Molecular Characterization and Nutritional Analysis of Certain Important Wild Edible Mushrooms of Nagaland, India

By

Ms. TOSHINUNGLA AO

THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE REQUIREMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY IN BOTANY

OF

DEPARTMENT OF BOTANY

NAGALAND UNIVERSITY, LUMAMI - 798627

NAGALAND, INDIA

2018

CONTENTS

Nagaland University

(*A Central University Estd. By the Act of Parliament No. 35 of 1989*)
Lumami 798627, Nagaland, India

December 05, 2018

DECLARATION

I, Ms. Toshinungla Ao bearing Ph. D. Registration No. 579/2014 dated May 20, 2014 hereby declare that the subject matter of my Ph. D. thesis entitled 'Molecular Characterization and Nutritional Analysis of Certain Important Wild Edible Mushrooms of Nagaland, India' is the record of original work done by me, and that the contents of this thesis did not form the basis for award of any degree to me or to anybody else to the best of my knowledge. This thesis has not been submitted by me for any Research Degree in any other University/Institute.

This is further certified that the Ph. D. thesis is submitted in compliance with the UGC Regulation 2016 dated May 05, 2016 (Minimum Standard and Procedure for Award of M. Phil. /Ph. D. Degree). Further, it is certified that the content of the thesis is checked for 'Plagiarism' with licensed software 'Plagiarism Checker X' and satisfies with norms of 'University Grants Commision', Govt. of India'. This thesis is being submitted to the Nagaland University for the degree of 'Doctor of Philosophy in Botany'.

S.Rama Rao
Professor

North-Eastern Hill University
Department of Biotechnology &
Bioinformatics
Mawkynroh, Shillong – 793 022
Meghalaya, India

0364-2722404(C)
0364-2722402 (O)
0-9436332760 (M)
srrao22@yahoo.com
Fax: 0364-2550076

Certificate

This is to certify that the thesis entitled "Molecular Characterization and Nutritional Analysis of Certain Important Wild Edible Mushrooms of Nagaland, India" embodies the original research work carried out by Ms. Toshinungla Ao, Department of Botany, Nagaland University, Lumami. I certify that a part of the research work was carried out under my guidance. No part of the work has been submitted to any University/Institution for award of any Degree/ Certificate.

(Prof. S. R. Rao)
Co-Supervisor

Acknowledgement

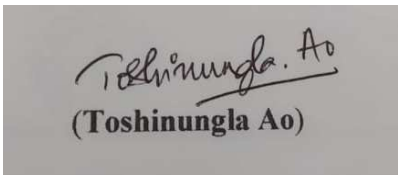
I would like to express my heartfelt gratitude to my Supervisor Prof. Chitta Ranjan Deb for his continuous support, expertise, guidance, motivation, patience and faith in me; and for providing all necessary facilities to carry out my research work. I could not have imagined having a better advisor and mentor for my Ph. D. study. I also express my appreciation and sincere thanks to my Co-Supervisor Prof. S. Rama Rao, North Eastern Hill University, Shillong for his valuable guidance, expertise, encouragement and support during the research period.

I extend my sincere thanks to the Head, Prof. S. K. Chaturvedi and former Head, Dr. Talijungla, Department of Botany, Nagaland University and all the faculty members for their constant encouragement and the necessary facilities provided by the department throughout the tenure of the study.

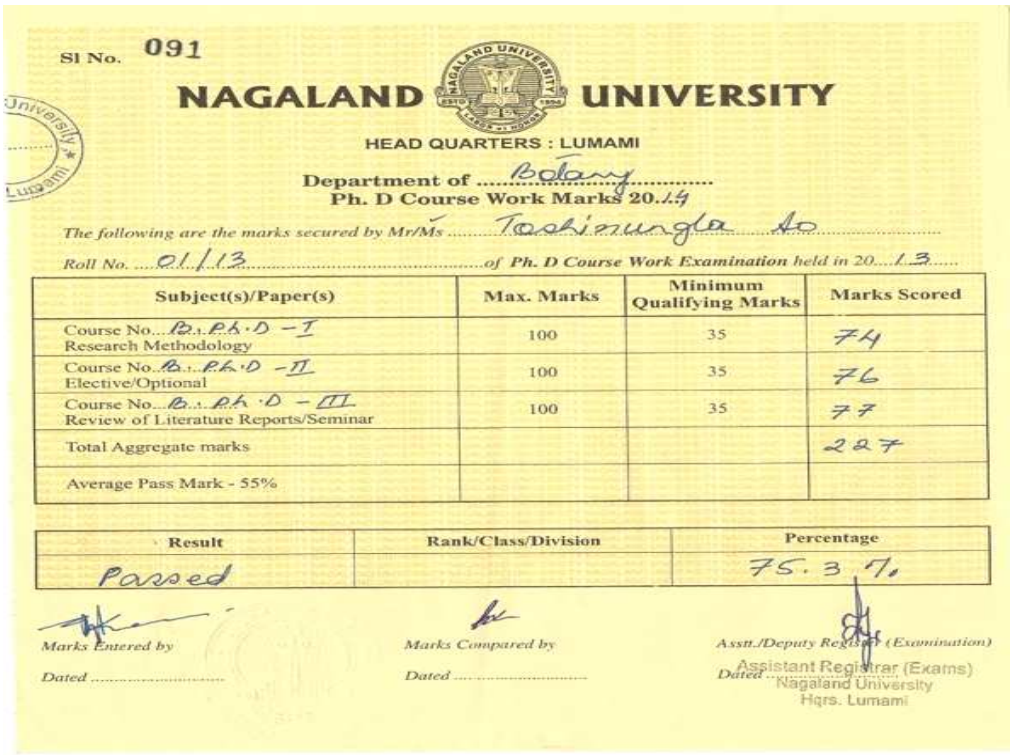
I express my gratitude to all the non-teaching staffs and research scholars of Department of Botany for their constant encouragement, help and wishes. I also sincerely thank all my labmates for their heartfelt support and encouragement throughout my study.

I am extremely thankful to the University Grants Commission, Govt. of India, New Delhi for the financial help through the UGC-BSR Fellowship, and Institutional Biotech Hub sponsored by DBT, New Delhi and UGC-SAP (DRS-III) programme for all the facilities provided for the present work.

My heartfelt gratitude goes to my family and friends for their prayers and constant support throughout the study period and to the Almighty God for His abundant grace and blessings on me which has helped me reach this far in life.


(Toshinungla Ao)

Ph. D. Coursework Mark Sheet and Certificate



Sl No. 091

NAGALAND UNIVERSITY

HEAD QUARTERS : LUMAMI

Department of Botany

Ph. D Course Work Marks 2014

The following are the marks secured by Mr/Ms Toshinungla Ao

Roll No. 01/13 of Ph. D Course Work Examination held in 2013

Subject(s)/Paper(s)	Max. Marks	Minimum Qualifying Marks	Marks Scored
Course No. Bot. Ph. D - I Research Methodology	100	35	74
Course No. Bot. Ph. D - II Elective/Optional	100	35	76
Course No. Bot. Ph. D - III Review of Literature Reports/Seminar	100	35	77
Total Aggregate marks			227
Average Pass Mark - 55%			

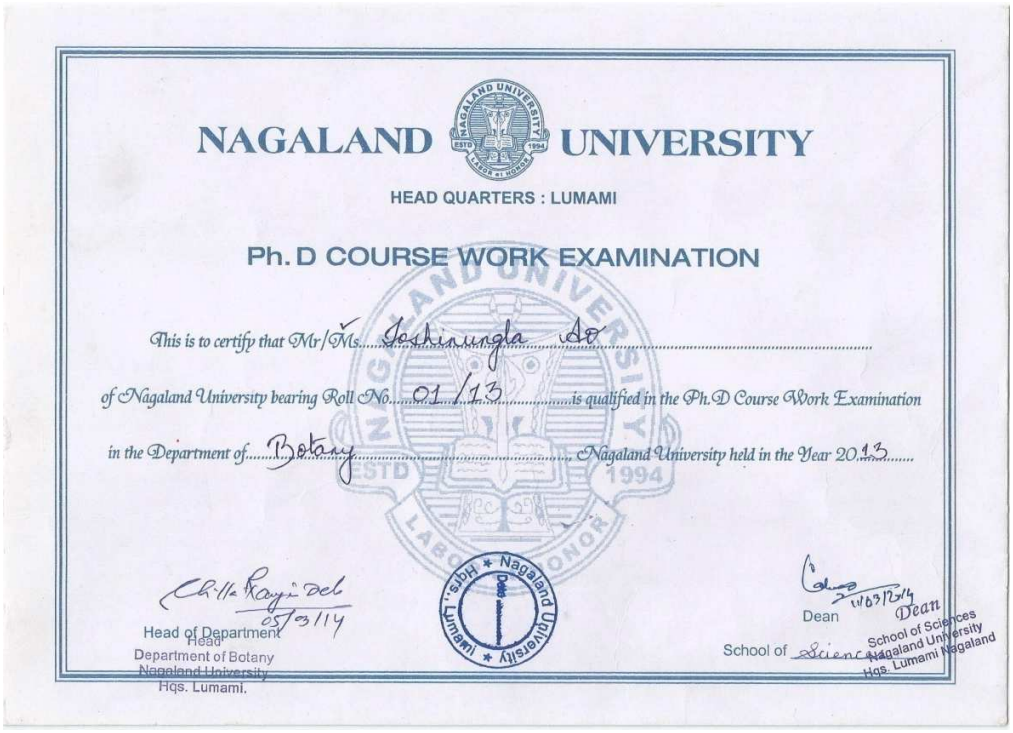
Result	Rank/Class/Division	Percentage
Passed		75.3 %

Marks Entered by
Dated

Marks Compared by
Dated

Asstt./Deputy Registrar (Examination)
Dated

Assistant Registrar (Exams)
Nagaland University
Hqrs. Lumami



NAGALAND UNIVERSITY

HEAD QUARTERS : LUMAMI

Ph. D COURSE WORK EXAMINATION

This is to certify that Mr/Ms Toshinungla Ao

of Nagaland University bearing Roll No. 01/13 is qualified in the Ph.D Course Work Examination

in the Department of Botany Nagaland University held in the Year 2013

Head of Department
Head
Department of Botany
Nagaland University
Hqs. Lumami.

Dean
Dean
School of Sciences
Nagaland University
Hqs. Lumami Nagaland

Abbreviations

Abbreviation	Expanded Form
-	Negative
+	Positive
%	Percentage
^{o}C	Degree Celsius
µg	Microgram
µl	Microlitre
µm	Micromolar
***p*H**	Potential of hydrogen
N	Normal
M	Molar
G	Gram
Ml	Millilitre
Mg	Milligram
Hr	Hour
min	Minute
mM	Millimolar
Ng	Nanogram
Nm	Nanometre
AFLP	Amplified Fragment Length Polymorphism
AOAC	Association of Official Analytical Chemists
Bp	Base pair
BSA	Bovine Serum Albumin
CI	Consistency Index
CTAB	Cetyl Trimethyl Ammonium Bromide
DAMD	Directed Amplification of Minisatellite DNA

DNA	Deoxyribonucleic acid
DNSA	Dinitrosalicyclic Acid
DPPH	2, 2-Diphenyl- 1-picrylhydazyl
EDTA	Ethylenediaminetetraacetic Acid
EMR	Effective Multiplex Ratio
EST	Expressed Sequence Tag
GAE	Gallic acid
Gst	Genetic Differentiation
H_2SO_4	Sulphuric acid
HCl	Hydrochloric Acid
Ht	Total Gene Diversity
Hs	Heterozygosity
HVR	Hyper Variable Region
IGS	Intergenic Spacer
ISSR	Inter Simple Sequence Repeat
ITS	Internal Transcribed Spacer
LSU	Large Subunit
ME	Minimum Evolution
MEGA	Molecular Evolutionary Genetics Analysis
$MgCl_2$	Magnesium Chloride
MI	Marker Index
MP	Maximum Parsimony
Na	Number of Observed Alleles
NaCl	Sodium chloride
NaOH	Sodium hydroxide
Ne	Effective Number of Alleles
NJ	Neighbor Joining

Nm	Estimate of Gene Flow
PCoA	Principle Coordinate Analysis
PCR	Polymerase chain reaction
PIC	Polymorphic Information Content
QE	Quercetin
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
rDNA	Ribosomal DNA
Rp	Resolving Power
Rpm	Revolutions per Minute
SPAR	Single Primer Amplification Reaction
SRAP	Sequence Related Amplified Polymorphism
SSR	Simple Sequence Repeat
SSU	Small Subunit
TAE	Tris Acetate EDTA
TBE	Tris Borate EDTA
TE	Tris EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Average
VNTR	Variable Number of Tandem Repeats
WEM	Wild Edible Mushrooms
LS	*Lentinus squarrosulus*
LX	*Lentinus sajor-caju*
LT	*Lentinus tigrinus*

List of Tables

List of Figures

Chapter - 1

Introduction

Fungi are extraordinarily widespread, diverse, abundant and ecologically important and occupy a prominent position in the biological world due to economical and environmental importance. Approximately 70,000 fungal species are identified while, it is reported that ~1.5 million fungal species are present on earth. Indeed, few works have been done on fungal diversity in the world (Nishida 1992; Ammirati *et al*., 1994). Documentation of fungal species in the world has been an important area of discussion and a number of works have concentrated on enumeration of world's fungal diversity. Fungi are not considered as plants as they lack chlorophyll and so are classified in a kingdom of their own which include mushrooms, toadstools, mould, mildew and yeast (Crous *et al*., 2006). Macro fungi or mushrooms are unique from fungal diversity point of view as they grow prolifically and found in many regions of the planet (Smith, 1963). Mushrooms are considered as indicators of ecosystem. Some species are indicators of sensitive habitats or specific environmental conditions (Kosztarb, 1983). Mushrooms are major portions of minor forest products which grow on cellulose, the biomolecule which is found abundantly in the

biosphere (Chang and Miles, 1992). Mushrooms are considered as seasonal fungi and occupy various niches in the ecosystem. Mushrooms belong to Kingdom Fungi and are classified under classes of Ascomycetes and Basidiomycetes. Mushrooms are macro fungi with a distinctive fruiting body such as brackets, bird's nest, jelly, boletes, corals, puffballs, stinkhorns and agarics. They are fleshy, leathery, sub-fleshy or woody and their fertile surface is found attached on lamellae or the tubes, which opens out through pores. The lamellate members are the agarics and the boletes and the polypores are the poroid members (Deshmukh, 2004).

Macro Fungal Diversity and Identification

The fungal diversity studies have been investigated world over and the mushrooms on earth is estimated to be 140,000, yet only ~10% are known (Wasser, 2002). Around 2000 fungal species are reported to be edible, but less than 25 mushroom species are accepted as food globally and very few have achieved the level of commercial item. *Agaricus bisporus*, *Auricularia* species, *Pleurotus* species, *Lentinula edodes*, *Volvariella volvacea* etc are some of the most accepted edible mushrooms globally (Bonatti *et al*., 2004). It is said that one third of global fungal population exists in India but only 50% are characterized so far (Manoharachary *et al*., 2005). It is reported that India has about 27,000 fungal species (Cowan, 2001; Chang and Miles, 2004) and approximately 850 mushroom species recorded so far (Deshmukh, 2004). In China and Japan, the information on the applications of both edible and medicinal mushrooms have been passed down from one generation to the next but it was not so in India. Despite this shortcoming, the traditional mycological information of Indian ethnic groups has proven to be knowledgeable and extensive, which describes about 283 wild mushroom species out of 2000 edible species recorded world over (Purkayastha and

Chandra, 1985). Macro fungi have the longest history of diversity studies of any mycota over the world, but are understudied. Mycologists continue to investigate the virgin, hidden and interesting fungal biodiversity because many macro fungi are on the verge of extinction due to anthropogenic factors and climate change (Swapana *et al*., 2008). Though indigenous mushroom resources are diverse in nature; morphological identification is limited for taxonomy (Zhang *et al*., 2006). Mushrooms play a vital role economically and biologically, so information on mushroom diversity is necessary because of the role they play to humans, animals and environment. They are considered as ecological indicators and used in reforestation programs (Wongchalee and Pukahute, 2012; Andrew *et al*., 2013). Mushroom taxonomic studies have been mainly based on the study of morphological characteristics like shape, size and color of caps and gills (Lee *et al*., 2006), which are considered unreliable (Lian *et al*., 2008) or limited. Mushroom poisoning is the outcome of misidentification and ingestion of a toxic wild mushroom as an edible species. The misidentification is mainly due to close resemblance of the poisonous mushroom species with the edible variety in terms of morphological traits (Berger and Guss, 2005). The use of morphological characteristics alone for mushroom identification is limited and not practical to have criteria to select only morphological traits as it is not clearly defined, which leads to constant disagreement and taxonomic rearrangements in fungi (Yang, 2011; Yang *et al*., 2012). Morphological characters are often influenced by environmental conditions and similarly ecological diversities of wild mushrooms may result in phenotypic plasticity within a few species or genotypes (Feng *et al*., 2012). Thus, the classification system for mushrooms and their allies is constantly relying on molecular data as morphological information is limited for fungal

systematics due to their evolutionary convergence, inherent simplicity, parallelisms, and phenotypic plasticity (Hofstetter *et al*., 2002).

With the advancement of biotechnology and mushroom science, molecular tools are more accurate, rapid and reliable method for identification of fungi (Fan *et al*., 2006; Moreau *et al*., 2006; Fonseca *et al*., 2008). The absence of a universally accepted DNA barcode marker for fungi is a significant limitation for diversity studies. DNA barcoding uses target (500 to 800) bp sequences for identifying species of all eukaryotic kingdoms using primers that are applicable for the widest possible taxonomic group (Blackwell, 2011; Mora *et al*., 2011; Schoch *et al*., 2012). Identification of mushrooms using molecular approaches has been widely accepted because they target the species at the DNA level. The primers 'ITS1' and 'ITS4' were the first PCR primers favored to work with fungal internal transcribed spacer (ITS) regions which code the highly variable regions i.e., ITS1 and ITS2 of the ribosomal operon (White *et al*., 1990). The ITS region is located between 18S or Small Subunit (SSU) and 28S or Large Subunit (LSU) in the rDNA cistron and is most commonly used for molecular phylogenetic studies as *rRNA* genes are known to be conserved at the species or genus level (Kim and Lee, 2000; Lee *et al*., 2000; Chen *et al*., 2004; Park *et al*., 2004). The ribosomal DNA (rDNA) coding genes is successfully used in species identification during evolutionary, environmental and taxonomic studies of fungi (Borneman and Hartin, 2000; Gurtner *et al*., 2001; Muruke *et al*., 2002). Similarly, during the last decade, molecular approach for identification of basidiomycetes had been attempted by amplification and restriction of special DNA sequences by PCR and restriction fragment length polymorphism (RFLP). This approach has been largely followed for identification of eukaryotes including fungi (Hopple and Vilgalys, 1994; Wesselink *et al*., 2002). With the

recent approval of large-scale changes to the naming procedure of fungi, sequence data from specimens/DNA barcodes will increasingly be crucial for fungal nomenclature (Hawksworth, 2011). The ribosomal RNA (*rRNA*) genes frequently used in studies of all organism groups, are also the most commonly sampled in fungi. The diversity of *rRNA* gene is a useful tool to study the taxonomy and phylogenetic classification in bacteria and fungi (Hur and Chun, 2004; Lim *et al*., 2005). DNA barcoding refers to sequencing of a particular locus that is regarded to be species specific and used to identify fungi based on the DNA sequence information at the species level. For fungal resolution at and below the generic level, the ITS region of the rDNA has always been the marker of choice for ecological studies (Seifert, 2009; Begerow *et al*., 2010). However, some workers (Kurzman and Robnett, 1998; Hong *et al*., 2000; Wesselink *et al*., 2002) have revealed that the LSU or 28S *rRNA* gene has variable regions with sequence divergence - D1, D2, and D3 which makes it sufficient to study the phylogeny between the fungal species.

The recent advancement in the field of molecular biology has introduced a new generation markers to facilitate dissection of plant genomes more easily and efficiently. Several molecular techniques have already been used for studying the genetic diversity in mushrooms such as isoenzymes (Lan *et al*., 1998), AFLP (Qi *et al*., 2003), RFLP (Park *et al*., 1996), ITS (Kindermann *et al*., 1998) and RAPD (Wang *et al*., 2003). These DNA based markers are versatile tools which are more advantageous over conventional phenotypic markers. DNA markers are known to be present in large numbers, stable across the developmental stages and least influenced by environmental factors, devoid of the pleiotropic and epistatic effects. Depending on the types of technique used, these markers are classified as PCR based markers and hybridization based markers. Some of the important PCR based

marker systems are random amplified polymorphic DNA (RAPD), amplified fragment polymorphism (AFLP), inter simple sequence repeats (ISSR), simple sequence repeats (SSR), directed amplification of mini-satellite DNA regions (DAMD), expressed sequence tag (EST) etc. Markers like RFLP, SSR and EST are co-dominant markers and can detect genetic variability at allelic level. However, the development and utilization of these marker systems are costly, laborious and time taking. While RAPD, ISSR, DAMD and AFLP are dominant marker systems, cost effective, easier to be developed and used. Therefore, for selecting a marker system, a number of factors like availability of equipment, time and expertise along with the nature of the crop, are to be taken into account (Williams *et al*., 1990; Heath *et al*., 1993; Zietkiewicz *et al*., 1994; Meyer *et al*., 1993; Vos *et al*., 1995; Vijayan, 2005). These markers have been developed successfully and effectively utilized in genetic diversity analysis. The evaluation of genetic variation and diversity would encourage the efficient use of genetic variations in the breeding program (Paterson *et al*., 1991). DNA finger printing has become an important tool in characterization of mushrooms (Shinwari *et al*., 1994a; 1994b). Large numbers of polymorphic markers are required to measure genetic diversity and relationships in a reliable manner. This limits the use of isozymes or morphological characters which lack adequate levels and often influenced by environmental factors in mushrooms. Molecular genetic markers are powerful tools to examine genetic relationships and diversity. Efforts have been initiated for the identification of cultivars and assessment of genetic diversity of many edible and medicinal mushrooms like *Agaricus bisporus, Auricularia auricula-judae, Lentinula edodes, Ganoderma lucidum, Tricholoma, Volvariella*, *Flammulina velutipes* etc by RAPD, ISSR and sequence-related amplified polymorphism (SRAP) (Welsh and Mc Clelland, 1990; Williams *et al*., 1990; Zietkiewicz *et*

al., 1994; Li and Quiros, 2001; Moore *et al*., 2001; Staniaszek *et al*., 2002; Yan *et al*., 2004; Sun *et al*., 2006; Zhang *et al*., 2007a; Fonseca *et al*., 2008; Guan *et al*., 2008; Tang *et al*., 2010; Tao *et al*., 2011; Sahoo *et al*., 2014; Liu *et al*., 2015; Dwivedi *et al*., 2017, Liu *et al*., 2018) however, only few works have explained the estimation of genetic diversity using multiple markers (Qin *et al.*, 2006; Yu *et al*., 2008).

