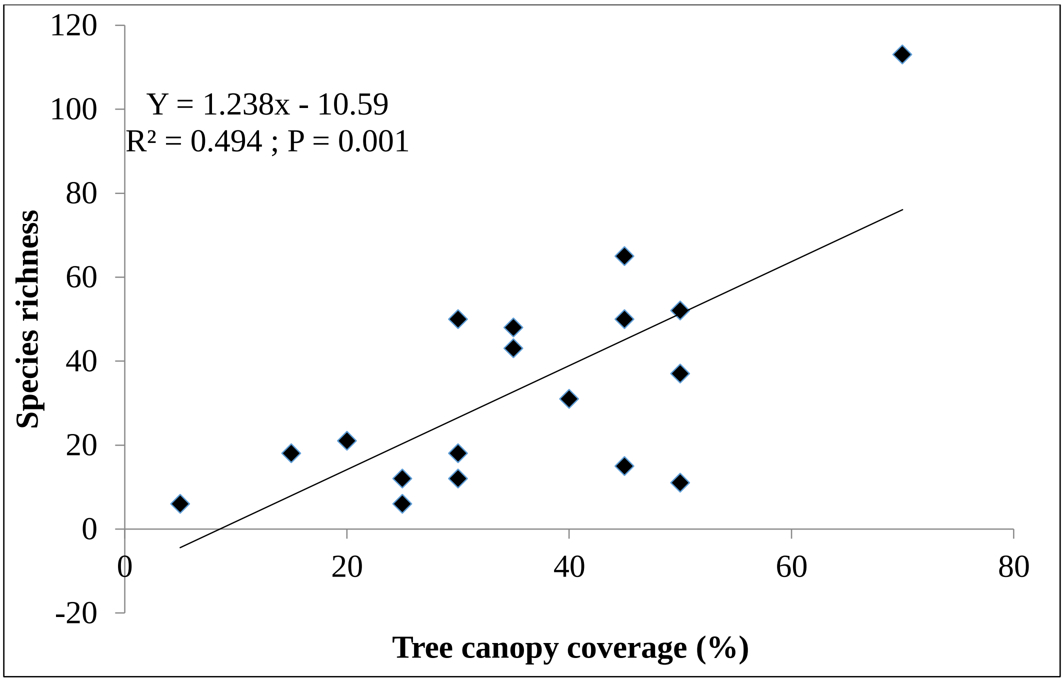

Figure 8: Relationships between macrofungal species richness and Tree canopy cover

C. Soil pH

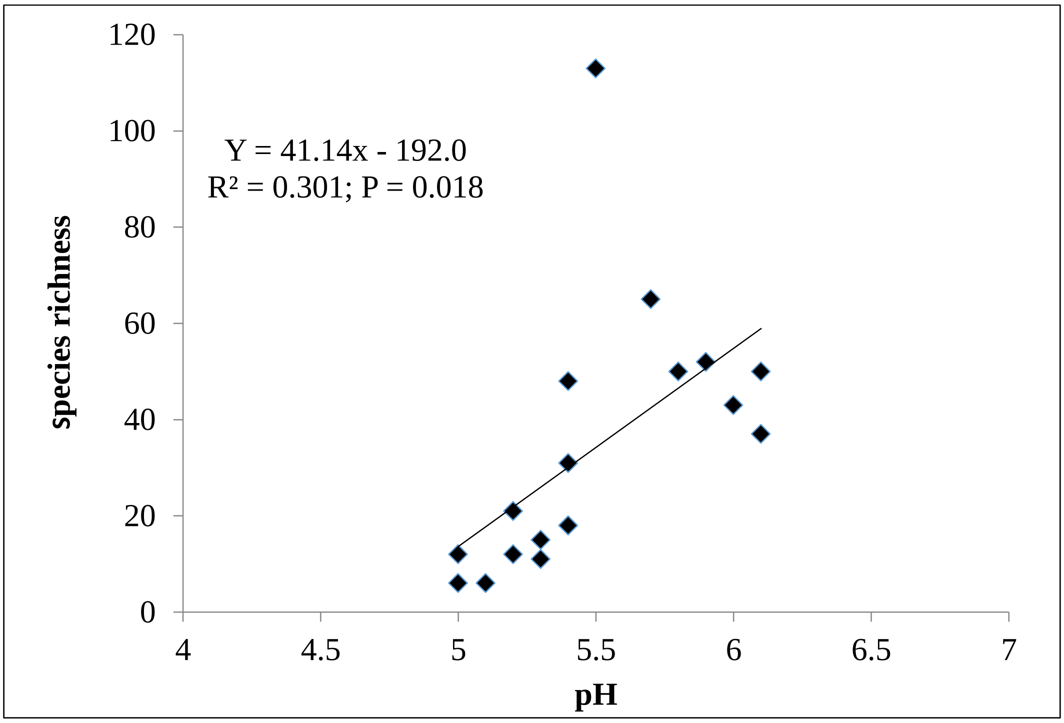

Figure 9: Relationships between macrofungal species richness and soil pH

4.3 Nutrient analysis

Three wild edible mushrooms namely *Laccaria laccata*, *Scleroderma cepa* and *Cantharellus cibarius* were selected to nutrient analysis to determine their moisture content, fat, protein, carbohydrate and ash. The study also estimated the macronutrients (calcium, magnesium, phosphorus and potassium) similarly, various micronutrients (copper, iron, manganese and zinc) also determined. All macro and micro nutrient estimation were determined on a dry weight basis. Each parameter was repeated thrice and mean of them was considered as the final result.

4.3.1 Macronutrient profile

a. Determination of Ash, Carbohydrate, Fat, Moisture and Protein

The highest ash content (13.38 %) was found in *Laccaria laccata* whereas lowest ash content was found in *Scleroderma cepa* (7.05). Figure number 10 shows that *Cantharellus cibarius* is rich in both carbohydrate (71.37%) and fat (1.94%) in comparison to *Laccaria laccata and Scleroderma cepa*. There was a significant difference ($P<0.05$) between these species in ash, carbohydrate, fat and protein. Moisture content of *Cantharellus cibarius* (13.66 %) *and Laccaria laccata* (13.63 %) was quite similar whereas *Scleroderma cepa* (12.37 %) had slightly lower value Which means that there was not significance difference ($P>0.05$) in term of moisture among these species. Among the sample evaluated, protein content was found to be highest in *Scleroderma cepa* (24.47 %) compared to the other two species of *Laccaria laccata* (23.3 %) and *Cantharellus cibarius* (16.18%).

