BIOPROSPECTING OF WILD MUSHROOM FLORA OF UPPER BRAHMAPUTRA VALLEY ZONE OF ASSAM

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Submitted by

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I hereby declare that the work embodied in this thesis entitled **"Bioprospecting of wild mushroom flora of Upper Brahmaputra Valley Zone of Assam"** is a research work done by me under the supervision and guidance of **Dr. Supriya Sharma**, Assistant Professor, Department of Plant Pathology, Assam Agricultural University, Jorhat. I further declare that this work has not been submitted earlier in full or in parts to any other university for the award of any other Degree, Diploma, Associateship, Fellowship or other similar tiltles.

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ABBREVIATIONS AND SYMBOLS

% : **Per cent**

°C : **Degree centigrade**

BOD : **Biological Oxygen Demand**

et al : **Et alia (and others)**

Fig : **Figure**

g : **Gram**

i.e : **Id est (That is)**

L : **Litre**

viz : **Namely**

PDA : **Potato dextrose agar**

c.m. : **Centimeter**

m.m. : **Milimeter**

Sp. : **Species**

Spp. : **Species (Plural)**

INTRODUCTION

CHAPTER I

INTRODUCTION

In the biological world diversity, economic value and environmental importance of mushrooms occupy a prominent place (Sarma, 2010). In developing countries like India, mushrooms are the source of progress in the fields of food, medicine and unemployment (Khatun *et al.,* 2011). Mushroom production represents one of the most commercially important steps towards diversification of agriculture based microbial technology for large-scale recycling of agro-wastes in an agricultural country like India. Mushrooms are usually placed above vegetables and below high proteins in meat, fish and therefore can solve world's food scarcity problem (Boa 2004). Mushrooms are globally appreciated for their nutritional value and medicinal properties. Mushrooms are great providers of several minerals and trace elements, as well as dietary fibers, and have low fat, high protein, and vitamin levels. In recent years, mushrooms have become recognized as a fantastic source of antioxidants, nutraceuticals, anticancer agents, prebiotics, immunomodulators, anti-inflammatory agents, and antibacterial and anti-diabetic agents. Mushrooms include bioactive substances in addition to the polysaccharides (-glucans) and proteins that make up their cell walls, as well as secondary metabolites such phenolic compounds, terpenes, and steroids. The species of mushroom, substrate, fruiting conditions, stage of development, age of the mushroom, storage conditions, and obviously cooking techniques all have an impact on the concentration and effectiveness of bioactive chemicals.

There are about 15,00,000 fungi present all over the world in which 14,000 are recognized as mushroom species (Mishra *et al.,* 2021). Out of 14,000 species of mushrooms reported worldwide, about 1,200 species belonging to the order Agaricales, Boletales and Russulales are described from India, which contributes 10 percent of the global mushroom diversity (Thiribhuvanamala*et al.,* 2011).Mushrooms are cosmopolitan in nature and occur seasonally in several habitats all over the world. In general, fungal diversity is greater in the tropics than that of temperate regions (Suryanarayanan*et al.* 2003). One third of total global fungal diversity exists in tropical region (Mishra *et al.,* 2021). India is one of the top 10 mega diverse nations of the world fortunate to have a varied agro climate, abundance of agro wastes, relatively low cost labor and a rich fungal diversity (Borkar *et al.,* 2015). Northeast India is also one of the biodiversity hotspots and

is very rich in mushroom flora (Verma *et al.,* 1995). Mushroom diversity studies have been carried out in some specific regions of India including Sikkim (Das 2010), Nagaland (Kumar *et al.,* 2013) and Meghalaya (Khaund2013). Assam, situated in the Northeast of India is a constituent unit of the Eastern Himalayan Biodiversity Region; one of the two biodiversity "Hot Spots" in the country (Parveen *et al.,* 2017). Assam is rich in diversity of biological habitats like woods, grasslands, and wetlands, which harbor and sustain a vast range of floral and animal species, because of its climatic conditions and diverse physical features. Assam has a sub-tropical monsoon climate with an average rainfall of around 1,500 mm per year (Parveen *et al.,* 2017). The day-time temperature in summer, the rainy season, rises to around 35ºC and in winter cools to 25ºC with a night time minimum of around 10ºC which favours the growth of most of the macrofungal species (Das *et al.,* 2005).

Wild edible fungi (WEF) have been collected and consumed by people for thousands of years. Of the 14,000 mushroom species, nearly 7000 species are well studied to possess varying degree of edibility and more than 3000 species spread over in 31 genera are regarded as prime edible. Thus far, only 200 of them are experimentally cultured, 100 economically cultivated, approximately 60 commercially grown and about 10 have reached an industrial scale (Chang and Miles, 2004). Mushrooms are consumed for their palatability and/or nutritional value. Palatability can be judged by color, texture, flavor, and taste. India is a diversified nation with numerous ethnic and tribal groupings, each of which manages its natural resources according to its own set of norms for everyday use.The eastern countries of Asia documented the traditional information on the utilization of cooked and therapeutic mushrooms had been accepted on generation to generation but it was not so much in India (Panda and Tayung, 2015). Traditional knowledge of wild edible and medicinal mushrooms might have lost all over the world due to lack of documentation. Traditional information of Indian tribal communities has demonstrated to be broad and profound, consuming nearly 283 species of wild macrofungi out of 2,000 species documented world over (Purkayastha and Chandra, 1985). Indigenous communities have been utilizing non-timber forest products by using their ethnomycological knowledge of wild mushrooms collection, preparations with food items and consuming since time immemorial for their different daily uses, *i.e.,* edible, medicinal and have been considered as the secondary food resources (Boa, 2004). Ethnomycological studies revealed that wild edible mushrooms have been reported from Odisha, West Bengal, Assam, Manipur,

Nagaland and Arunachal Pradesh of India (Sarma*et al.,* 2010; Tanti *et al.,* 2011). The North Eastern Himalayan Region of India is a biodiversity hotspot which covers eight states, *viz.,* Arunachal Pradesh, Manipur, Assam, Meghalaya, Nagaland, Mizoram, Tripura and Sikkim (Myers, 2003) but very less amount of research have been carried out from different parts of the Northeastern states of India. The negative site of various traditional knowledge is proper identification, addressing poisoning, various utilization process, spreading of traditional knowledge and market value for selling wild mushrooms due to which many people died in various part of the world (Agrahar-Murugkar and Subbulakshmi, 2005; Basumatary and Gogoi, 2016; Khaund and Joshi, 2014; Sarma*et al.*, 2010; Singh and Chhetry, 2010; Paul *et al.,* 2015)

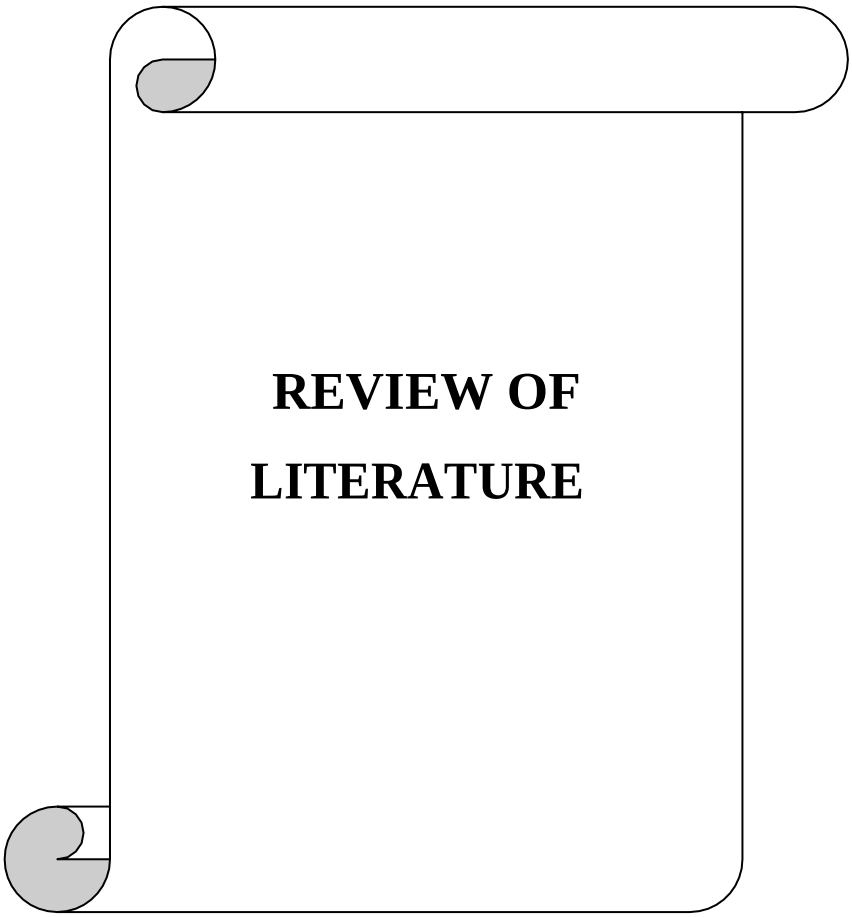
Among the ethnic tribes in Assam,Garos uses at least seven species of mushrooms followed by *Adivashis, Bodos* and *Rajbangsi's* of Western Assam. But the potentialities of such species are yet to be studied in detail. Though the state is rich in mushroom diversity, owing to the lack of adequate knowledge on edible and poisonous nature, extensive consumption is hampered. Edible fungi of the state still need to be scientifically explored. Besides these, global warming, habitat destruction or overexploitation may pose negative effects on wild edible mushrooms, therefore their artificial cultivation technologies need to be standardised.The range of wild species that have been identified and analysed is still small and little is known about variation within species that occur in different districts of the state. Research is needed on species that have greatest market potential and efforts should be made to highlight the nutritional properties and its advantages. Therefore, identification of new wild species of mushroom would pave the way for harnessing more of its nutritional as well as its medicinal value in dividends. Hence, the present study entitled "***Bioprospecting of wild mushroom flora of Upper Brahmaptura Valley Zone of Assam*"** attempted to document the macrofungal diversity of some districts of Upper Brahmaputra Valley Zone of Assam with special emphasis on their identification and their uses.

1.1. AIM AND OBJECTIVES

Therefore, in line with the above established facts, the present research programme has been designed with the following objectives-

I. Survey and collection of wild mushroom flora of Upper Brahmaputra Valley Zone of Assam

II. Identification of the collected wild mushroom flora based on morphological, anatomical and cultural characteristics


REVIEW OF
LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Mushroom: Structure, Growth, and Composition

In broad sense, "mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand" (Chang *et al.,* 1982). It is perhaps the most well-known and documented edible forest product(Chamberlin *et al.,* 1998*)* . Mushrooms have been widely used as foods (Falconer *et al.,* 1990, Gilbert *et al.,* 1957) and very often as delicious and nutritious foods (Vincent *et al., 2013)*. Approximately 14,000 described species of the 1.5 million fungi estimated in the world produce fruiting bodies that are large enough to be considered as mushrooms (Chang *et al.,* 2006*)*. Mushrooms belong to basidiomycetes and ascomycetes with a cell cycle including the formation of sexual spores and have two growth phases, i.e., the vegetative phase (mycelia) and the reproductive phase (fruit bodies). The fungal spores are located in a special structure called the basidium (for Basidiomycetes) or the ascus (for Ascomycetes). The mushroom continues its life cycle in three key stages. viz., vegetative growth, reproductive growth, and spore production by fruit bodies of the mushrooms. Fungi lack the most important feature of plants, i.e., the ability to use energy from the sun directly through chlorophyll. Thus, fungi depend on other organisms for food, absorbing nutrients from the organic material in which they live. The living body of the fungus is mycelium made out of a tiny web of threads (or filaments) called hyphae. Hyphae absorb digestive products, penetrating the substrate to some extent. Under specific conditions, sexually compatible hyphae will fuse and start to form spores. The larger spore-producing structures are considered as mushrooms. The spores released from the gills again germinate and develop to form hyphae, which is the main mode of fungal vegetative growth. The mushroom produces several million spores in its life, and this life cycle is repeated each time the spores germinate to form the mycelium. Mycelial growth is generally coupled with increased enzyme production and respiration.

2.2 Global Trends of Mushroom Cultivation

Mushrooms are the common components in folk medicine, especially in Africa, the Middle East, China, and Japan since ages. Earlier, edible mushrooms were only harvested

wild and were difficult to domesticate and cultivate. Collection from wild woodlands is still important in the world and particularly in southern Asia (*Arora D,* 2008) and other developing countries (Fanzo *et al.,* 2012). Mushrooms such as Auricularia, Flammulina, and Lentinula were most likely cultivated for the first time around the year 600–800 AD in China and other Asian countries (Chang *et al.,* 2017). Their cultivation at large scale started only at the beginning of the twentieth century when pure cultures of mushroom were prepared from spore and tissue. As the amount of wild mushrooms shrink from boththe degraded environment and natural resources and more costly labor, cultivated mushrooms would not only provide food security but also sustainable and more nutritious diets (Vincent *et al.,* 2013). The commercial production of edible mushrooms represents the unique exploitation of a microbial technology for the bioconversion of agricultural, industrial, forestry, and household wastes into nutritious food (mushrooms). Mushrooms have the capacity to breakdown the lignin and utilize it as a food source, thus exposing the underlying cellulose and hemicellulose for food use by other organisms. Thus, mushroom cultivation represents a very basic natural process of fungal decay.

With the world's increasing population and its decrease in per capita arable land, along with rapid urbanization and industrialization, climate change, and a demand for quality and functional foods, it will be necessary to focus on secondary agriculture and novel crops, such as mushrooms. Mushroom cultivation could also be an important part of sustainable agriculture and forestry. Huge quantities of wide varieties of organic waste are generated from agriculture, forestry, and food processing. The impacts of the mushroom business on livelihoods and poverty reduction are significant and widespread. Mushroom cultivation does not require a lot of land, significant capital investment, but is a viable and attractive activity for both rural farmers and semi-urban dwellers. Mushroom cultivation strengthens the livelihood of poor and marginal farmers by generating constant farm income and reduces the vulnerability to poverty. The scale of cultivation can be large or small based on the capital and labor availability. It can be cultivated on a part-time basis with little maintenance. Indirectly, mushroom cultivation also provides opportunities for improving the sustainability of small farming systems through the recycling of organic matter, which can be used as a growing substrate and then returned to the land as fertilizer. There are hundreds of identified species of fungi which have made a significant contribution to human food and medicine. The total number ofdescribed fungi of all kinds is currently 110,000 species (Kirk *et al.,* 2008) of which 16,000 (15%) species are mushrooms (Kirk *et*

al., 2008, Hawksworth *et al.,* 2012, Wasser, 2010). Out of these, more than 3000 species from 231 genera are regarded as prime edible mushrooms (Wasser *et al.,* 1999) of which only about 200 are experimentally grown, 100 economically cultivated, around 60 commercially cultivated, and more than 10 produced on an industrial scale in many countries (Chang *et al.,* 2017). Approximately 700 mushroom species out of the known 16,000 are considered to be safe and have medicinal properties (Wasser *et al.,* 2010). The number of poisonous mushrooms approximates 500 species. The most acceptable varieties among the cultivated types are Agaricusbisporus (button mushroom), Pleurotus spp. (oyster mushroom), Lentinus edodes (Shiitake), and Volvariella spp. (paddy straw mushrooms). In the second half of the twentieth century, there were rapid changes in rate of growth of mushroom production and number of species like shiitake, oyster mushrooms, and wood ear mushroom, and Flammulina were brought under commercial cultivation. By the end of the twentieth century, the share of button mushroom in total world production was less than 40%, which in next 10 years became around 30%. Presently shiitake, oyster, wood ear, and button mushrooms contribute 22%, 19%, 18%, and 15%, respectively in terms of total mushroom production in the world (Singh *et al.,* 2017). The contribution of medicinal mushrooms in world trade has also increased over last few decades.

Mushroom farming is today being practiced in more than 100 countries, and its production is increasing at an annual rate of 6–7%. Cultivated mushrooms have now become popular all over the world. In 1999, the world production of cultivated edible mushrooms was estimated to be >7 million tons, showing a steady increase over the last two decades. China is the largest producer, consumer, and exporter of mushrooms in the world, followed by the United States and the Netherlands . In China, mushroom is the 6th important crop in the country as far as revenue generation for the nation is concerned. Mushroom production in China in 2010 was 21,524,473 t (Gupta *et al.,* 2012). The last few decades have witnessed a sharp rise in diversification in number of mushroom species that have been cultivated, world mushroom production, commercialization accompanied with mechanization, and in many cases automation. Mushroom cultivation and its processing have been beneficial to millions of people in China, India, and other developing countries in terms of financial, social, and health improvement. The global mushroom industry has expanded very rapidly in the last two decades by the addition of newer types of mushrooms for commercial cultivation. In addition, cultivation and development of mushroom industries have positively impacted on economic growth, and this impact of

mushroom cultivation and mushroom derivatives and products on human welfare in the twenty-first century can be considered globally as a “nongreen revolution.”