The ISSR markers are regarded as reliable and economical DNA marker system (Zietkiewicz *et al*., 1994; Tsumura *et al*., 1996). The ISSR markers have variable microsatellite regions and easy application at low cost which makes these markers highly useful for systematic and ecological evaluations (Reddy *et al*., 2002) and broadly used for population genetics, strain identification and genetic diversity of many edible and medicinal mushrooms (Yu *et al*., 2008; Fu *et al*., 2010; Tang *et al*., 2010; Du *et al*., 2011a; Shao *et al*., 2011). The DAMD technique was reported first by Heath *et al*. (1993). The mini-satellite regions are DNA tandem repeat regions which exhibits high levels of variations in the number of repeat units. DAMD-PCR techniques uses mini-satellite core sequences as primers which contains hyper variable regions (HVR) or variable number of tandem repeats (VNTR). These tandem repeats are known to be present in diverse plants and animal species. Because of this, DAMD primers are fruitful as PCR primers in diverse organisms at relatively high stringencies (Jeffreys *et al*., 1985; Heath *et al*., 1993; Karaca *et al*., 2002; Karaca and Ince, 2008). However, there is no report of genetic diversity studies using these primers so far in mushrooms. The RAPD is another convenient method to evaluate the genetic diversity in diverse organisms including mushrooms. The use of RAPD markers has enabled the rapid generation of reliable reproducible DNA fragments in many mushroom species. The RAPD technique based on random genomic studies is therefore, reliable to

indicate the overall genetic variation then sequence analysis of a single region of genome (Achenbach *et al*., 1996; Akbar *et al.*, 2011). RAPD markers have already been used for strain identification of several mushrooms, discrimination of mushroom cultivars and helpful in protection of elite strains (Moore *et al*., 2001; Ravash *et al*., 2009; Agarwal *et al*., 2013; Rehman *et al*., 2015). RAPD technique is still the quickest and most reliable method for assessing the variability at DNA level, especially being useful for intra-specific analysis. These markers have the advantage of amplifying both transcript/translated and non-coding regions of the genome. This is vital when the objective is to examine the variation within species genome (Williams *et al*., 1990; Ferreira and Grattapaglia, 1996; Ro *et al*., 2007). On the other hand, RAPD markers have limitations for its low experimental reproducibility, but the problem can be overcomed with the usage of many primers and more strict criteria in examining the results (Carvalho and Vieira, 2001). Now a day's strain identification can be effectively achieved with the help of DNA marker technologies developed for this purpose (Bian and Song, 2005), which has also been used to study genetic diversity and population genetics in a variety of agricultural products (Simioniuc *et al*., 2002; Ferriol *et al*., 2003) including mushrooms (Zhang *et al*., 2007; Yu *et al*., 2008; Guan *et al*., 2008). Sequence related amplified polymorphism (SRAP) (Li and Quiros, 2001) and ISSR (Zietkiewicz *et al*., 1994) DNA marker technologies has proved to be highly reliable and effective, especially when we combine the data from both marker analysis. In the past, these techniques were used by different workers *viz*., RFLP technique was used for genotyping of *Agaricus brunnescens* (Castle *et al*., 1987), RAPD technique was used for differentiating cultivars of button mushroom (Moore *et al*., 2001), for genotype identification of *Pleurotus* species used ITS-RFLP technique (Ma and Luo, 2002), ITS and IGS regions were used for genetic

diversity evaluation in *Armillaria* mushroom (Chillali *et al*., 1998) and ISSR markers in strain identification of *Lentinula edodes* (Zhang *et al*., 2007) and differentiation of strains in *A. bisporus* (Guan *et al*., 2008).

Mushrooms are healthy and natural foods which originate from an organic farming system (Moore and Chiu, 2001). For sustainable mushroom cultivation and production, novel strains with enhanced characteristics are essential. But, mushroom strains are not so easy to discriminate due to absence of clearly distinguishable characters which impedes strain improvement and makes strain protection problematic. Molecular markers like rDNA sequencing, ISSR, RAPD, RFLP, SSR, SRAP, microsatellite and mitochondrial genotypes have all been used to discriminate mushrooms or strains of *Agaricus* (Moore *et al*., 2001; Nazrul and Bing, 2010; Malekzadeh *et al*., 2011), *Auricularia* (Yan *et al*., 2004; Tang *et al*., 2010; Du *et al*., 2011; Du *et al*., 2013), *Ganoderma* (Rolim *et al*., 2011; Dwivedi *et al*., 2017), *Lentinula* (Qin *et al*., 2006; Fu *et al*., 2010; Liu *et al*., 2015), *Lentinus* (Dwivedi *et al*., 2017), *Flammulina velutipes* (Liu *et al*., 2018) and *Volvariella* (Sahoo *et al*., 2014) etc. This provides reliable information for strain identification and protection in mushrooms. Over the years, the methods for detection and assessment of genetic diversity have extended from study of discrete morphological to biochemical and molecular characters. Several PCR based markers have been applied to determine the genetic variation among populations and genetic resources. Molecular markers has made it possible to evaluate genetic diversity by recognizing the polymorphic nucleotide sequences dispersed throughout the genome and to determine inter- and intra-species genetic relationship (Gostimsky *et al*., 2005; Karakas *et al*., 2010). The ISSR and RAPD molecular markers are among the most widely used to assess the genetic diversity, genetic map construction, linkage analysis and DNA

fingerprinting in plants (Darvasi *et al*., 1993; Neale and Savolainen, 2004). Molecular markers are also used to locate drought induced genes in the genome and as these markers are not influenced by environment, they are often considered to be superior to morphological markers (Maric *et al*., 2004).

Nutritional Value of Mushrooms

The usage of mushrooms as food is probably as old as human civilization and the early history regarding the use of mushrooms in different countries has been reviewed by many past workers (Butler, 1915; Singer, 1961; Atkinson, 1961; Abou *et al*., 1987). The oriental knowledge of using mushrooms is older than the Europeans (Lambert, 1938). The early pre-historic man began collecting wild mushrooms by trial and error over time for eating purpose (Atri *et al*., 1997). Since time immemorial, societies around the world have valued wild mushrooms as a nutritious food (Breene, 1990; Chang and Miles, 1992; Manzi *et al*., 1999). Mc-Connell and Esselen (1947) reported that fresh mushroom contains 0.9% mannitol, 0.28% reducing sugar, 0.59% glycogen and 0.91% hemicelluloses. The first successful research uncovered the anti-tumour effects of the hot water extracts from some mushrooms (Ikekawa *et al*., 1969). Chihara *et al*. (1969) isolated anti-tumor polysaccharide which was named lentinan from shiitake mushroom. Carbohydrates of *A. bisporus* were reported by Crisan and Sands (1978). Mushrooms are often regarded as one of the best available source of vitamins especially vitamin B (Breene 1990; Mattila *et al*., 1994; Zrodlowski, 1995; Chang and Buswell, 1996; Mattila *et al*., 2001). The mineral content of wild edible mushrooms (WEM) was reported to be higher than the cultivated ones (Aletor, 1995; Mattila *et al*., 2001; Rudawska and Leski, 2005). Mushrooms are regarded as biological response modifiers and the fourth principle form of the conventional cancer

treatment (Yang *et al*., 1993). Compounds present in mushrooms reduce blood sugar levels and blood pressure, reduce cholesterol and inhibit platelet aggregation (Liu, 1999; Mizuno *et al*., 1995; Zhu and Mori, 1993). Mushrooms are nature's greatest untapped source of nutritious enzyme complex which enables them to develop successfully on a large variety of inexpensive substrates such as lignin, pectin, cellulose, hemicelluloses, and other industrial wastes which are not suitable even for animal feed (Bano *et al*., 1963). Although mushrooms have been appreciated for their flavor and unique taste; medicinal and tonic attributes, recognition of mushrooms as a good source of nutritious food and physiologically important source of medicinal compounds is recent (Li and Chang, 1982). Gruen and Wong (1982) indicated that the nutritional value of edible mushrooms is comparable with meat, milk and egg food sources. The world mushroom production has a market value of U.S $10 billion. The major exporting countries of fresh mushrooms are Netherlands, Poland, Ireland and Belgium. China is the largest exporter of preserved mushrooms with a market share of 41.82%. Netherlands (25.11%) and Spain (7.37%) are the other major countries. Germany, U.S.A and France are the major importing countries of prepared and preserved mushrooms. India has the potential to be a major producer of mushroom, ranks 54th as producer and ranks 6th as an exporter (Harsh and Joshi, 2008).

Mushroom research gained momentum because of the role played by mushrooms in food industry and medicines. Mushrooms are popularly used to enhance immunity as nutrient supplements in the form of tablets, as anticancer drug and to fight HIV effectively (King, 1993). Many edible and medicinal mushrooms have been traditionally used in China, Korea and Japan for their medicinal and tonic properties. Examples include *Auricularia* species (jelly mushrooms) which have traditionally been used to treat piles and various stomach

ailments; *Tremella fuciformis* (jelly mushroom) for maintaining healthy lung tissue; *Hericium erinaceus* (monkey head mushroom) for treating gastric ulcers; *Volvariella volvacea* (straw mushroom) for lowering blood pressure and accelerating the healing of wounds and *Lentinula edodes* (shiitake) in the prevention of rickets and lowering gastric acidity (Chang, 1996). Mushrooms are regarded as the most important among the horticultural crops in developed countries (Baruah, 2008). Mushrooms are highly popular as functional food and as an important source to develop drugs and nutraceuticals (Lakhanpal and Rana, 2005) which is responsible for the antioxidant, antimicrobial and antitumor properties of mushrooms (Jones and Janardhaanan, 2000). Modern pharmacological research confirms large parts of traditional knowledge regarding the medicinal effects of mushrooms due to their antifungal, antibacterial, antioxidant and anti-viral properties (Barros *et al*., 2007; Ferreira *et al*., 2007; Wani *et al*., 2010). Besides the pharmacological properties, mushrooms have become crucial in human diet due to rich nutritional properties like high protein, low fat etc (Murugkar and Subbulakshmi, 2005). The WEM are important non-timber forest resource used by mycophilic societies and their use has been documented in many countries around the world (Jones and Whalley, 1994; Roberto *et al*., 2006). Wild edible mushroom is popularly used as food and medicines in many African and Asian countries and is appreciated for its unique flavor and nutritional compositions (Boa, 2004). Tibuhwa (2013) documented the use of WEM in Tanzania and assessed its importance as a source of healthy food and income generation for the disadvantaged rural dwellers and reported that WEM are among the non-wood forest products, not well reported in many countries, badly collected, poorly studied and relatively underutilized in the country. There is no detailed information on

how much is harvested, no market orders and also lack of general awareness on income generation potential and its contribution to food security.

Edible mushrooms are considered as healthy food as they are rich in proteins, fibers, minerals, vitamins, low/no calories and cholesterol lowering properties and the food value of mushrooms lies between meat and vegetables (Bano, 1976; Wani *et al*., 2010) apart from the flavor they impart. Protein content of fresh mushrooms is about twice that of vegetables and contains all the 9 amino acids essentials for the health of humans (Sumbali, 2005). For example, *Ganoderma* is broadly consumed around the world as a health food, being regularly used by many Asians for the advancement of health and longevity and as a remedy for illness (Jong and Birmingham, 1991; Wachtel-Galor *et al*., 2003). Mushrooms have been found effective against cancer, cholesterol reduction, skin diseases, stress, insomnia, asthma, allergies and diabetes (Bahl, 1983). Recently, mushrooms have gained much attention due to its antioxidant, hypocholesterolaemic, anticancer, antiviral, immuno-potentiating and hepatoprotective properties. These new compounds termed 'Mushroom Neutriceuticals' are extracted either from the fungal fruiting body or mycelium and represent a major component of the increasing mushroom biotechnology industry (Chang and Buswell, 1996). Mushrooms are considered to possess therapeutic properties and at present, over 270 mushroom species are reported with known therapeutic properties (Ooi and Liu, 1999). Studies on mushrooms have been an important area for the scientists in particular and the people in common because of their role in human welfare, food industry, medicines, and biodegradation (Ozturk *et al*., 2003). The existence of secondary metabolites in mushrooms like polyphenols, terpenes, steroids and polyketides which have antioxidant properties and pharmacological applications play vital role in boosting the health of consumers (Cheung *et al*., 2003; Barros

et al., 2007). Polyphenols are aromatic compounds which include complex structures like flavonoids and anthocyanins and show antifungal, anti-inflammatory, antioxidant, anticancer, antimutagenic, antimicrobial, immune modulator and many other pharmacological activities (Ramesh and Pattar, 2010; Keles *et al*., 2011). Patel and Goyal (2013) reported that mushrooms act as anti-cancer compounds, plays significant role as angiogenesis inhibitor, mitotic kinase inhibitor, reactive oxygen species inducer, anti-mitotic, topoisomerase inhibitor, leading to apoptosis and also checking cancer proliferation. Oxidation is an important process for all living organisms for the production of energy and oxygen-centered free radicals and other reactive oxygen species produced continuously *in vivo*, result in tissue damage and cell death. Free radicals cause oxidative damage which is related to aging, cirrhosis, diabetes, atherosclerosis and cancer (Halliwell and Gutteridge, 1984). Natural sources of antioxidants are considerably studied for their properties to protect organisms and cells from damage brought about by oxidative stress (Cazzi *et al*., 1997). Most organisms possess antioxidant defense and repair systems for protection against free radical damage by oxidative enzymes like superoxide dismutase (SOD) and catalase (CAT) and by chemical compounds like ascorbic acid, carotenoids, α-tocopherol, glutathione and polyphenolic compounds (Mau *et al*., 2001), yet these systems are not sufficient. Mau *et al*. (2004) described that mushrooms are rich sources of antioxidants.

Fungi are obviously non-toxic as they have been in close human consumption by natives and tribals, since history (Pandey and Srivastava, 1994). The Indian subcontinent comprises of several eco-climatic zones and is a treasure house of fungal diversity. 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 rich fungal diversity world, including

wild mushrooms. Due to diverse climatic, geographical and ecological conditions, India is home to a large number of fungal species. As such, explorations in Rajasthan, Indo Gangetic Plain, Eastern Himalayan, Western Ghats, Northwest Himalayan and Southern India have been carried out by a number of workers throughout the country as hotspots of mushroom diversity, since 1825 (Borkar *et al*., 2015). These factors combined make India a potential major producer of temperate, tropical and subtropical mushroom species. There is the need to investigate the Indian mushrooms for its bioactive compounds, which can possibly be used in the treatment of various diseases like cancer, AIDS etc. (Deshmukh, 2006). The information on the food value of mushrooms in India has been provided by many workers (Hira *et al*., 1990; Murugkar and Subbulakshmi, 2005). In India, the nutritional contents of the different species of mushrooms such as *Agaricus*, *Russula*, *Lentinus*, *Pleurotus*, *Volvariella*, *Calvatia* etc were studied by various researchers (Murugkar and Subbulakshmi, 2005; Pushpa and Purushothama, 2010; Manikandan, 2011). According to Pushpa and Purushothama (2010), *A. bisporous* contains 41.06% protein, 28.38% carbohydrate, 2.12% fat, 7.01% ash and 18.23% fiber whereas Manikandan (2011) revealed that 33.48% protein, 46.17% carbohydrate, 3.1% fats, 5.7% ash and 20.9% fiber in *A. bisporous*. Similarly, the nutritional compositions of mushrooms such as *L. edodes* (32.93% protein, 47.6% carbohydrates, 3.73% fat, 5.2% ash, and 28.8% fiber), *P. ostreatus* (30.4% protein, 57.6% carbohydrate, 2.2% fat, 9.8% ash, 8.7% fiber) and *V. volvacea* (37.5% protein, 54.8% carbohydrate, 2.6% fat, 1.1% ash, 5.5% fiber) were extensively studied by Manikandan (2011). Rai (1997) discussed the status of medicinal mushrooms, their pharmacologically active principles and mechanism of action. Ajith and Janardhanan (2007) and Thekkuttuparanbil *et al.* (2007) studied the antioxidant and anti-tumor activities of mushrooms found in South India. Vishwakarma *et al*. (2011)

studied and described the medicinal properties of some medicinally important wild mushrooms of Garhwal Himalaya, Uttarakhand, namely *Ganoderma lucidum, Agaricus campestris, Coprinus comatus* etc. *Sachan et al*. (2013) documented the WEM flora of Similipal Biosphere Reserve (SBR) of Odisha, India and their uses by the indigenous tribes for medicine and food. The mushrooms identified in the SBR are native to many parts of India which was reported by several authors in the North-Eastern hills (Verma *et al*., 1995; Singh *et al*., 2007); North Western Himalayas (Atri *et al*., 1997) and Kanyakumari district (Davidson *et al*., 2012).

The North-Eastern region of India being the transitional zone between the Indian, Indo-Malayan and Indo-Chinese bio-geographical regions makes the gate way for many of India's flora and fauna. Moreover, Northeast India is one of the biodiversity hotspots and is very rich in mushroom diversity (Verma *et al*., 1995). Baruah *et al*. (1971) reported some basidiomycetous mushrooms of Sibsagar district. Gogoi and Prakash (2015) reported 138 species of mushrooms belonging to 48 genera, 23 families and 5 orders of the class Agaricomycetes under Basidiomycota from Hollongapar Gibbon Wildlife Sanctuary, Assam. Basumatary and Gogoi (2016) reported 5 wild edible macrofungi species viz., *Volveriella volvacea, Agaricus semotus, Lentinus polychrous, Stropharia semiglobata* and *Termitomyces eurrhizus* belonging to family Plutaceae, Agaricaceae, Lentinaceae, Strophariaceae and Tricholomataceae as most widely consumed among all the edible varieties available in Kokrajhar district of Assam by the ethnic community. A total of 44 macro fungal species were collected from various areas of Assam, *viz*., Nalbari, Barpeta, Jorhat, Sibasagar, Karbi Anglong, Kamrup, Golaghat, Goalpara, Tezpur, Tinsukia, Rangia and Nagaon (Parveen *et al*., 2017). Verma *et al*., 1987 described the mushroom flora of the NEH India from Manipur

and Meghalaya belonging to the family Auriculariaceae, Clavariaceae, Cantharellaceae, Tricholomataceae, Pluteaceae, Paxillaceae, Cortinariaceae, Cycoperdaceae, Sclerodermataceae of Basidiomycotina and fan-lily, Halvellaceae of Ascomycotina. Some WEM species have been reported from Manipur and Arunachal Pradesh of N.E India (Sing and Sing, 1993; Sing *et al*., 2002). In Arunachal Pradesh, different varieties of mushrooms were sold abundantly in the market (Angami *et al*., 2006).

Singh *et al.* (2008) reported that species like *Lentinus edodes*, *Lactarius piperatus*, *Auricularia auricula*, *Cantherallus cibarius*, *Schizophyllum commune*, *Boletus edulis*, *Volvariella volvacea*, *Agaricus bisporus* and *Pleurotus sajor-caju* were prevalent in Manipur areas while Talukdar (2009) reported 34 species of mushrooms from Manipur. A total of 8 WEM species namely *Auricularia delicata*, *Cantharellus cibarius*, *Lactarius volemus*, *Lentinula edodes*, *Pleurotus citrinopileatus*, *Pleurotus ostreatus*, *Schizophllum commune* and *Termitomyces eurhizus* were reported to be sold in the markets of Senapati and Kangpokpi districts of Manipur (Apshahana and Sharma, 2018). Murugkar and Subbulakshmi (2005) studied and analyzed 7 WEM commonly consumed in the Khasi Hills of Meghalaya for its nutritional properties. Eleven wild edible macro fungal species belonging to 9 genera and 8 families consumed by the Khasi tribe of Meghalaya was reported by Khaund and Joshi (2013). Kumar *et al.* (2015) documented and identified a total of 77 mushroom species from Narpuh Reserve Forest (Block-I) of Meghalaya out of which, 44 mushroom species were identified up to the species level and the remaining species were identified up to the genus level. Kalita *et al.* (2016) documented wild edible macro fungi from Shyrwat and Upper Shillong Reserve Forests of Meghalaya where a total of 22 macro fungi were collected during the rainy season. Lalrinawmi *et al.* (2017) reported a total of 27 edible mushroom

species representing 18 genera, 13 families and 7 orders growing in both soil and wood from Mizoram. Das *et al*. (2017) reported 13 wild edible macro fungi belonging to 8 families under 8 genera from forests and local markets of Tripura, India. Das (2010) reported 126 wild mushrooms from Barsey Rhododendron Sanctuary of Sikkim.

Nagaland is one of the Northeast Indian states with total geographic area of 16,579 sq km and is bounded by Myanmar in the East, Assam in the West, Arunachal Pradesh and a part of Assam in the North and Manipur in the South. It lies between 93^{o}20′ to 95^{o}15′ E and 25^{o}10′ to 27^{o}40′ N. The state fall under one agro-climatic zone of Mild Tropical Hill Zone and receives South West Monsoon rain in summer and North East monsoon rain in winter with an average recorded annual rainfall ranges between 2000-2500 mm. The temperature during summer ranges from 16-31^{o}C and drops as low as 4^{o}C during winter. Due to the current environmental issues like global warming, climate change, deforestation and unsystematic collection of wild mushrooms in forest areas, the macro fungal diversity is depleting at alarming rate and in the near future, the bio-resource will be lost.