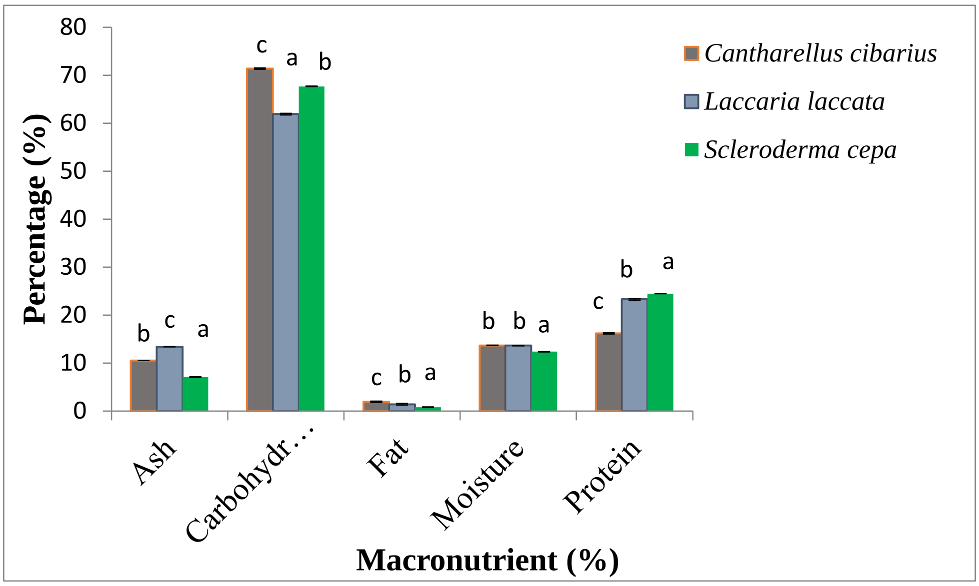

Figure 10: Macronutrients profile of 3 wild edible mushrooms

b. Determination of calcium, magnesium, phosphorus and potassium (Macrominerals)

In all 3 sample potassium (1.41 - 3.62 µg/g) was dominant macro element followed by Phosphorous (0.33 - 0.38 µg/g), calcium (0.13 - 0.15 µg/ g) and magnesium (0.9 - 0.11µg/ g). There was a significant difference in potassium of three species but no significant difference among calcium, magnesium and phosphorus of three species.

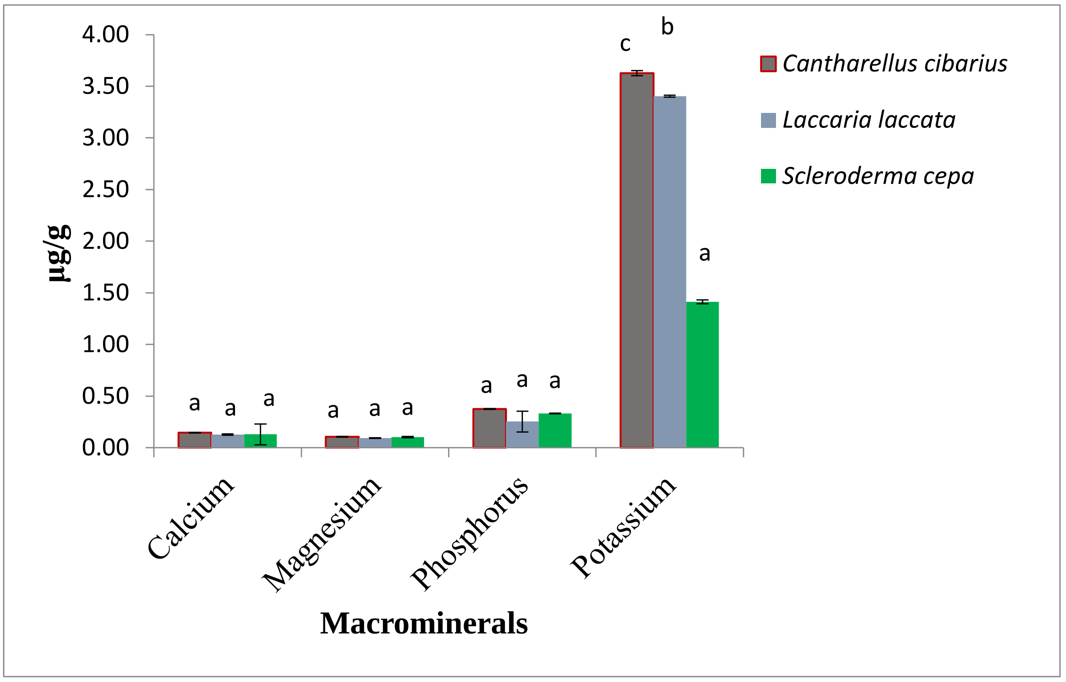

Figure 11: Macrominerals profile of 3 wild edible mushrooms

4.3.2 Microminerales profile

a. Determination of Copper, Iron, Manganese, Zinc

Copper (30.94 g/ g) and manganese (16.06 g/ g) were highest in *Laccaria laccata*, whereas copper (2.40 g/ g) and manganese (7.22 g/ g) were lowest in *Scleroderma cepa*. In case of iron *Laccaria laccata* dominated over *Scleroderma cepa* (0.16 µg/ g) and *Cantharellus cibarius* (0.08 µg/ g) with the value of (0.20 µg/ g). Similarly, *Scleroderma cepa* (77.35 µg/ g) dominated over *Laccaria laccata* (56.67 µg/ g) and *Cantharellus cibarius* (45.70 µg/ g) in context of zinc. Significance differences (P<0.05) were observed in all mushrooms species.