2.3 History and Status of Mushroom Cultivation in India

The history of mushroom cultivation in India goes back to the ages of “Vedas,” wherein the mention was made in the classical religious scriptures like “Rig Veda” and “Atharva Veda” about the use of juice from fly agaric mushroom (Amanita muscaria) as an intoxicating drink, named “Soma.” Vegetarianism is assumed to bethe norm of Indian diet and is primarily based on cereals (wheat, rice, and maize) which are deficient in protein. Incorporation of mushrooms in Indian diet has the potential to bridge this protein gap and reduce the problem of malnutrition to a great extent. For people in a developing country like India, the two main issues are the quality food and the level of unemployment, in addition to environmental concerns, which can be very well resolved by popularizing mushroom cultivation among rural masses and the younger generation (Gupta *et al.*, 2019). Although time to time, small efforts and simple research were conducted to introduce mushroom cultivation in India, scientific and systematic research only started in 1961, when the Indian Council of Agricultural Research, New Delhi, first cultivated Agaricusbisporus at Solan in Himachal Pradesh, a hilly state of North India. India, primarily being an agrarian economy, is rich in terms of agrowastes that are not properly utilized by the nation’s farmers. India produces nearly 700 million tons of agricultural residues which can profitably be utilized for mushroom cultivation. Even if India uses 2% of its total agroresidues for mushroom production, the production would be 7.0 million tons of fresh mushrooms, which will be equal to current global button mushroom production. Currently, India is using only 0.03% of these residues to produce about 0.13 million tons of mushrooms and contributesthe norm of Indian diet and is primarily based on cereals (wheat, rice, and maize) which are deficient in protein. Incorporation of mushrooms in Indian diet has the potential to bridge this protein gap and reduce the problem of malnutrition to a great extent. For people in a developing country like India, the two main issues are the quality food and the level of unemployment, in addition to environmental concerns, which can be very well resolved by popularizing mushroom cultivation among rural masses and the younger generation (Gupta *et al.*, 2019). Although time to time, small efforts and simple research were conducted to introduce mushroom cultivation in India, scientific and systematic research only started in 1961, when the Indian Council of Agricultural Research, New Delhi, first cultivated Agaricusbisporus at

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2.4 Relative Contribution of Different Mushroom Species

In India, there are five mushroom species, viz., white button mushroom (Agaricusbisporus), oyster (Pleurotus spp.), paddy straw (Volvariellavolvacea), milky (Calocybe indica), and shiitake (Lentinula edodes), which are under commercial cultivation. Even though cultivation technologies of many exotic mushrooms have been standardized, the commercial markets are still dominated by Agaricusbisporus, Pleurotus spp., and Volvariellavolvacea. These three mushrooms are contributing about 96% of total mushroom produced in India. Milky mushroom (Calocybe indica) is indigenous tropical mushroom of the country (Satish *et al.*, 2017). However, the commercial cultivation is restricted to southern states of India and contributing up to 3% to the total mushroom production. Production of paddy straw mushroom became more popular in the states of Odisha and Chhattisgarh, and its production was registered at 7% to the total mushroom production. Two to three crops of button mushroom are grown seasonally in temperate regions with minor adjustments of temperature in the growing rooms, while one crop of button mushroom is raised in the northwestern plains of India seasonally. Oyster, paddy straw, and milky mushrooms are grown seasonally in the tropical/subtropical areas. In Northeastern states,Uttarakhand and Chhattisgarh, oyster mushroom cultivation is emerging as one of the leading cottage industry. Hence, for choosing a species for commercial cultivation, the grower must consider the availability of waste materials to use as a growth medium, the prevailing environmental conditions, available expertise, available resources, and market demand.

2.5 Diversity of Mushrooms of Nutraceutical and Therapeutic Significance

It has become increasingly apparent in recent years that the organisms traditionally referred to as fungi are not all closely related. This prompted some authors to use term "fungi" in a very general sense and the term "Fungi" with a capital "F" specifically for the so-called true fungi that appear to be related to one another. The true fungi are placed in

the kingdom Fungi. Modern systematics, based on morphological characters and analysis of rDNA sequences, divides the kingdom Fungi into four major phyla or divisions: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota.The two fungal phyla that produce large, visible fruit bodies are the Ascomycota and Basidiomycota. The Ascomycota contains at least 40,000 different species worldwide, many of them rather inconspicuous, but including such familiar groups as the morels and truffles, the cup fungi, and most of the lichens, as well as many microscopic molds and yeasts. They all produce their spores within macroscopic cells called asci, which typically open under pressure when mature, shooting the spores out into the air currents (Roberts *et al.,* 2013). The Basidiomycota contains at least 30,000 different species worldwide and includes many of our most familiar fungi, including all the agarics (mushrooms and toadstools), puffballs and stinkhorns, bracket fungi, chanterelles, and club and coral fungi, as well as the plant-parasitic rusts and smuts (Robert *et al.,* 2013). All of them produce their spores on the external surface of microscopic cells called basidia and their fruit bodies have evolved in many ingenious ways in order to liberate these spores. The fungi comprising the phylum Basidiomycota commonly are known as basidiomycetes. Basidiomycota includes classes Hymenomycetes, Ustilaginomycetes, and Urediniomycetes. Hymenomycetes includes orders Agaricales, Aphyllophorales, Auriculariales, Dacrymycetales, Ceratobasidiales, and Tuslasnellales. Among Hymenomycetes, mainly the orders Agaricales, Aphyllophorales, and Auriculariales and class Gasteromycetes contain the mushrooms of pharmaceutical importance. The rest of the orders, that is, Dacrymycetales, Ceratobasidiales, and Tuslasnellales, contain parasitic and disease causing forms. Hymenomycetes also includes another subgroup (order) Tremellales. Ustilaginomycetes includes orders Ustilaginales and Exobasidiales which include smut causing forms, while Urediniomycetes include orders Uredinales, Septobasidiales, Sporidiales, and Eocronatirum that are responsible for causing rusts in various crop plants (Alexopolous *et al.,* 2002).

2.6 Nutrition in Mushrooms

Edible mushrooms have been widely utilized as human food for centuries and appreciated for texture, flavor, as well as medicinal and tonic attributes (Manzi *et al.,* 2001). In general, mushrooms contain 90% water and 10% dry matter (Sanchez *et al.,* 2010). They have a chemical composition, which is attractive from the nutritional point of view (Dundar *et al.,* 2008). Mushrooms are nutritionally important as they are rich in protein, fibers, and minerals, while poor in fats. The mushroom protein contains all the

nine essential amino acids required by humans. Mushrooms are considered as a potential substitute of muscle protein on account of their high digestibility (Pavel, 2009). Besides this, mushrooms are also rich source of vitamin B1, B2, B12, C, D, and E (Heleno *et al., 2010, Mattile,* 2001) and a relatively good source of nutrients like phosphorus, iron, and vitamins, including thiamine, riboflavin, ascorbic acid, ergosterol, and niacin. Mushrooms are also an excellent source of vitamin D which is otherwise not available in other food supplements. Mushrooms are low in calories, fat-free, cholesterol-free, gluten-free, and very low in sodium. Minerals such as potassium, iron, copper, zinc, and manganese are high in fruit bodies. They also have ash, glycosides, volatile oils, tocopherols, phenolic compounds, flavonoids, carotenoids, folates, organic acids, etc. (Sánchez C, 2004). Mushrooms are also important from nutraceutical point of view, as they contain several compounds like unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid, and carotenoids. The nutritional attributes of edible mushrooms and the health-benefiting effects of the bioactive compounds they contain make mushrooms a health food. Consumers are now deeply interested in food bioactives that provide beneficial effects to humans in terms of health promotion and disease risk reduction. Mushrooms can be considered as functional food which provides health benefits in addition to nutritional value (Vaz JA *et al*., 2010). The concept of "functional foods" was first introduced as a factor in the analysis of foods after nutrients (Sadler M *et al.,* 1998)

2.7 Therapeutic Potential of Mushrooms

The major attribute of mushrooms is their medicinal properties and presence of bioactive compounds. The pharmacological properties of mushrooms include immunity enhancement, maintenance of homeostasis, regulation of biorhythm, and most importantly cure and prevention of various life-threatening diseases such as cancer, stroke, and heart diseases. Medicinal properties of mushrooms including antiinflammatory, antioxidant, immunomodulatory, anticarcinogenic, antiviral, antibacterial, antifungal, hepatoprotective, antidiabetic, anti-angiogenic, hypoglycemic, etc. have been reported (Xu T *et al.,* 2015)Immunomodulatory and antitumor activities of polysaccharide–protein complex (PSPC) from mycelial cultures of mushrooms have been extensively studied (Yip KP *et al.,* 2015). The pharmacological potential of mushrooms includes the following: as antioxidants, as hypocholesterolemicagents, as hypoglycemic agents, as antitumor agents, as immunomodulators, as antimicrobial agents, as antiviral agents, as antiallergic agents, as anti-inflammatory agents and as hepatoprotective agents.

2.8 Bioactive Compounds in Mushroom

Mushrooms are widely used for their high nutritional value as a functional food. Additionally, they have been highly appreciated for their medicinal and therapeutic applications (Chang *et al.,* 2004). Interestingly, mushrooms are a rich source of biologically active compounds providing medicinal or health benefits such as the prevention and treatment of diseases to humans. Edible mushrooms produce a vast diversity of bioactive compounds such as polysaccharides, proteoglycans, terpenoids, phenolic compounds, steroids, and lectins. These compounds have a wide range of therapeutic effects and can act as immunomodulatory, anticarcinogenic, antiviral, antioxidant, and anti-inflammatory agents (Badalyan SM, 2012). Specific bioactive compounds in mushrooms are responsible for improving human health in a number of ways. Bioactive compounds can be found in mushrooms as well as their cell wall components as polysaccharides (β-glucans) and proteins or as secondary metabolites such as phenolic compounds, terpenes, and steroids. The concentration and efficacy of bioactive compounds are varied and depend on the type of mushroom, substrate, fruiting conditions, stage of development, age of mushroom, storage conditions, and of course cooking procedures (Guillamon *et al.,* 2010). On the basis of their chemical structure, bioactive compounds of mushrooms may be classified as peptides and proteins, phenolic compounds, polysaccharides, polysaccharides protein complexes, terpenes, terpenoids, etc.

2.9 Ethnomedicinal practices of wild mushroom by the local tribes of India

Ethnomycology is the study of wild macrofungi in folklore and rituals, from prehistoric times to that time (Charaya and Mehrotra, 1999) with their morphometric identification by the tribe along with documentation of nutritional properties and indigenous knowledge regarding their uses as food, medicine and in some other cultural traditional uses also. Edible and medicinal practices of mushrooms in India is quite common, some of which dates back to 1700–1100 BC (Wasson, 1971). India is a diverse country whichbelongs to different types of tribal people or ethnic groups and each group has own management practices of natural resources for their daily uses. The eastern countries of Asia documented the traditional information on the utilization of cooked and therapeutic mushrooms had been accepted on generation to generation but it was not so much in India (Panda and Tayung, 2015). Traditional knowledge of wild edible and medicinal mushrooms might have lost all over the world due to lack of documentation.

Traditional information of Indian tribal communities has demonstrated to be broad and profound, consuming nearly 283 species of wild macrofungi out of 2,000 species documented world over (Purkayastha and Chandra, 1985). Indigenous communities have been utilizing non-timber forest products by using their ethnomycological knowledge of wild mushrooms collection, preparations with food items and consuming since time immemorial for their different daily uses, i.e., edible, medicinal and have been considered as the secondary food resources (Boa, 2004). Ethnomycological studies revealed that wild edible mushrooms have been reported from Odisha, West Bengal, Assam, Manipur, Nagaland and Arunachal Pradesh of India (Baruah *et al.,* 1971; Sarma *et al.,* 2010; Sing and Sing, 1993; Sing *et al.,* 2002; Tanti *et al.,* 2011).

The North Eastern Himalayan Region of Indiais a biodiversity hotspot which covers eight states, viz., Arunachal Pradesh, Manipur, Assam, Meghalaya, Nagaland, Mizoram, Tripura and Sikkim (Myers, 2003) but very less amount of research have been carried out from different parts of the Northeastern states of India. The negative site of various traditional knowledge is proper identification, addressing poisoning, various utilization process, spreading of traditional knowledge and market value for selling wild mushrooms due to which many people died in various part of the world (Agrahar-Murugkar and Subbulakshmi, 2005; Basumatary and Gogoi, 2016; Khaund and Joshi, 2014; Sarma *et al.,* 2010; Singh and Chhetry, 2010; Paul *et al.,* 2015). In ancient time, the alertness of wild edible macrofungi and their significance to people of the developing countries have often been ignored but it is only in recent years that initiatives on non-wood forest products have begun to clarify their action use and functions in livelihoods (Devi, 2017; Kumar *et al.,* 2014). Systematics of wild macrofungi has accepted more awareness than other endangered aspects like conservation (Kumar *et al.,* 2014).

2.9.1 Records of Ethnomedicinal uses of mushrooms

Wild medicinal mushrooms are edible, medicinal, poisonous, edible with medicinal properties and poisonous with medicinal activity and these mushrooms play a considerable role in sustaining the livelihood of the rural people. (Chauhan *et al.,* 2014) recorded twelve wild edible macrofungi, viz., Agaricus campestris, Helvellacompressa, Morchellaconica, Morchella esculenta, Morchella deliciosa, Ramaria botrytis, Lactariusdeliciosus, Rhizopogon vulgaris, Sparassiscrispa, Gyromitra sp. Hygrophorus sp. and Lycoperdon sp. which were used by the local tribes of Kinnaur district, Himachal Pradesh,India as food by

various modes of preparation. (Dutta and Acharya, 2014) found 34 macrofungal species from West Bengal, India which were eat up or used by the locals and tribals peoples among which 31 macrofungi species were found to be edible and 5 were used as traditional medicine, while some of them were used for both the purposes. (Srivastava *et al.,* 2011) conducted an ethnobotanical survey for use of Termitomyces species in Gorakhpur forest division of Uttar Pradesh, India and they found that tribal people and forest dwellers use Termitomyces species as food as well as medicinal purposes but other uses were not clearly known. Earlier findings revealed that macrofungi were used as food supplements and also important due to their healing capacities used by the traditional people (Valverde *et al.,* 2015). (Hernández-Santiago *et al.,* 2016) documented a sum of 106 macrofungal species which were growing in oak and pine forest, grassland, deciduous tropical forest, among the identified macrofungi 26 species consumed, 18 recorded as toxic, 6 having ludic application, left over 56 species not individually used but 56 species used as food and 28 species as medicine from Southeastern Mexico. (Adhikari *et al.* 2005) also worked on ethnomedicinal knowledge of wild mushrooms from the vicinities of Lumle and Kathmandu valley of Nepal. They found a total of 24 species of which 18 macrofungi were used as culinary, 8 for various medicinal uses and 3 for their other reason.

(Okigbo and Nwatu, 2015) reported six different species of macrofungi which were used by the people of Anambra State, Nigeria for various purposes, such as Daldiniaconcentrica in cure of stomach upset, Auricularia auricular-judae and Lentinus squarrosulus in medication of infertility and anaemia, Trichobatrachus robustus in remedies of Anaemia and high blood pressure, Termitomyces sp. for curing Anaemia, weakness, and high blood pressure and V. volvacea only used for the cure of Anaemia. Ethnomycological study of the use of mushrooms in North central Nigeria reveals that the majority of citizens consume macrofungi mainly due to their nutritional, palatability and medicinal characteristics (Ayodele *et al.,* 2009). (Kimn and Song, 2014) reported 38 species of wild mushrooms belonging 33 genera, 22 families by the traditional knowledge recorded from the tribal communities and 158 types of practices were classified and ideal families were Tricholomataceae, Pleurotaceae, Polyporaceae and Hymenochaetaceae. This result revealed 24 methods of preparation for the cooked mushrooms, such as soups, teas, simmered and roasted along with different medicinal uses. Each tribe of India use their own methods for preparation of traditional medicine, such as some fungus or fungal spores

used directly, some of them are used in powdered form, mixed with water, milk, tea, sugar, butter, chili, egg and oil, mixed with other plant parts and vegetables for various remedies.