The rich biodiversity of northeastern region of India needs extensive exploration and this applies to Nagaland too. The rich mushroom diversity of the state needs intensive exploration to widen the commercial application of mushrooms as there is immense scope for mushroom industry in the state. The nutritional and medicinal properties of mushrooms can be further exploited, development of mushroom spawns, skill development trainings, awareness campaigns etc can all lead to cultivation, conservation, commercialization and development of mushroom industry in the state. Nevertheless, all the above developments can be achieved only when the mushroom diversity of the state is known for which proper documentation, identification and characterization of the wild mushrooms available in the

state is of utmost importance. The rich mushroom diversity provides huge socio-economic potential for the state. Not much work has been initiated on mushroom research in Nagaland. Only recently, some works have been carried out on wild mushrooms of Nagaland (Ao *et al*., 2016a; Ao *et al*., 2016b). Due to absence of digital or conventional database/Herbarium on mushroom flora of the state, there is the need to explore the mushroom diversity of Nagaland. Therefore, the present study was undertaken for survey of different parts of Nagaland, India, collection, identification and documentation of mushrooms available especially the WEM. There is also no information available of any previous reports on molecular identification and characterization of any WEM species from the state. Thus, in this study, some popular WEM species were subjected to molecular characterization using ITS, 18S and 28S *rRNA* genes which generated the molecular barcodes for that particular WEM. The molecular phylogenetic studies were also carried out for the selected WEM species. Up until now, there is no information and records available on application of ISSR, RAPD and DAMD markers in genetic diversity analysis on any WEM species of Nagaland, as such, the objective of the present study was also to study the inter- and intra-specific genetic diversity of the available wild edible *Lentinus* species which is a very popular wild edible mushroom globally. Furthermore, there is very few works reported on inter- and intra-specific genetic diversity of *Lentinus* at both the national and international level using ISSR, RAPD and DAMD markers (Dwivedi *et al*., 2017). Interestingly, the edibility and medicinal properties of few *Lentinus* species had already been reported by past workers like Watling (1993) who had reported *L. tuber-regium* (Fr.) Fr. and *L. squarrosulus* Mont. as popular food sources across central Africa and Isikhuemhen *et al*. (2012) had reported that *L. squarrosulus* is available in sub-Saharan Africa and in parts of Asia. Fangkratok *et al*. (2013) had reported

Lentinus polychrous Lev. as a wild edible mushroom available in Thailand. Therefore, in this study, genetic diversity assessment was conducted for three wild edible species of *Lentinus viz*. *L. sajor-caju*, *L. squarrosulus* and *L. tigrinus*. For genetic diversity analysis, three markers were used - ISSR, RAPD and DAMD. As these primers are randomly distributed across the genome, there are huge chances of amplifying high numbers of polymorphic bands (Hamada and Kakunaga 1982; Tautz and Renz 1984; Heath *et al*., 1993; Zietkiewicz *et al*., 1994; Zhou *et al*., 1997). The main advantages of using these primers are that they are quick, highly polymorphic, randomly distributed throughout the genome and inexpensive. As the markers are PCR-based, therefore, require very small amount of template DNA and also do not require any sequence information for primer construction. The evaluation of nutritional values and antioxidant properties was also carried out for ten WEM species in this study.

Hence, the present study is an important first step towards producing a checklist of mushrooms available in Nagaland and an insight to the nutritional qualities of some popular WEM species. Keeping in mind the importance of mushrooms to humans and environment, the present work was proposed with the following objectives –

- To survey, document and identify the wild edible mushroom species found in Nagaland.
- As Nagaland has a rich diversity of mushrooms, few popular WEM species were subjected to molecular phylogenetic analysis using ITS, 18S and 28S ribosomal RNA genes.
- Genetic diversity assessment of three wild edible species of the genus *Lentinus* using ISSR, RAPD and DAMD markers (SPAR approach).

- The nutritional composition and antioxidant properties of ten popular WEM species were also determined as part of the research work.

The present research work is the first of its kind for Nagaland and will play an important role for future researchers. Globally, mushrooms are regarded as important horticultural and medicinal crop. Therefore, it is the need of the hour to popularize WEM species as sources of healthy food because presently the world is facing food insecurity and many nations are going hungry facing protein deficiency. Moreover, mushrooms are in high demand because of their medicinal and therapeutic potential for pharmaceutical applications.

Chapter - 2

Documentation of Wild Mushroom

Introduction

The fungal diversity studies have been investigated world over and the mushrooms on earth is estimated to be 140,000, yet only ~10% are known (Wasser, 2002). Mycologists continue to investigate the virgin, hidden and interesting fungal biodiversity because many macrofungi are on the verge of extinction due to anthropogenic factors and climate change (Swapana *et al*., 2008). Mushrooms belong to Kingdom Fungi and are classified under classes of Ascomycetes and Basidiomycetes. Mushrooms are macro fungi with a distinctive fruiting body such as brackets, bird's nest, jelly, boletes, corals, puffballs, stinkhorns and agarics. They are fleshy, leathery, sub-fleshy or woody and their fertile surface is found attached on lamellae or the tubes, which opens out through pores. The lamellate members are the agarics and the boletes and the polypores are the poroid members (Deshmukh, 2004). It is said that one third of global fungal population exists in India but only 50% are characterized so far (Manoharachary *et al*., 2005). It is reported that India has about 27,000 fungal species

(Cowan, 2001; Chang and Miles, 2004) and approximately 850 mushroom species recorded so far (Deshmukh, 2004). Around 2000 fungal species are reported to be edible, but less than 25 mushroom species are accepted as food globally and very few have achieved the level of commercial item. *Agaricus bisporus*, *Auricularia* species, *Pleurotus* species, *Lentinula edodes*, *Volvariella volvacea* etc. are some of the most accepted edible mushrooms globally (Bonatti *et al*., 2004). In China and Japan, the information on the applications of both edible and medicinal mushrooms have been passed down from one generation to the next but it was not so in India. Despite this shortcoming, the traditional mycological information of Indian ethnic groups has proven to be knowledgeable and extensive, which describes about 283 wild mushroom species out of 2000 edible species recorded world over (Purkayastha and Chandra, 1985).

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 rich fungal diversity world, including wild mushrooms. Due to diverse climatic, geographical and ecological conditions, India is home to a large number of fungal species. As such, explorations in Rajasthan, Indo Gangetic Plain, Eastern Himalayan, Western Ghats, Northwest Himalayan and Southern India have been carried out by a number of workers throughout the country as hotspots of mushroom diversity, since 1825 (Borkar *et al*., 2015). The North-Eastern region of India being the transitional zone between the Indian, Indo-Malayan and Indo-Chinese bio-geographical regions makes the gate way for many of India's flora and fauna. The region comprises of the states- Arunachal Pradesh, Assam, Meghalaya, Manipur, Tripura, Mizoram, Nagaland and Sikkim. Northeast India is among the top 34 global biodiversity hotspots of the world and is rich in mushroom flora. The region is known for high endemism and is an

important sub-centre for mushroom origin, the most important macrofungi. The study on the taxonomy of mushrooms are gaining momentum as mushrooms are on the verge of extinction because of the effects of climate change and habitat destruction (Verma *et al*., 1995; Bhattacharjee *et al*., 2015). Mushrooms have already been elaborately studied in the western countries, but in tropical countries like India especially the North-East India is still unexplored. So, it is high time for explorations to be carried out to conserve this valuable natural resource. Till date not much work has been initiated on mushroom research in Nagaland. The state of Nagaland is very rich in biodiversity including wild mushrooms. Only recently, some works have been initiated on wild mushrooms of Nagaland (Ao *et al*., 2016a; Ao *et al*., 2016b). Due to lack of digital or conventional database/Herbarium on mushroom flora of the state, there is the need to explore the mushroom biodiversity of Nagaland. The present study was thus, undertaken for survey of different parts of Nagaland, collection, identification and documentation of mushrooms available especially the wild edible mushrooms (WEM).

Materials and Methods

Survey Area and Sample Collection

Nagaland is one of the North-East Indian states with total geographic area of 16,579 sq km. Nagaland is bounded by Myanmar in the East, Assam in the West, Arunachal Pradesh and a part of Assam in the North and Manipur in the South (**Figure 2.1**). It lies between $93^{o}15'$ to $95^{o}15'$ E and $25^{o}10'$ to $27^{o}4'$ N. Nagaland has a typical monsoon climate. The year can be divided into 4 seasons- winter (December-February), pre-monsoon (March-April), monsoon (May-September) and retreating monsoon (October-November). The average

annual rainfall is recorded to be between 2000-2500 mm. The temperature during summer ranges from 16-34^{o}C and drops as low as 4^{o} C during winter.

The state forests types are broadly categorized into three groups based on climatic conditions and altitudes as Tropical, Sub-tropical and Temperate types which are further sub-divided into 9 major types:

1. Tropical forests - Tropical Wet Evergreen Forest, Tropical Semi- Evergreen Forest, Tropical Moist Evergreen Forest (up to1000m).
2. Sub-Tropical Forests - Sub-Tropical Evergreen Broadleaved Forests, Sub-Tropical Mixed Deciduous Broadleaved Forests, Sub- Tropical Alpine Forests (between 1000-1800m).
3. Temperate Forests – Montane Wet Temperate Forests, Temperate Rhododendron Forests, Sub- Alpine Forests (between 1800-3848m).

Regular explorations and collections were undertaken in forest areas of Dimapur, Longleng, Kohima, Mokokchung, Phek, Tuensang, Wokha and Zunheboto districts of Nagaland, India during each season i.e., winter, spring, summer and autumn. Mushrooms samples were collected on site and morphological characters like texture and fruiting body color; size, shape and color of the stipe, pileus and lamellae position and color; presence of annulus; color of spore print, type of volva, its habitat, host and location were recorded prior to preservation of the collected mushroom. The local markets were surveyed during the season of availability to gather knowledge on the wild mushroom species sold (Ao *et al*., 2016a; Ao *et al*., 2016b).

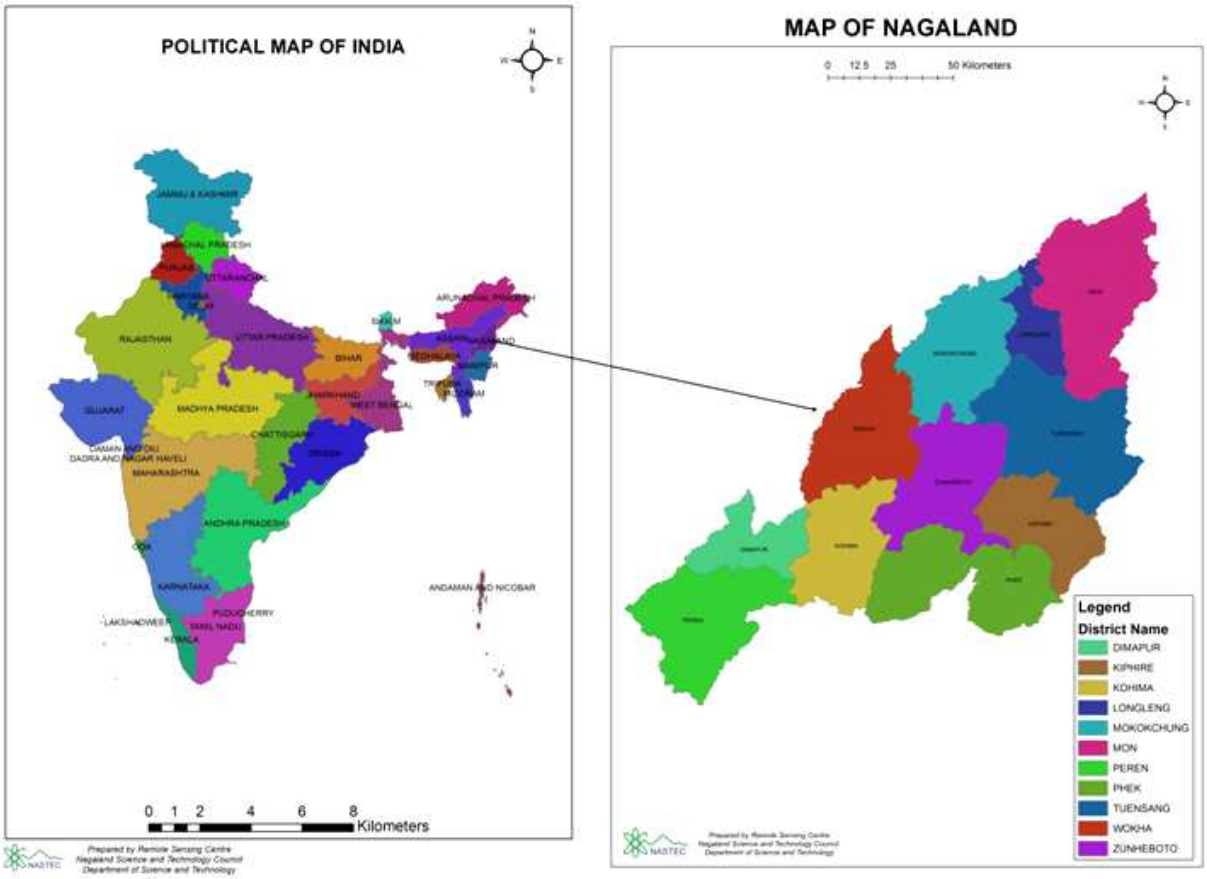


Figure 2.1: shows the survey area for sample collection

Phenotypic identification of the collected mushroom specimen was done based on morphological parameters mentioned above. The specimens were maintained in formaldehyde solution (2%, v/v) and stored as herbarium for future references. All the voucher specimens were deposited in the herbarium of Department of Botany, Nagaland University, Lumami, India. For identification of mushrooms, standard manuals and keys were consulted (Crawshay, 1930; Christensen, 1943; Pegler, 1983; Roy and De, 1996; Das and Sharma, 2005; Das, 2009; Mortimer *et al*., 2014; Philips, 2006). Mycokeys at www.mushroomexpert.com and www.mycokey.com were also consulted for identification.

Results

Nagaland has a forest cover of 12,489 sq. km approx. which is 75.33% of the state's geographical area based on the data released by India State of Forest Report, 2017. Due to various anthropogenic factors, forest cover is declining in the state. This affects the survivability of biodiversity present. Wild mushrooms were collected in forest areas of Dimapur, Longleng, Kohima, Mokokchung, Phek, Tuensang, Wokha and Zunheboto covering an altitudinal range of 150 m ASL to 3000 m ASL. The GPS coordinates were recorded during field collections and is given in **Annexure I**. The mushroom populations decreased in number as the altitudinal range increased. The highest number of mushroom species was collected between the altitudinal range of 900 m and 1400 m ASL.

A total of 141 mushroom species belonging to 80 genera under 44 families – Russulaceae, Hygrophoraceae, Amanitaceae, Polyporaceae, Boletaceae, Agaricaceae, Lyophyllaceae, Auriculariaceae, Dacrymycetaceae, Inocybaceae, Suillaceae, Ganodermataceae, Tricholomataceae, Clavariaceae, Pleurotaceae, Tremellaceae, Omphalotaceae, Hericiaceae, Hymenochaetaceae, Psathyrellaceae, Cortinariaceae,

Sclerodermataceae, Hydnangiaceae, Mycenaceae, Strophariaceae, Gomphaceae, Geastraceae, Phallaceae, Pyronemataceae, Leotiaceae, Xylariaceae, Clavicipitaceae, Schizophyllaceae, Nidulariaceae, Sparassidaceae, Physalacriaceae, Exidiaceae, Bulgariaceae, Entolomataceae, Hymenogastraceae, Clavulinaceae, Helvellaceae, Sarcoscyphaceae and Cantharellaceae have been identified correctly in the present study (**Table 2.1, Figure 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 2.10, 2.11, 2.12, 2.13, 2.14, 2.15, 2.16, 2.17**). Out of 141 mushroom species, only 12 species belonged to Ascomycetes class and the remaining mushrooms belonged to Basidiomycetes class. Thus, the field surveys revealed that the Basidiomycetes mushroom dominates over Ascomycetes mushroom. For identification, morphological observations were performed as described above and spores were collected through spore prints and observed under microscope. A total of 52 mushroom species were identified as edible, 10 mushrooms were found to be poisonous and the remaining 79 mushrooms were found to be inedible. *Schizophyllum commune*, *Auricularia auricula-judae*, *A. polytricha*, *Lactifluus piperatus*, *L. volemus*, *Sceleroderma citrinum*, *Termitomyces heimii*, *Tremella fuciformis*, *Tricholoma imbricatum*, *Hygrocybe conica* and *Agaricus moelleri* was found to be the most abundant during the season of availability. The collected mushroom species were predominantly found to be parasitic, saprophytic and ecto-mycorrhizal in habitat.

Table 2.1: List of wild mushrooms collected and identified during the study

Mushroom species	Family	Habitat	Edibility	Accession No.
Auricularia auricula-judae (Bull.) Quél.	Auriculariaceae	On dead stumps and branches of sub-tropical and temperate trees especially *Alnus*. Grows on both dead and living trees, very common.	Edible	NUBOT-TA-AA-01
Auricularia polytricha (Mont.) Sacc.	Auriculariaceae	In clusters on rotten or dead and decaying stumps and twigs, very common.	Edible	NUBOT-TA-AP-02
Cantharellus cibarius (Fr.)	Cantharellaceae	Found under *Lithocarpus* in sub-tropical forests, frequent.	Edible	NUBOT-TA-CC-03
Lactifluus piperatus (L.) Pers.	Russulaceae	Under sub-tropical semi-evergreen forests, common.	Edible	NUBOT-TA-LP-04
Lactifluus volemus (Fr.) Kuntze	Russulaceae	Under sub-tropical semi-evergreen forests including pine, common.	Edible	NUBOT-TA-LV-05
Lentinula edodes (Berk.) Pegler	Omphalotaceae	On trunks of Oak trees, common.	Edible	NUBOT-TA-LE-06
Hericium cirrhatum (Pers.) Nikol.	Hericiaceae	On trunks of semi-evergreen and temperate trees, uncommon, vulnerable on Red Data List.	Edible	NUBOT-TA-HC-07
Dacryopinax spathularia (Schwein) G. W. Martin	Dacrymycetaceae	On dead and decaying logs in large groups, common.	Edible	NUBOT-TA-DS-08
Schizophyllum commune Fr.	Schizophyllaceae	On branches of dead wood and cut timber, very common.	Edible	NUBOT-TA-SC-09
Rusulla senecis S. Imai	Russulaceae	Found under *Lithocarpus* and *Castanopsis* in sub-tropical forests, frequent.	Inedible	NUBOT-TA-RS-10
Russsula natarajanii K. Das, J.R. Sharma & Atri	Russulaceae	Found under *Lithocarpus* and *Castanopsis* in sub-tropical forests, occasional.	Inedible	NUBOT-TA-RN-11
Strobilomyces strobilaceus. (Scop.) Berk	Boletaceae	Grows in association with semi-evergreen and coniferous trees, uncommon.	Edible	NUBOT-TA-SS-12
Pcynoporus cinnabarinus (Jacq.) P. Karst.	Polyporaceae	Grows on fallen, dead and decaying stumps of trees like *Alnus*, common.	Inedible	NUBOT-TA-PC-13
Microporus xanthopus (Fr.) Kuntze	Polyporaceae	Grows on fallen branches and twigs, very common.	Inedible	NUBOT-TA-MX-14
Hymenochaete rubiginosa (Dicks.) Lev.	Hymenochaetaceae	On old rotting tree stumps, uncommon.	Inedible	NUBOT-TA-HR-15
Trametes gibbosa (Pers.) Fr.	Polyporaceae	On dead tree stumps, common.	Inedible	NUBOT-TA-TG-16
Trametes hirsuta (Wilfen) Pilat	Polyporaceae	On dead tree stumps, common.	Inedible	NUBOT-TA-TH-17

Coprinus disseminatus (Pers.) J.E.Lange	Psathyrellaceae	In troops around the stumps of dead, decaying wood, frequent.	Inedible	NUBOT-TA-CD-18
Amanita strobiliformis (Paulet ex Vittad.) Bertill	Amanitaceae	Under sub-tropical semi-evergreen forest trees, uncommon.	Edible	NUBOT-TA-AS-19
*Ganoderma lucidum (*Curtis) P. Karst.	Ganodermataceae	On trunks and roots of *Quercus* species, uncommon.	Inedible	NUBOT-TA-GL-20
Daldinia concentrica (Bolton) Cesati & de Notaris	Xylariaceae	On dead wood logs, frequent.	Inedible	NUBOT-TA-DC-21
Boletus edulis Bull.	Boletaceae	Under coniferous and semi-evergreen forest types, occasional.	Edible	NUBOT-TA-BE-22
Cortinarius purpurascens Fr.	Cortinariaceae	Grows in association with semi-evergreen and coniferous woods, occasional.	Inedible	NUBOT-TA-CP-23
Scleroderma citrinum Pers.	Sclerodermataceae	On mossy or peaty ground in any forest type, common.	Inedible	NUBOT-TA-SC-24
Calocera viscosa (Pers.) Fr.	Dacrymycetaceae	Grows on dead stumps in temperate evergreen forests, uncommon.	Inedible	NUBOT-TA-CV-25
Cordyceps militaris (L.) Fr.	Clavicipitaceae	Grows singly on ground after parasitizing on the larvae or pupae of butterflies and moths, frequent.	Inedible	NUBOT-TA-CM-26
Tricholoma imbricatum (Fr.) P. Kumm.	Tricholomataceae	In coniferous woods, especially with pine, frequent.	Edible	NUBOT-TA-TI-27
Pleurotus pulmonarius (Fr.) Quél.	Pleurotaceae	In clusters on cut timber and fallen logs, frequent.	Edible	NUBOT-TA-PP-28
Crepidotus mollis (Schaeff.) Staude	Inocybaceae	In groups or overlapping tiers on fallen branches and tree trunks, common.	Inedible	NUBOT-TA-CM-29
Crucibulum laeve (Huds.) Kambly	Nidulariaceae	On twigs, fallen branches and other vegetable remains, frequent.	Inedible	NUBOT-TA-CL-30
Auricularia mesenterica (Dicks.) Pers.	Auriculariaceae	On dead stumps and wood logs, frequent.	Inedible	NUBOT-TA-AM-31
Leotia lubrica (Scop.) Pers.	Leotiaceae	In damp areas in almost all forest types, occasional.	Inedible	NUBOT-TA-LL-32
Amanita vaginata (Bull.) Lam.	Amanitaceae	Grows singly or numerous in all forest types including coniferous, common.	Inedible	NUBOT-TA-AV-33
Clavulinopsis fusiformis (Sowerby) Corner.	Clavariaceae	Grows in tufts on ground amongst grasses and leaf litters, frequent.	Inedible	NUBOT-TA-CF-34
Clavaria fragilis Holmsk.	Clavariaceae	Grows in clusters on ground amongst leaf litters and in fields, common.	Edible	NUBOT-TA-CF-35