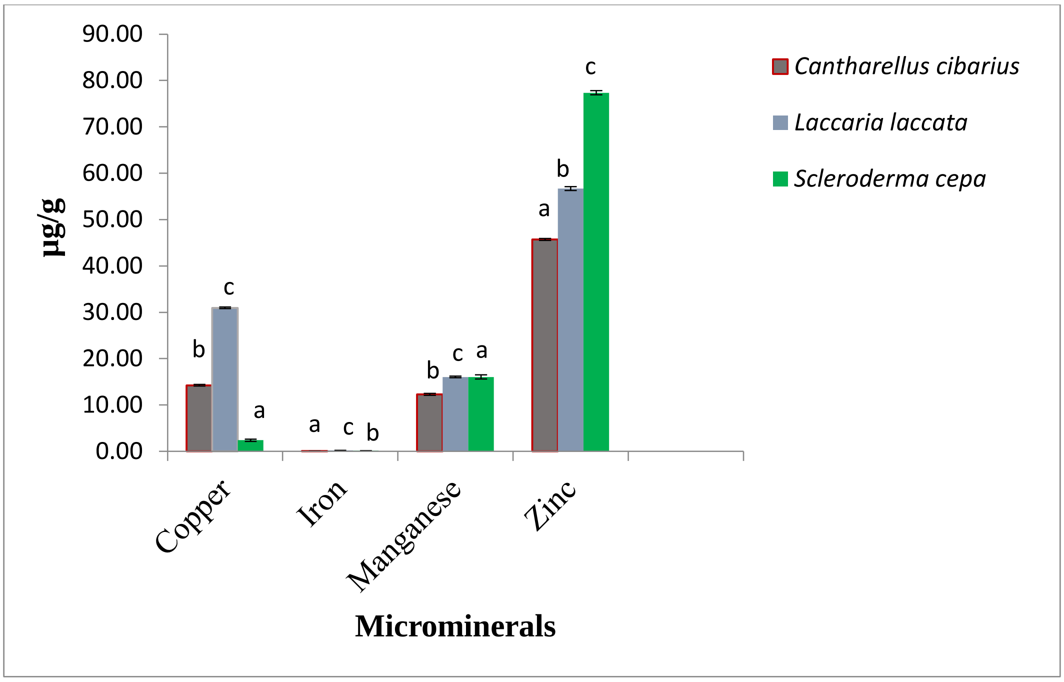

Figure 12: Microminerals profile of 3 wild edible mushrooms

CHAPTER V: DISCUSSION

5.1 Macrofungal diversity

In the present study a total of 70 species of macrofungi species were collected, of which three were ascomycota and 67 were basidiomycota belonging to 26 families and 12 orders. Among them 60 were identified at the species level, and 10 were identified at the generic level. In this study, it was found that Russulaceae was the dominant family, which is consistent with the results provided by (Shrestha *et al*., 2021). Macrofungal species collected in the study area were grown on different habitats, i.e. soil, stump, tree trunk, pine cone etc. Soil was found as main habitat for mushrooms growth, maximum number of macrofungi found in soil remaining all were found in other habitat. The variation observed in occurrence of mushrooms species in different habitat may be due to their particular mode of nutrition. (Parveen *et al*., 2017). Certain species of macrofungi are associated with specific type of trees and plants (Hawkworth, 2001). Pinus has a strong association with ectomycorrhizal fungi that might the reason the study found 50 species mycorrhizal species out of 70 species. The Shannon diversity index and Simpson index were 3.49 and 0.95. These values indicate that the study area had high diversity of macrofungal species. The study area contained high macrofungal diversity which might be because of the study area had favorable environmental condition for macrofungal growth and development.

5.2 Relationship between macrofungal species richness and environmental variables

Moisture content and soil pH are two important abiotic factors that influence fungal growth. Appearances of macrofungal fruiting bodies are highly dependent on these factors. The majorities of mushroom grow and perform well at pH levels that are close to neutral or slightly basic (Khan *et al*., 2013). (Yamanaka, 2003) revealed that most ectomycorrhizal species grew best at pH 5 or 6, whereas saprotrophic species grew best at pH 7 or 8. Our study found pH of that community forest was 5 – 6 and most of the macrofungal species were ectomycorrhizal. Similarly, (Zhang *et al*., 2010) found highest macrofungal diversity in shaded forests than in more exposed/sunny forest slopes. In our research work Soil moisture found to have positive effect on macrofungal diversity. (Trudell and Edmonds, 2004; Bhandari & Jha, 2017; Shah *et al*., 2020) also prove that fungal growth increases as soil moisture increases.

Tree canopy of trees is an important factor in habitat formation (Nakamura *et al*., 2017). Higher canopy cover provides shade and reduces moisture loss, and increased canopy cover resulted in more litter on the forest floor, which provides additional habitat for fungal growth (Gabel and Gabel, 2007). The result shows that the richness of macrofungal species increases with increased canopy cover; this finding is consistent with the findings of (Santos-Silva *et al*., 2011)

5.3 Nutrient analysis

Thirteen parameters: ash, carbohydrate, fat, moisture, protein, calcium, magnesium, phosphorus, potassium, copper, iron, manganese, and zinc, were tasted for nutrient analysis of three wild edible mushrooms *(Cantherellus cibarius, Laccaria laccata* and *Scleroderma cepa).*

5.3.1 Macronutrient profile

Fresh mushrooms generally contain 85-95% moisture, whereas air-dried specimens contain 5-20% moisture, depending on duration and storage (Crisan and Sands, 1978). In present investigation, the moisture contain ranged between 12.37 – 13.66 %. Moisture content of the mushrooms varied with the type of mushrooms. (Cuptapun *et al*., 2010) worked on moisture content of four edible mushrooms and documented 7.21-7.5 % moisture content in dry weight basis. The high moisture content is an indication that fresh mushrooms cannot keep for long time. This is because high water activity enhances microbial growth (Bano, 1976). The average crude protein content of edible mushrooms ranges between 19-40 % (Kurtzman, 1978). The present study found protein content in *Laccaria laccata* was 23.30% which is lower than the values reported by Jha and Tripathi (2012) but higher than study done by (Egwim *et al*., 2011) similarly protein content reported in this study in *Cantharellus cibarius* was 16.18% lower than the values reported by (Egwim *et al*., 2011) whereas *Scleroderma cepa* composed 24.47% protein. The ash contents among three wild mushrooms range from 7.5 – 10.5 % of the mushrooms. These results were similar to the result reported by (Singha *et al*., 2017). In general, mushrooms are low calorie foods because they contain small amount of fat. In mushrooms, fat content is very low as compared to carbohydrates and proteins. Fat in mushrooms contains all classes of lipid compounds including free fatty acids, mono-, di-, and triglycerides, sterols, sterol esters and phospholipids (Manzi *et al*., 1999). The fat content in *Scleroderma cepa*, *Laccaria* *laccata and Cantherellus cibarius* ranged from 0.78 - 1.94%. *Scleroderma cepa* had low fat content as comparison to other hence this may makes it a suitable health food. The results showed that carbohydrate were abundance in all 3 species. The obtained value of carbohydrate indicate that the mushrooms are good energy food resources**.** *C. cibarius*, *L. laccata* and *S. cepa* are similar in terms in their calcium, magnesium and phosphorous content but differ in terms of potassium. The nutrition composition of different mushroom species had varied probably due to species difference and their bioaccumulation ability of minerals and other nutrient into their tissue (Teke, *et al*., 2021).