2.9.2 Traditional uses of wild mushrooms as medicine in India

Wild mushrooms are used as herbal medicine, namely, wound healing, burning, itching, staunch bleeding eye disorders (pain, redness, conjunctivitis), fever and vomiting, typhoid, frost bites, ear puss, skin infections, curing stomach upset, brain tonic, against anger, constipation, weakness, eczema and mouth freshener (Devi, 2017; Dutta and Achariya, 2014; Kumar *et al.,* 2014; 2017; Lahiri *et al.,* 2010; Malik *et al.,* 2017; Manna *et al.,* 2014; Panda and Tayung, 2015; Rai *et al.,* 1993; Thangaraj *et al.,* 2017). Some mushrooms are used for a common illness, such as an antidepressant, treating lumbago, leg pains, numbness in limbs and tendon discomfort, nervous system colds, sore throats, sore eyes and stop bleeding (Malik *et al.,* 2017; Pala *et al.,* 2013; Panda and Tayung, 2015). Some major traditional uses of mushrooms against various scared diseases, viz, pneumonia, respiratory problems, asthma, jaundice, pox, goiters, diabetes, cancer, aphrodisiac, invigorative, revitaliser, anti-aging, kidney stones and partial paralysis (Kumar *et al.,* 2014; 2017; Manna *et al.,* 2014; Pala *et al.,* 2013; Panda and Swain, 2011; Panda and Tayung, 2015; Rai *et al.,* 1993). Wild mushrooms are also used by the tribal people for private diseases, such as aphrodisiac, menstrual cramps, to enhancing the lactation and prescribed to women after deliveries, treat male infertility and male visceral organ infections (Kumar *et al.,* 2017; Thangaraj *et al.,* 2017).

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

Assam is situated in the north eastern part of India. Assam is rich and diverse in flora and fauna. The study area is located in the Upper Brahmaputra Valley Zone of Assam comprises of Tinsukia, Jorhat, Dibrugarh, Sivasagar and Golaghat district. The Upper Brahmaputra Valley Zone of Assam is lies between 94°30′ E to 96°0′ E longitude and 27°0′ N to 27°45′ N latitude. This area has total 16193 km^2 of geographical area and a huge amount of total forest area. There are three National Parks, many wildlife sanctuaries and reserve forests present in this area which indicate the diversity of flora and fauna and importance of landscapes in the conservation of flora and fauna. Humidity is high in monsoon season and temperature goes around 9°C to 37°C. The average annual rainfall is 1478.1mm to 2771.7mm.

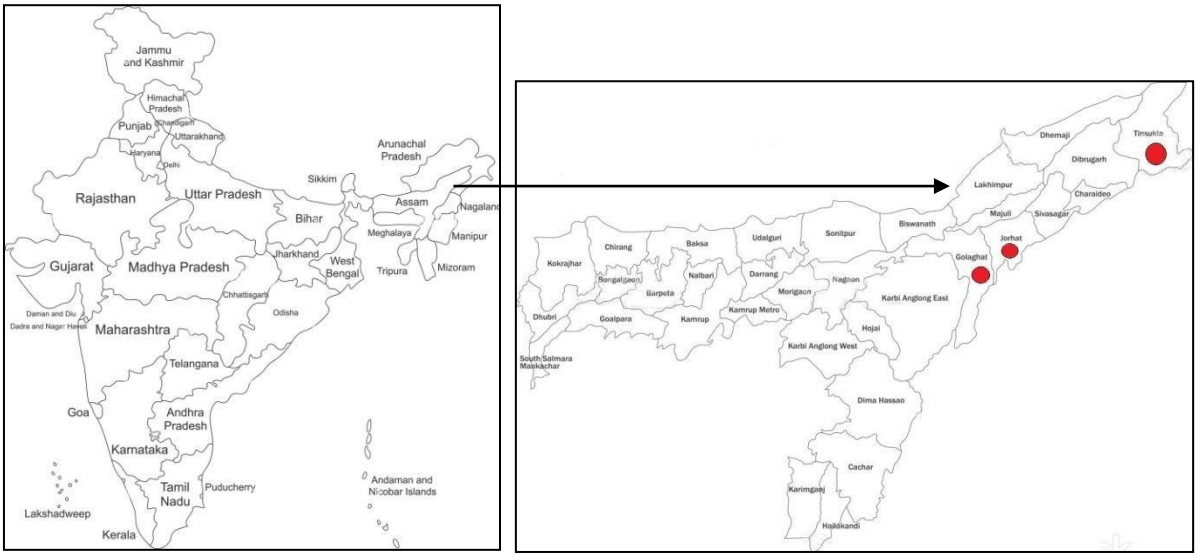


Fig.- 3.1 Map of the study area

Districts visited for sample collection are indicated as

3.2 Surveys

Surveys are depends upon timing and location of presence of mushrooms. Macrofungi exhibit pattern of diversity that are related to largely to substratum and host availability (Natarajan et al 2005). Surveying is best just after the period of rain but some

macrofungi can be found any time in some locations (Susan metzler and Van metzler 1992). There is a difficulty to find mushrooms in a single visit. So on the basis of information given by local people and tribal people systematic and regular surveys were carried out during the month of May 2022 to July 2022. From the same sources location of surveys were selected. Five sites altogether were chosen for collection and they are Gelapukhuri, Senchoa Gaon, Lichubari, Barbheta and Golaghat.

3.3 Collection

Collection is carried out from the selected areas on the basis of information given by local people and tribal people and tried to collect in proper time when fruiting bodies are approximately fully developed. Regular visits for collection of mushrooms were carried out during the month of May 2022 to July 2022 after every period of rain.

At the time of collection a sharp knife, cellophane bags, forceps, brush, camera, notebook, pen etc. were carried and they were used at the time of collection. Before collecting the mushroom samples photographs with habitat were captured and after collection a proper photograph of upper and lower body were captured on black cloth. The knife was used to incise to a depth sufficient to enable identification of the host and collecting mushrooms from the soil. Forcep was used to pick the mushroom and Soil was removed using a soft brush (Stojchev et al 1998). Mushrooms were removed from soil with a great care to avoid damage of the stipe and volva. After collecting mushroom samples were puted in cellophane bags and brought them to the lab. Along with these equipments labels were also carried where some parameter are mentioned like Collection No., date, location, habitat, brief note on distinguishing macroscopic features and collectors name. Along with that codes of collected samples were written like WMS-1, WMS-2, WMS-3, etc. by order from the first collection to the last collection accordingly.

3.4 Preservation

The collected samples were preserved in a preservative liquid like formaldehyde as soon as possible before it got decomposed and as a dried specimen or in a deep fridge in -20°C after isolation.

3.5 Questionnaire

A specific questionnaire to gather ethnomycological and edibility information was prepared separately. Questionnaire of parameter used for evaluation of wild mushrooms by observations and information by ethnic group/tribe was prepared. Separate questionnaire was prepared for each mushroom. The sample questionnaire is given in Table 3.1

Table 3.1

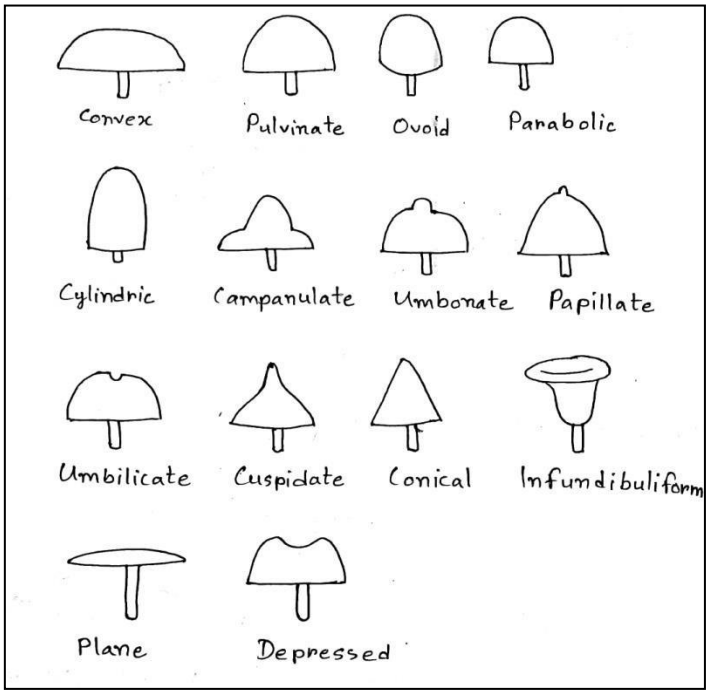
Sample No.	?
Code of the Collected Sample	?
Season of Survey	?
Vernacular name of mushroom	?
Habitat of mushroom	?
Substrate of mushroom	Single/multiple and name
Extent of occurrence	Rare/frequent and more frequent
Name of ethnic group/tribe	?
Edible part	Cap/stipe, entire part
Edibility flavour	Cheese/vegetable/fish/chicken
Chewing	Softness/hardness
Important feature	?

3.6 Morphological and anatomical characterization

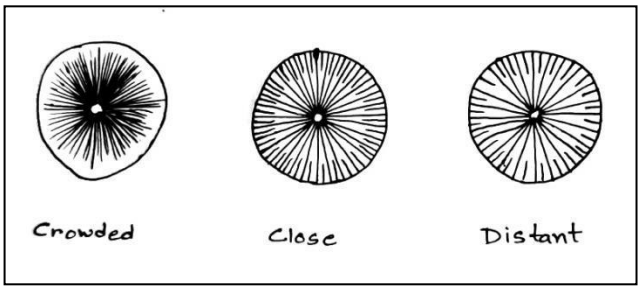
A study of mushroom was carried out after the time of collection. The fresh collected mushrooms were observed on the basis of morphological and anatomical data and recorded in the questionnaire prepared. For anatomical study mushroom were sectioned longitudinally an transversely with the help of blade. Some micro parts that are not visible clearly were observed by the help of dissecting microscope. Mushrooms were damaged and decomposed very fastly, therefore observations were taken carefully before drying.

Parameters of morphological and anatomical characters were acquired at the time of going through different literature before the start of project. To describe the morphological characters, parameters like Shape, size, colour, texture, heights, gill, mushroom smell, stipe etc. were considered and to describe the anatomical characters like Thickness of cap, attachment of gills to the cap, types of stipes (T.S) and stalk attachment etc. were considered. Characters were puted in the questionnaire and made a separate questionnaire for characterization.

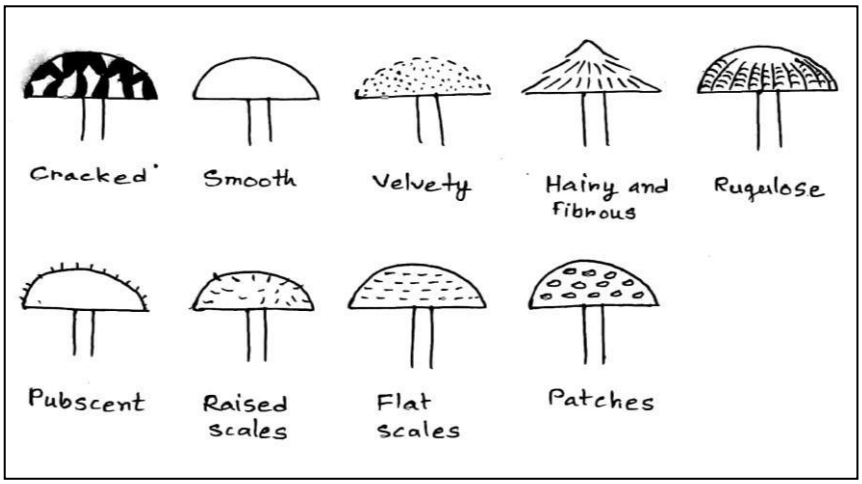
The considered parameters for morphological and anatomical characters were acquired from different literature and internet with diagram which were helpful at the time of characterization. They are Shape, size, colour, texture, heights, warts (present/absent), substrate (single/multiple and name), hymanophore (smooth/tooth like/ridged/porous), gill shape and name of type, gill colour, stem shape, mushroom smell,unique feature of morphology, thickness of cap, veil (universal/partial), margin of cap, marginal lines, edge of gill, attachment of gills to the cap, types of stipe (T.S), types of veils, surface of stem, stalk attachment. Unique feature of anatomy etc.