Russula cyanoxantha (Schaeff.) Fr.	Russulaceae	Found under *Lithocarpus* and *Castanopsis* in sub-tropical forests, frequent.	Inedible	NUBOT-TA-RC-36
Tremella fuciformis Berk.	Tremellaceae	On dead or fallen branches of broadleaved trees, common.	Edible	NUBOT-TA-TF-37
Trametes versicolor (L.) Lloyd	Polyporaceae	On dead and decaying tree stumps, frequent.	Inedible	NUBOT-TA-TV-38
Amanita phalloides (Vaill. ex Fr.) Link	Amanitaceae	Under sub-tropical semi-evergreen forests, common.	Poisonous	NUBOT-TA-AP-39
Lentinus squarrosulus Mont. Singer	Polyporaceae	On dead stumps of trees like Oak, frequent.	Edible	NUBOT-TA-LS-40
Hygrocybe conica (Schaeff.) P. Kumm.	Hygrophoraceae	In grass fields after fresh burning of forest, frequent.	Edible	NUBOT-TA-HC-41
Russula fragilis Fr.	Russulaceae	Found under *Lithocarpus* and *Castanopsis* in sub-tropical forests and conifers, common.	Inedible	NUBOT-TA-RF-42
Russula nobilis Velen	Russulaceae	Found under *Lithocarpus* and *Castanopsis* in sub-tropical forests, common.	Poisonous	NUBOT-TA-RN-43
Russula heterophylla (Fr.) Fr.	Russulaceae	Found under *Lithocarpus* and *Castanopsis* in sub-tropical forests, occasional.	Edible	NUBOT-TA-RH-44
Amanita cokeri E.-J. Gilbert & Kühner ex E.-J. Gilbert	Amanitaceae	Under sub-tropical semi-evergreen forests, uncommon.	Poisonous	NUBOT-TA-AC-45
Suillus luteus (L.) Roussel	Suillaceae	Under coniferous especially pine, frequent.	Edible	NUBOT-TA-SL-46
Hygrocybe vitellina (Fr.) P. Karst.	Hygrophoraceae	Amongst damp moss, frequent.	Inedible	NUBOT-TA-HV-47
Xerocomellus chrysenteron (Bull.) Šutara	Boletaceae	Under sub-tropical semi-evergreen forests including pine, frequent.	Edible	NUBOT-TA-XC-48
Suillus pictus (Peck) A.H. Sm. & Thiers	Suillaceae	Under sub-tropical semi-evergreen forests, frequent.	Edible	NUBOT-TA-SP-49
Lichenomphalia umbellifera (L.) Redhead, Lutzoni, Moncalvo & Vilgalys	Hygrophoraceae	On fallen twigs in moist woods, common.	Inedible	NUBOT-TA-LU-50
Laccaria tortilis (Bolton) Cooke	Hydnangiaceae	On bare soil in damp woods, common.	Edible	NUBOT-TA-LT-51
Dacrymyces stillatus Nees	Dacrymycetaceae	On damp decaying wood, frequent.	Inedible	NUBOT-TA-DS-52
Aureoboletus auriporus (Peck) Pouzar	Boletaceae	Under sub-tropical semi-evergreen forests, frequent.	Inedible	NUBOT-TA-AA-53
Crepidotus applanatus (Pers.) P. Kumm.	Inocybaceae	On fallen branches and twigs in semi-evergreen forests, occasional.	Inedible	NUBOT-TA-CA-54
Mycena erubescens Höhn.	Mycenaceae	Under semi-evergreen forests including pine, occasional.	Inedible	NUBOT-TA-ME-55

Crepidotus luteolus Sacc.	Inocybaceae	Grows on fallen branches and twigs in semi-evergreen forests, occasional.	Inedible	NUBOT-TA-CL-56
Lepiota cristata (Bolton) P. Kumm.	Agaricaceae	Found in woods and leaf litter, very common.	Inedible	NUBOT-TA-LC-57
Hypholoma capnoides (Fr.) P. Kumm.	Strophariaceae	Under sub-tropical semi-evergreen forests, common.	Inedible	NUBOT-TA-HC-58
Agaricus moelleri Wasser	Agaricaceae	Under sub-tropical semi-evergreen forests, occasional.	Poisonous	NUBOT-TA-AM-59
Amanita fulva Fr.	Amanitaceae	Under *Castanopsis* and *Lithocarpus* species in sub-tropical forests, common.	Inedible	NUBOT-TA-AF-60
Melanoleuca grammopodia (Bull.) Murrill	Tricholomataceae	Grows on leaf mulch or composted soil in fields, common.	Edible	NUBOT-TA-MG-61
Aleuria aurantia (Pers.) Fuckel	Pyronemataceae	Grows in groups on soil amongst grasses or on bare soil or at roadside, common.	Edible	NUBOT-TA-AA-62
Macrolepiota albuminosa (Berk.) Pegler	Agaricaceae	Grows on termite mounds in grassy fields, common.	Edible	NUBOT-TA-MA-63
Termitomyces heimii Natarajan	Lyophyllaceae	Grows on termite mounds and clayey soil, common.	Edible	NUBOT-TA-TH-64
Pleurotus ostreatus (Jacq) P. Kumm	Pleurotaceae	Grows in clusters on dead or leaving trees, common.	Edible	NUBOT-TA-PO-65
Amanita rubrovolvota S. Imai	Amanitaceae	Grows in association with *Castanopsis*, *Lithocarpus and Rhododendron*, *Quercus*, etc, occasional.	Inedible	NUBOT-TA-AR-66
Ramaria stricta (Pers.) Quél	Gomphaceae	Grows on ground in association with dead tree stumps, frequent, Vulnerable on Red Data List.	Inedible	NUBOT-TA-RS-67
Russula sp.	Russulaceae	Found with deciduous and broad-leaved trees, frequent.	Inedible	NUBOT-TA-R-68
Lentinus tigrinus (Bull.) Fr.	Polyporaceae	Grows on dead stumps of trees like Oak, frequent.	Edible	NUBOT-TA-LT-69
Boletus sp.	Boletaceae	Under *Castanopsis* and *Lithocarpus* in sub-tropical forests	Inedible	NUBOT-TA-B-70
Termitomyces eurhizus (Berk.) R. Heim	Lyophyllaceae	Grows in groups on ground near termite mounts, common.	Edible	NUBOT-TA-TE-71
Lycoperdon perlatum Pers.	Agaricaceae	Grows in fields, roadsides, in woods and amongst fallen leaf litter in mixed wood and conifers, common.	Edible when young and white in color	NUBOT-TA-LP-72

Laetiporus sulphureus (Bull.) Murr.	Polyporaceae	Grows on dead stumps as well as living tree trunk of hardwoods and oaks, uncommon.	Edible	NUBOT-TA-LS-73
Coprinus comatus (O.F. Müll.) Pers.	Agaricaceae	Grows singly or in clusters or lines on lawns, wood pieces or on ground, common.	Edible	NUBOT-TA-CC-74
Pleurotus citrinopileatus Singer	Pleurotaceae	In clusters on cut timber and fallen logs, frequent.	Edible	NUBOT-TA-PC-75
Termitomyces microcarpus (Berk. & Broome) R. Heim	Lyophyllaceae	Grows in groups on termite mounts, frequent.	Edible	NUBOT-TA-TM-76
Termitomyces sp.	Lyophyllaceae	Grows in groups on termite mounts, frequent.	Edible	NUBOT-TA-T-77
Hygrocybe miniata (Fr.) P. Kumm.	Hygrophoraceae	Grows in fields, woods or grassy fields, common.	Inedible	NUBOT-TA-HM-78
Gloioxanthomyces nitidus (Berk. & M.A. Curtis) Lodge, Vizzini, Ercole & Boertm.	Hygrophoraceae	Grows in clusters in woods and damp soils, common.	Inedible	NUBOT-TA-GN-79
Ganoderma applanatum (Pers.) Pat.	Ganodermataceae	On trunks and roots of trees, occasional.	Inedible	NUBOT-TA-GA-80
Amanita virosa (Fr.) Bertill.	Amanitaceae	In mixed forests, occasional.	Poisonous	NUBOT-TA-AV-81
Geastrum sp.	Geastraceae	In coniferous forests, occasional.	Inedible	NUBOT-TA-G-82
Lactifluus glaucescens (Crossl.) Verbeken	Russulaceae	Under *Quercus* and *Lithocarpus* in sub-tropical forests, frequent.	Inedible	NUBOT-TA-LG-83
Phallus indusiatus Vent.	Phallaceae	Grows singly on soil in woods, common.	Unknown	NUBOT-TA-PI-84
*Hygrocybe cantharellus (*Schwein.) Murrill	Hygrophoraceae	Grows in damp soils and moss, frequent.	Inedible	NUBOT-TA-HC-85
Tremella mesenterica Retz.	Tremellaceae	Grows on dead woods, common.	Inedible	NUBOT-TA-TM-86
Lentinus sajor-caju (Fr.) Fr.	Polyporaceae	Grows on dead stumps of trees like Oak, common.	Edible	NUBOT-TA-LS-87
Suillus sp.	Suillaceae	Under *Castanopsis* and *Lithocarpus* in sub-tropical forests	Inedible	NUBOT-TA-S-88
Lycoperdon pyriforme Schaeff.	Agaricaceae	Grows on deadwood of hardwoods or conifers, found scattered or in dense clusters, occasional.	Edible	NUBOT-TA-LP-89
Helvella crispa (Scop.) Fr.	Helvellaceae	Found in deciduous woods, uncommon.	Poisonous	NUBOT-TA-HC-90

Otidea alutacea (Pers.) Massee	Pyronemataceae	Found in clusters on ground in woods, uncommon.	Inedible	NUBOT-TA-OA-91
Sparassis crispa (Wulfen) Fr.	Sparassidaceae	Found at the base of tree trunks, uncommon.	Edible	NUBOT-TA-SC-92
Cookeina tricholoma (Mont.) Kuntze	Sarcoscyphaceae	Found in clusters on trunks or stumps with deciduous or coniferous trees, uncommon.	Inedible	NUBOT-TA-CT-93
Clavulina coralloides (L.) J. Schröt.	Clavulinaceae	Found with deciduous or coniferous trees, uncommon.	Inedible	NUBOT-TA-CC-94
Agaricus augustus Fr.	Agaricaceae	Found with deciduous or coniferous trees, occasional.	Edible	NUBOT-TA-AA-95
Hypholoma fasciculare (Huds.) P.Kumm.	Hymenogastraceae	Found in dense clusters on trunks or stumps with deciduous or coniferous trees, common.	Inedible	NUBOT-TA-HF-96
Flammulina velutipes (Curtis) Singer	Physalacriaceae	Found to grow in clusters on decaying trees, survive cold weather, common	Edible	NUBOT-TA-FV-97
Mycena inclinata (Fr.) Quél.	Mycenaceae	Found in dense tufts or clusters on tree stumps, common.	Inedible	NUBOT-TA-MI-98
Hemimycena lactea (Pers.) Singer	Mycenaceae	Found in large groups in forest, occasional.	Inedible	NUBOT-TA-HL-99
Russula rosea Pers.	Russulaceae	Found near deciduous trees like beech, frequent.	Inedible	NUBOT-TA-RR-100
Russula ochroleuca Fr.	Russulaceae	Found with conifers, very common	Edible	NUBOT-TA-RO-101
Russula puellaris Fr.	Russulaceae	Found with conifers, frequent.	Edible	NUBOT-TA-RP-102
Lepiota felina (Pers.) P. Karst.	Agaricaceae	Found with conifers, uncommon.	Poisonous	NUBOT-TA-LF-103
Leucocoprinus birnbaumii (Corda) Singer	Agaricaceae	Found in tropical areas, uncommon.	Inedible	NUBOT-TA-LB-104
Lepiota lilacea Bres.	Agaricaceae	Found with deciduous and conifers, uncommon.	Poisonous	NUBOT-TA-LL-105
Leucocoprinus brebissonii (Godey) Locq.	Agaricaceae	Found in deciduous woods and flowerpots, occasional.	Inedible	NUBOT-TA-LB-106
Leucocoprinus fragilissimus (Berk. & M. A. Curtis) Pat.	Agaricaceae	Found singly or scattered, in humus or litter, in woods, frequent.	Inedible	NUBOT-TA-LF-107
Scleroderma areolatum Ehrenb.	Sclerodermataceae	Found in moist places on ground under sub-tropical areas, uncommon.	Inedible	NUBOT-TA-SA-108
Laccaria laccata (Scop.) Cooke	Hydnangiaceae	Found in troops in woods, common.	Edible	NUBOT-TA-LL-109
Xerula radicata (Relhan) Dörfelt	Physalacriaceae	Found under grasses or leaf debris, common.	Inedible	NUBOT-TA-XR-110
Mycena galopus (Pers.) P. Kumm.	Mycenaceae	Found under leaf litter, dead and old tree stumps in woods, common.	Inedible	NUBOT-TA-MG-111

Lactarius deliciosus (L.) Gray	Russulaceae	Found under sub-tropical semi-evergreen forests including pine, frequent.	Edible	NUBOT-TA-LD-112
Pholiota aurivella (Batsch) P. Kumm.	Strophariaceae	Found in clusters on living and dead tree stumps of conifers and hardwoods, occasional.	Inedible	NUBOT-TA-PA-113
Lactarius deterrimus Gröger	Russulaceae	Found under sub-tropical semi-evergreen forests, frequent.	Edible	NUBOT-TA-LD-114
Arrhenia onisca (Fr.) Redhead, Lutzoni, Moncalvo & Vilgalys	Tricholomataceae	Found scattered in small groups on ground in litter or moss, occasional.	Inedible	NUBOT-TA-AO-115
Tremella foliacea Pers.	Tremellaceae	Found on dead and decaying logs like *Lithocarpus*, uncommon.	Inedible	NUBOT-TA-TF-116
Octospora rutilans (Fr.) Dennis & Itzerott	Pyronemataceae	Found in clusters amongst mosses, uncommon.	Inedible	NUBOT-TA-OR-117
Coprinellus micaceus (Bull.) Vilgalys, Hopple & Jacq. Johnson	Psathyrellaceae	Found in clusters in decaying woods, common.	Edible	NUBOT-TA-CM-118
Entoloma murrayi (Berk. & M.A.Curtis) Sacc.	Entolomataceae	Found singly or in small groups on ground in litter or humus with conifers and hardwoods, uncommon.	Inedible	NUBOT-TA-EM-119
Psilocybe semilanceata (Fr.) P. Kumm.	Strophariaceae	Found in dense clusters in woods, common, hallucinogenic, common.	Inedible	NUBOT-TA-PS-120
Bulgaria inquinans (Pers.) Fr.	Bulgariaceae	Found in clusters on fallen trunks like *Lithocarpus*, uncommon.	Inedible	NUBOT-TA-BI-121
Xylaria hypoxylon (L.) Grev.	Xylariaceae	Found in groups under dead woods, frequent.	Inedible	NUBOT-TA-XH-122
Xylaria polymorpha (Pers.) Grev.	Xylariaceae	Found in groups under dead or decaying woods, frequent.	Inedible	NUBOT-TA-XP-123
Pseudohydnum gelatinosum (Scop.) P. Karst.	Exidiaceae	Found under rotting and decaying tree stumps, common.	Edible	NUBOT-TA-PG-124
Phallus impudicus L.	Phallaceae	Found singly or scattered associated with rotting wood in gardens and woods, common.	Inedible	NUBOT-TA-PI-125
Pholiota highlandensis (Peck) Quadr. & Lunghini	Strophariaceae	Found on ground among debris in forests, occasional.	Inedible	NUBOT-TA-PH-126
Armillaria mellea (Vahl) P. Kumm.	Physalacriaceae	Found in dense clusters on or around tree trunks or stumps with deciduous or coniferous trees, common.	Edible	NUBOT-TA-AM-127

Amanita flavoconia G.F. Atk.	Amanitaceae	Found to grow with oaks and other hardwoods including conifers; growing alone or scattered, uncommon.	Poisonous	NUBOT-TA-AF-128
Dacrymyces chrysospermus Berk. & M.A. Curtis	Dacrymycetaceae	Found under coniferous woods, uncommon.	Inedible	NUBOT-TA-DC-129
Lepista nuda (Bull.) Cooke	Tricholomataceae	Found to grow under hardwoods even in gardens, common.	Edible	NUBOT-TA-LN-130
Cerioporus leptocephalus (Jacq.) Zmitr.	Polyporaceae	Found under dead and decaying tree stumps, common.	Inedible	NUBOT-TA-CL-131
Hericium erinaceus (Bull.) Pers.	Hericiaceae	Found on living deciduous trees, occasional, vulnerable on Red Data List.	Edible	NUBOT-TA-HE-132
Crepidotus variabilis (Pers.) P. Kumm.	Inocybaceae	Found in groups on fallen tree trunks, twigs and dead stumps, frequent.	Inedible	NUBOT-TA-CV-133
Psathyrella piluliformis (Bull.) P.D. Orton	Psathyrellaceae	Found in groups under dead or decaying stumps in sub-tropical forests, common.	Inedible	NUBOT-TA-PP-134
Sarcoscypha sp.	Sarcoscyphaceae	Found on dead tree trunks and old stems, uncommon	Inedible	NUBOT-TA-S-135
Lactarius subdulcis (Pers.) Gray	Russulaceae	Found in woods mostly near broad leaved trees, common	Edible	NUBOT-TA-LS-136
Marasmiellus ramealis (Bull.) Singer	Omphalotaceae	Found on old stems, broken twigs, common.	Inedible	NUBOT-TA-MR-137
Marasmiellus candidus (Fr.) Singer	Omphalotaceae	Found on fallen branches, amongst leaf litter, uncommon	Inedible	NUBOT-TA-MC-138
Xeromphalina kauffmanii A.H. Sm.	Mycenaceae	Found in clusters on coniferous stumps and logs, frequent.	Inedible	NUBOT-TA-XK-139
Agaricus xanthodermus Genev.	Agaricaceae	Found with deciduous or coniferous trees, meadows or gardens, occasional, vulnerable on Red Data List.	Poisonous	NUBOT-TA-AX-140
Ductifera sucina (Möller) K.Wells	*Incertae sedis*	Found on decayed wood of hardwoods and old stems, uncommon.	Inedible	NUBOT-TA-DS-141

Figure 2.2: Wild edible mushrooms of Nagaland: 1. *Aleuria aurantia*, 2. *Auricularia polytricha*, 3. *Auricularia auricula judae*, 4. *Agaricus augustus*, 5. *Amanita strobiliformis*, 6. *Armillaria mellea*, 7. *Boletus edulis*, 8. *Cantharellus cibarius*, 9. *Clavaria fragilis*.

Figure 2.3: Wild edible mushrooms of Nagaland: 10. *Coprinus comatus*, 11. *Coprinellus micaceus*, 12. *Dacryopinax spathularia*, 13. *Flammulina velutipes*, 14. *Hericium cirrhatum*, 15. *Hygrocybe conica*, 16. *Hericium erinaceus*, 17. *Lentinula edodes*, 18. *Lycoperdon perlatum*.

Figure 2.4: Wild edible mushrooms of Nagaland: 19. *Lactifluus piperatus*, 20. *Lentinus sajor-caju*, 21. *Lentinus squarrosulus*, 22. *Lentinus tigrinus*, 23. *Lactifluus volemus*, 24. *Laccaria tortilis*, 25. *Laetiporus sulphureus*, 26. *Laccaria laccata*, 27. *Lactarius deliciosus*.

Figure 2.5: Wild edible mushrooms of Nagaland: 28. *Lactarius deterrimus*, 29. *Lactarius subdulcis*, 30. *Lepista nuda*, 31. *Lycoperdon pyriforme*, 32. *Macrolepiota albuminosa*, 33. *Melanoleuca grammopodia*, 34. *Pleurotus citrinopileatus*, 35. *Pleurotus ostreatus*, 36. *Pleurotus pulmonarius*.

Figure 2.6: Wild edible mushrooms of Nagaland: 37. *Pseudohydnum gelatinosum*, 38. *Russula ochroleuca*, 39. *Russula puellaris*, 40. *Russula heterophylla*, 41. *Schizophyllum commune*, 42. *Suillus luteus*, 43. *Strobilomyces strobilaceus*, 44. *Sparassis crispa*, 45. *Suillus pictus*.

Figure 2.7: Wild mushrooms of Nagaland (Edible - 46-52; Inedible - 53-54): 46. *Tremella fuciformis*, 47. *Termitomyces heimii*, 48. *Termitomyces eurrhizus*, 49. *Termitomyces microcarpus*, 50. *Termitomyces* sp., 51. *Tricholoma imbricatum*, 52. *Xerocomellus chrysenteron*, 53. *Aureoboletus auriporus*, 54. *Amanita fulva*.

Figure 2.8: Wild mushrooms of Nagaland: 55. *Amanita rubrovolvata*, 56. *Amanita vaginata*, 57. *Auricularia mesenterica*, 58. *Arrhenia onisca*, 59. *Boletus* sp., 60. *Bulgaria inquinans*, 61. *Crepidotus applanatus*, 62. *Coprinus disseminatus*, 63. *Clavulinopsis fusiformis*.

Figure 2.9: Wild mushrooms of Nagaland: 64. *Crucibulum laeve*, 65. *Crepidotus luteolus*, 66. *Cordyceps militaris*, 67. *Cortinarius purpurascens*, 68. *Calocera viscosa*, 69. *Cerioporus leptocephalus*, 70. *Clavulina coralloides*, 71. *Cookeina tricholoma*, 72. *Crepidotus mollis*

Figure 2.10: Wild mushrooms of Nagaland: 73. *Crepidotus variabilis*, 74. *Dacrymyces stillatus*, 75. *Dacrymyces chrysospermus*, 76. *Daldinia concentrica*, 77. *Entoloma murrayi*, 78. *Ganoderma applanatum*, 79. *Ganoderma lucidum*, 80. *Gloioxanthomyces nitidus*, 81. *Geastrum* sp.