5.3.2 Microminerals profile

The element content of mushrooms is determined by the element content of the soil (Mleczek *et al*., 2016). Zinc is widespread among living organisms due to its biological significance and mushrooms are known as Zinc accumulators (Mendil *et al*., 2004). The study revealed high content of zinc. It may be due to higher accumulation capacity of these mushrooms. Copper and manganese contents were higher in *L. laccata* and lower in *S. cepa* but iron content was low in all 3 species. It might be due to the elemental content varied not only with respect to the regions of the mushrooms where they grow, but also depends on the substratum, atmospheric conditions, age and part of the fructification (Manzi *et al*., 2001). Several trace minerals in mushroom are considerably higher than those in agricultural crop plants, vegetables and fruit. Concentration was found to depend on the physiology of the species and particularly on its ecosystem pattern (Duarte *et al*., 2006).

CHAPTER VI: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The current study provides a broad picture of macrofungal diversity of Myagdi district. During the survey, 70 macrofungal species were identified and soil moisture, tree canopy cover and soil pH had positive relationship with macrofungal species richness. *Cantharellus cibarius* had the highest frequency of 44.44 % whereas *Mycena* sp. had the highest density of 11.88 %. Macrofungi play a crucial role in the forest ecosystem by decomposing organic matter, cycling nutrients, and forming mutualistic relationships with other plants. Therefore, it is inevitable to explore, conserve and document this natural wealth. Mushrooms are a chief source of nutrient as well. From this research it concluded that *Cantharellus cibarius*, *Sclerodrma cepa* and *Laccaria laccata* posses small amount of fat and high amount of carbohydrates and proteins. Hence, this makes it a highly nutritive and good energetic food.

6.2 Recommendation

- Heavy metals, antioxidant, antimicrobial, anticancer, and anti-diabetic properties of wild mushroom are recommended for further study.
- Proper identification are needed in order to reduce death rate through mushrooms poisoning
- Unexplored area should be explored to know the actual diversity of macrofungi in Nepal
- Mushrooms have very good nutritional value they should be further studied to develop dietary supplement.

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APPENDICES

APPENDIX I

Equipment

Camera – To take photographs

Digger and Zipper bag - To collect and keep soil sample

Rope – To make quadrate

Basket or bag for keeping stuff

Fungus drier – To dry fungus

GPS - To note geographic location

Knife – To collect sample

Stationeries

Scissors for cutting papers

Brush for cleaning specimens

Field note book, pen and pencils, eraser, sharpener, scale for recording field data white and Black or other colour paper for taking spore print

Labels & Microscope

Chemicals and other materials

Ethanol, formalin	Hot air oven
Cotton blue, lactophenol and emerson oil	Stands and conical flasks
Conc. H_2SO_4	Crucible and Beakers
Copper sulphate	Test tubes
Hydrogen peroxide	Atomic absorption Spectrophotometer
NaOH	Kjeldahl digestion flask
HCL	Soxhlet extraction apparatus
Sodium Sulphate	Electrical balance
Petroleum ether	Autoclave
Boric acid	Grinders and desiccators
Sodium Hydroxide	pH meter

APPENDIX II

List of macrofungi collected from Thulo Ban Community Forest during rainy season with their ecology, habitat, order, family, frequency and density.

S. N	Scientific Name	Ecology	Habitat/ Substrate	Order	Family	Edibility	Frequency (%)	Density (%)
1	*Amanita abrupta*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Unknown	11.11	0.72
2	*Amanita caesarea*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Edible	5.55	0.36
3	*Amanita fulva*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Inedible	5.55	0.18
4	*Amanita multisquamosa*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Inedible	5.55	0.54
5	*Amanita pantherina*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Poisonous	5.55	0.18
6	*Amanita phalloides*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Poisonous	5.55	0.72
7	*Amanita porphyria*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Inedible	5.55	0.18
8	*Amanita rubescens*	Mycorrhizal	Soil	Agaricales	Hydnangiaceae	Edible	5.55	0.54
9	*Amanita spissacea*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Poisonous	5.55	0.36
10	*Amanita vaginata*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Edible	5.55	0.18
11	*Auriscalpium vulgare*	Saprotrophic	Fallen *Pinus roxburghii* cone	Russulales	Auriscalpiaceae	Inedible	11.11	0.72

12	*Boletus edulis*	Mycorrhizal	Soil	Boletales	Boletaceae	Edible	27.77	2.99
13	*Boletus eximius*	Mycorrhizal	Soil	Boletales	Boletaceae	Inedible	5.55	0.54
14	*Cantharellus cibarius*	Mycorrhizal	Soil	Cantharellales	Cantharellaceae	Edible	44.44	7.92
15	*Coltricia cinnamomea*	Mycorrhizal	Soil	Hymenochaetales	Hymenochaetaceae	Inedible	5.55	0.18
16	*Cortinarius laniger*	Mycorrhizal	Soil	Agaricales	Cortinariaceae	Unknown	5.55	0.18
17	*Cortinarius purpurascens*	Mycorrhizal	Soil	Agaricales	Cortinariaceae	Edible	5.55	0.9
18	*Cortinarius rubellus*	Mycorrhizal	Soil	Agaricales	Cortinariaceae	Poisonous	5.55	0.18
19	*Cortinarius callisteus*	Mycorrhizal	Soil	Agaricales	Cortinariaceae	Inedible	5.55	0.18
20	*Cortinaria* sp.	Mycorrhizal	Soil	Agaricales	Cortinariaceae	Inedible	5.55	0.9
21	*Cortinarius* sp.	Mycorrhizal	Soil	Agaricales	Cortinariaceae	Inedible	5.55	0.72
22	*Cyathus olla*	Saprotrophic	Soil	Agaricales	Agaricaceae	Poisonous	11.11	6.48
23	*Entoloma* sp.	Mycorrhizal	Soil	Agaricales	Entolomataceae	Poisonous	5.55	0.36
24	*Favolus* sp.	Saprotrophic	Decaying wood of *Schima wallichii*	Polyporales	Polyporaceae	Unknown	5.55	5.76
25	*Geastrum sp.*	Saprotrophic	Soil	Phallales	Geastraceae	Inedible	5.55	0.54
26	*Glutinoglossum*	Saprophytic	Soil	Geoglossales	Geoglosaceae	Unknown	11.11	2.34