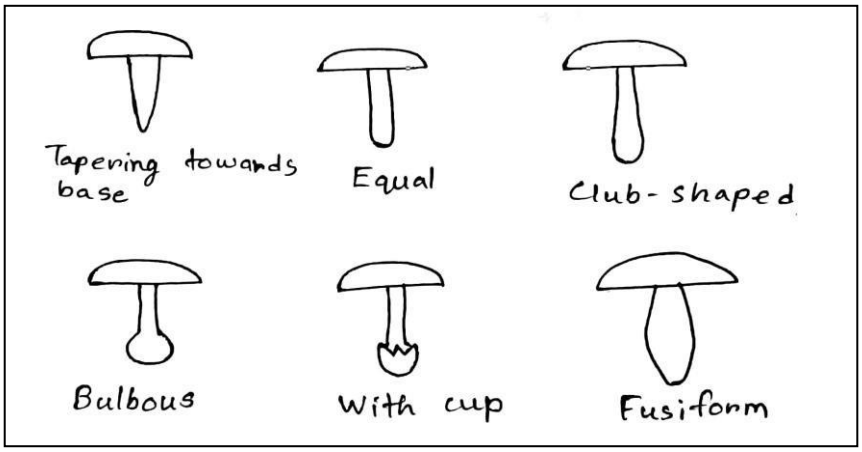
a- Shape



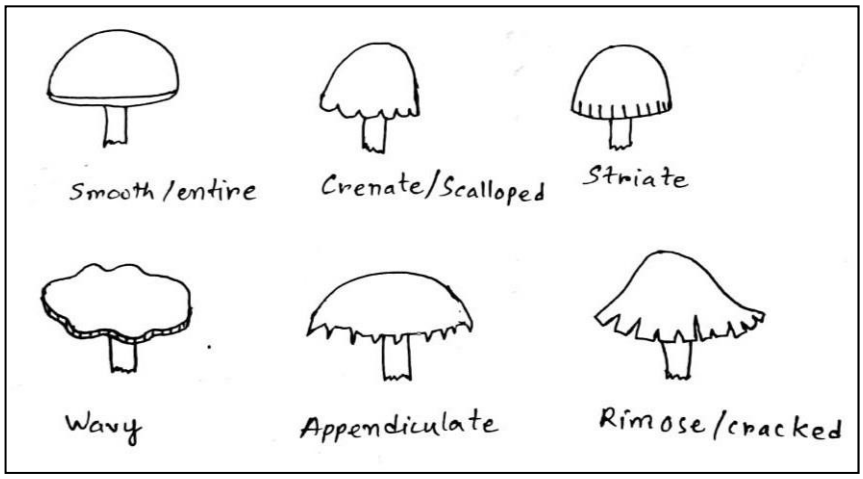
b- Type of gill



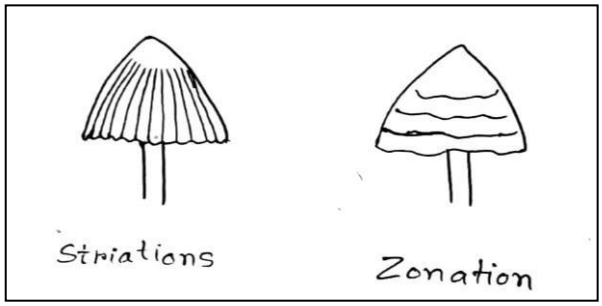
c- Texture



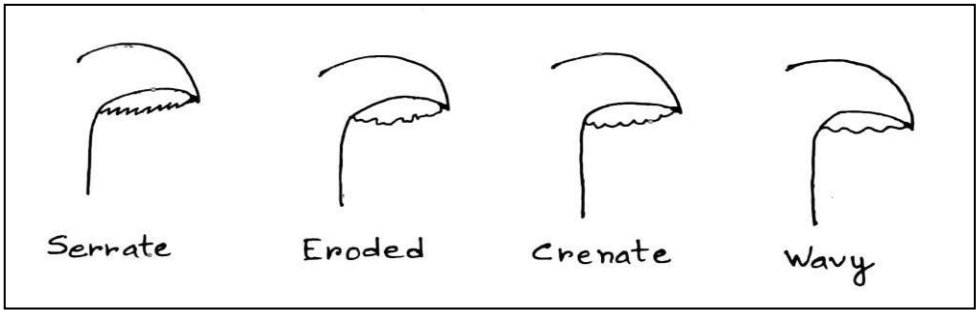
d- Stem shape



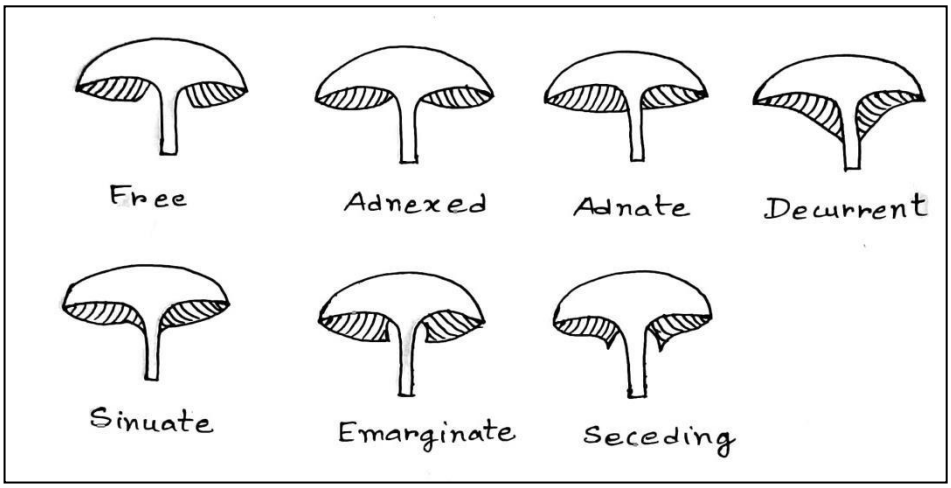
e- Margin of cap



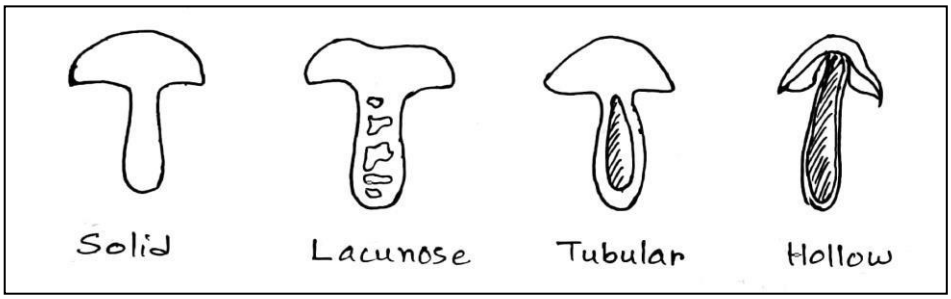
f- Marginal lines



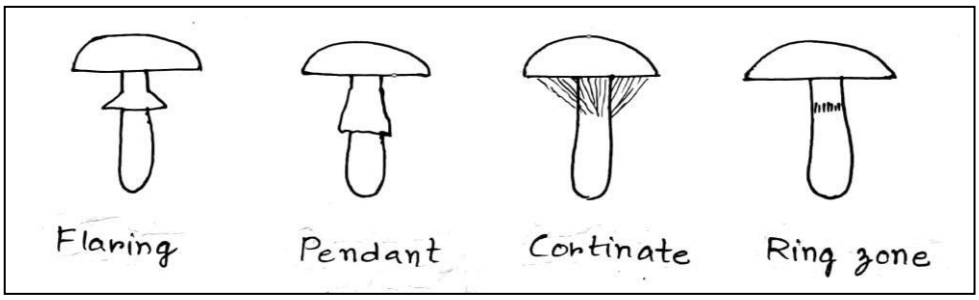
g- Edge of gill



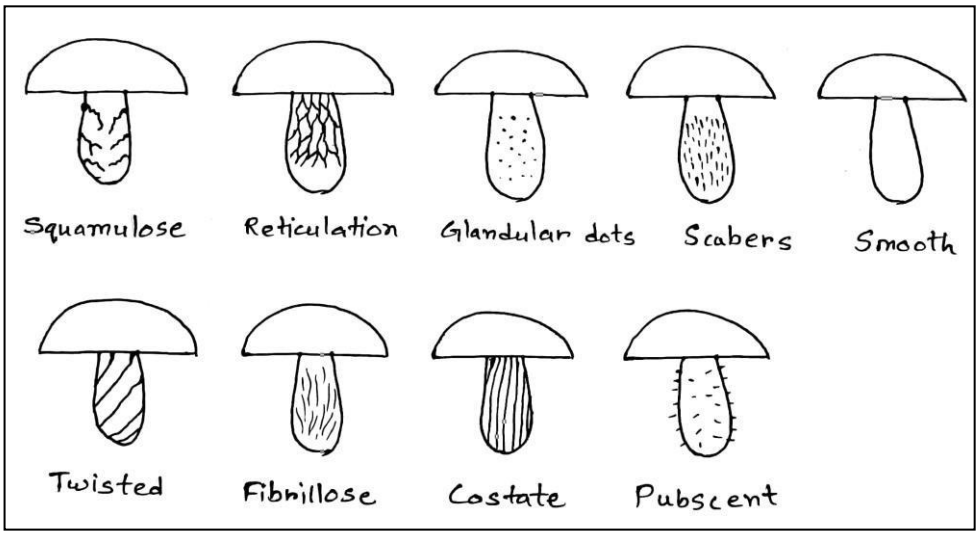
h- Attachment of gills to the cap



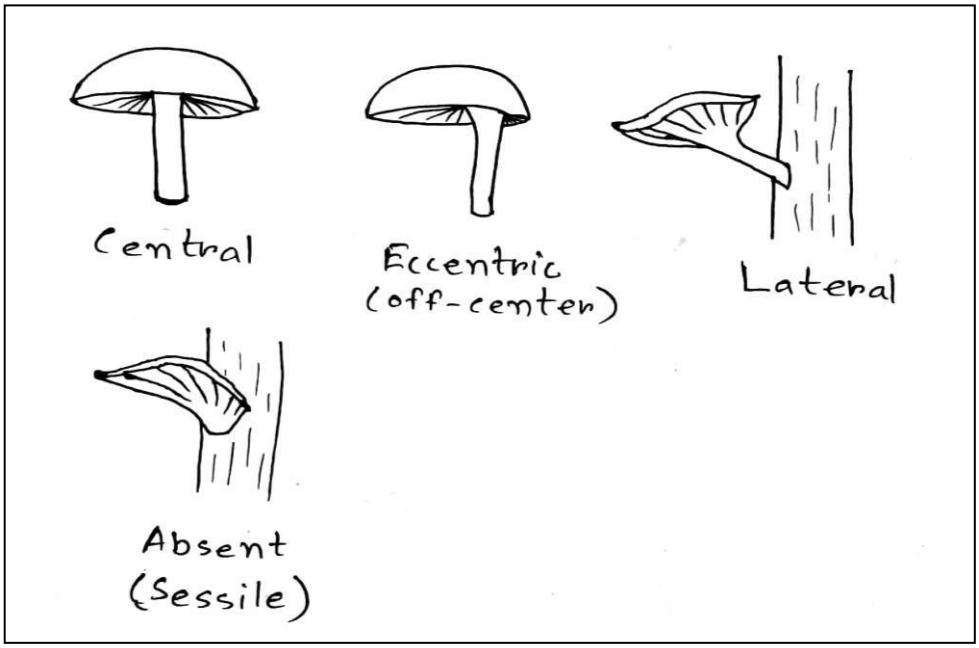
i- Types of stipes (T.S)



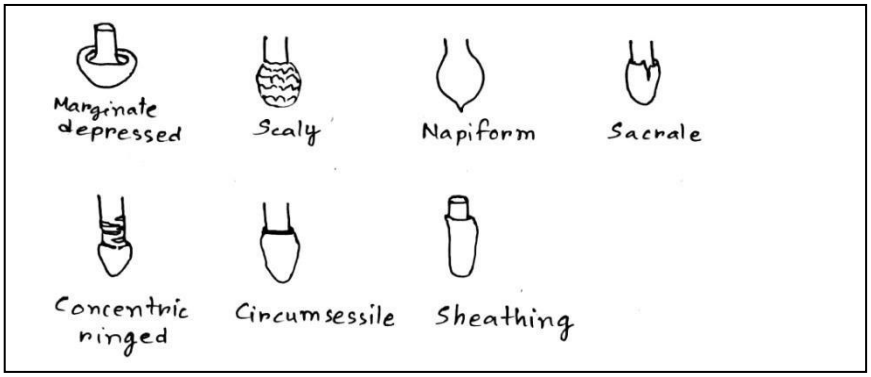
j- Types of veils



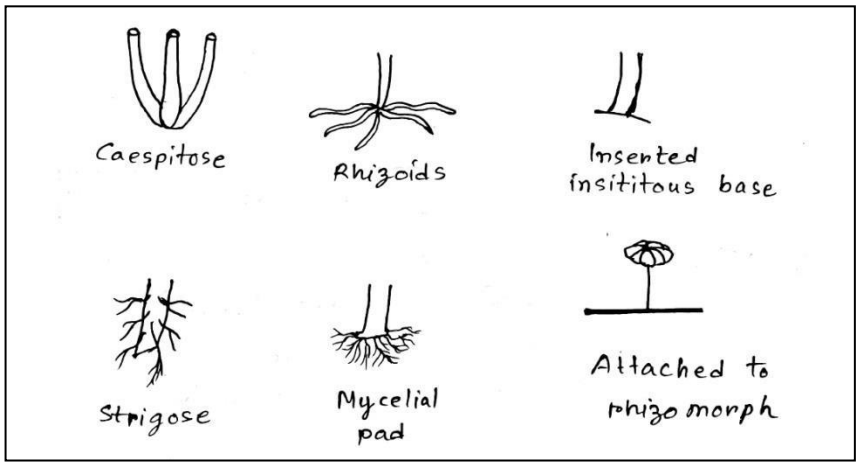
k- Surface of stem



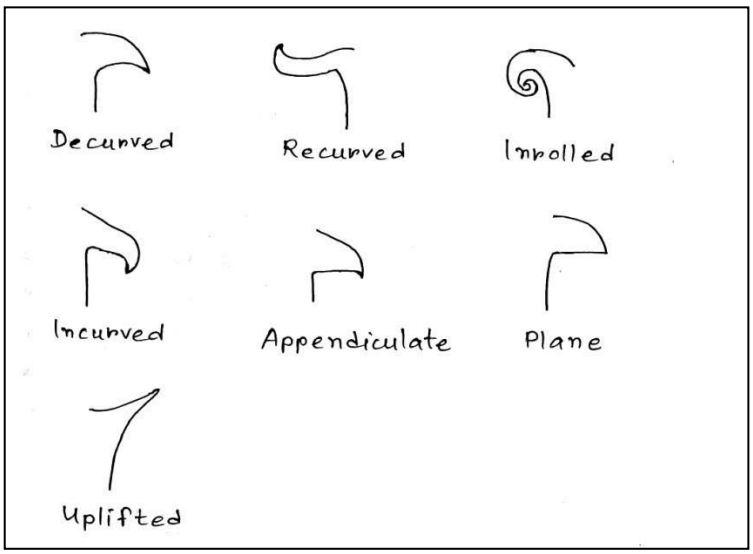
l- Stalk attachment



m- Volva type



n- Stipe base



o- Pileus margin

Fig.- 3.2 (a-o)- Some parameters of morphological and anatomical characterization for reference at the time of identification

3.7 Identification

Identification of the specimens were carried out by standard microscopic methods (Roy and De, 1996). Microscopic characters were observed under dissecting microscope carefully and try to find the similarities and dissimilarities and puted information in the questionnaire for further evaluation and identification. Identification of mushrooms was done with the help of literature and expertise available in the department. The collected edible mushrooms were listed as date wise as for as possible correct scientific name with citation of authors name and taxonomy position (Nage et al., 1991).

3.8 Isolation

The method used for isolation of mycelium of mushroom was Tissue Culture Technique (Kapoor and Sharma, 2014).

3.8.1 Glasswares

During the isolation glasswares viz., Petri plates, conical flasks, beaker, measuring cylinder were used. For different laboratory experiments, the glassware was washed properly, rinsed with tap water after cleaning with detergent. After wshing they were allowed to dry.

3.8.2 Sterilization of glasswares

All the glasswares used during the experiment was sterilized after wiped with ethanol in a hot air oven at 180°C for two hours.

3.8.3 Culture media

The suitable media for the mushroom species was Potato Dextrose Agar (Himedia).

Composition:

PDA: 39g

Distilled water: 1000ml

39g of PDA was boiled in distilled water in a beaker in an oven and boiled it until the PDA was dissolved in the water. Then the media was poured in conical flasks of 500ml and 250ml. The flasks were plugged with non-absorbent cotton and covered with brown paper. Then the media was sterilised in autoclave in 121°C for 15minutes. The media was preserved until use.

3.8.4 Tissue Culture

Tissue culture was done in tissue culture lab in a laminar air flow which is sterilise to minimize the chance of contamination.

Tissue culture of 5 samples were done and they are- WMS-12, WMS-13, WMS-14, WMS-15 and WMS-16.

3.8.4.1 Requirements:

Media, Cotton, Ethanol, Spirit lamp, Lighter, Antibiotic, Petri plates, Parafilm, Inoculation needle, Distilled water, Sodium hypoclorite, Blade, Blotting paper, Marker, Forcep, Celophane bag, Rubber band etc.

3.8.4.2 Procedure:

I. Clean the laminar air flow chamber with ethanol.
II. Turn on the UV ray for 15 to 20 minutes after putting the equipments except the sample.
III. After 15 to 20 minutes UV ray was off and hands were sterilised.
IV. Lit up the spirit lamp to reduce the contamination. In teh chamber.
V. Antibiotic was added to the media to get the desired culture.
VI. Media was poured on the petriplate and allowed to solidify.
VII. Mushrooms were teared and extracted inner part from the stipe and gill with the help of blade and cutted into 2-3mm pieces.
VIII. The cut-portion was surface sterilised by dipping in 1 Per cent sodium hypoclorite for 1 minute
IX. The treated pieces were washed in two changes of sterile water to remove the sterilant and allowed to dry on blotting paper.
X. The pieces were transferred aseptically, excess water was removed and transferred onto culture medium for mycelium growth, usually 1-4 pieces per plate.
XI. Closed the petri plate and sealed with parafilm.
XII. Wrote date and sample name or sample code on the cover of petri plate.
XIII. Put all plate in a cellophane bag and tied with rubber band and wrote date on the celophane bag.
XIV. Inoculated petri-plateswere incubated in B.O.D (Biological Oxygen Demand) incubator at 27±1˚C.

3.8.5 Sub culture

Sub culture is done to get the pure form of desired sample.

For sub culture of mycelium- Media, Cotton, Ethanol, Spirit lamp, Lighter, Antibiotic, Petri plates (Diameter 9 c.m.), Parafilm, Inoculation needle, Marker, Cork borer, Forcep, Celophane bag, Rubber band etc. Are kept in laminar air flow for 15-30 minutes after turning on the UV light. Then the media is poured onto petriplates and allowed to solidify.

Inside the laminar air flow chamber, The plates containing the mushroom culture are taken. Then the cork borer and inoculation loop are made contamination free by wiping it with ethanol and burning it in spirit lamp. With the help of cork borer, a disc of mycelium was cutout from the plate and transferred to the centre of a new petriplate containing PDA of 10-20 ml with the help of inoculation needle. Then the petriplates are sealed with parafilm and incubated for 3-10 days.

3.9 Cultural characterization

Regular observations were carried out after every 24 hours. A separate questionnaire was prepared to note some characteristic features like growth in every 24 hours. Other characters like colour, density, type were noted. For detailed observation photograph were taken at the time of observation and after full growth microscopic photos were taken at 10x and 40x zoom with the help of Coslabview software after connecting the microscopre with a laptop.