Figure 2.11: Wild mushrooms of Nagaland: 82. *Hypholoma capnoides*, 83. *Hygrocybe miniata*, 84. *Hymenochaete rubiginosa*. 85. *Hygrocybe vitellina*, 86. *Hemimycena lactea*, 87. *Hygrocybe cantharellus*, 88. *Hypholoma fasciculare*, 89. *Lepiota cristata*, 90. *Lichenomphalia umbellifera*.

Figure 2.12: Wild mushrooms of Nagaland: 91. *Lactifluus glaucescens*, 92. *Leotia lubrica*, 93. *Leucocoprinus birnbaumii*, 94. *Leucocoprinus brebissonii*, 95. *Leucocoprinus fragilissimus*, 96. *Marasmiellus candidus*, 97. *Marasmiellus ramealis*, 98. *Microporus xanthopus*, 99. *Mycena erubescens*.

Figure 2.13: Wild mushrooms of Nagaland: 100. *Mycena galopus*, 101. *Mycena inclinata*, 102. *Octospora rutilans*, 103. *Otidea alutacea*, 104. *Pcynoporus cinnabarinus*, 105. *Phallus indusiatus*, 106. *Phallus impudicus*, 107. *Pholiota aurivella*, 108. *Pholiota highlandensis*.

Figure 2.14: Wild mushrooms of Nagaland: 109. *Psathyrella piluliformis*, 110. *Psilocybe semilanceata*, 111. *Russula cyanoxantha*, 112. *Russula fragilis*, 113. *Russula natarajanii*, 114. *Russula senecis*, 115. *Ramaria stricta*, 116. *Russula rosea*, 117. *Russula* sp.

Figure 2.15: Wild mushrooms of Nagaland: 118. *Scleroderma citrinum*, 119. *Sarcoscypha* sp., 120. *Scleroderma areolatum*, 121. *Suillus* sp., 122. *Trametes gibbosa*, 123. *Trametes hirsuta*, 124. *Tremella mesenterica*, 125. *Trametes versicolor*, 126. *Tremella foliacea*.

Figure 2.16: Wild mushrooms of Nagaland (Inedible - 127-130; Poisonous - 131-135): 127. *Xeromphalina kauffmanii*, 128. *Xerula radicata*, 129. *Xylaria hypoxylon*, 130. *Xylaria polymorpha*, 131. *Amanita cokeri*, 132. *Agaricus moelleri*, 133. *Amanita phalloides*, 134. *Amanita virosa*, 135. *Agaricus xanthodermus*.

Figure 2.17: Wild mushrooms of Nagaland (Poisonous - 136-140, Inedible- 141): 136. *Amanita flavoconia*, 137. *Helvella crispa*, 138. *Lepiota felina*, 139. *Lepiota lilacea*, 140. *Russula nobilis*, 141. *Ductifera sucina*.

Table 2.2: Price of some popular wild edible mushrooms sold in local markets of Nagaland

Mushroom Species	Avg. Approx. Rate per Plate/Packet (INR)
Schizophyllum commune	Dried- 150 Fresh- 50
***Pleurotus* species**	Fresh- 200
***Agaricus* species**	Fresh- 150
***Termitomyces* species**	Fresh- 100
***Russula* species**	Fresh- 100
***Auricularia* species**	Dried- 100 Fresh- 50
***Lactarius* species**	Fresh- 100
***Lentinus* species**	Fresh- 100
***Lentinula* species**	Dried- 250 Fresh- 100
***Hygrocybe* species**	Fresh- 100

Source: Survey of local markets in Nagaland on the present study

Figure 2.18: Photo plate shows WEM species sold at local markets of Nagaland (Ao *et al*., 2016a).

Wild edible mushrooms (WEM) are highly coveted food resource in Nagaland. During the mushrooming season, the rural population throughout the state collects the mushrooms from the forest areas. Market surveys revealed the popularity and demand of WEM. The WEM are sold at the local markets at a price range of 50-250 INR per packet (**Table 2.2 and Figure 2.18**). They are sold both in fresh as well as dry form. The local people prepare soups, chutney, salads and various side dishes from mushrooms. *Lentinula edodes, Lentinus squarrosulus, L. sajor-caju, L. tigrinus, Lactarius volemus, S. commune, T. heimii, T. microcarpus, T. eurhizus, A. auricula-judae, A. polytricha, Pleurotus ostreatus, P. pulmonarius* etc are among the most popularly sold WEM species in the local markets of Nagaland every year (Ao *et al*., 2016a). The local people most commonly dry the mushrooms in order to store them for longer duration so that they can sell them at the markets even after the mushroom season has finished and also, there is no other method of processing available to the local people for storage. This is mainly because of lack of scientific knowledge of post harvest processing and packaging. This guarantees awareness programme among the local communities on collection, processing and packaging of WEM species in order to grab good market price. Ten WEM species were selected for the present work. These ten WEM are the most popular mushroom species consumed in the state and are also recognized popularly worldwide. Therefore, the morphological descriptions given below are of the ten WEM used in the present research work:

***Auricularia auricula-judae* (Bull.)**

Common name: Jew's ear; Wood ear; Jelly ear.

Fruit body 3-8 cm normally but can be as much as 12 cm across; typically ear shaped with minute, greyish, downy hairs; wavy and irregular; gathered together and attached at a

central and lateral position; gelatinous; leathery/rubbery; bright reddish to tan with a purplish hint; lower surface wrinkled with folds and ridges; when dry the color changes to brownish black, hard and brittle. Spore print white. Found on the branches of deciduous trees especially Alder, and on dead and decaying logs/branches or main trunk. It is found on both living and dead trees.

***Auricularia polytricha* (Mont.) Sacc.**

Common name: Cloud ear fungus; Hairy wood ear.

Fruit body 1-9 cm, resupinate, gelatinous, lateral, loosely attached, sometimes a very short stalk present, elastic; sterile surface dark brown, hairy, silky. Hymenium smooth or wrinkled, brown to blackish brown. Spore print white. Found in clusters on dead or rotten and decaying stumps and twigs.

***Cantharellus cibarius* (Fr.)**

Common name: Chanterelles.

Pileus 3-10 cm across, pale to deep egg yellow fading with age, flattened with an irregular, incurved margin, later becoming wavy and lobed; depressed at the centre. Stipe 2.9-8.0 cm × 0.5-2.0 cm, tapering towards base, color same as cap, sometimes paler, solid. Flesh yellowish, watery; smell faint, fragrant of apricots. Lamellae primitive or pseudo-gills, egg-yellow in color, decurrent, narrow, irregularly forked and vein-like. Spore print cream to yellowish. Found in all kinds of woods and with deciduous and coniferous trees.

***Lactifluus piperatus* (L.) Roussel**

Common name: Peppery milk-cap

Pileus 4-16 cm across, creamy white, matt and glabrous, convex with concave to widely funnel-shaped centre. Lamellae whitish, decurrent, radial and crowded. Stipe 3-7cm ×

0.9-3 cm, cylindrical, white, firm, tapering towards the base. Flesh white, thick. Milk white in color. Spore print white. Found under deciduous and mixed forest types.

***Laetiporus sulphureus* (Bull.) Murr.**

Common name: Chicken of the woods; Sulphur shelf

Fruiting body up to 50-60 cm across, fan shaped, irregular to semicircular in over-lapping series; bright orange-yellow to bright orange when young, fading on maturity; tubes up to 0.5 cm, sulphur-yellow to buff. Flesh thick, soft and watery when young, becoming tough on maturity. Stipe absent. Spore print white. Found on living matured and dead hardwoods in forests such as *Quercus*, *Prunus*, etc and occasionally also on conifers.

***Lentinula edodes* (Berk.) Pegler**

Common name: Shiitake.

Pileus 1-10 cm across, solitary, deep brownish red to tan brown, darker color at the centre of cap, sub-umbonate and convex. Lamellae radial, white in color, later developing reddish brown tints/spots. Stipe 1.5-6 cm × 0.3-0.7cm, short, color same as pileus but paler and tough. Volva absent. Spore print white. The mushroom grows in clusters or groups on decaying woods of deciduous trees like oak, beech, poplar, mulberry, *Castanopsis* etc.

***Lentinus sajor-caju* (Fr.) Fr.**

Common name: Not available.

Pileus 1.5-11cm across, greyish to yellowish to whitish, depressed, convex. Lamellae deeply decurrent, crowded, narrow. Stipe 1-3.5 cm × 0.2-0.9 cm, cylindrical, central to lateral, yellowish white, solid, firm, annulus present attached towards stipe apex but falls down on maturity. Spore print white. Habitat in groups attached to dead and dying trees.

***Lentinus squarrosulus* Mont. Singer**

Common name: Not available.

Pileus 1.5-12 cm across, solitary, convex then later depressed, umbilicate to infundibuliform, whitish to yellowish. Lamellae decurrent, crowded, narrow, white to cream to yellowish. Stipe 2–6 × 0.4–l cm, central to excentric, concolorous with the pileus, tapering downward and often bent, pale yellowish to cream with sub-bulbous base. Odor mushroomy. Spore print pale cream to yellowish. Grows singly or more commonly in groups or clusters on water-soaked hardwood, logs or stumps. One variety of *L. squarrosulus* was also collected and identified during the study.

Lentinus squarrosulus* Mont. var. *squarrosulus

Common name: Not available.

Pileus 1.5-9.5 cm across, solitary, convex then later depressed, umbilicate to infundibuliform, pale yellow to yellowish white, splitting at maturity. Lamellae deeply decurrent, crowded, unequal, narrow. Stipe 2–4 × 0.4–1 cm, concolorous with the pileus, tapering downward and often bent. Odor mushroomy. Spore print pale yellowish to cream.

***Lentinus tigrinus* (Bull.) Fr.**

Common name: Not available.

Pileus 1-10 cm across, fleshy, depressed when young, infundibuliform when matured, surface at first greyish brown than blackish brown owing to crowded, innate, fibrillose squamules, radially arranged, becoming pale ochraceous cream colored to almost white on expansion with the squamules crowded at the centre. Lamellae decurrent, crowded, concolourous with the cap. Stipe 1-5 cm × 0.2-1 cm, central, cylindrical, solid, whitish to yellowish and hairy. Spore print cream.

***Schizophyllum commune* Fr.**

Common name: Split-gill fungus.

Fruiting bodies fan shaped, leathery, pileus greyish-white, ranging from (1-4) cm broad, lobed, fused with others and attached to the substratum laterally. Lamellae greyish–violet radiating from the point of attachment and rolling back to cover the space between the gills, protecting the hymenium from desiccation; flesh pale and tough. Stem absent or rudimentary. Spore print white. Habitat on branches of dead and decaying wood and cut timber.

***Termitomyces heimii* Natarajan**

Common name: Termite mushrooms.

Pileus upto 10cm across, creamy, umbonate, incurved margin and smooth. Lamellae crowded, free, whitish, turn pinkish with age, fissile and serrulate margin. Stipe up to 10 cm long and -1.6 cm wide, white, cylindrical and fairly thick near the base with hollow pseudorrhiza. Annulus present, white in color, thick and persistent. Spore print cream to yellowish brown. Habitat on termite mounds or clayey soil.

Discussion

The wild mushrooms were found almost throughout the year but collections by the local people in the state were high in the months of May, June, July, August, September and lasts till October. The peak season for mushroom collection is end of April to July. Mushroom hunting is not gender oriented in the state i.e., both men and women are equally involved. Wild edible mushrooms are generally identified based on the indigenous knowledge passed on from generation to generation and unfortunately, at times; there are cases of mushroom poisoning due to misidentification. During various field studies, it was

observed that the presence and abundance of mushrooms has decreased over time due to anthropogenic factors like shifting cultivation, developmental activities and unsystematic collection of mushrooms by mushroom gatherers and climate change. For the formation of mushroom fruit body, climate plays an important factor and change in the distribution, abundance and morphology of fungi is often linked to climatic changes (Diez *et al*., 2013). Macrofungi are mostly found in the wet season than the dry season. During the present study too, most of the mushrooms were collected during the wet season. The dry season is mostly dominated by polypore and bracket fungi like *Trametes gibbosa, T. versicolor, Pycnoporus cinnabarinus, Microporus xanthoporus, Daldinia concentrica, Ganoderma lucidum* and *Schizophyllum commune* etc. During the dry season, due to decreased rainfall and increase in temperature and sunlight, the fleshy macrofungi are not able to withstand these conditions (Andrew *et al*., 2013). As we know that fungi helps the growth of plants by their ecto-mycorrhizal associations with the roots of plants in the forests. Thus, measures need to be taken to conserve the fungal diversity including mushrooms as unsystematic collection will lead to extinction of these mushrooms in the forest. The research on macrofungi is of importance because of the role played by macrofungi in human welfare, biodegradation, food and medicines. Also, the presence or absence of mushrooms indicates the damage caused to the forest through external factors (Ozturk *et al*., 2003). In the present study also, it was observed that the population of wild mushrooms decreased in the collection areas over time which is a clear indication that mushrooms are useful biological indicator to assess the ecosystem damage.

Wild edible mushrooms (WEM) are considered as traditionally important nutritious food which forms a vital part of the food culture of all tribes and is highly appreciated as a

delicacy in the state. Present study identified 52 species of mushrooms out of 141 species to be edible after due consultation of literature, manuals and information collected from the local mushroom harvesters. Besides, the number of medicinally important mushrooms available in the state is high, e.g., *Auricularia auricula-judae*, *A. polytricha*, *Lentinula edodes*, *Schizophyllum commune*, *Trametes gibbosa*, *T. versicolor*, *Pycnoporus cinnabarinus*, *Microporus xanthoporus*, *Coprinus disseminatus*, *Daldinia concentrica*, *Ganoderma lucidum*, *Aleuria aurantia*, *Cantharellus cibarius*, *Hygrocybe conica*, *Lentinus squarrosulus*, *L. sajor-caju*, *Pleurotus pulmonarius*, *Lycoperdon perlatum*, *Flammulina velutipes*, *Cordyceps militaris*, *Tremella mesenterica*, *T. fuciformis* etc. which are reported to have medicinal properties (Mizuno, 1999; Zhang *et al*., 1999; Chang and Miles, 2004; Wani *et al*., 2010; Ao *et al*., 2016a). Wild mushrooms represent one of the most important non-wood forest resource globally and also in India. As mushrooms grow easily on low cost substratum in the wild, WEM can be cheap source of nutritional food and often regarded as 'poor man's meat'. It is very clear that mushrooms have the capacity to provide healthy diet to rural tribal population in the form of cheap source of proteins, dietary fiber, low cholesterol, minerals and antioxidants. The demand for edible and medicinal mushrooms has increased over the years. Mushrooms provide the local people with seasonal food and alternative source of income (Sysouphanthong *et al*., 2010) during the mushrooming season. The time has arrived to identify and explore the wild mushroom diversity as this biodiversity plays an integral part in food security and checking environmental quality. Therefore, efforts need to be made to conserve and promote this valuable natural bio-resource. Due to the recent awareness of the nutritional and therapeutic value of mushrooms, there is consumer

demand for different mushroom varieties which urges the farmers to exploit different wild mushrooms for usage.

Mushrooms are considered to be rich sources of proteins equivalent to animal proteins and hence popularly advocated as health food. Present day world is facing human nutritional problems especially protein deficiency and the FAO (Food and Agriculture Organization) has emphasized the adoption of mushrooms as an ideal food for the protein hungry masses of developing countries to combat protein deficiency. There is immense scope to popularize mushrooms as a valuable non-timer forest bio-resource which will provide a means of occupation to the rural people especially targeting the under privileged group in the society. Thus, mushrooms are essential to humans for its many uses as food, in pharmaceutical applications, biodegradation, bioremediation and as ecological indicators of climate change. The WEM available in Nagaland are under-studied and under-exploited so it becomes imperative to thoroughly screen and explore the potential mushrooms available in the state. This study promotes awareness to harvest and exploit this underutilized local resource, which will not only provide nutritious food but employment opportunities especially to the disadvantaged groups (i.e., unemployed and old people).

Summary and Conclusion

This chapter reports the documentation of WEM available in Nagaland. In the present study, a total of 52 mushroom species were identified as edible, 10 mushrooms were found to be poisonous and the remaining 79 mushrooms were found to be inedible. *Schizophyllum commune, Auricularia auricula-judae, A. polytricha, Lactifluus piperatus, L. volemus, Sceleroderma citrinum, Termitomyces heimii, Tremella fuciformis, Tricholoma imbricatum, Hygrocybe conica* and *Agaricus moelleri* were found to be the most abundant during the

season of occurrence. The collected mushroom species were predominantly found to be parasitic, saprophytic and ecto-mycorrhizal in habitat. The scope of mushroom is endless and it is high time to survey, collect, identify, record and conserve the macrofungal biodiversity. A systematic study of the wild edible mushrooms will explore the possibility for the scientific cultivation of mushrooms in the state. The local people can be encouraged to work in this field by making them aware of the nutritional and medicinal benefits of mushrooms and how it can be an income generator for them. This can be achieved by organizing public workshops, seminars, campaigns etc and through media. Thus, commercialization of mushrooms in Nagaland is only possible when systematic research and work is initiated in this field with a proper vision to improve the socio-economic status of the people. Therefore, there is the need to develop a viable scale of operation to sustain and enhance the production of mushroom spawns and develop techniques to domesticate and cultivate the WEM to meet the demands of both rural and urban dwellers. Moreover, a thorough screening of the wild mushrooms is required to study its bioactivity and medicinal properties.

The findings of this study will be regarded as a database of wild mushrooms of the state and will help in future research works. Because of the alarming food crisis faced in the world today, nations have joined hands to overcome food insecurity. The State of Food Security and Nutrition in the World (SOFI) has called upon all countries and stakeholders to act together to end hunger and prevent all forms of malnutrition by 2030. There is the need to identify the rich mushroom biodiversity of the state which will help in creating strategies for management and conservation as various anthropogenic factors and climate change has affected the survivability of mushrooms in nature. For the betterment of society, WEM should be domesticated for sustainable and commercial production. The WEM needs to be

popularized as a nutritious food in order to have market acceptability which will indirectly help the locals to earn their livelihoods.

Chapter - 3

Molecular Phylogeny and Genetic Diversity Analysis

Introduction

Mushroom research has gained prominence due to the role played by mushrooms in food and pharmaceutical industries but it is projected that only ~10% mushrooms in the world are so far studied (Wasser, 2002). Mushroom taxonomic studies have been mainly based on morphological characters like shape, size and color of caps and gills (Lee *et al.*, 2006), which sometimes fails to identify some mushrooms correctly. Due to inherent simplicity of fungi, convergent evolution, similarities and phenotypic flexibility; morphological information is of restricted use for proper identification and classification of these macrofungi and their families and therefore, needs molecular data (Hofstetter *et al.*, 2002). The misidentification of poisonous mushrooms to edible species is most commonly due to close resemblance in terms of general morphology (Berger and Guss, 2005). For

example, the edible *Volvariella volvacea* mushroom is often confused with *Amanita phalloides*, a highly poisonous mushroom as they have similar morphological appearance. Morphological characters are plastic in nature and influenced by environmental conditions. Similarly ecological diversities of wild edible mushrooms (WEM) may also lead to phenotypic plasticity which may or may not reflect true phylogenetic diversity (Feng *et al*., 2012) in true sense. Therefore, proper characterization and identification of mushroom is of utmost priority to avoid identification errors and consumption of toxic mushrooms. In different regions of the world, distinction between edible and non-edible mushroom is mostly based on cultural traditions and vernacular naming systems (Tibuhwa, 2012). Though indigenous mushroom resources are diverse in nature, morphological identification often influence taxonomy (Zhang *et al*., 2006) leading to improper identification.

The normal method of mushroom identification is the study of fruiting body, preparing cultures on various media to differentiate the mycelia belonging to diverse fungal species. Even though these methods are adopted routinely, however, they are time consuming and lack accuracy in discriminating between closely related species (Rosendahl, 2008; Appiah *et al*., 2017). With the advancement of biotechnology and mushroom science, molecular tools are more accurate, rapid and reliable method for identification of fungi as compared to traditional methods (Moreau *et al*., 2006; Fonseca *et al*., 2008). The absence of a universally accepted DNA barcode marker for fungi in the eukaryotic kingdom is a major limitation for diversity studies. DNA barcoding uses 500 to 800 bp of target sequences to identify species of all eukaryotic taxa using primers that are applicable for the broadest possible taxonomic group (Blackwell, 2011; Mora *et al*., 2011; Schoch *et al*., 2012). The diversity of *rRNA* gene is a useful tool to study the taxonomy and phylogenetic classification

in bacteria and fungi (Hur and Chun, 2004; Lim *et al*., 2005). Identification of mushrooms using molecular approaches has been widely accepted because they target the species at the DNA level. The rDNA coding genes are successfully used in species identification during evolutionary, environmental and taxonomic studies of fungi including mushrooms (Hopple and Vilgalys, 1994; Wesselink *et al*., 2002; Borneman and Hartin, 2000; Gurtner *et al*., 2001). During the last decade, molecular approach for identification of Basidiomycetes has been attempted by amplification and restriction of special DNA sequences by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The primers 'ITS1' and 'ITS4' were the first PCR primers favored to work with fungal internal transcribed spacer (ITS) regions which code the highly variable regions i.e., ITS1 and ITS2 of the ribosomal operon (White *et al*., 1990). The ITS region is located between 18S or Small Subunit (SSU) and 28S or Large Subunit (LSU) in the rDNA cistron and is most commonly used for molecular phylogenetic studies as *rRNA* genes are known to be conserved at the species or genus level (Kim and Lee, 2000; Lee *et al*., 2000; Muruke *et al*., 2002; Chen *et al*., 2004; Park *et al*., 2004). This provides a great opportunity to workers to study the genome relationship between any two taxa (Hillis and Dixon, 1991; Salazar *et al*., 1999). However, some workers have revealed that the LSU or 28S *rRNA* gene has variable regions with sequence divergency- D1, D2, and D3 which makes it appropriate to study the phylogeny between the fungal species (Kurzman and Robnett, 1998; Hong *et al*., 2000; Wesselink *et al*., 2002). Molecular identification procedures are fast, specific and sensitive for studying the genetic relationships among mushrooms (Singh *et al*., 2003; Guglielmo *et al*., 2008). Molecular methods used in the identification of mushrooms include rDNA-ITS region sequence analysis, internal transcribed spacer-restriction fragment length polymorphism

(ITS-RFLP), random amplified polymorphic DNA (RAPD) and matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (Jasalavich *et al*., 2000; Adair *et al*., 2002; Schmidt and Low, 2005). Several PCR based markers have been developed and applied to assess the genetic variation among populations and genetic resources.