	glutinosum							
27	*Habeloma sinapizans*	Myorrhizal	Soil	Agaricales	Hymenogastraceae	Poisonous	5.55	0.18
28	*Heterobasidion annosum*	Parasitic	Stump of *Pinus roxberghii*	Agaricales	Bondarzewiaaceae	Inedible	5.55	1.62
29	*Hortiboletus rubellus*	Myorrhizal	Soil	Boletales	Boletaceae	Edible	5.55	0.72
30	*Hypoloma fasciculare*	Saprotrophic	Tree trunk of *Lyonia ovalifolia*	Agaricales	Strophariaceae	Poisonous	11.11	11.7
31	*Inocybe* sp.	Mycorrhizal	Soil	Agaricales	Inocybaceae	Poisonous	5.55	0.54
32	*Laccaria amethystine*	Mycorrhizal	Soil	Agaricales	Amanitaceae	Edible	11.11	0.9
33	*Laccaria laccata*	Mycorrhizal	Soil	Agaricales	Hydnangiaceae	Edible	38.88	9.54
34	*Lactarius cinereobrunneus*	Mycorrhizal	Soil	Russulales	Russulaceae	Unknown	5.55	1.62
35	*Lactarius deterrimus*	Mycorrhizal	Soil	Russulales	Russulaceae	Edible	5.55	0.18
36	*Lactifluus piperatus*	Mycorrhizal	Soil	Russulales	Russulaceae	Inedible	5.55	1.44
37	*Lactarius volemus*	Mycorrhizal	Soil	Russulales	Russulaceae	Edible	5.55	0.9
38	*Leotia lubrica*	Saprotrophic	Soil	Leotiales	Leotiaceae	Inedible	5.55	5.58
39	*Lycoperdon molle*	Saprotrophic	Soil	Agaricales	Agaricaceae	Inedible	5.55	0.18
40	Marasmius sp.	Saprotrophic	Fallen twig of	Agaricales	Marasmiceae	Inedible	5.55	0.72

			Rhododendron arboretum					
41	*Microporus xanthopus*	Saprotrophic	Fallen branches of *Egelhardia spicata*	Polyporales	Polyporaceae	Inedible	5.55	1.26
42	*Mycena sp.*	Saprotrophic	Stump of ***S****chima wallichii*	Agaricales	Mycenaceae	Inedible	5.55	0.18
43	*Mycena sp.*	Saprotrophic	Stump of***S****chima wallichii*	Agaricales	Mycenaceae	Inedible	5.55	11.88
44	*Nyctalis agaricoides*	Parasitic	Cap of *Russula nigiricans*	Russulales	Lyophyllaceae	Inedible	11.11	1.08
45	*Oudemansiella* sp.	Saprotrophic	Tree trunk of *Schima wallichii*		Physalacriaceae	Inedible	5.55	0.54
46	*Oudemansiella radicata*	Saprotrophic	Soil	Agaricales	Physalacriaceae	Edible	27.77	1.26
47	*Phellinus tremulae*	Parasitic	Stump of *Rhododendronar boretum*	Hymenochaeta les	Hymenochaetaceae	Poisonous	5.55	0.54
48	*Pulveroboletus ravenelii*	Mycorrhizal	Soil	Boletales	Boletaceae	Edible	5.55	0.54
49	*Phylloporus*	Mycorrhizal	Soil	Boletales	Boletaceae	Edible	5.55	0.36

	rhodoxanthus							
50	*Ramaria stricta*	Mycorrhizal	Soil	Gomphales	Gomphaceae	Edible	11.11	0.9
51	*Retiboletus nigerrimus*	Mycorrhizal	Soil	Botetales	Boletaceae	Unknown	5.55	0.36
52	*Russsula compacta*	Mycorrhizal	Soil	Russulales	Russulaceae	Unknown	11.11	0.72
53	*Russula cyanoxantha*	Mycorrhizal	Soil	Russulales	Russulaceae	Edible	38.88	2.34
54	*Russula densifolia*	Mycorrhizal	Soil	Russulales	Russulaceae	Poisonous	5.55	0.36
55	*Russula earlei*	Mycorrhizal	Soil	Russulales	Russulaceae	Unknown	5.55	0.72
56	*Russula emetica*	Mycorrhizal	Soil	Russulales	Russulaceae	Poisonous	5.55	0.36
57	*Russula* fellea	Mycorrhizal	Soil	Russulales	Russulaceae	Inedible	11.11	0.9
58	*Russula nigiricans*	Mycorrhizal	Soil	Russulales	Russulaceae	Poisonous	27.77	2.52
59	*Russula rosea*	Mycorrhizal	Soil	Russulales	Russulaceae	Inedible	5.55	0.36
60	*Russula sanguinea*	Mycorrhizal	Soil	Russulales	Russulaceae	Inedible	5.55	0.36
61	*Russula ochricompacta*	Mycorrhizal	Soil	Russulales	Russulaceae	Unknown	5.55	0.72
62	*Russula virescens*	Mycorrhizal	Soil	Russulales	Russulaceae	Edible	33.33	1.8
63	*Scleroderma Cepa*	Mycorrhizal	Soil	Boletales	Sclerodermataceae	Edible	27.77	1.8
64	*Scleroderma verrucosum*	Mycorrhizal	Soil	Botetals	Sclerodermataceae	Poisonous	5.55	0.18
65	*Strobilomyces strobilaceus*	Mycorrhizal	Soil	Botetales	Boletaceae	Edible	11.11	0.72