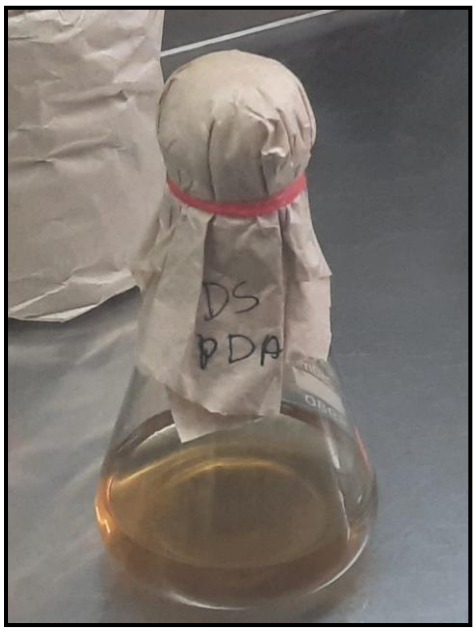
a

b

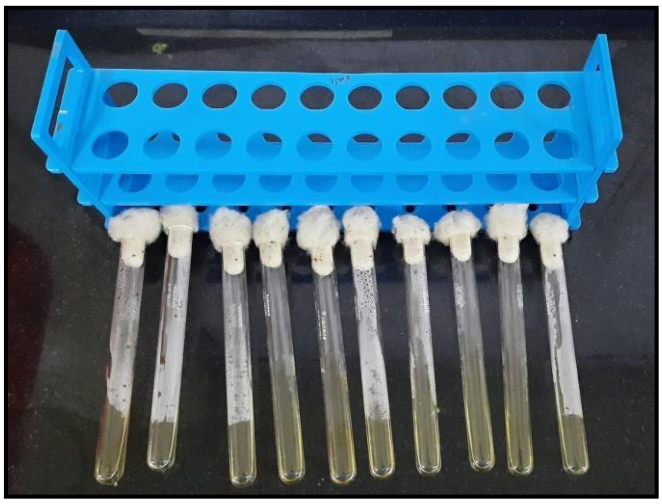
c

d

Photoplate 3.1 a,b- Collection of mushrooms, c- Preservation of mushroom in formaldehyde solution, d- Preservation of mushroom in -20°C



a



b

C

Photoplate 3.2 a- Prepared PDA media, b- PDA slant, c- Sterilization of petriplates in Hot air oven

a

b

c

Photoplate 3.3 a- Laminar air flow for isolation, b- BOD incubator, c- Product of tissue culture

a

b

c

Photoplate 3.4 a- Laminar air flow for sub-culture, b- Product of sub-culture, c- Slant culture

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Survey and collection of wild mushroom flora from Upper Brahmaputra Valley Zone of Assam

A random roving survey was carried out in the forests and tea gardens of the districts in Upper Brahmaputra Valley Zone (Tinsukia, Jorhat and Golaghat)for collection of wild mushroom floraat different time intervals. The time of collection ranged from May to August as the rainy season is considered ideal for wild mushroom flushing. From the surveyed districts of Upper Brahmaputra Valley Zone *i.e.,* Tinsukia, Jorhat and Golaghat, a total of **20** wild mushroom species were collected fromsix different locations*viz.*,Gelapukhuri, Senchoa Gaon, Lichubari, Barbheta, Titabarand Golaghat (**Table 4.1, Plate 4.1**)

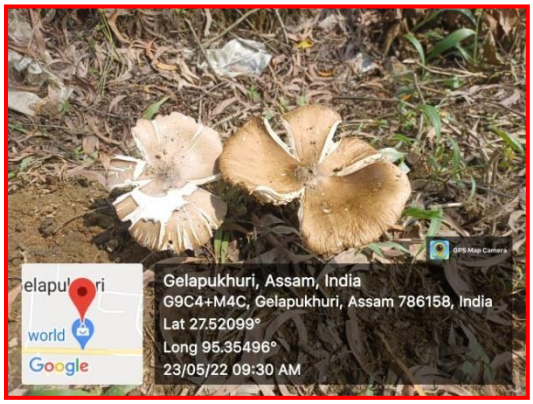
Table 4.1 Collection of wild mushroom flora from Upper Brahmaputra Valley Zone, Assam

Sl. No.	Codes of the Collected Samples	Date and Time of Collection	Area and District of Collection (Longitude and Latitude)
1	WMS-1	20/05/2022 (3:08PM)	Gelapukhuri, Tinsukia(95.354735°E and 27.521641°N)
2	WMS-2	20/05/2022 (3:23PM)	Gelapukhuri, Tinsukia(95.35281° E and 27.52264°N)
3	WMS-3	23/5/2022 (9:30AM)	Gelapukhuri, Tinsukia(95.35496°E and 27.52099°N)
4	WMS-4	25/5/2022 (8:42AM)	Senchoa Gaon, Jorhat(94.197647°E and 26.715285°N)
5	WMS-5	25/5/2022 (8:57AM)	Barbheta, Jorhat (94.200156°E and 26.724917°N)
6	WMS-6	25/5/2022 (9:00AM)	Senchoa Gaon, Jorhat(94.199843°E and 26.715076°N)

7	WMS-7	25/5/2022 (9:19AM)	Lichubari, Jorhat(94.197443°E and 26.716993°N)
8	WMS-8	25/5/2022 (9:28AM)	Barbheta, Jorhat(94.198592°E and 26.716941°N)
9	WMS-9	25/5/2022(9:32AM)	Barbheta, Jorhat(94.19873°E and 26.717168°N)
10	WMS-10	25/5/2022 (9:39AM)	Barbheta, Jorhat(94.196974°E and 26.719898°N)
11	WMS-11	26/5/2022(2.35PM)	Lichubari, Jorhat(94.197465°E and 26.71748°N)
12	WMS-12	31/5/2022(1:48PM)	Golaghat(93.977445°E and 26.121293°N)
13	WMS-13	10/6/2022(5:35PM)	Barbheta, Jorhat(94.194343°E and 26.723629°N)
14	WMS-14	10/6/2022(5:35PM)	Barbheta, Jorhat(94.194342°E and 26.723587°N)
15	WMS-15	16/6/2022(5:18PM)	Barbheta, Jorhat(94.194351° E and 26.723575°N)
16	WMS-16	19/6/2022 (10:58AM)	Barbheta, Jorhat(94.194351°E and 26.723575°N)
17	WMS-18	27/8/2022(1:34 PM)	Titabar, Jorhat (94.304325°E and 26.585203°N)

WMS-1

WMS-2



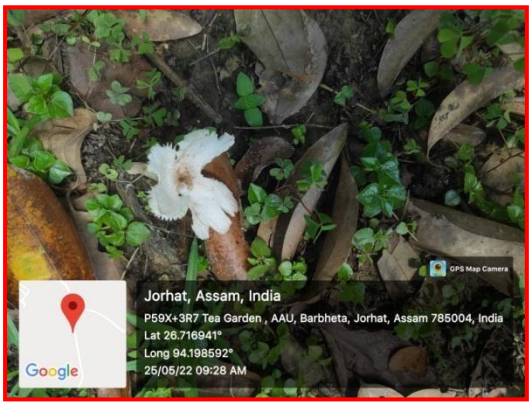
WMS-3

WMS-4

WMS-5

WMS-6

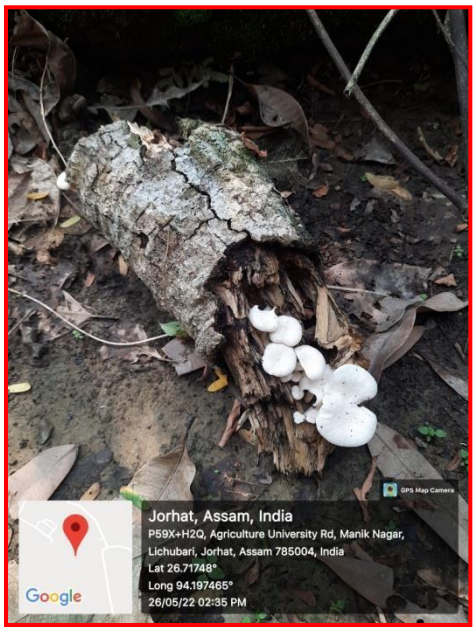
WMS-7



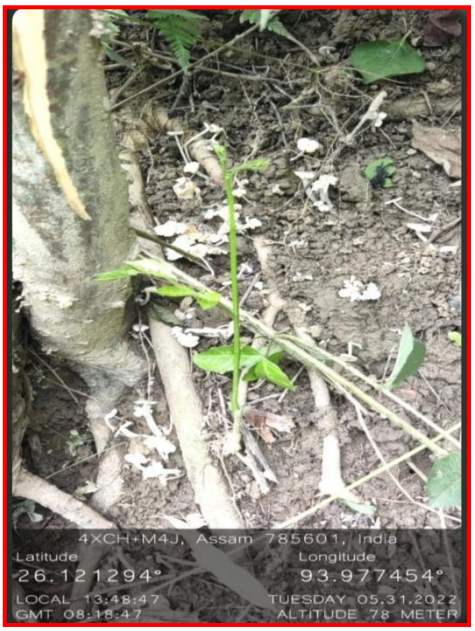
WMS-8

WMS-9

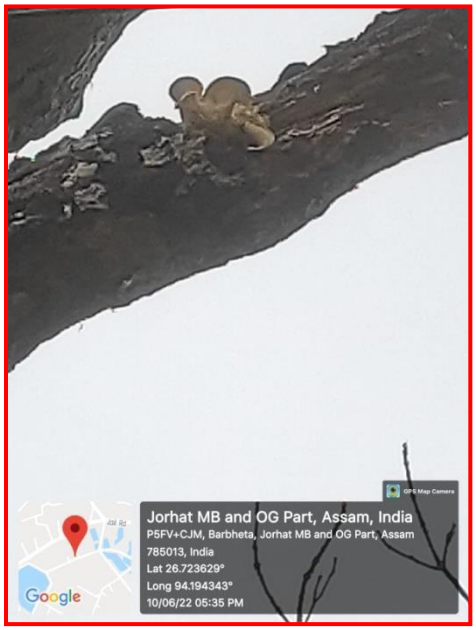
WMS-10



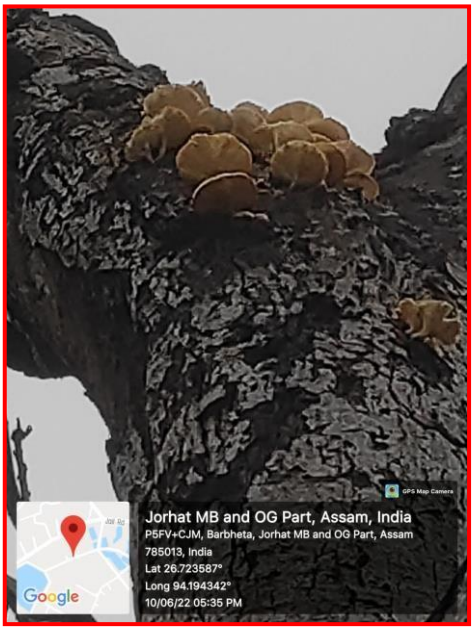
WMS-11



WMS-12



WMS-13



WMS-14

WMS-15

WMS-16

WMS-17

Photoplate 4.1. (WMS-1 – WMS-17): Samples of wild mushroom flora collected from different locations of Upper Brahmaputra Valley Zone of Assam

4.2 Identification of collected wild mushroom flora

4.2.1 Morphological Identification

Morphological characteristics of wild mushrooms obtained from the study area are described in Table 4.2.1 (a-f)

Table 4.2.1 a-Morphological characters of the collected wild mushroom samples (WMS-1, WMS-2, WMS-3)

Sl No.	Morphological Traits/ Characters	WMS-1	WMS-2	WMS-3
1	Shape	Umbonate	Convex	Umbonate
2	Size	4 c.m.	5 c.m.	5 c.m.
3	Colour	White to Ivory	White and purple patches	Brown
4	Texture	Smooth	Smooth	Smooth
5	Height	9 c.m.	4 c.m.	7 c.m.
6	Warts (Present/Absent)	Absent	Absent	Absent
7	Hymanophore	Ridged	Ridged	Ridged
8	Gill Shape (Thick/Thin) and (Name of type)	Thin and Crowded	Thin (Crowded)	Thin (Crowded)
9	Gill Colour (Same as cap colour or not and colour name)	Same as cap colour	Brown	White
10	Stem Shape	Equal	Equal	Equal
11	Mushroom smell	Chlorine	Chlorine	Chlorine
12	Volva Type	Unknown	Unknown	Unknown
13	Stipe Base	Unknown	Unknown	Unknown
14	Pileus Margin	Decurved	Decurved	Decurved

Table 4.2.1 b-Morphological characters of the collected wild mushroom samples (WMS-4, WMS-5, WMS-6)

Sl No.	Morphological Traits/ Characters	WMS-4	WMS-5	WMS-6
1	Shape	Pulvinate	Plane	Infundibuli-form
2	Size	4 c.m.	4 c.m.	3.5 c.m.
3	Colour	Maroon	Light brown	Light brown
4	Texture	Smooth	Rough	Smooth
5	Height	8 cm	4 c.m.	4.5 cm
6	Warts (Present/Absent)	Absent	Absent	Absent
7	Hymanophore	Ridged	Porous	Ridged
8	Gill Shape (Thick/Thin) and (Name of type)	Thin and Crowded	Absent	Thin (Closed)
9	Gill Colour (Same as cap colour or not and colour name)	Pale white	Same as cap colour	Same as cap colour
10	Stem Shape	Equal	AbsentEqual	Equal
11	Mushroom smell	Coconut	Chlorine	Chlorine
12	Volva Type	Sacrale	Unknown	Absent
13	Stipe Base	Caespitose	Inserted insititous base	Inserted insititous base
14	Pileus Margin	Recurved	Plane	Incurved

Table 4.2.1 c-Morphological characters of the collected wild mushroom samples (WMS-7, WMS-8, WMS-9)

Sl No.	Morphological Traits/ Characters	WMS-7	WMS-8	WMS-9
1	Shape	Infundibuliform	Infundibuliform	Infundibuliform
2	Size	4 c.m.	5.5 c.m.	8.7 c.m.
3	Colour	Light brown	White	Brownish grey
4	Texture	Smooth	Rugulose	Cracked and patches
5	Height	0.5-1 c.m.	7 c.m.	17 c.m.
6	Warts (Present/Absent)	Absent	Absent	Absent
7	Hymanophore	Smooth	Ridged	Ridged
8	Gill Shape (Thick/Thin) and (Name of type)	Absent	Thick (Distant)	Thin (Distant)
9	Gill Colour (Same as cap colour or not and colour name)	Absent	Pale yellow	White
10	Stem Shape	Absent	Equal	Club shaped
11	Mushroom smell	Coconut	Chlorine	Decaying banana
12	Volva Type	Absent	Napiform	Napiform
13	Stipe Base	Absent	Rhizoids	Caespitose
14	Pileus Margin	Uplifted	Upliftd	Inrolled

Table 4.2.1 d-Morphological characters of the collected wild mushroom samples (WMS-10, WMS-11, WMS-12)

Sl No.	Morphological Traits/ Characters	WMS-10	WMS-11	WMS-12
1	Shape	Infundibuliform	Depressed	Convex
2	Size	9 c.m.	5.3 c.m.-6.3 c.m.	1.5 c.m.
3	Colour	Orange	White	Pale yellow
4	Texture	Smooth	Flat scales and Squamose	Smooth
5	Height	7.5 c.m.	4.3 c.m.	6 c.m.
6	Warts (Present/Absent)	Absent	Absent	Absent
7	Hymanophore	Porous	Ridged	Ridged
8	Gill Shape (Thick/Thin) and (Name of type)	Absent	Thin (Closed)	Thin (Closed)
9	Gill Colour (Same as cap colour or not and colour name)	Absent	Yellowish white	Same as cap colour
10	Stem Shape	Absent	Equal	Eual
11	Mushroom smell	Coconut	Chlorine	Chlorine
12	Volva Type	Absent	Circumsessile	Unknown
13	Stipe Base	Absent	Caespitose	Strigose
14	Pileus Margin	Uplifted	Decurved	Plane

Table 4.2.1 e-Morphological characters of the collected wild mushroom samples (WMS-13, WMS-14, WMS-15)

Sl No.	Morphological Traits/ Characters	WMS-13	WMS-14	WMS-15
1	Shape	Infundibuliform	Infundibuliform	Plane
2	Size	5 C.M	4 C.M	6 c.m.
3	Colour	Pale brown	Light brown	White
4	Texture	Smooth	Flat Scales	Smooth
5	Height	4 c.m.	5 c.m.	7 c.m.
6	Warts (Present/Absent)	Absent	Absent	Absent
7	Hymanophore	Ridged	Ridged	Ridged
8	Gill Shape (Thick/Thin) and (Name of type)	Thin (Crowdwd)	Thin (Closed)	Thin (Crowded)
9	Gill Colour (Same as cap colour or not and colour name)	White	Same as Cap Colour	Ivory
10	Stem Shape	Equal	Equal	Equal
11	Mushroom smell	Chlorine	Chlorine	Chlorine
12	Volva Type	Absent	Absent	Napiform
13	Stipe Base	Inserted Insititous Base	Caespitose	Strigose
14	Pileus Margin	Inrolled	Decurved	Plane

Table 4.2.1 f-Morphological characters of the collected wild mushroom samples (WMS-16, WMS-17)

Sl No.	Morphological Traits/ Characters	WMS-16	WMS-17
1	Shape	Numerous caps fanning out and dividing from a common base	Infundibuliform
2	Size	20 c.m.	10 c.m.
3	Colour	Pale yellow	Light brown
4	Texture	Rough	Flat scales
5	Height	30 c.m.	12 c.m.
6	Warts (Present/Absent)	Absent	Absent
7	Hymanophore	Smooth	Ridged
8	Gill Shape (Thick/Thin) and (Name of type)	Absent	Thin and crowded
9	Gill Colour (Same as cap colour or not and colour name)	Absent	White
10	Stem Shape	Absent	Equal
11	Mushroom smell	Chlorine	Chlorine
12	Volva Type	Absent	Circumsessile
13	Stipe Base	Absent	Strigose
14	Pileus Margin	Plane	Recurved