The recent advancement in the field of plant molecular biology gave a new class of markers which facilitates the dissection of genomes efficiently and with ease (Vijayan, 2005). Several molecular techniques have already been used for studying the genetic diversity and variation in basidiomycetes such as isozymes (Lan *et al*., 1998), AFLP (Qi *et al*., 2003), RFLP (Park and Ryu, 1996), ITS (Kindermann *et al*., 1998) and RAPD (Wang *et al*., 2003). These DNA based markers have more advantages over the conventional phenotypic markers and known to be stable across the developmental stages, present in more numbers and least influenced by environment, devoid of the pleiotropic and epistatic effects. Some of the important PCR based marker systems are RAPD, AFLP, inter simple sequence repeats (ISSR), simple sequence repeats (SSR), directed amplification of minisatellite DNA (DAMD), expressed sequence tag (EST) etc. Markers like RFLP, SSR and EST are co-dominant markers and can detect genetic variability at allelic level. However, the development and utilization of these marker systems are costly, laborious and time taking. While RAPD, ISSR, DAMD and AFLP are dominant marker systems but they are cost effective, easier to be developed and used. Therefore, for selecting a marker system, a number of factors like availability of equipment, time and expertise along with the nature of the crop are to be taken into account (Zietkiewicz *et al*., 1994; Vos *et al*., 1995; Vijayan, 2005). Such markers have been developed successfully and effectively utilized in genetic

diversity assessment of fungi including mushrooms (Tao *et al*., 2011; Du *et al*., 2013; Dwivedi *et al*., 2017). The determination of genetic diversity promotes the efficient utilization of genetic variations in breeding programs (Paterson *et al*., 1991). DNA finger printing has become an important tool in characterization of mushrooms (Shinwari *et al*., 1994). Large numbers of polymorphic markers are required to measure genetic diversity and relationships in a reliable manner. This limits the use of isozymes or morphological characters which lack adequate levels and often influenced by environmental factors in mushrooms. Molecular genetic markers are powerful tools to examine genetic relationships and diversity. Efforts have been initiated for the identification of cultivars and assessment of genetic diversity of many edible and medicinal mushrooms like *Agaricus bisporus, Auricularia auricula-judae, Lentinula edodes, Ganoderma lucidum, Tricholoma* spp. etc by using RAPD, ISSR and sequence-related amplified polymorphism (SRAP) markers (Sun *et al*., 2006; Zhang *et al*., 2007a; Fonseca *et al*., 2008; Guan *et al*., 2008; Tang *et al*., 2010; Tao *et al*., 2011; Du *et al*., 2013) however, only some reports have described the estimation of genetic diversity using multiple markers (Qin *et al*., 2006; Yu *et al*., 2008).

The ISSR markers have variable microsatellite regions and easy application at low cost which makes these markers highly useful for systematic and ecological evaluations (Reddy *et al*., 2002) and broadly used for population genetics, strain identification and genetic diversity of several mushrooms (Yu *et al*., 2008; Fu *et al*., 2010; Tang *et al*., 2010; Shao *et al*., 2011; Du *et al*., 2013). The DAMD technique was first reported by Heath *et al*. (1993) which use minisatellite core sequences as primers which contains hyper-variable regions (HVR) or variable number of tandem repeats (VNTR). These tandem repeats are known to be present in diverse plants and animal species but not reported in fungi or

mushrooms. Because of this, DAMD primers are effective as PCR primers in diverse organisms at relatively high stringencies (Karaca *et al*., 2002; Karaca and Ince, 2008). Besides, RAPD is another convenient method to study the genetic variations and diversity in various organisms including mushrooms. The use of RAPD markers has allowed the rapid regeneration of reliable and reproducible DNA fragments in wide varieties of mushroom species. RAPD markers have been used successfully for discrimination of several mushroom cultivars, in mushroom strain identification and protection of elite strains (Ravash *et al*., 2009; Agarwal *et al*., 2013; Rehman *et al*., 2015). RAPD is still the quickest, cost effective and most reliable method for assessing the variability at the DNA level, especially being useful for intra-specific analysis. These markers have the added advantage to amplify both the coding and non-coding regions and this is vital when the objective of the research is to assess the variation in the genome at inter-specific level (Williams *et al*., 1990; Ferreira and Grattapaglia, 1996; Ro *et al*., 2007).

Wild edible mushrooms (WEM) are highly popular as a great source of functional food in Nagaland. The ethnic people mostly collect the WEM species during different growing seasons for consumption as well as to sell them at the local markets. Since, the WEM species are identified on the traditional knowledge passed down from generation to generation, most of the times, wrong identification of the species leads to poisoning and death of consuming populace. The chapter deals with the molecular phylogenetic analysis of six WEM species and one variety of *L. squarrosulus* of Nagaland using molecular markers *viz*. ITS, 18S *rRNA* and 28S *rRNA* genes. As the genus *Lentinus* is an important edible species globally and represented by 120 species, in this study, three edible species of *Lentinus viz*., *L. sajor-caju*, *L. squarrosulus*, *L. squarrosulus var. squarrosulus* and *L.*

tigrinus collected from different areas of Nagaland was subjected to inter- and intra-specific genetic diversity assessment using ISSR, RAPD and DAMD markers (SPAR method) and the cumulative approach.

Materials and Methods

Sample collection and DNA extraction

For the present study, samples were collected from different forest locations and a few from local markets of Nagaland. Sun dried or oven dried (at ±60^{o}C) samples were used for isolation of genomic DNA using standard CTAB protocol (Doyle and Doyle, 1987) (**ANNEXURE II**). The DNA samples were tested for quality on agarose gel (0.8%, w/v) and quantified by Thermo Scientific Multiskan Go Spectrophotometer as per the normal procedure.

PCR amplification

Fungal specific primers (ITS1F and ITS4B) and universal ITS primers (ITS5 and ITS4) (White *et al*., 1990) were used for amplifying the fungal ITS region. During the study, universal ITS primers (ITS5 and ITS4) gave better results and hence, were used for amplifying the ITS region. For amplifying the 18S *rRNA* region of mushroom species, NS1 and NS2 primers were used (White *et al*., 1990). However, for amplification of 28S *rRNA* gene, LROR and LR5 primers were used (Vilgalys and Hester, 1990) (**ANNEXURE III**). The PCR master mix and the thermal cycling conditions are summarized in (**ANNEXURE IV**). The PCR amplification reaction was performed using Thermo Scientific Arktik Thermal Cycler.

Varying concentrations of DNA (20-100 ng) and $MgCl_2$ (1-2 mM) were used for optimizing the PCR reaction conditions for ISSR, RAPD and DAMD primers. For PCR amplification reaction, 50 ng template DNA and 1.75 mM $MgCl_2$ was observed to be ideal for amplification. The sequence information of ISSR, RAPD and DAMD primers used in the study is summarized in **Annexure VI, Annexure VII and Annexure VIII**. Final amplification reactions were performed in 20 µl volume which contained 50 ng of template DNA, 2 µl of 10X *Taq* Buffer, 1.75 mM $MgCl_2$, 0.4 mM each dNTPs, 0.5 mM each primer for ISSR, RAPD and DAMD, 3 units ml^{-1} of *Taq* polymerase and sterile pure water. The PCR amplification reaction was performed using a thermal cycler and the cycling conditions is summarized in **Annexure IX**. The PCR amplicons were separated by gel electrophoresis (1.5%, w/v) with 100 bp and 500 bp ladder (Genei) as the size standard and amplification was repeated twice in order to confirm the results.

Sequence analysis

The PCR amplicons were confirmed by gel electrophoresis (1.2%, w/v) with 100 bp ladder (Genei) as the size standard. A commercial kit (HiPura PCR Product Purification Kit, Make: HiMedia, India) was used to purify the amplicons before sequencing. The successful PCR products were sent to SciGenom Labs Private Ltd., Cochin, India and 1st Base, Singapore for sequencing. Search for homologous nucleic acid sequences was performed using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/) and the UNITE (Koljalg *et al*., 2013) (https://unite.ut.ee/analysis.php#) database for fungal ITS sequences was referred to compare the results with those obtained from NCBI GenBank. The ITS, 18S and 28S *rRNA* target DNA sequences and those nucleotide sequences retrieved from GenBank after blasting were subjected to multiple sequence alignment using ClustalW algorithm (Thompson *et al*.,

1994) through MEGA-X (Kumar *et al*., 2018) software. A separate alignment scheme was produced separately for each region i.e., ITS, 18S and 28S *rRNA* region. The DNA sequences were submitted at NCBI GenBank to obtain accession numbers (**Table 3.1**). The GenBank accession numbers and nucleotide sequence information is given in **ANNEXURE V**.

Phylogenetic analysis

The evolutionary history of the WEM species presently studied was inferred through the construction of phylogenetic trees using Neighbor Joining (NJ) (Kimura, 1980), Minimum Evolution method (ME) (Rzhetsky and Nei, 1992) and Maximum Parsimony (MP) (Nei and Kumar, 2000) in MEGA-X. For NJ based model, the evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. For ME analysis, the evolutionary distances were computed using the Tamura 3-parameter method (Tamura K, 1992) and are in the units of the number of base substitutions per site. The ME tree was estimated using the Close-Neighbor-Interchange (CNI) algorithm (Nei and Kumar, 2000) at a search level of 1. The MP tree was generated using the Tree-Bisection-Regrafting (TBR) algorithm (Nei and Kumar, 2000). In all the methods of phylogenetic analysis, the bootstrap consensus tree was inferred from 1000 replicates and is taken to represent the evolutionary history of the taxa analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985).

Table 3.1: GenBank Accession Numbers of the WEM species

WEM specimen	Name of species	Strain	GenBank Accession Number		
			ITS	**18S *rRNA***	**28S *rRNA***
NUBOT-TA-LE-06	*Lentinula edodes*	AO-DEBCR-1	KU343186	KT207467	KT459341
NUBOT-TA-LS-40	*Lentinus squarrosulus*	AO-DEBCR-2	KT207468	KT207469	KU170488
NUBOT-TA-LS-40	*Lentinus squarrosulus*	AO-DEBCR-3	KT207470	KT207471	KT459340
NUBOT-TA-LS-87	*Lentinus sajor-caju*	AO-DEBCR-4	KX342920	KX342921	KX342922
NUBOT-TA-LT-69	*Lentinus tigrinus*	AO-DEBCR-5	MG462731	MG722855	MG462732
NUBOT-TA-TH-64	*Termitomyces heimii*	AO-DEBCR-6	KT459337	KT459336	KT459338
NUBOT-TA-SC-09	*Schizophyllum commune*	AO-DEBCR-7	KT229567	KT253193	KT459339

Scoring and data analysis

Reproducible and non-ambiguous bands generated by each primer were scored manually as presence (1) or absence (0) across all samples and a binary data matrix was constructed which is used for all the analysis. Faint bands were not considered for data analysis. To evaluate the genetic diversity of the *Lentinus* species, parameters like total number of bands, number of polymorphic and monomorphic bands, and percentage of polymorphic bands were calculated from the binary data for each marker system. To evaluate the discriminatory power and efficiency of each of the three markers (ISSR, RAPD and DAMD), parameters like Polymorphic Information Content (PIC), Resolving Power (*Rp*) and Marker Index (MI) was also calculated. The PIC value was calculated by the formula, PIC = $1 - \Sigma pi^2$ where pi is the frequency of the i^{th} allele (Roldan-Ruiz *et al*., 2000). The *Rp* of each primer was calculated according to Prevost and Wilkinson (1999); which is $Rp = \Sigma Ib$ where Ib (band informativeness) = $1–[2|0.5–p|]$, where p is the proportion of individuals containing the band. MI was calculated according to the formula given by Varshney *et al*. (2007) i.e., MI = EMR X PIC where EMR is the product of the total number of fragments per primer and the fraction of polymorphic fragments.

To estimate the genetic diversity and population genetic structure parameters like percentage of polymorphic loci (Lynch and Milligan, 1994), observed number of alleles, effective number of alleles (Kimura and Crow, 1964), Nei's gene diversity (Nei, 1973) and Shannon index (Lewontin, 1972) were calculated using the software PopGene, version 1.32 (Yeh *et al*., 1999). The estimate of gene flow (Nm) between the populations was calculated as $Nm = 0.5 (1−Gst)/Gst$ (McDermott and McDonald, 1993), where Gst is the gene differentiation.

Pair-wise similarity matrix was calculated by DICE similarity coefficient using the SIMQUAL module and a dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) with the sequential, agglomerative, hierarchical, and nested (SAHN) module for analysis of genetic relationships using NTSYS version 2.21 (Rohlf, 2000) for each marker system. The degree of confidence of the dendrogram obtained was checked by bootstrapping using 1000 replications in FreeTree and TreeView software. Mantel's correlation (Mantel, 1967) of the similarity matrices for the marker systems was done by using the MXCOMP module and Principle Coordinate Analysis (PCoA) was performed by using the EIGEN program of NTSYS version 2.21software.

Results

Molecular phylogenetic studies of *Lentinula edodes, Lentinus squarrosulus, L. squarrosulus* var. *squarrosulus, L. sajor-caju, L. tigrinus, Schizophyllum commune, Termitomyces heimii*

The preliminary identification of the collected WEM was based on observation of the phenotypic characters which has been described in chapter 2. One different variety was recorded and identified as *L. squarrosulus* var. *squarrosulus*, was a matter of concern but the same was amicably resolved with the utilization of molecular markers that helped in identifying correctly and conclusively at the species level. The amplification of ITS1-5.8S rDNA-ITS2 regions by universal primers ITS5 and ITS4 produced DNA fragment band between 500-800 bp with a mean GC content of 23.2%.The amplification of 18S *rRNA* or small subunit produced amplicons between 440-533 bp with a mean GC content of 22.8%, using primers NS1 and NS2. Primers LROR and LR5 for large subunit or 28S *rRNA* produced amplified PCR product between 394-947 bp with a mean GC content of 24.8%.

Figure 3.1 shows the representative of the PCR products photograph taken through gel documentation system. During the present investigation, *Schizophyllum commune* showed highest blast hit of 100% identity for ITS region and *Lentinus squarrosulus* showed highest blast hit of 100% identity for 28S *rRNA* using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/) at NCBI GenBank. The blast hit for 18S nucleotide sequences did not give good homology with the target sequences which may be due to very few nucleotide sequences deposited in GenBank and also the 18S *rRNA* region might have lacked hyper variability. The phylogenetic relationship of the WEM was studied by constructing the phylogenetic trees using amplified sequences of ITS, 18S and 28S *rRNA* with similar sequences retrieved from NCBI GenBank after blasting. Neighbor Joining (NJ), Minimum Evolution method (ME) and Maximum Parsimony (MP) were used to estimate phylogeny of the WEM species presently studied. From the MP tree, the Consistency Index (CI) was calculated for ITS, 18S and 28S *rRNA* regions using MEGA-X software. Consistency Index (CI) is defined as a measure of the amount of homoplasy on a tree i.e., the independent evolution of the same character state and not derived from a common ancestor. High CI measures the overall homoplasy and is used as a standard to compare the levels of homoplasy (Kluge and Farris, 1969). The CI ranges between 1.0 (no homoplasy) and 0.0. During the present study, the CI for ITS, 18S *rRNA* and 28S *rRNA* was 0.713501, 0.859070 and 0.743169 which indicated the low homoplasy nature of the studied WEM species.

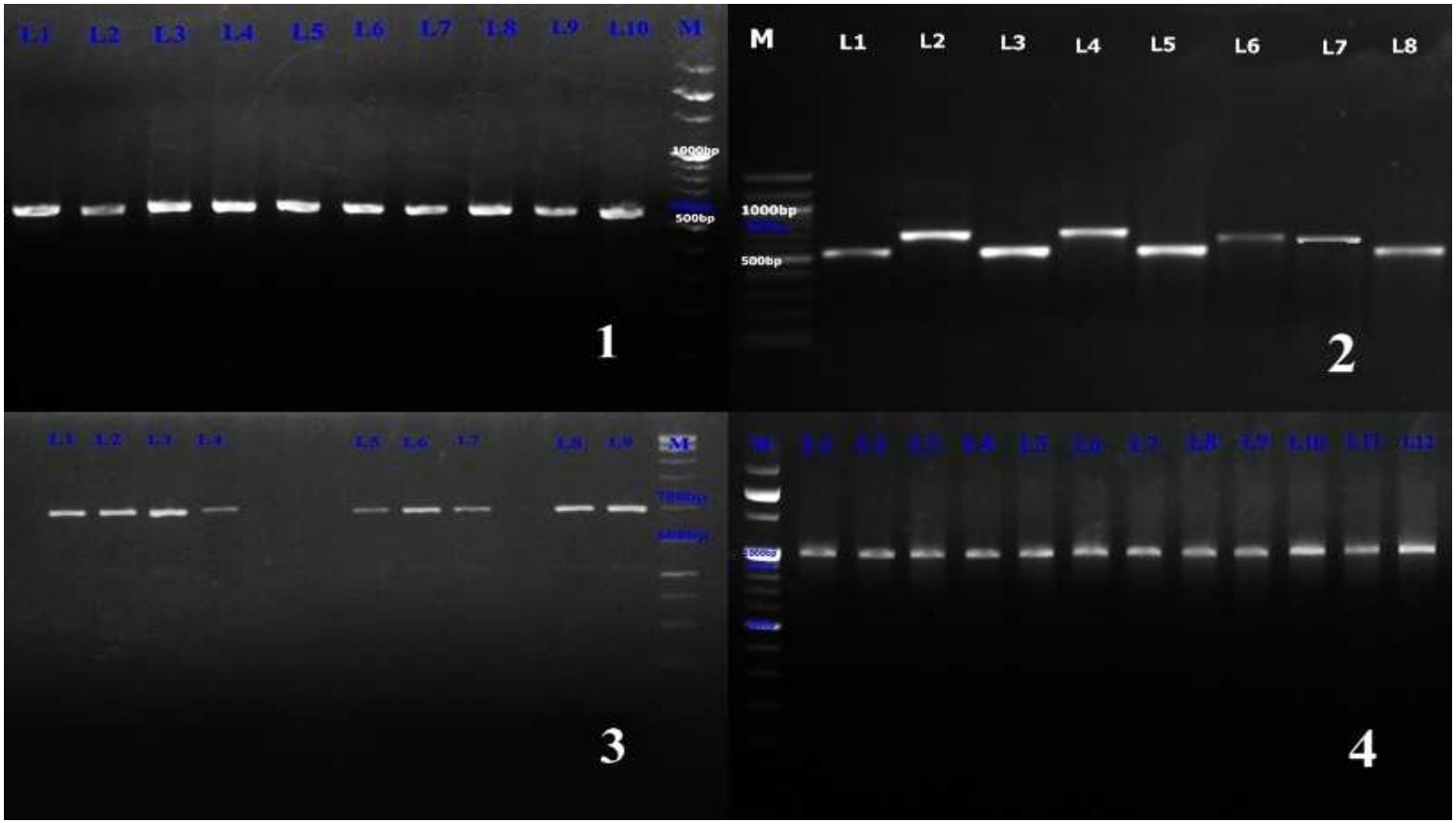


Figure 3.1 shows the representative of the PCR products photograph taken through gel documentation system. The gel image shows the amplified PCR products of investigated mushrooms with different markers: **1-** 18S *rRNA* gene, **2 & 3-** ITS region, **4-** 28S *rRNA* gene.

ITS region

The phylogenetic tree generated from ITS sequences using NJ method is shown in **Figure 3.2**. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and the optimal tree with branch length of 2.484 was obtained. The tree generated revealed two major clades and four main sub-clades.

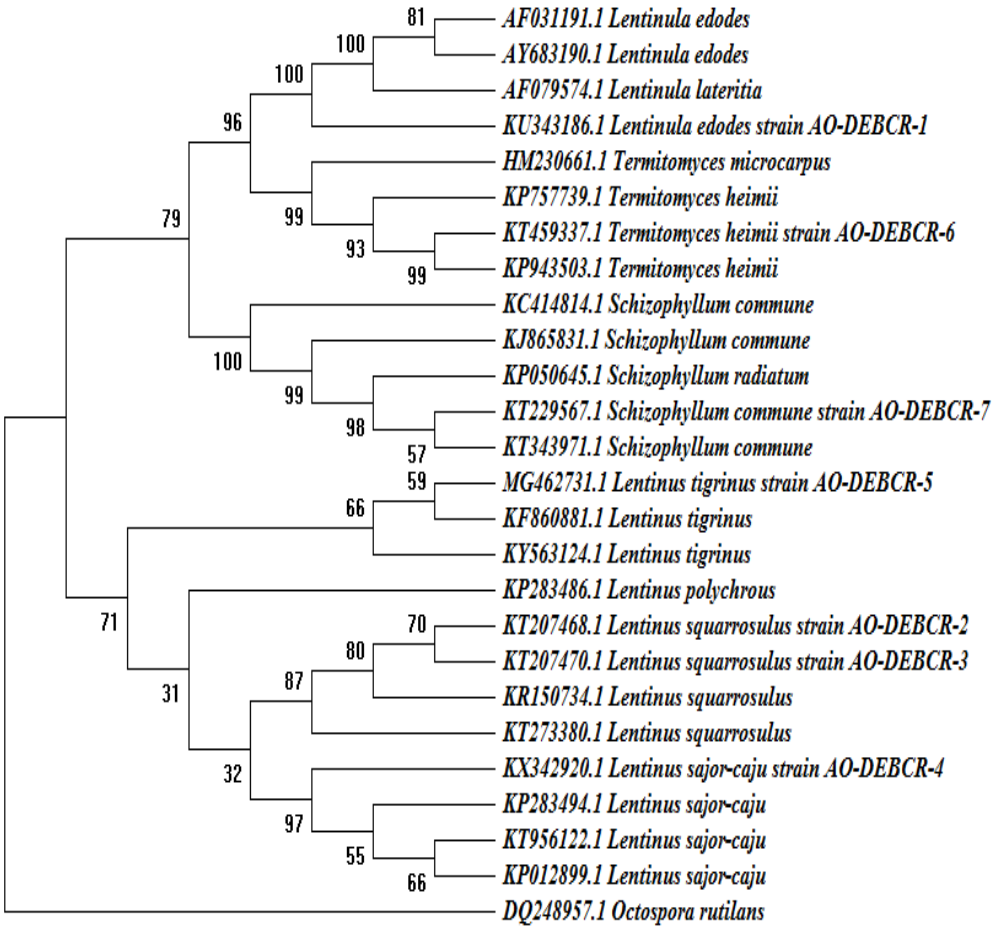


Figure 3.2: Consensus phylogenetic tree generated from ITS sequences using Neighbor Joining method. Numbers on the node of the branches are bootstrap value.