66	*Suillus granulatus*	Mycorrhizal	Soil	Boletales	Suillaceae	Edible	11.11	1.08
67	*Tapinella panuoides*	Saprotrophic	Stump of *Pinus roxberghii*	Agaricales	Tapinellaceae	Poisonous	5.55	2.34
68	*Tremella mesenterica*	Mycorrhiza	Tree stump of *Lyoniaovalifolia*	Tremellales	Tremellaceae	Edible	5.55	1.08
69	*Tylopilus felleus*	Saprotrophic	Soil	Boletales	Boletaceae	Inedible	5.55	1.08
70.	*Xylaria polymorpha*	Saprotrophic	Stump of ***S**chima wallichii*	Xylariales	Xylariaceae	Inedible	5.55	0.54

APPENDIX III

The instrumental parameters for determination of Fe, Cu, Zn, K, Ca, Mg & Mn by flame atomic absorption spectrometry

Element	Wavelength (nm)	Lamp current (mA)	Slit width (nm)	Fuel
Iron (Fe)	248.3	4	0.2	Acetylene
Copper (Cu)	324.8	4	1.4	Acetylene
Zinc (Zn)	285.2	5	0.5	Acetylene
Potassium (K)	766.5	350 or optimum	0.7	Acetylene
Calcium (Ca)	239.9	10	0.2	Acetylene
Magnesium (mg)	213.9	10	0.2	Acetylene
Manganese (Mn)	279.5	10	0.2	Acetylene
Phosphorus	400	10	1.0	Acetylene