4.2.2 Anatomical Identification

Anatomical characteristics of wild mushrooms obtained from the study area are described in Table 4.2.2 (a-f)

Table 4.2.2 a-Anatomical characters of the collected wild mushroom samples (WMS-1, WMS-2, WMS-3)

Sl No.	Anatomical Traits/ Characters	WMS-1	WMS-2	WMS-3
1	Thickness of Cap	4 m.m.	5 m.m.	3 m.m.
2	Veil (Universal/Partial)	Partial	Absent	Absent
3	Margin of Cap	Smooth	Smooth	Cracked
4	Marginal Lines (Striations/Zonation)	Striations	Absent	Absent
5	Edge of Gill	Smooth	Smooth	Smooth
6	Attachment of Gills to The Cap	Free	Adnexed	Adnate
7	Types of Stipes (T.S)	Tubular	Solid	Solid
8	Types of Veils	Flaring	Absent	Absent
9	Surface of Stem	Costate	Smooth	Smooth
10	Stalk Attachment	Central	Central	Central

Table 4.2.2 b- Anatomical characters of the collected wild mushroom samples (WMS-4, WMS-5, WMS-6)

Sl No.	Anatomical Traits/ Characters	WMS-4	WMS-5	WMS-6
1	Thickness of Cap	4 m.m.	1 m.m.	1 m.m.
2	Veil (Universal/Partial)	Absent	Absent	Absent
3	Margin of Cap	Cracked	Smooth	Striated
4	Marginal Lines (Striations/Zonation)	Absent	Zonation	Striations
5	Edge of Gill	Marginal	Absent	Smooth
6	Attachment of Gills to The Cap	Adnate	Absent	Adnexed
7	Types of Stipes (T.S)	Hollow	Solid	Hollow
8	Types of Veils	Absent	Absent	Absent
9	Surface of Stem	Costate	Smooth	Costate
10	Stalk Attachment	Central	Lateral	Central

Table 4.2.3 c- Anatomical characters of the collected wild mushroom samples (WMS-7, WMS-8, WMS-9)

Sl No.	Anatomical Traits/ Characters	WMS-7	WMS-8	WMS-9
1	Thickness of Cap	1 m.m.	2 m.m.	5 m.m.
2	Veil (Universal/Partial)	Absent	Absent	Absent
3	Margin of Cap	Smooth	Rimose	Smooth and big cracked
4	Marginal Lines (Striations/Zonation)	Absent	Striations	Absent
5	Edge of Gill	Absent	Smooth	Smooth
6	Attachment of Gills to The Cap	Absent	Free	Decurrent
7	Types of Stipes (T.S)	Absent	Hollow	Solid
8	Types of Veils	Absent	Absent	Absent
9	Surface of Stem	Absent	Smooth	Fibrilose
10	Stalk Attachment	Absent	Central	Central

Table 4.2.4 d- Anatomical characters of the collected wild mushroom samples (WMS-10, WMS-11, WMS-12)

Sl No.	Anatomical Traits/ Characters	WMS-10	WMS-11	WMS-12
1	Thickness of Cap	1 c.m.	2-3 m.m.	3 M.M
2	Veil (Universal/Partial)	Absent	Absent	Absent
3	Margin of Cap	Cracked	Crenate	Crenate
4	Marginal Lines (Striations/Zonation)	Absent	Absent	Striations
5	Edge of Gill	Absent	Smooth	Smooth
6	Attachment of Gills to The Cap	Absent	Decurrent	Adnexed
7	Types of Stipes (T.S)	Absent	Solid	Solid
8	Types of Veils	Absent	Absent	Absent
9	Surface of Stem	Absent	Scabers	Smooth
10	Stalk Attachment	Absent	Lateral	Central

Table 4.2.5 e- Anatomical characters of the collected wild mushroom samples (WMS-13, WMS-14, WMS-15)

Sl No.	Anatomical Traits/ Characters	WMS-13	WMS-14	WMS-15
1	Thickness of Cap	2 m.m.	2 m.m.	8 m.m.
2	Veil (Universal/Partial)	Universal Veil	Absent	Universal
3	Margin of Cap	Smooth	Cracked	Crenate
4	Marginal Lines (Striations/Zonation)	Absent	Absent	Absent
5	Edge of Gill	Marginate	Marginate	Smooth
6	Attachment of Gills to The Cap	Decurrent	Decurrent	Adnexed
7	Types of Stipes (T.S)	Solid	Solid	Tubular
8	Types of Veils	Absent	Absent	Flaring
9	Surface of Stem	Rough	Squamulose	Smooth
10	Stalk Attachment	Lateral	Lateral	Central

Table 4.2.6 f- Anatomical characters of the collected wild mushroom samples (WMS-16, WMS-17)

Sl No.	Anatomical Traits/ Characters	WMS-16	WMS-17
1	Thickness of Cap	3 m.m.	5 m.m.
2	Veil (Universal/Partial)	Absent	Absent
3	Margin of Cap	Smooth	Cracked
4	Marginal Lines (Striations/Zonation)	Zonation	Absebt
5	Edge of Gill	Absent	Smooth
6	Attachment of Gills to The Cap	Absent	Free
7	Types of Stipes (T.S)	Absent	Solid
8	Types of Veils	Absent	Absent
9	Surface of Stem	Absent	Squamulose
10	Stalk Attachment	Absent	Central

The samples with identification are presented in Table 4.2.3

Table 4.2.3 Identification of Wild mushrooms

Sl No.	Codes of collected wild mushromsamples	Family	Genus	Species
1.	WMS-1	Lyophyllaceae	*Termitomyces*	*globules*
2.	WMS-2	Lyophyllaceae	*Volvariella*	*volvocca*
3.	WMS-3	Lyophyllaceae	*Termitomyces*	*radicatus*
4.	WMS-4	Boletaceae	*Boletus*	*edulis*
5.	WMS-5	Polyporaceae	*Trametes*	*versicolor*
6.	WMS-6	Bolbitiaceae	*Bolbitius*	*sp.*
7..	WMS-7	Auriculariaceae	*Auricularia*	*auricular-judae*
8	WMS-8	Russulaceae	*Russula*	*sp.*
9.	WMS-9	Lyophyllaceae	*Termitomyces*	*eurrhizus*
10.	WMS-10	Polyporaceae	*Lentinus*	*critinus*
11.	WMS-11	Pleurotaceae	*Pleurotus*	*cornucopiae*
12.	WMS-12	Lyophyllaceae	*Termitomyces*	*microcarpus*
13.	WMS-13	Pleurotaceae	*Pleurotus*	*sp.*
14.	WMS-14	Polyporaceae	*Lentinus*	*tigrinus*
15.	WMS-15	Physalacriaceae	*Hymenopellis*	*megalospora*
16.	WMS-16	Sparassidaceae	*Sparassis*	*sp.*
17.	WMS-17	Lyophyllaceae	*Termitomyces*	*macrocarpus*

4.3 Diversity and edibility of collected mushrooms based on ethnomycological knowledge

Species diversity of macrofungi is related to the particular habitats and ecosystem. It was found that environmental factors like light, temperature and RH greatly influenced the growth and development of macrofungi. During the surveyed period of four months (May to August) in the three different districts, the outcome of the regular survey was 17 different species of macrofungi belonging to 11 genera and 9 families were identified (Table 4.2.3). Out of the 17 species identified, 6 belongs to the family *Lyophyllaceae*, 3 belongs to *Polyporaceae*, 1 belongs to *Boletaceae*, 1 belongs to *Bolbitiaceae*, 1 belongs to *Auriculariaceae*, 1 belongs to *Russulaceae*, 2 belongs to *Pleurotaceae*, 1 belongs to *Physalacriaceae*and 1 belongs to *Sparassidaceae*.

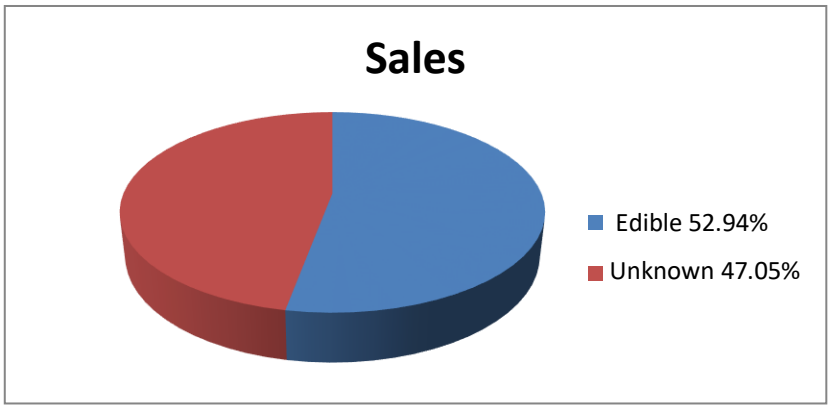
Based on the ethnomycological knowledge of the local tribes encountered during the study, which were mostly Adivashis, out of 17 collected specimens about 9 wild mushroom samples were found to be edible which is represented in the following table (**Table 4.3**).

Table 4.3 Traits of collected wild mushroom samples based on diversity and ethnomycological knowledge

Code of the Collected Sample	Season of Survey	Scientific name	Habitat of mushroom	Substrate of mushroom	Extent of occurrence	Name of ethnic group/tribe	Important feature
WMS-1	Spring	*Termitomyces globules*	Land and humid environment	Single and soil	Frequent	Tea Garden community	Edible
WMS-2	Spring	*Volvarilla volvocca*	Land and humid environment	Single and soil	Frequent	Tea Garden community	Edible
WMS-3	Spring	*Termitomyces aurantiacus*	Land and humid environment	Single and soil	Frequent	Tea Garden community	Edible
WMS-4	Spring	*Boletus edulis*	Land and humid environment	Single and soil	Rare	Unknown	Edible
WMS-5	Spring	*Trametes versicolor*	Decaying wood and humid environment	Single and wood	Rare	Unknown	Unknown
WMS-6	Spring	*Bolbitius sp.*	Decaying wood and humid environment	Single and wood	Rare	Unknown	Unknown
WMS-7	Spring	*Auricularia auricular-judae*	Decaying wood and humid environment	Single and wood	Frequent	Tea Garden community	Edible
WMS-8	Spring	*Russula sp.*	Land and humid environment	Single and soil	Rare	Unknown	Unknown
WMS-9	Spring	*Termitomyces eurrhizus*	Land and humid environment	Single and soil	Frequent	Tea Garden community	Edible
WMS-10	Spring	*Lentinus critinus*	Decaying wood and humid environment	Single and wood	Rare	Unknown	Unknown

WMS-11	Spring	*Pleurotus cornucopiae*	Decaying wood and humid environment	Single and wood	Frequent	Unknown	Unknown
WMS-12	Summer	*Termitomyces indicus*	Land and humid environment	Single and soil	Frequent	Tea Garden community	Edible
WMS-13	Summer	*Pleurotus sp.*	Decaying wood and humid environment	Single and wood	Rare	Unknown	Unknown
WMS-14	Summer	*Lentinus tigrinus*	Decaying wood and humid environment	Single and wood	Frequent	Tea Garden community	Edible
WMS-15	Summer	*Hymenopellisn megalospora*	Land and humid environment	Single and soil	Frequent	Unknown	Unknown
WMS-16	Summer	*Sparassis sp.*	Living wood and hot and humid environment	Single and soil	Rare	Unknown	Unknown
WMS-17	Summer	*Termitomyces macrocarpus*	Land and humid environment	Single and soil	Frequent	Tea Garden community	Edible

Fig. 4.1 Economic value of collected Wild Mushrooms of Upper Brahmaputra Valley zone of Assam



4.4 Study on cultural characteristics of few identified wild mushroom flora

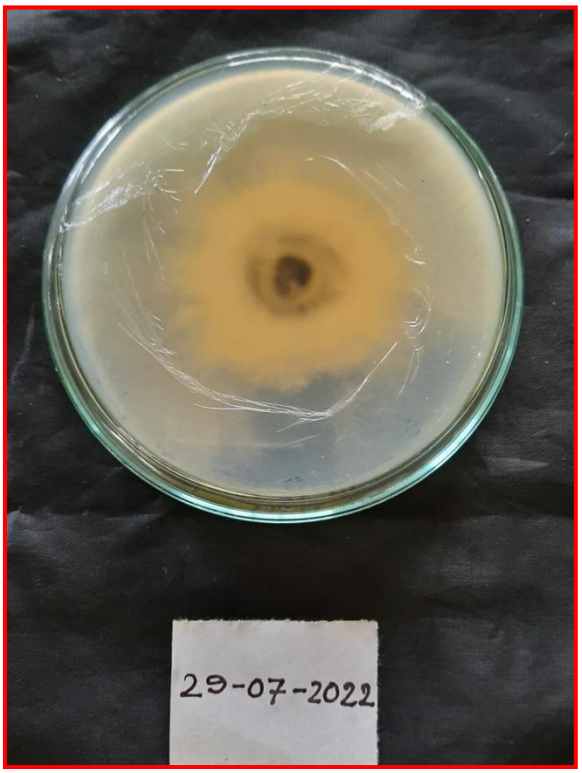
The collected wild mushroom flora were identified based on morphological and anatomical characteristics. As isolation or tissue culture of fleshy fungi is a difficult task due to their highly perishable nature, therefore, only five wild mushroom samples could be isolated *viz.*, WMS-12, WMS-13, WMS-14, WMS-15 and WMS-16 (**Plate 4.2. a-e**)

The cultural characteristics of the five mushroom species are described below and the diametric growth patterns are listed in **Table 4.4.1 and Table 4.4.2**. The colour of mycelia varied from white to off white, density of mycelia varied from low to high and type is smooth to velvety. All cultures were grown in PDA media and maintained in B.O.D. incubator at 27±1 ^{o}C. The cultural description of the isolated mushroom samples are given as follows:

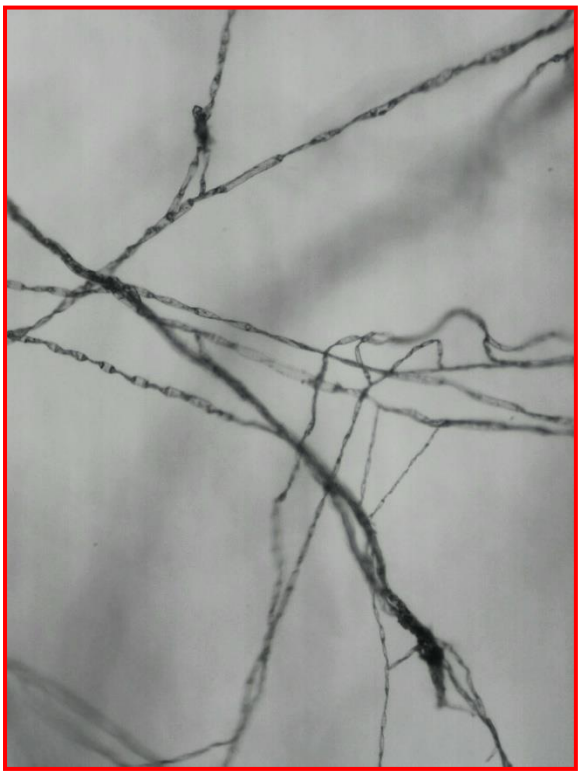
1. **Sub-culture WMS-12-** It showed off white to pale yellow colour on the top and dark yellow colour on the back. Mycelium was not uniformfly scattered. Mycelium is highly dense in the centre and gradually low dense to the side. Mycelium has cotton like texture. It took almost ten days for full growth.

2. **Sub-culture WMS-13-** It showed white colour on the top and white colour on the back. Mycelium is low dense. Mycelium was uniformfly scattered. Mycelium has cotton like texture. It took almost five days for full growth.
3. **Sub-culture WMS-14-** It showed white colour on the top and light yellow colour on the back. Mycelium is low dense. Mycelium was not uniformfly scattered. Mycelium has velvety texture. It took almost four days for full growth.
4. **Sub-culture WMS-15-** It showed yellowish white colour on the top and pale yellow colour on the back. Mycelium was not uniformfly scattered. Mycelium is highly dense to the centre and low dense to the side. Mycelium made some ring like structure alternately. Mycelium has cotton like texture. It took almost ten days for full growth.
5. **Sub-culture WMS-16-** It showed off white colour on the top and white colour on the back. Mycelium is low dense. Mycelium is low dense to the centre and highly dense to the side. Mycelium was not uniformfly scattered. Mycelium has cotton like texture. It took almost three days for full growth.