Mushroom species belonging to genera *Lentinula*, *Termitomyces* and *Schizophyllum* formed a single monophyletic clade with a bootstrap value of 79 which is again sub-divided into three distinct sub-clades. This is because they all belong to the order Agaricales under class Agaricomycetes. However, genera *Lentinula* and *Termitomyces* formed a clade together with a bootstrap value of 96 separated from the genus *Schizophyllum* which may infer that they share more common ancestry. It is seen that *T. heimii* AO-DEBCR-6 belongs to the cluster of genus *Termitomyces* with a boot strap value of 99, *L. edodes* AO-DEBCR-1 belongs to the cluster of genus *Lentinula* with a boot strap value of 100 and *S. commune* AO-DEBCR-7 belongs to the cluster of genus *Schizophyllum* with a boot strap value of 100. The genus *Lentinus* form a single major clade with a boot strap value of 71 which is further sub-divided into four distinct sub-clades - *L. tigrinus*, *L. polychrous*, *L. squarrosulus* and *L. sajor-caju*. This is because *Lentinus* belong to the order Polyporales under class Agaricomycetes. It is observed that *L. tigrinus* AO-DEBCR-5, *L. squarrosulus* AO-DEBCR-2, *L. squarrosulus* AO-DEBCR-3 and *L. sajor-caju* AO-DEBCR-4 belongs to the cluster of genus *Lentinus* respectively with a bootstrap value of 71.

The ME tree generated from ITS datasets is shown in **Figure 3.3**. The evolutionary distances were computed using the Tamura 3-parameter method (Tamura K, 1992) and the optimal tree with branch length of 2.5002 was obtained. The tree generated showed very similar topology to that of NJ analysis which resolved the tree into two major clades- *Lentinula, Termitomyces* and *Schizophyllum* forming a monophyletic clade and the genus *Lentinus* forming the other major clade and four main sub-clades.

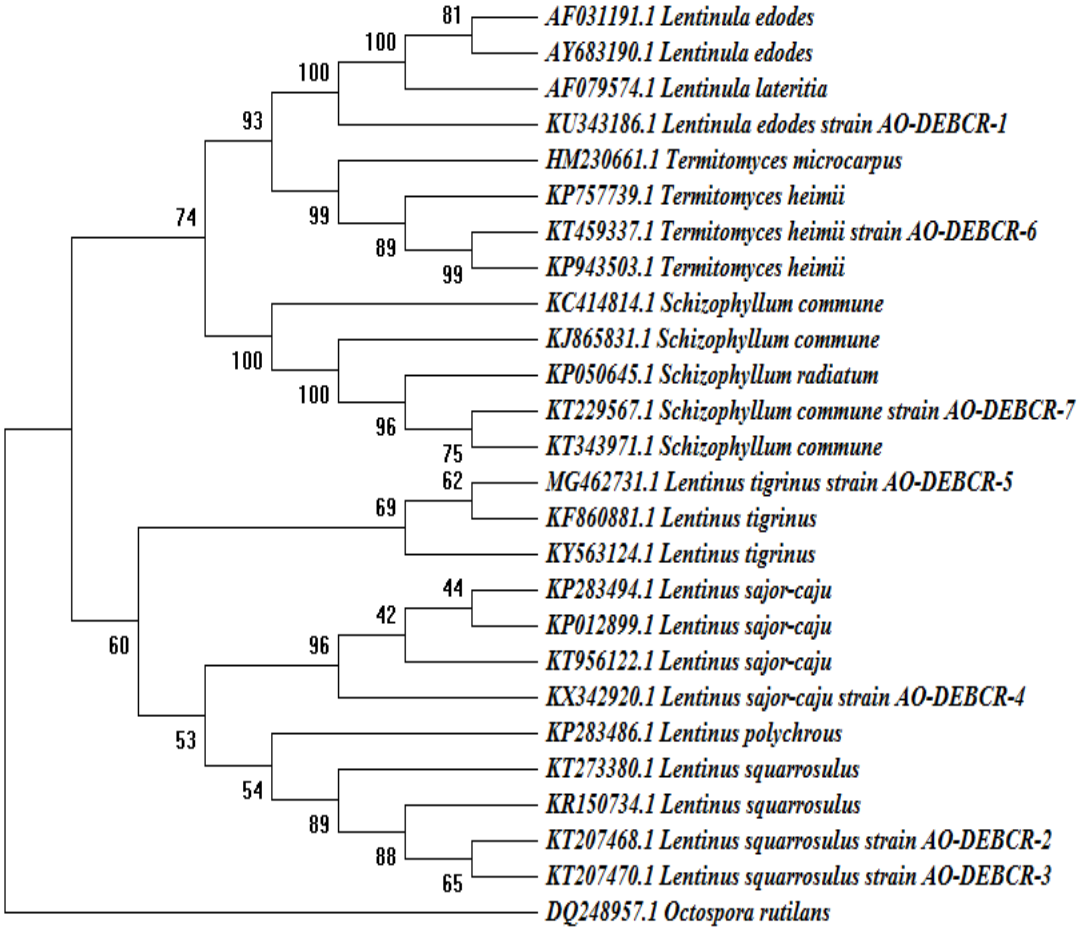


Figure 3.3: Consensus phylogenetic tree generated from ITS sequences using Minimum Evolution method. Numbers on the node of the branches are bootstrap value.

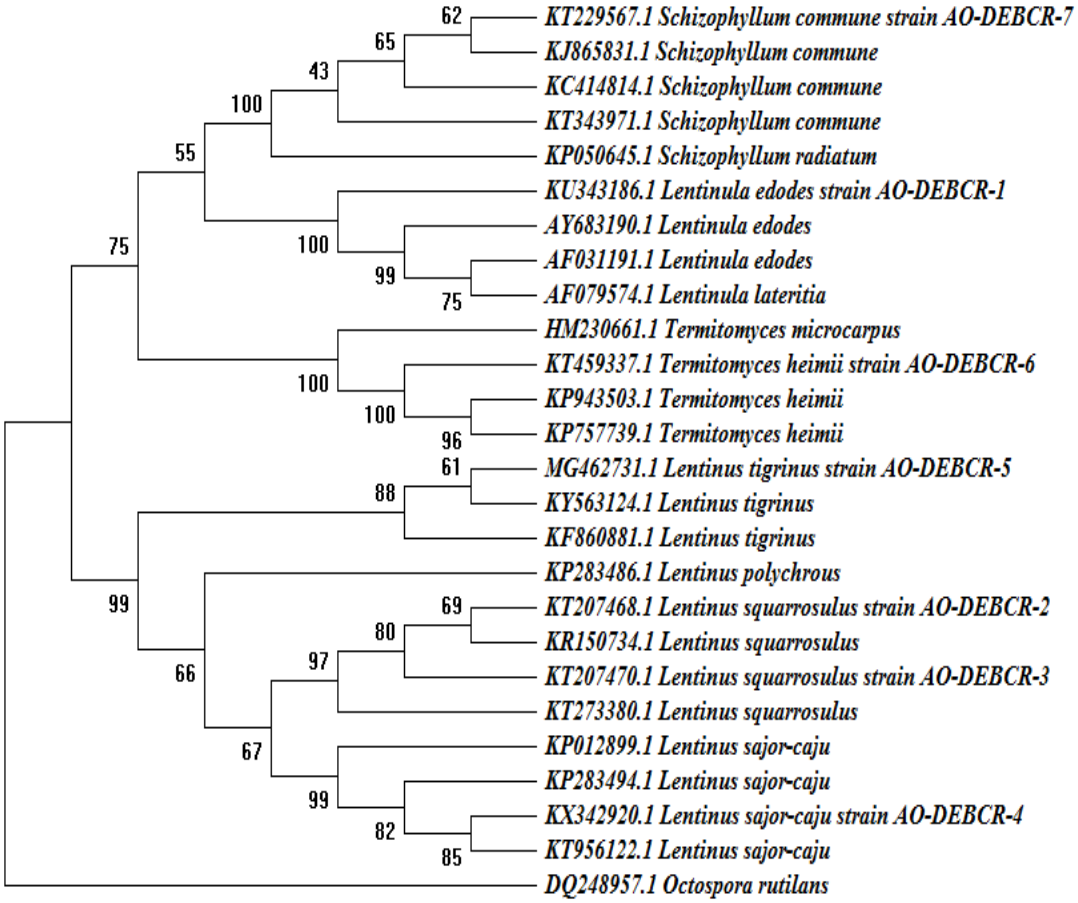


Figure 3.4: Consensus phylogenetic tree generated from ITS sequences using Maximum Parsimony method. Numbers on the node of the branches are bootstrap value.

The MP tree generated from ITS datasets is shown in **Figure 3.4**. The evolutionary history inferred using the MP method yielded 5 most parsimonious trees of length 1630 using the Tree-Bisection-Regrafting (TBR) algorithm. The consistency index (CI) was 0.713501 and the retention index (RI) was 0.866983 for all sites and parsimony-informative sites. The MP tree generated also resolved the studied WEM into two major clades and four sub-clades like the tree topologies inferred from NJ and ME analysis. The genera *Lentinula*, *Termitomyces* and *Schizophyllum* forming one monophyletic clade which is further sub-divided into three distinct sub-clades. However, here the genus *Lentinula* and *Schizophyllum* form a clade together with a bootstrap value of 55 and the genus *Termitomyces* forms a separate sub-clade with a bootstrap value of 100. This may be due to the reason that the genus *Lentinula* shares common evolutionary history with both *Termitomyces* and *Schizophyllum*. The genus *Lentinus* formed the other major clade dividing into sub-clades of its own genus.

All the phylogenetic trees generated are rooted to *Octospora rutilans* which is an Ascomycetes mushroom and differs from the studied WEM species which are all Basidiomycetes mushrooms.

18S *rRNA* gene

The 18S *rRNA* phylogenetic trees were constructed using amplified sequences of 18S *rRNA* sequences and similar sequences retrieved from GenBank through NCBI blast. The phylogenetic tree generated from 18S *rRNA* sequences using NJ method is shown in **Figure 3.5.** The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and the optimal tree with branch length of 2.986 was obtained. The tree generated resolved into seven clades. The WEM species belonging to genera *Termitomyces*,

Lentinula and *Schizophyllum* formed separate clades with very less bootstrap values. However, the genus *Lentinus* did not form a clade together instead the species *L. tigrinus* and *L. squarrosulus* each formed a separate clade. Also, *L. sajor-caju* and *L. squarrosulus* shared a common clade but interestingly, *S. radiatum* and *L. squarrosulus* all formed separate individual clades and did not cluster with its respective clade.

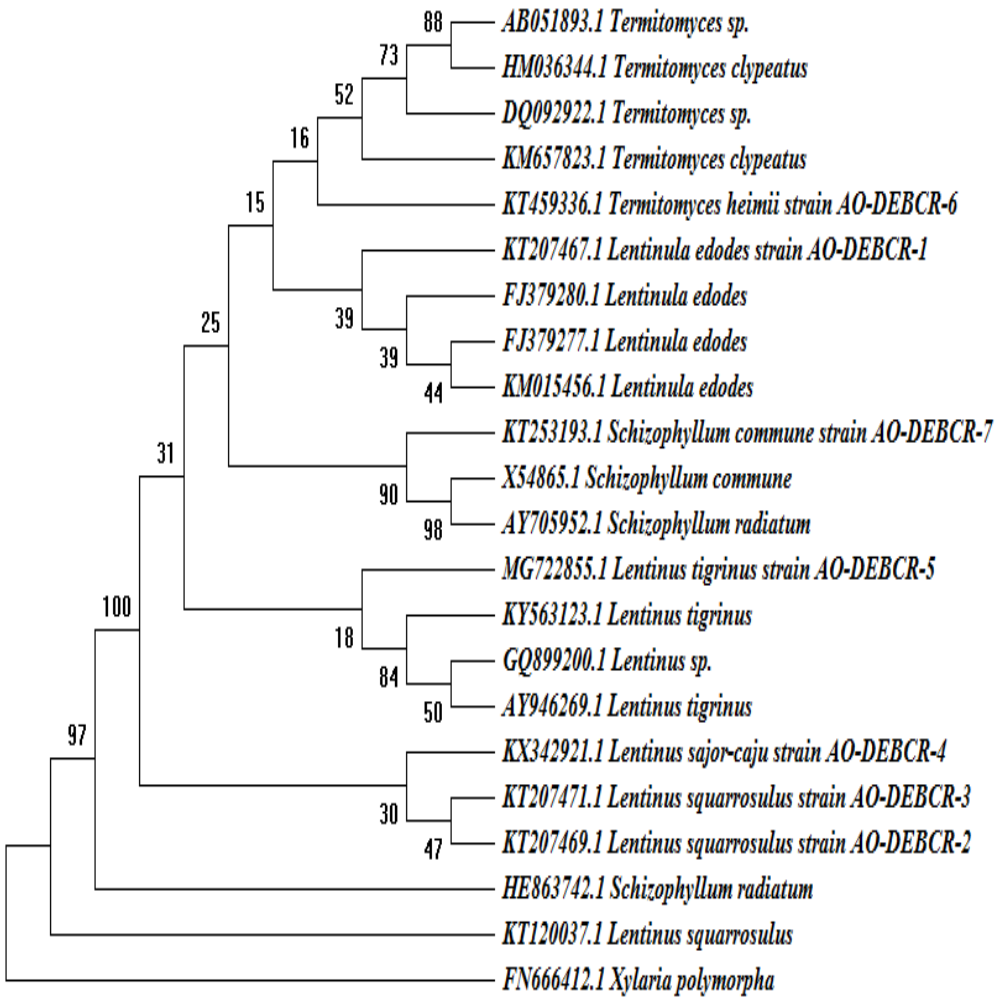


Figure 3.5: Consensus phylogenetic tree generated from 18S *rRNA* sequences using Neighbor Joining method. Numbers on the node of the branches are bootstrap value.

The ME tree generated from 18S *rRNA* datasets is shown in **Figure 3.6**. The evolutionary distances were computed using the Tamura 3-parameter method (Tamura K, 1992) and the optimal tree with branch length of 3.043 was obtained. The tree generated resolved into eight clades and was somewhat similar to the NJ tree generated. The genera *Lentinus, Termitomyces, Lentinula* and *Schizophyllum* all formed separate clades with low bootstrap value. The WEM species *L. tigrinus* and *L. squarrosulus* formed separate clades. However, *T. heimii* AO-DEBCR-6 and *L. edodes* AO-DEBCR-1 formed individual clades and did not form cluster with its own genus.

The MP tree generated from 18S *rRNA* datasets is shown in **Figure 3.7**. The evolutionary history inferred using the MP method yielded 2 most parsimonious trees of length 1344 using the Tree-Bisection-Regrafting (TBR) algorithm. The consistency index (CI) was 0.859070 and the retention index (RI) was 0.854714 for all sites and parsimony-informative sites. The tree generated resolved the studied WEM into eight clades. The WEM species belonging to genera *Termitomyces, Lentinula, Schizophyllum* and *Lentinus* all formed separate clades with bootstrap value of 60, 84, 79 and 70. However, the genus *Lentinus* separated into different clades, *L. tigrinus* AO-DEBCR-5 formed a separate clade of its own, and than *L. squarrosulus* and *L. sajor-caju* formed a clade together with low bootstrap value.

All the phylogenetic trees generated are rooted to *Xylaria polymorpha* which is an Ascomycetes mushroom and differs from the studied WEM species which are all Basidiomycetes mushrooms.

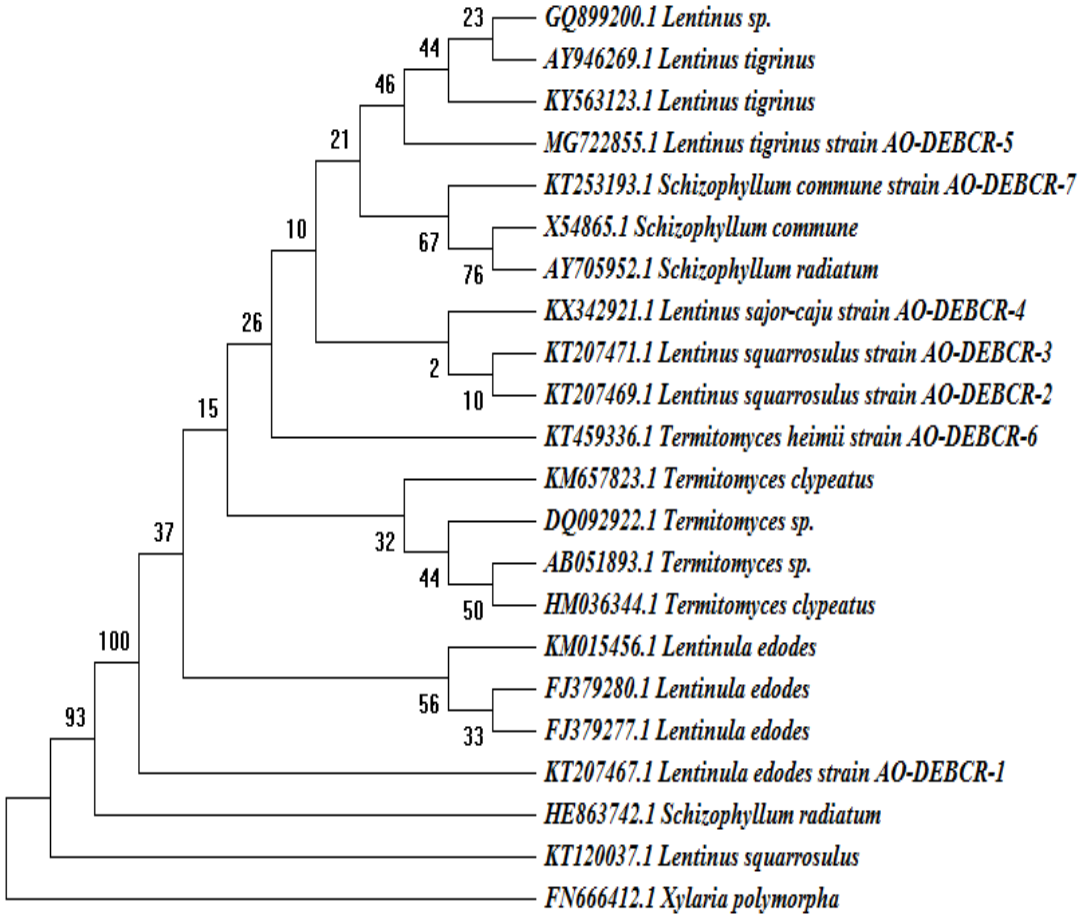


Figure 3.6: Consensus phylogenetic tree generated from 18S *rRNA* sequences using Minimum Evolution method. Numbers on the node of the branches are bootstrap value.

.

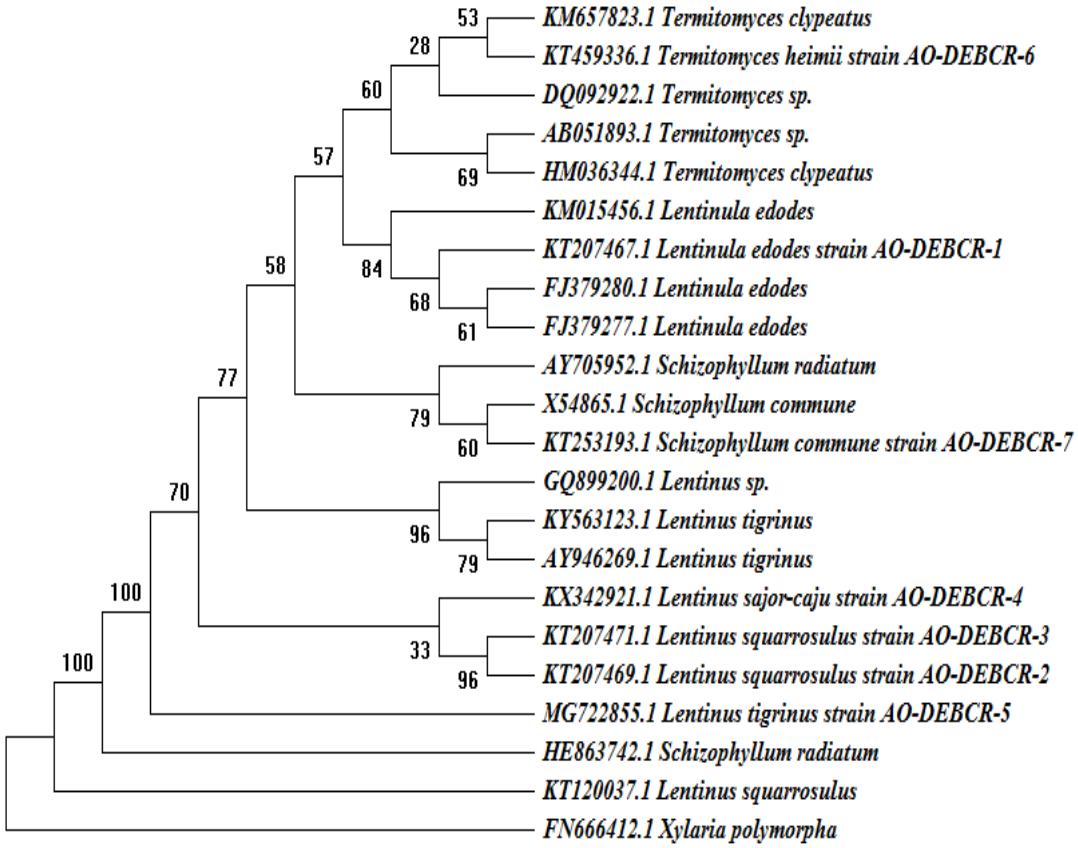


Figure 3.7: Consensus phylogenetic tree generated from 18S *rRNA* sequences using Maximum Parsimony method. Numbers on the node of the branches are bootstrap value.