APPENDIX IV

CALIBRATION CURVE OF METALS

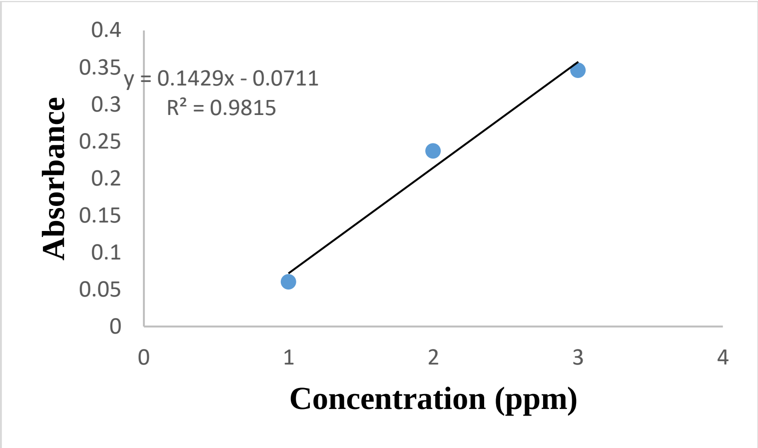

Figure: Calibration curve of Copper

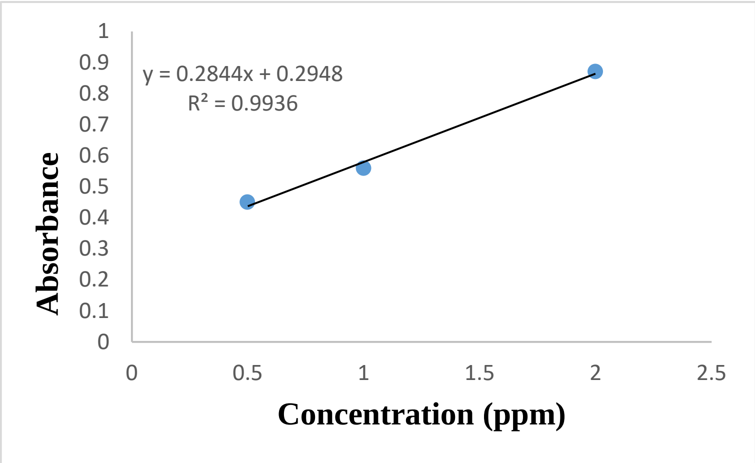

Figure: Calibration curve of Magnesium

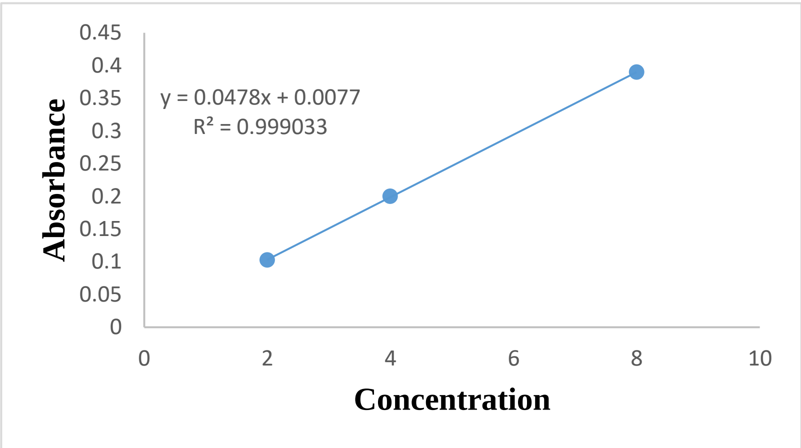

Figure: Calibration curve of Iron

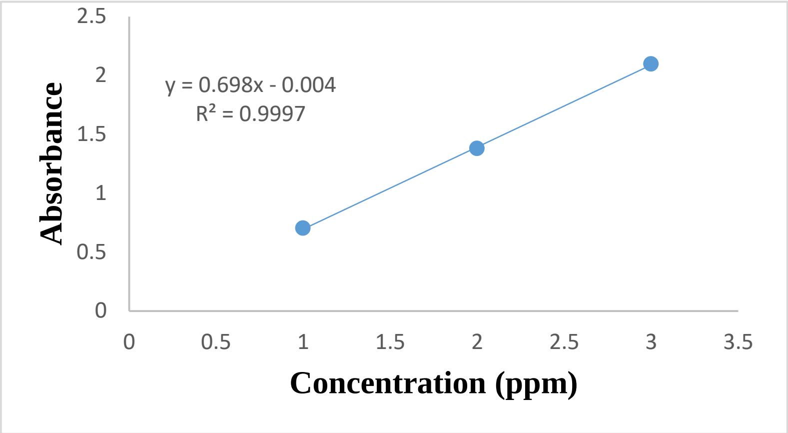

Figure: Calibration curve of Phosphorous

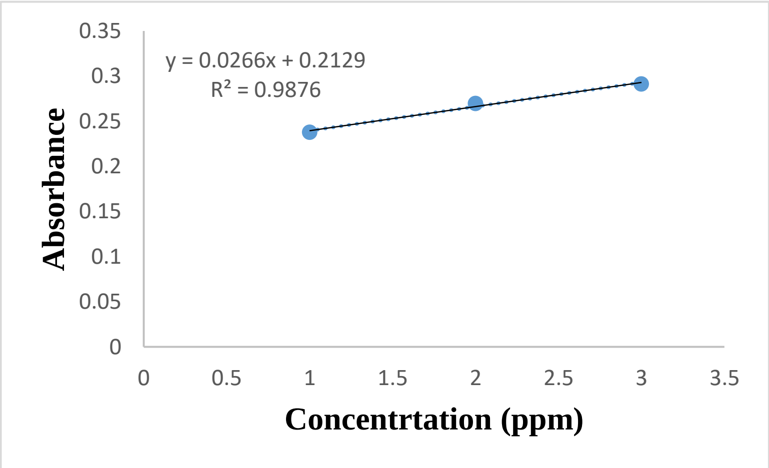

Figure: Calibration curve of Manganese

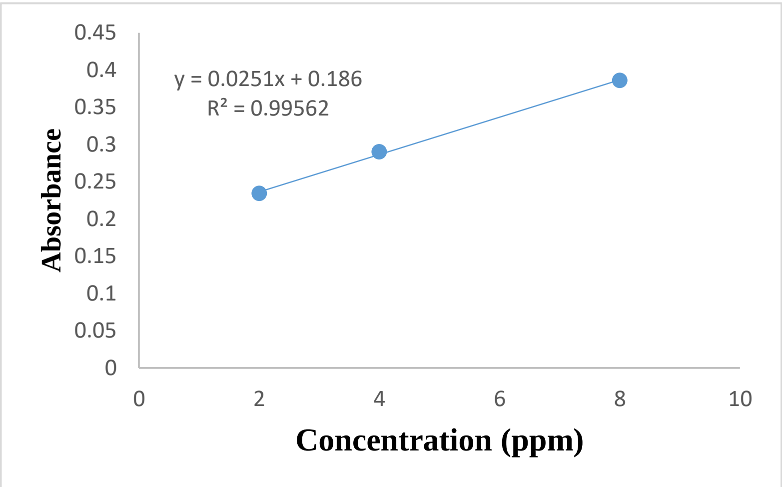

Figure: Calibration curve of Zinc

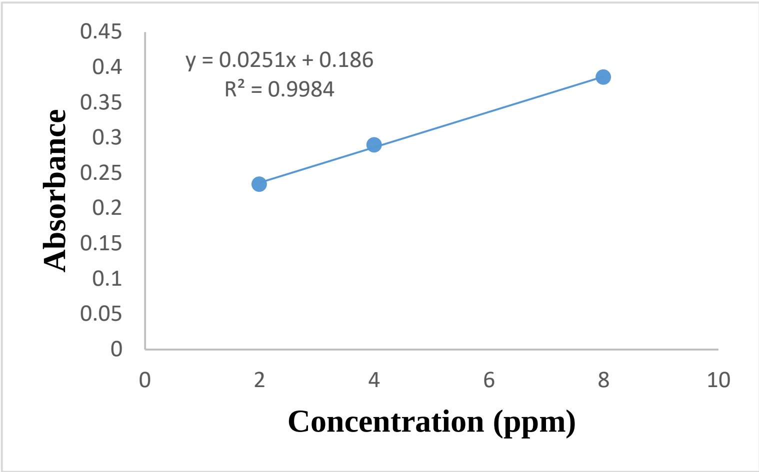

Figure: Calibration curve of Potassium

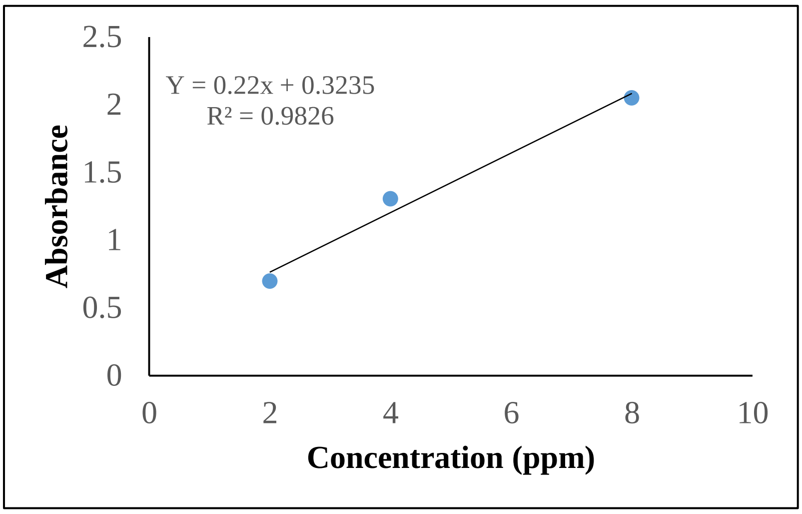

Figure: Calibration curve of Calcium

APPENDIX V

Proximate chemical composition of 3 three wild edible mushrooms; Values expressed as mean± standard error

S.N	**Species**	**Ash (%)**	**Carbohydrate (%)**	**Fat (%)**	**Moisture (%)**	**Protein (%)**
1	*Laccaria laccata*	13.38±0.008	61.89±0.16	1.41±0.04	13.63±0.04	23.30±0.15
2.	*Scleroderma cepa*	7.05±0.03	67.68±0.21	0.78±0.01	12.37±0.02	24.47±0.16
3.	*Cantherellus cibarius*	10.50±0.01	71.37±0.15	1.94±0.03	13.66±0.02	16.18±0.13
4.	***P-value***	0.00	0.00	0.00	0.63	0.02

S.N	**Species**	**Calcium (µg/g)**	**Magnesium (µg/g)**	**Phosphorus (µg/g)**	**Potassium (µg/g)**
1	*Laccaria laccata*	0.12±0.006	0.09±0.003	0.25±0.10	3.40±0.008
2.	*Scleroderma cepa*	0.13±0.01	0.10±0.006	0.33±0.003	1.41±0.017
3.	*Cantharellus cibarius*	0.14±0.003	0.10±0.003	0.37±0.003	3.62±0.024
4.	***P - value***	0.18	0.19	0.05	0.02