Front view of myclium



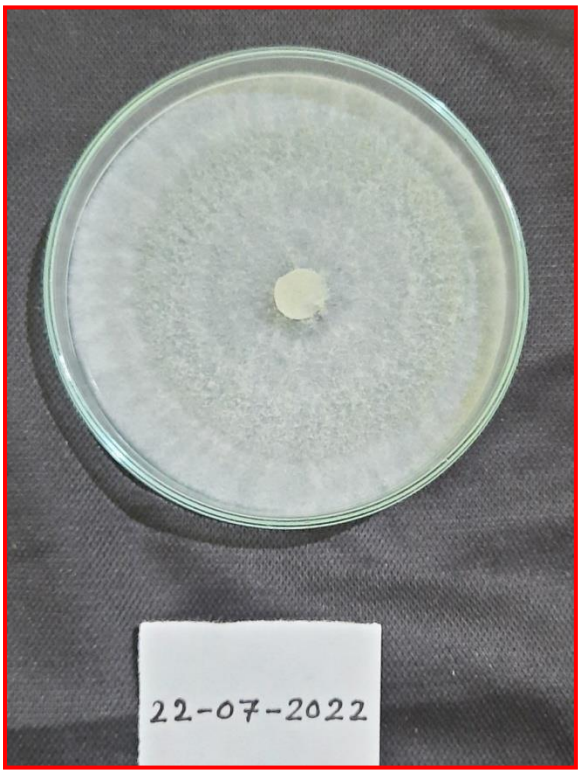
Back view of mycelium



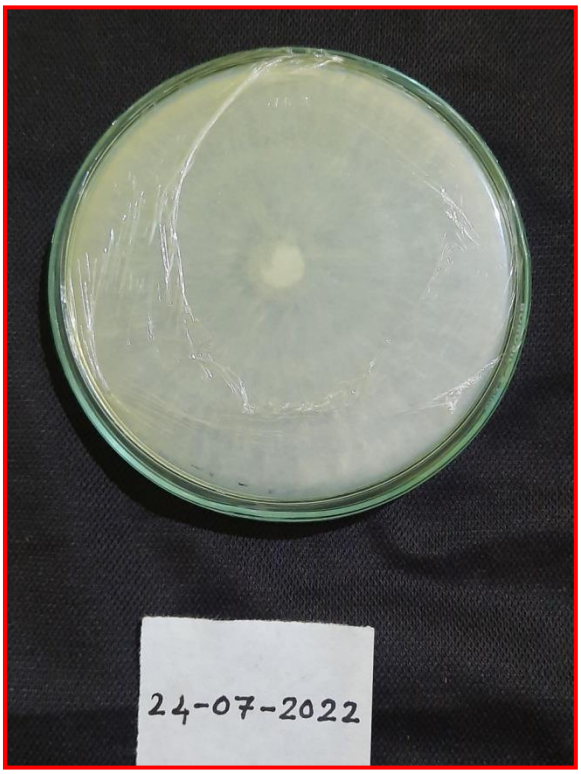
Mycelium at 10x

Mycelium at 40x

Photoplate 4.2. a- Mycelium of wild mushroom collected sample (WMS-12)

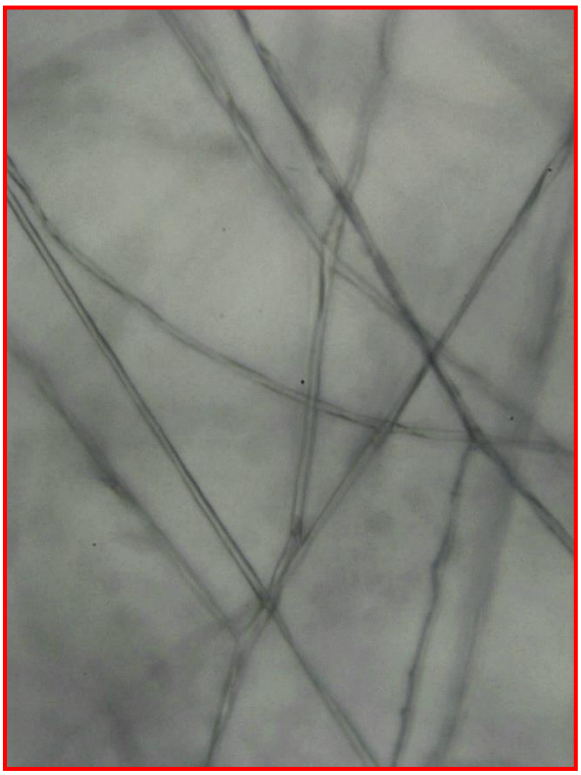


Front view of myclium



Back view of mycelium

Mycelium at 10x



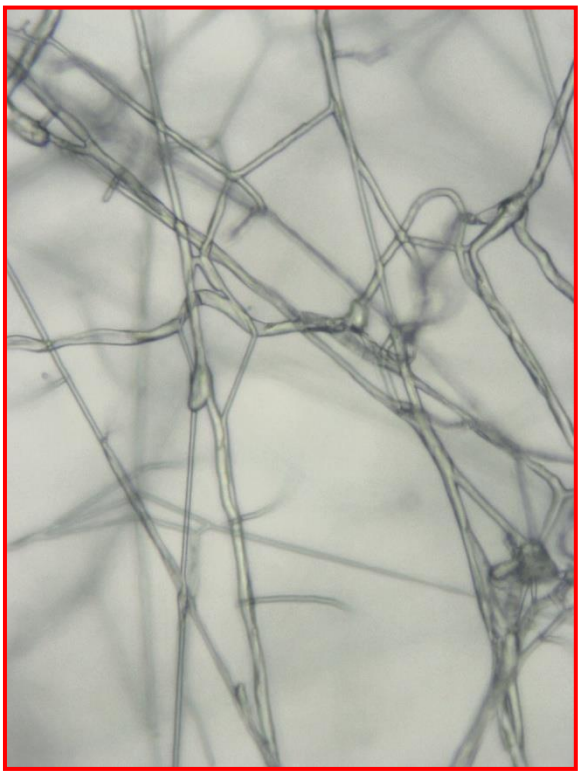
Mycelium at 40x

Photoplate 4.2. b- Mycelium of wild mushroom collected sample (WMS-13)

Front view of myclium

Back view of mycelium

Mycelium at 10x



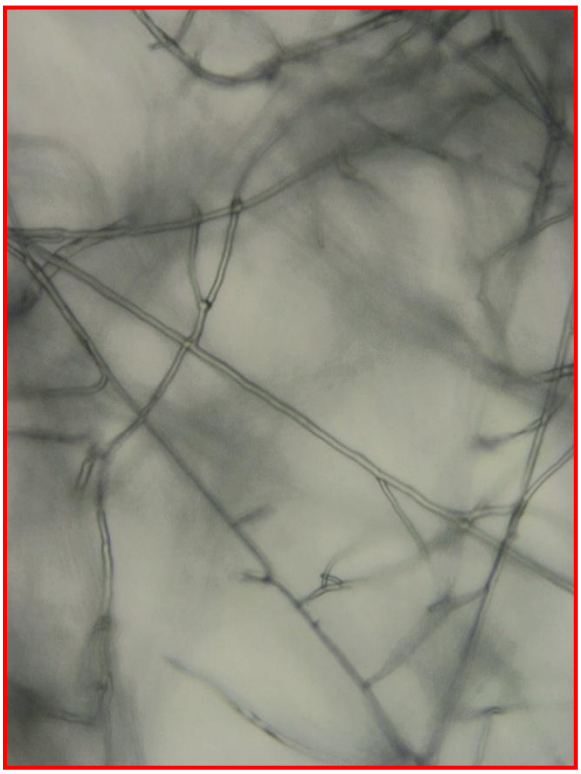
Mycelium at 40x

Photoplate 4.2.c. Mycelium of wild mushroom collected sample (WMS-14)

Front view of myclium

Back view of mycelium

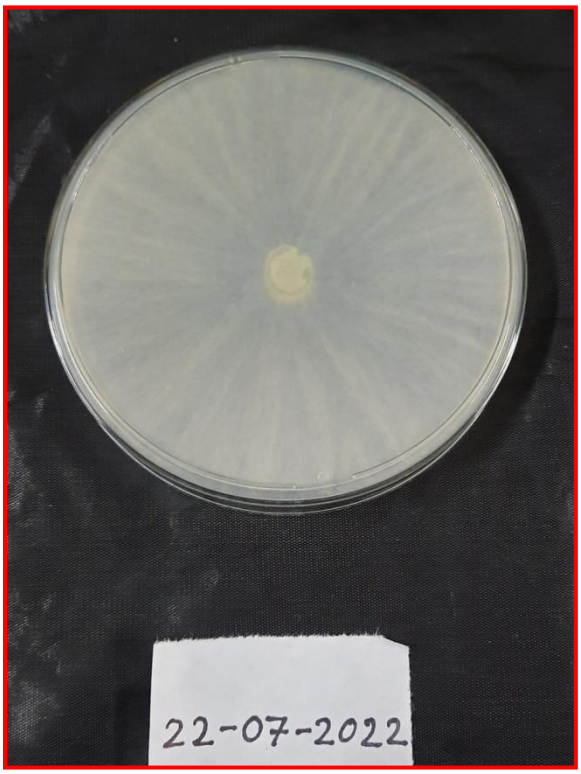
Mycelium at 10x



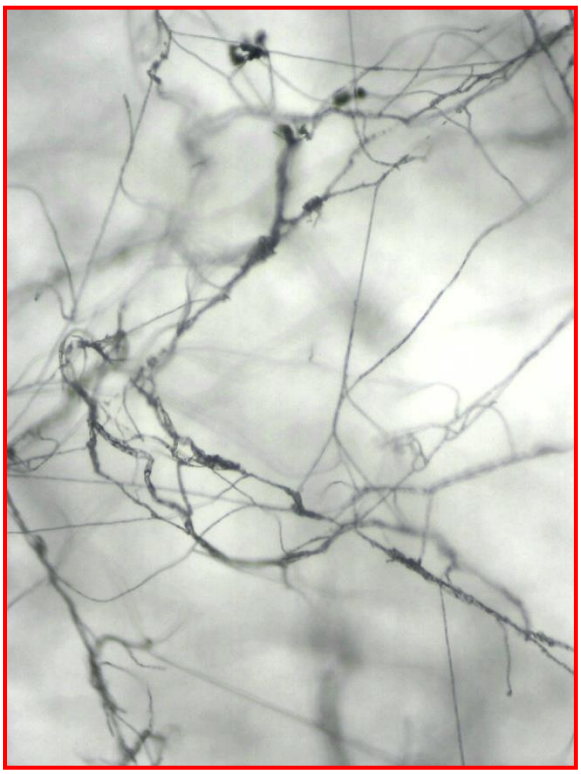
Mycelium at 40x

Photoplate 4.2. d. Mycelium of wild mushroom collected sample (WMS-15)

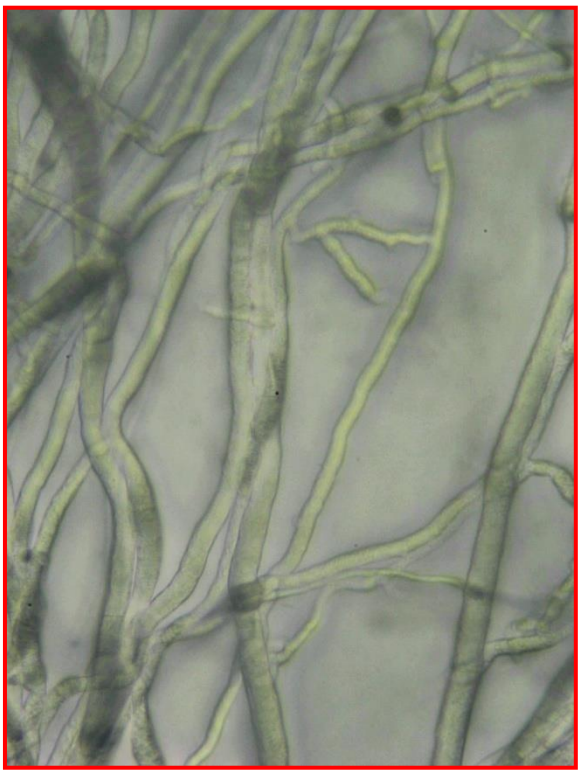
Front view of myclium



Back view of mycelium



Mycelium at 10x



Mycelium at 40x

Photoplate 4.2. e. Mycelium of wild mushroom collected sample (WMS-16)

Table 4.4.1 Cultural characteristics of sub-cultures of five mushroom samples on PDA media

Sl No.	Sub-cultures	Mycelium character		
		Colour	Density	Type
1	WMS-12	Off white	High	Cottony
2	WMS-13	White	Low	Cottony
3	WMS-14	White	Low	Velvety
4	WMS-15	Yellowish white	High	Cottony
5	WMS-16	Off white	Low	Cottony

Table 4.4.2 Diametric record of full growth of mycelium of five mushroom samples

Day	Diameter of mycelium of mushroom samples (c.m.)				
	WMS-12	WMS-13	WMS-14	WMS-15	WMS-16
1	0	0	0	0	0
2	0	1.7	3.5	1.1	3
3	2.5	4.3	6	1.5	9 (Full growth)
4	3.5	6.5	9 (Full growth)	2.5	
5	4.8	9 (Full growth)		3.6	
6	5.3			4.5	
7	6.8			5.5	
8	8.5			6.6	
9	8.8			8.5	
10	9 (Full growth)			9 (Full growth)	

The diametric growth patterns of five mushroom cultures were listed in **Fig. 4.2 (a-e)**