S.N	**Species**	**Copper (µg/g)**	**Iron (µg/g)**	**Manganese (µg/g)**	**Zinc (µg/g)**
1	*Laccaria laccata*	30.94±0.18	0.20±0.003	16.06±0.18	56.67±0.40
2.	*Scleroderma cepa*	2.40±0.26	0.16±0.003	7.22±0.44	77.34±0.43
3.	*Cantharellus cibarius*	14.26±0.16	0.07±0.003	12.28±0.21	45.70±0.22
4.	***P- value***	0.00	0.00	0.00	0.00

APPENDIX VI

1. MACROFUNGAL SPORE PRINT

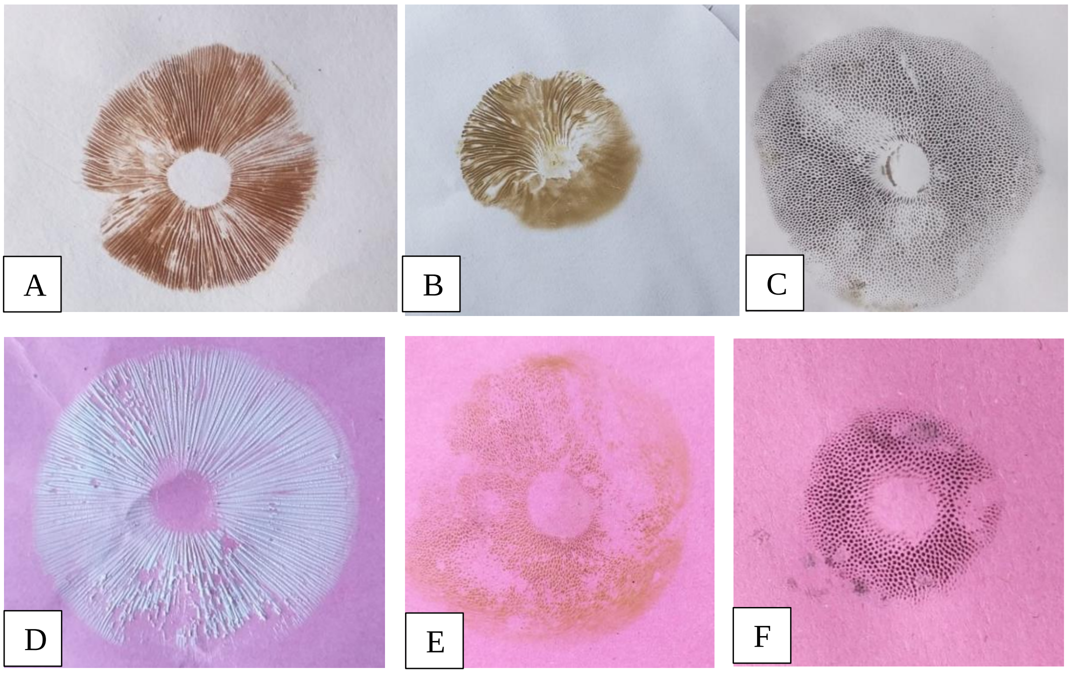

Figure: Spore prints of (A) *Hebeloma sinapizans* (B) *Phylloporus rhodoxanthus* (C) *Retiboletus nigerrimus* (D) *Amanita rubescens* (E) *Suillus granulatus* (F) *Strobilomyces strobilaceus*

2. SAMPLE USED FOR NUTRIENT ANALYSIS

Figure: (A-B) Fresh and dried sample of *Laccaria Laccata* (C-D) *Dried sample of Scleroderma cepa and Cantharellus cibarius* respectively

3. FIELD WORK AND LAB WORK

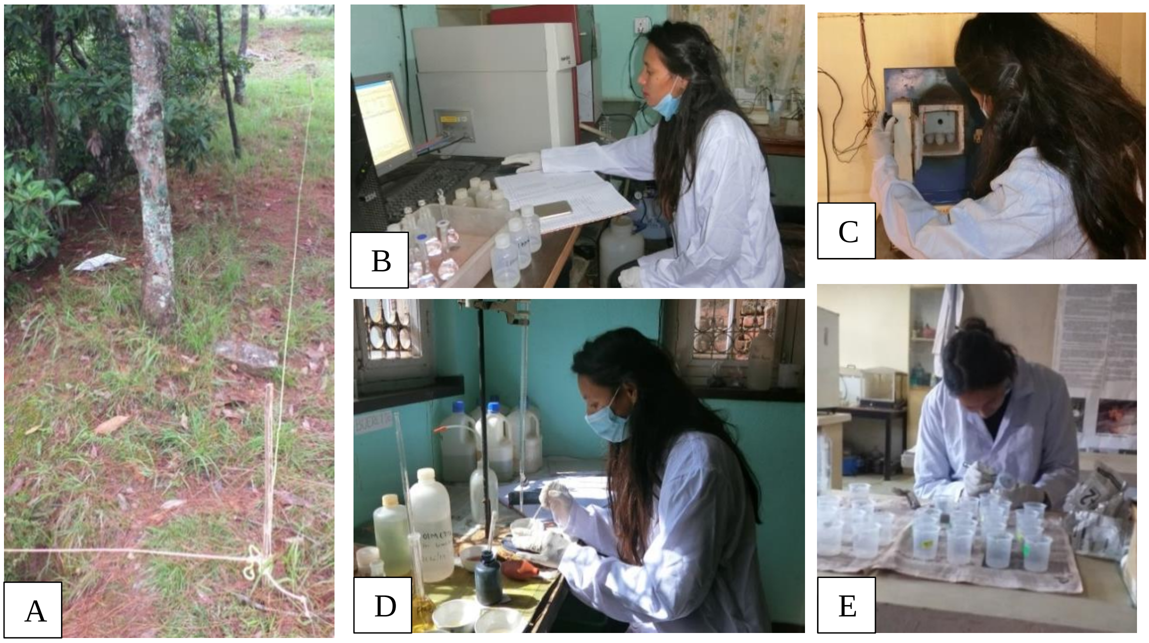

Figure: (A) Quadrate (B) Estimation of minerals by AAS (C) Determination of Ash in muffle furnace (D) Determination of protein by titration (E) Tagging while determining soil pH