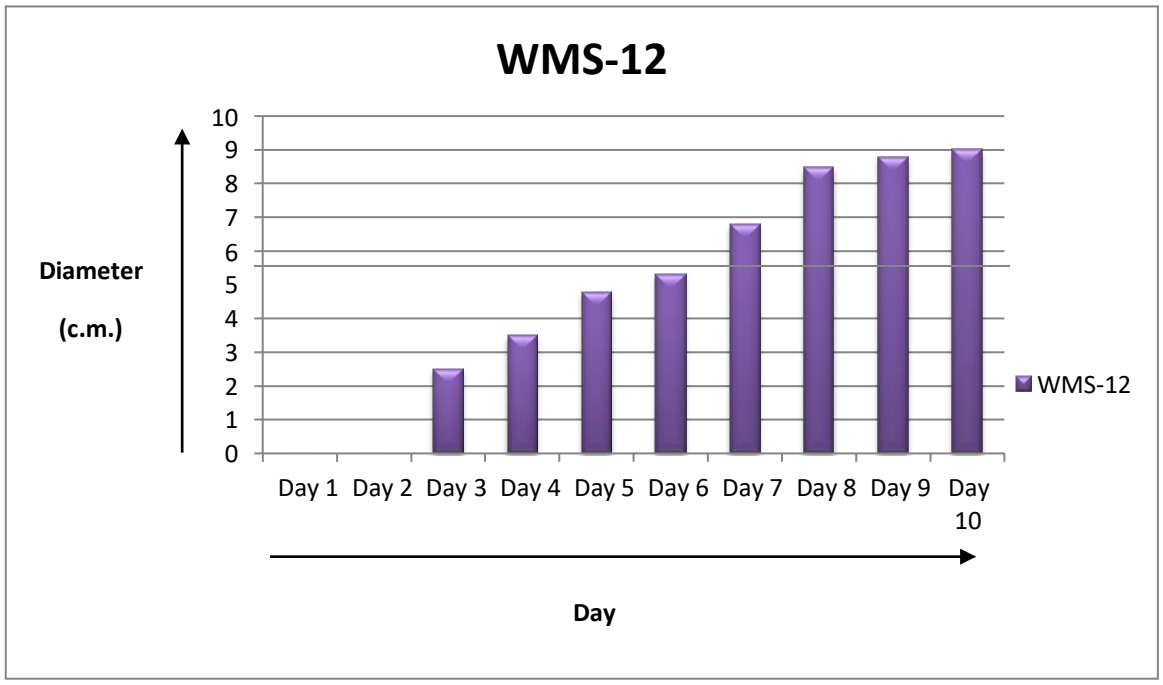


Fig. 4.2 a- Diametric growth pattern of wild mushroom culture WMS-12

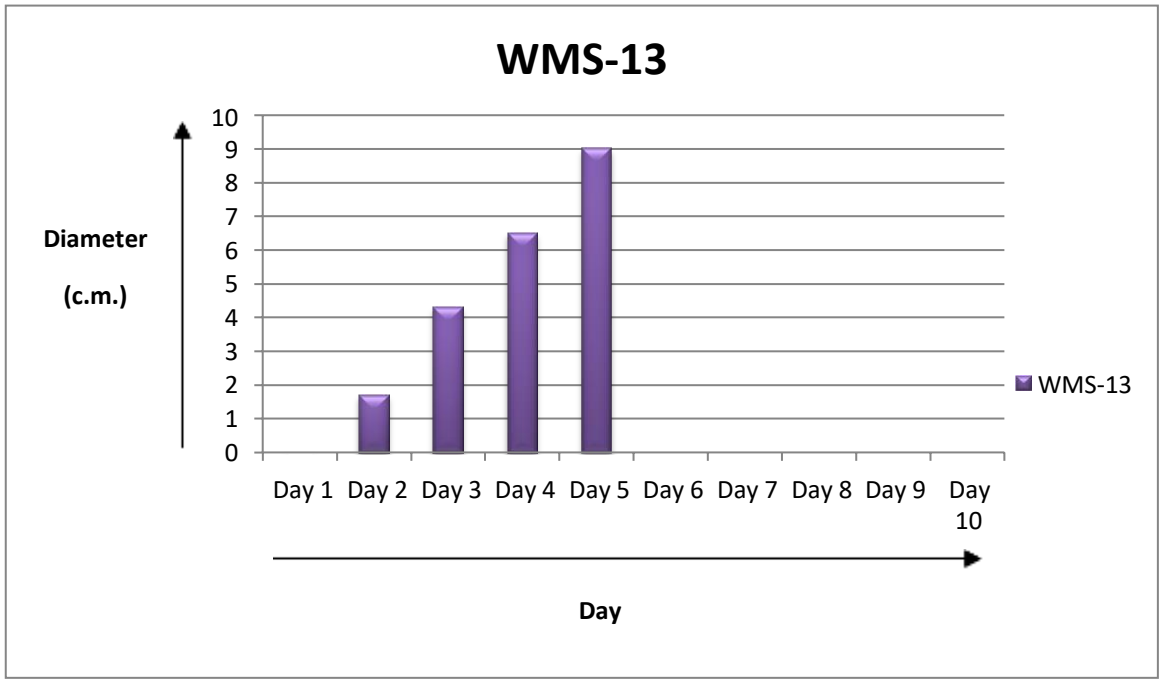


Fig. 4.2 b- Diametric growth pattern of wild mushroom culture WMS-13

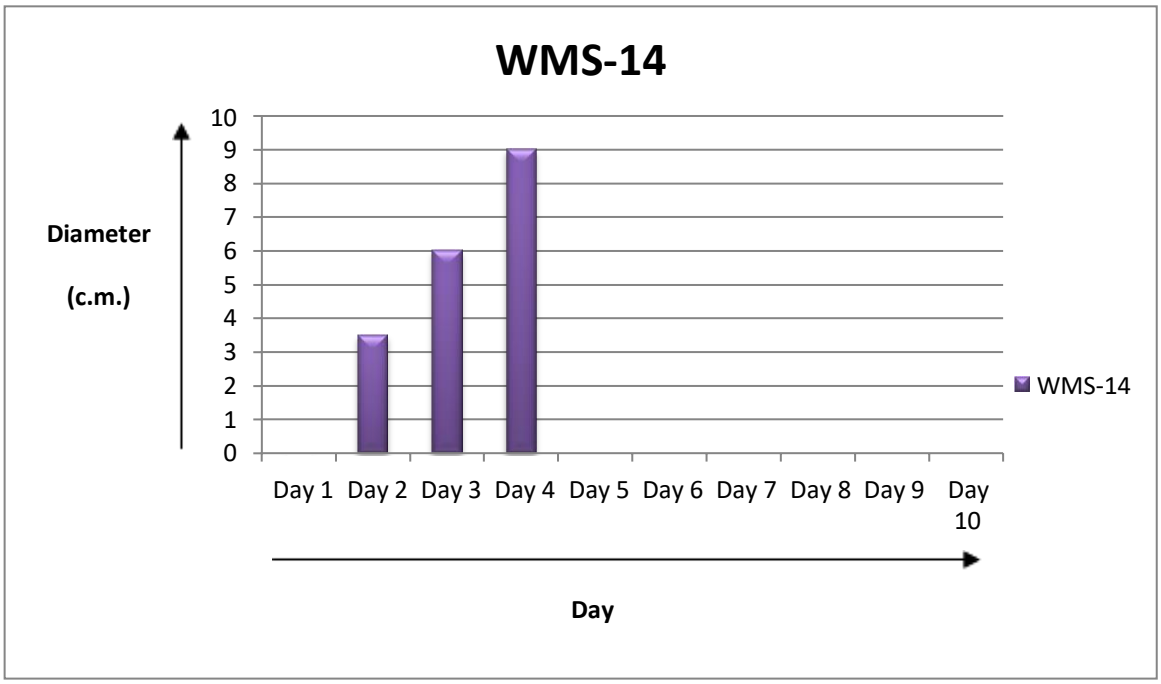


Fig. 4.2 c- Diametric growth pattern of wild mushroom culture WMS-14

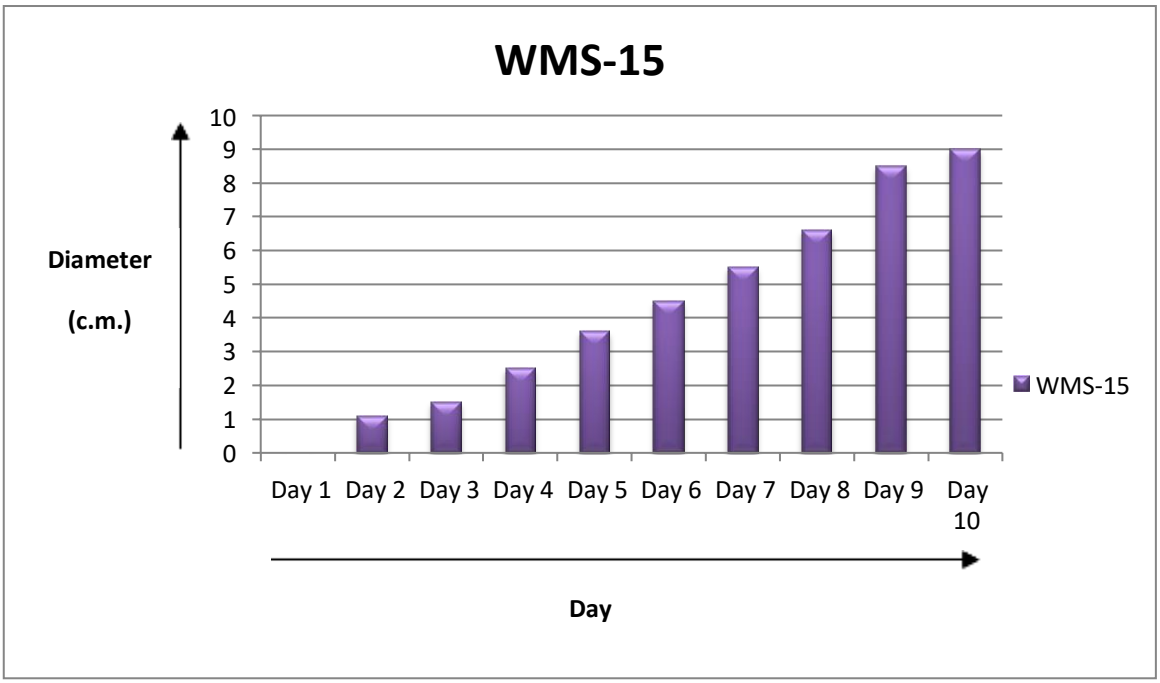


Fig. 4.2 d- Diametric growth pattern of wild mushroom culture WMS-15

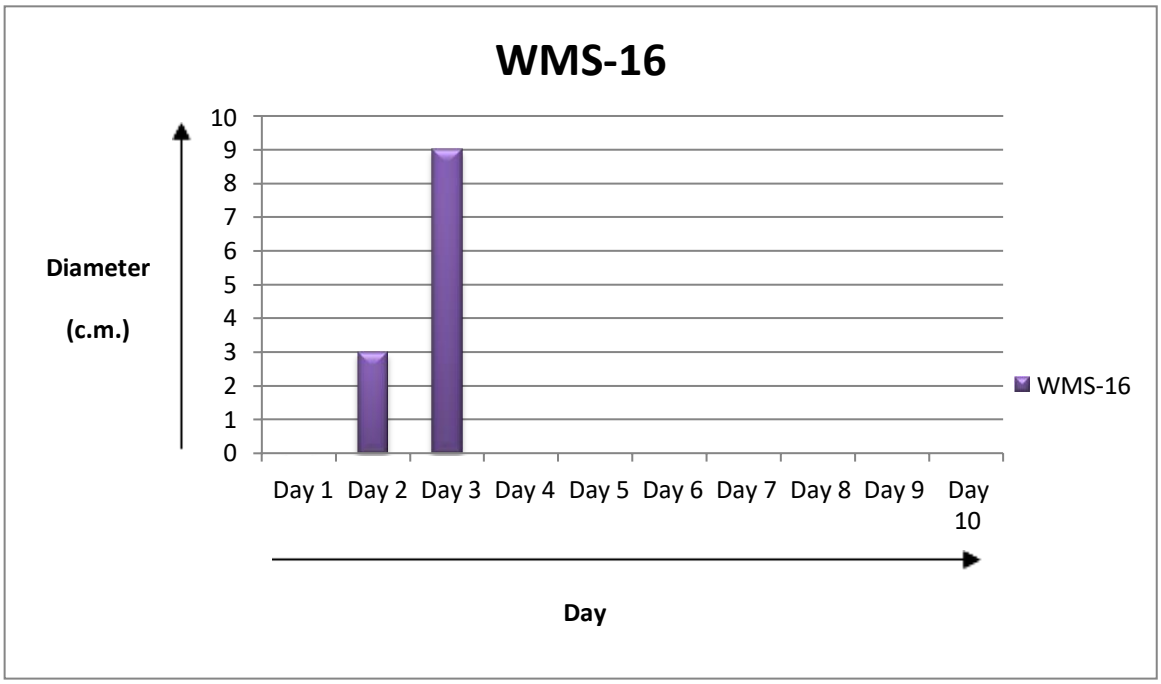


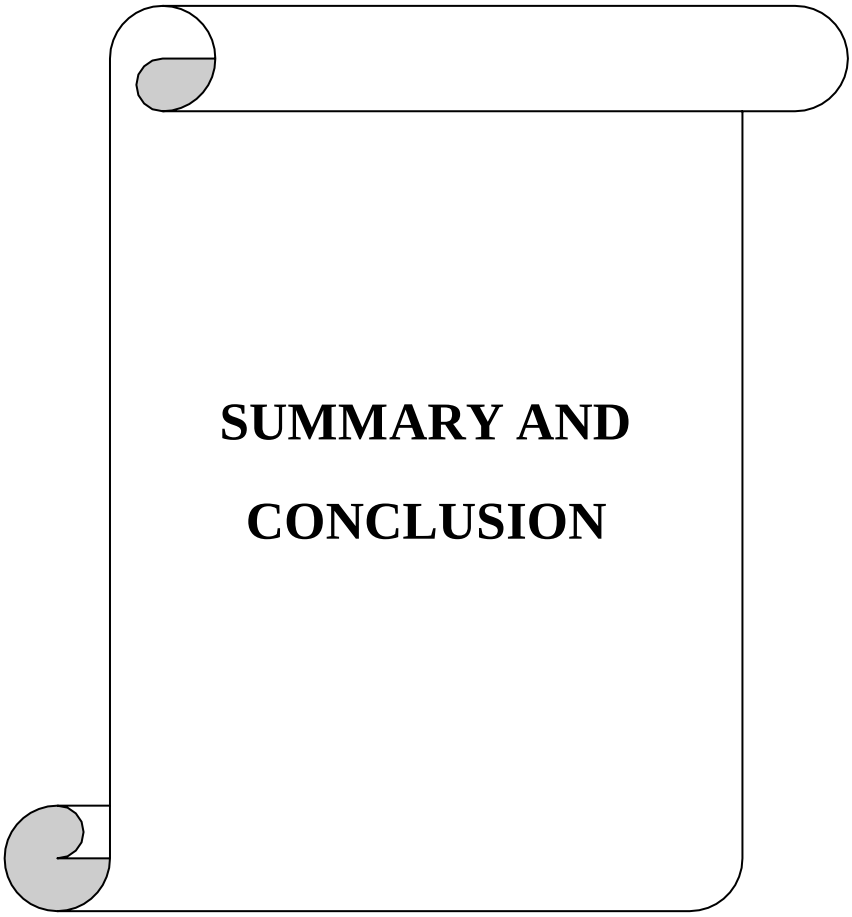
Fig. 4.2 e- Diametric growth pattern of wild mushroom culture WMS-16

It is visible from the above graphs that every mushroom has different growth pattern. Some are taking three days to grow fullyon Petri plate and other mushroom sample culturesare taking ten days to reach full growth stage. From the selected wild mushroom growth patterns we can infer that different wild mushroom samples have their specific requirement for nutrients and growth. Isolated wild mushroom samples like WMS-12, WMS-13, WMS-14, WMS-15 and WMS-16 which are identified as *Termitomyces microcarpus, Lentinus polychrous, Lentinus tigrinus,Hymenopellis megalospora* and *Sparassis* sp., and include both edible and non-edibile types, were all grown on PDA which acted as a basal media for any fungus isolation. Therefore, the pattern in their growth characteristics were not found to be same.

4.5 Discussion

A total of 17 Mushroom samples were collected and identified during 2022. The collected mushrooms were discovered to grow naturally in various research environments with various environmental conditions. Due to the ideal temperatures and humidity levels for their fruiting bodies, it was discovered that the majority of the gathered mushrooms were present from May to August. Out of 17 mushrooms 4 were identified to genus level. The highest species diversity was found in the family *Lyophyllaceae (6 spp.), followed by Polyporaceae (3 spp.), followed by Pleurotaceae (2 spp.), Boletaceae, Bolbitiaceae, Auriculariaceae, Russulaceae,Physalacriaceae*and and *Sparassidacea* were represented by one species each.

Sarma *et al.,* 2010, also reported wild edible mushrooms used by some ethnic tribes of western Assam like Garos, Bodos, Adivashis and Rajbangshi. During their period of study, they identified 26 different species of macrofungi belonging to 14 genera and 13 families. Out of these 26 species, about 23 species were found to be edible except *Ganoderma lucidum, Cantherallus cibarius*and *Lenzites betulina.*In western Assam, frequency of occurrence of macrofungi like *Ganoderma lucidum*(100%) was followed by *Cantharellus tubaeformis* (83.33%)and *Agaricus bisporus*(83.33%), *Schizophyllum commune, Auricularia delicata, Boletus luteus, Cantherallus cibarius, Lycoperdon cladopus, Termitomycesclypeatus*(66.66%), *Auricularia auricula, Lentinus edodes, Laetiporus sulphureus, Morchella esculanta, Termitomycesmammiformies, Auricularia polytricha, Agaricus sylvetica, Calvatia gigantia, Lentinus sajor-cajo, Lentinusostreatus, Tricholoma terreum*(33.33%), *Agaricus campestries, Boletus edulis, Lenzites betulina, Lycoperdon pyriforme, Termitomyces robustus, Termitomyces microcarpus* (16.66%).



SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

The present research work under this project aimed to identify the wild mushroom speciesdistributed in the Upper Brahmaputra Valley Zone in Assam. A random roving survey in the mentioned agricultural belt, resulted in identification of 17 wild mushroom species from the districts of Tinsukia, Jorhat and Golaghat which grew naturally in different habitats. The high rainfall from mid-June to July contributed a favorable environment for their appearance and growth.

These 17 wild mushroom species were identified based on their typical morphological and anatomical characteristics. Among the identified wild mushrooms, 9 (52.94%) out of 17, were identified as edible.Their edibility was primarily based on ethnomycological knowledge of the ethnic groups who consumed these mushrooms from generation to generation. The main reason for using these wild mushrooms by the ethnic group can be attributed to their nutraceutical and pharmaceutical properties which are present in wild mushrooms by default.

The identified edible wild mushrooms *viz.,Termitomyces globules, Termitomycesaurantiacus, Termitomyces indicus, Termitomycesmacrocarpus, Termitomyceseurrhizus, Volvarillavolvocca, Boletus*sp., *Auricularia auricular-judae, Lentinus critinus*and*Lentinus tigrinus,* are not being exploited commercially upto now like oyster or button mushroom. As these wild mushrooms possess commendable nutritional and pharmaceutical properties, therefore bioprospecting the same would bring a revolution to human health and pharmaceutical industry as well.

The findings of this study,will thereby, serve as a reference database for the state's wild mushrooms and assist in exploration of these edible wild mushroom in future research work.

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CHAPTER VI

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