28S *rRNA* gene

The phylogenetic tree generated from 28S *rRNA* gene sequences showed similarity with the tree topology generated from ITS sequences. The tree topology generated from 28S *rRNA* sequences using NJ method is shown in **Figure 3.8**. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and the optimal tree with branch length of 0.608 was obtained. The tree generated resolved into two major clades which further sub-divided into respective sub-clades. The first clade consisted of the mushroom species belonging to genera *Lentinula*, *Termitomyces* and *Schizophyllum* forming a single monophyletic clade with a bootstrap value of 97 which further sub-divided into three sub-clades between them. The genus *Lentinula* and *Termitomyces* formed a clade together with a bootstrap value of 84 while *Schizophyllum* formed a separate sub-clade with a bootstrap value of 100. Further, genus *Lentinula* formed two sub-clades of its own. It is observed that *T. heimii* AO-DEBCR-6 belongs to the cluster of genus *Termitomyces* with a boot strap value of 100, *L. edodes* AO-DEBCR-1 belongs to the cluster of genus *Lentinula* with a boot strap value of 100 and *S. commune* AO-DEBCR-7 belongs to the cluster of genus *Schizophyllum* with a boot strap value of 100. The genus *Lentinus* forms the second major clade with a boot strap value of 100 which is again sub-divided into sub-clades - *L. tigrinus*, *L. squarrosulus*, *L. badius*, and *L. sajor-caju.* It is observed that *L. tigrinus* AO-DEBCR-5, *L. squarrosulus* AO-DEBCR-2, *L. squarrosulus* AO-DEBCR-3 and *L. sajor-caju* AO-DEBCR-4 belongs to the cluster of genus *Lentinus* respectively with a bootstrap value of 100. As inferred from the study, the genera *Lentinula*, *Termitomyces* and *Schizophyllum* will always form a clade together as they belong to order Agaricales and *Lentinus* belong to order Polyporales under class Agaricomycetes of division Basidiomycota of Fungi.

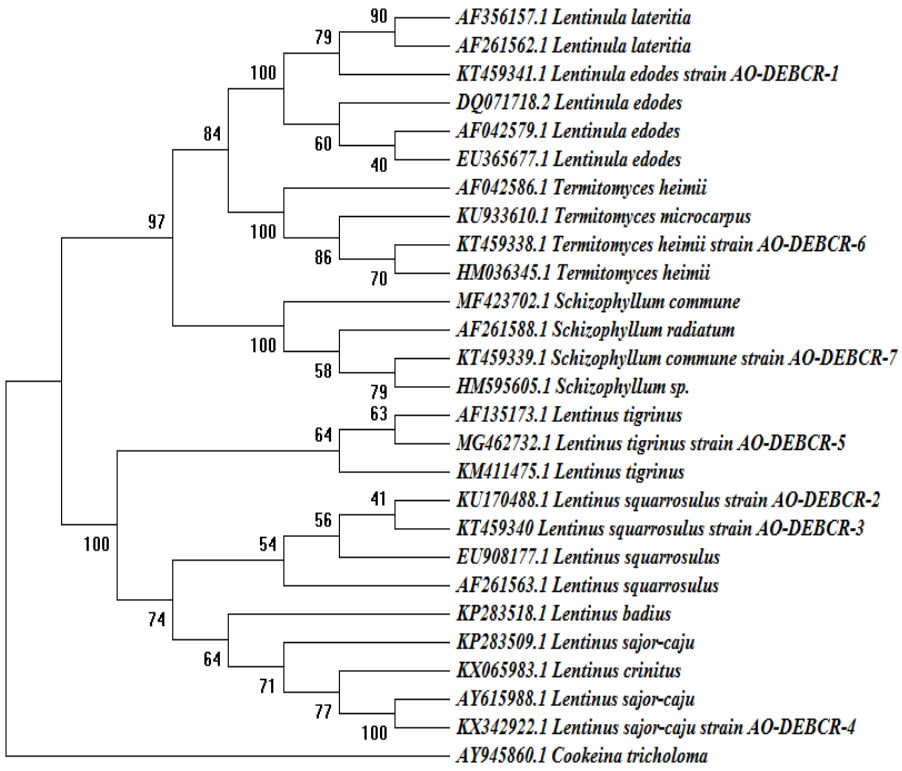


Figure 3.8: Consensus phylogenetic tree generated from 28S *rRNA* sequences using Neighbor Joining method. Numbers on the node of the branches are bootstrap value.

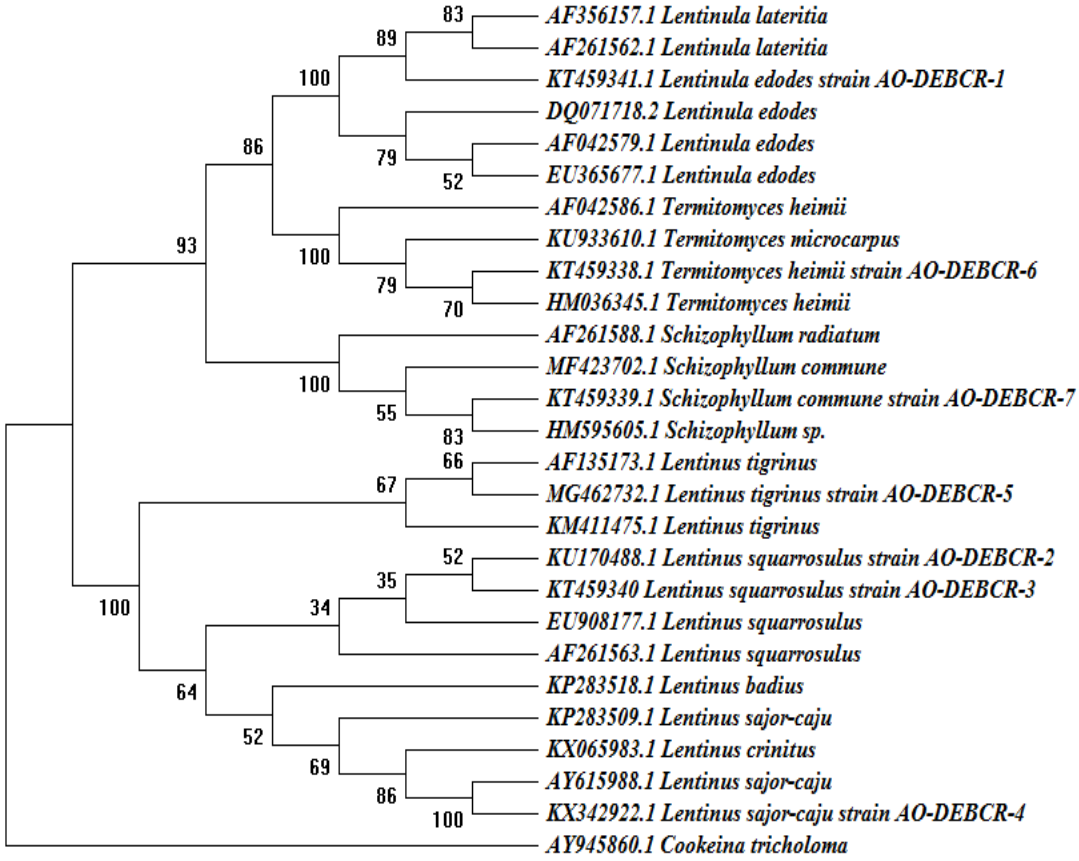


Figure 3.9: Consensus phylogenetic tree generated from 28S *rRNA* sequences using Minimum Evolution method. Numbers on the node of the branches are bootstrap value.

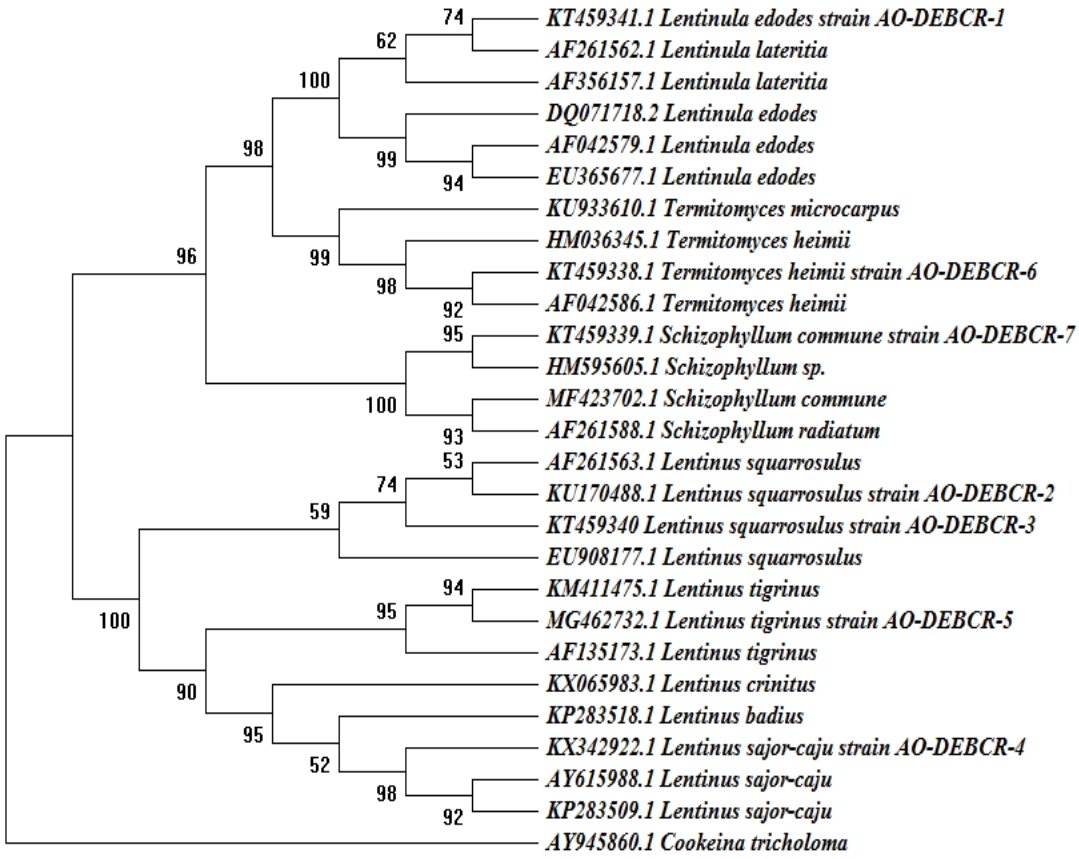


Figure 3.10: Consensus phylogenetic tree generated from 28S *rRNA* sequences using Maximum Parsimony method. Numbers on the node of the branches are bootstrap value.

The ME tree generated from 28S *rRNA* datasets is shown in **Figure 3.9**. The evolutionary distances were computed using the Tamura 3-parameter method (Tamura K, 1992) and the optimal tree with branch length of 0.608 was obtained. The tree generated showed very similar topology to that of NJ analysis which resolved the tree into two major clades- *Lentinula*, *Termitomyces* and *Schizophyllum* in one clade and the genus *Lentinus* forming the other major clade.

The MP tree generated from 28S *rRNA* datasets is shown in **Figure 3.10**. The evolutionary history inferred using the MP method yielded 9 most parsimonious trees of length 693 using the Tree-Bisection-Regrafting (TBR) algorithm. The consistency index (CI) was 0.743169 and the retention index (RI) was 0.921008 for all sites and parsimony-informative sites. The MP tree generated also resolved the studied WEM into two major clades and four sub-clades like the tree topologies inferred from NJ and ME analysis of 28S *rRNA* and also the tree topologies inferred from ITS datasets.

All the phylogenetic trees generated are rooted to *Cookeina tricholoma* which is an Ascomycetes mushroom and differs from the studied WEM species which are all Basidiomycetes mushrooms.

Genetic Diversity Analysis of the three edible *Lentinus* species

ISSR Analysis

Inter-specific natural genetic variation in three *Lentinus* species as revealed by ISSR markers

Out of 25 primers screened, 10 primers resulted clear and polymorphic bands. **Figure 3.11** shows the representative of the banding pattern. A total of 84 amplicons were produced out of which 79 were found to be polymorphic with an average of 7.9 polymorphic bands per primer and 5 were found to be monomorphic band. The percentage of polymorphic bands ranged from 85.71% to 100% with an average of 94.19% polymorphism among the 18 accessions of *Lentinus* species belonging to *L. sajor-caju, L. squarrosulus, L. squarrosulus* var. *squarrosulus* and *L. tigrinus* respectively. Primers 807, 842 and ISSR13 yielded the highest number of polymorphic bands which is 10, while primer 810 yielded the lowest number of polymorphic bands i.e. 5. The PIC value ranged from 0.56 to 0.77 with an average PIC value of 0.7 per primer. The *Rp* values ranged from 15.8 to 22 with an average of 17.84 per primer and the highest *Rp* value of 22 was observed in primer ISSR18. The MI values ranged from 4.18 to 6.47 with an average of 5.51 per primer and the highest MI was observed in primer 807. The number of observed alleles (Na) was 1.98 (±0.31) and the effective number of alleles (Ne) was 1.62 (±0.34). The Nei's gene diversity (H) and Shannon's index (I) among all the populations were 0.35 (±0.16) and 0.52 (±0.2) respectively. The total gene diversity (Ht) and Heterozygosity (Hs) within the populations was found to be 0.35 (±0.02) and 0.23 (±0.02). The mean coefficient of genetic differentiation (Gst) was found to be 0.36 while the estimate of gene flow (Nm) was found to be 0.91 (**Table 3.2 and 3.3**).

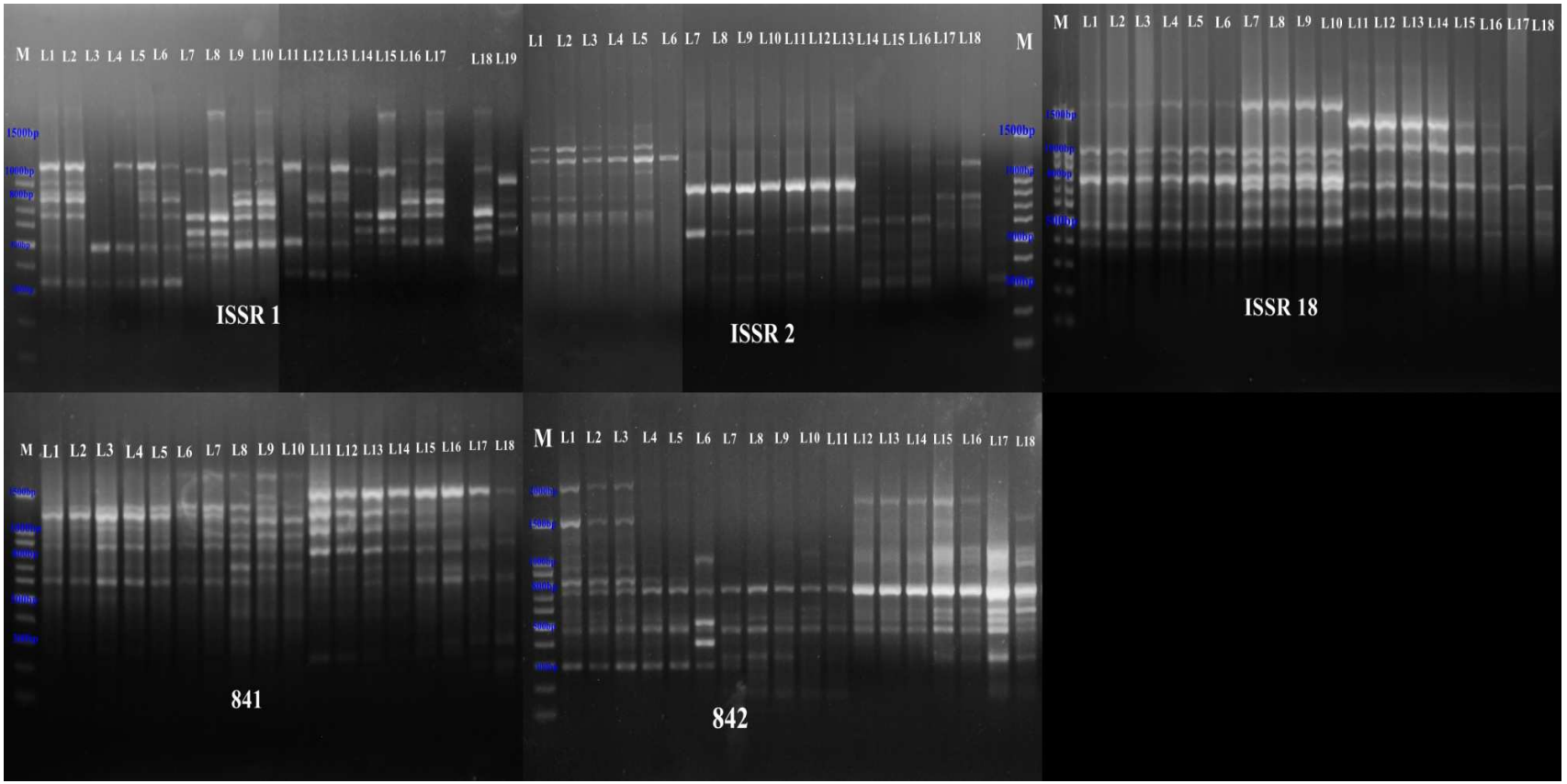


Figure 3.11 shows the representative of the banding pattern obtained for 18 accessions of *Lentinus* species with ISSR primers - ISSR1, ISSR2, ISSR18, 841 & 842 (L1-L6 *Lentinus squarrosulus*, L7-L12 *L. sajor-caju* and L13-L18 *L. tigrinus* in gel photographs of DAMD and RAPD also).

Table 3.2: Data on polymorphism obtained by ISSR primers used to assess genetic diversity of *Lentinus* species

Sl. No.	Marker	Primer Name	TB	PB	MB	PPB	PIC	*Rp*	MI
1.	ISSR	ISSR1	9	9	0	100	0.72	17.8	6.04
2.		ISSR2	8	7	1	87.5	0.69	16.3	5.07
3.		ISSR13	10	10	0	100	0.74	16.4	6.21
4.		ISSR11	7	6	1	85.71	0.66	18.6	4.75
5.		ISSR18	9	8	1	88.89	0.56	22	4.18
6.		807	10	10	0	100	0.77	15.8	6.47
7.		810	5	5	0	100	0.74	16.8	6.22
8.		841	9	8	1	88.89	0.67	18	5
9.		842	11	10	1	90.91	0.67	18	5.12
10.		834	6	6	0	100	0.7	18.7	5.88
TOTAL			84	79	5				
AVERAGE/PRIMER			8.4	7.9	0.5	94.19	0.7	17.84	5.51

Notes: TB = Total Band; PB = Polymorphic Band; MB = Monomorphic Band; PPB = Percentage of Polymorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index

Table 3.3: Summary of the various genetic diversity indices analyzed in *Lentinus* species using ISSR marker

Species Name	N	PPB	Average PIC	Average *Rp*	Average MI	Na (Mean±SD)	Ne (Mean±SD)	H (Mean±SD)	I (Mean±SD)	Ht (Mean±SD)	Hs (Mean±SD)	Gst	Nm
Lentinus squarrosulus	6	73.05	0.51	7.91	2.96	1.68±0.54	1.47±0.38	0.27±0.21	0.41±0.31	0.35±0.02	0.23±0.02	0.36	0.91
Lentinus sajor-caju	6	61.4	0.41	9.11	1.34	1.42±0.5	1.33±0.42	0.21±0.22	0.26±0.31				
Lentinus tigrinus	6	86.21	0.63	6.85	3.06	1.57±0.4	1.4±0.38	0.23±0.21	0.34±0.3				
Lentinus squarrosulus +Lentinus sajor-caju+ Lentinus tigrinus	18	94.19	0.7	17.84	5.51	1.98±0.31	1.62±0.34	0.35±0.16	0.52±0.2				

Notes: N= No. of accessions; PPB = Percentage of Polymorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index; Na = Observed no. of alleles; Ne = Effective no. of alleles; H = Nei's gene diversity; I = Shannon's Index; Ht = Total gene diversity; Gst = Genetic differentiation; Nm = Gene flow

Table 3.4: Intra-specific diversity in *Lentinus squarrosulus* species as revealed by ISSR, DAMD and RAPD markers

MARKER	TB	Average No. of Bands/Primer	Total No. of PB	Average No. of Polymorphic Bands/Primer	Total No. of MB	Average Polymorphism (%)	Average PIC	Average *Rp*	Average MI
ISSR	76	7.6	54	5.4	22	73.05	0.51	7.91	2.96
DAMD	67	6.7	38	3.8	29	59.39	0.41	8.63	1.81
RAPD	48	6	40	5.0	08	82.68	0.56	7.42	2.92
ISSR+DAMD+RAPD	191	6.82	132	4.71	59	70.92	0.49	8.02	2.53

Notes: TB = Total Band; PB = Polymorphic Band; MB = Monomorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index

Table 3.5: Intra-specific diversity in *Lentinus sajor-caju* species as revealed by ISSR, DAMD and RAPD markers

MARKER	TB	Average No. of Bands/Primer	Total No. of PB	Average No. of Polymorphic Bands/Primer	Total No. of MB	Average Polymorphism (%)	Average PIC	Average *Rp*	Average MI
ISSR	57	5.7	35	3.5	22	61.4	0.41	9.11	1.34
DAMD	46	4.6	32	3.2	14	70.94	0.48	7.94	1.74
RAPD	61	7.63	40	5.0	21	69.33	0.51	7.68	2.94
ISSR+DAMD+RAPD	164	5.86	107	3.82	57	66.2	0.45	8.28	1.94

Notes: TB = Total Band; PB = Polymorphic Band; MB = Monomorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index

Intra-specific natural genetic variation in three *Lentinus* species as revealed by ISSR markers

Lentinus squarrosulus

At intra-specific level among the 3 accessions of *L. squarrosulus* and 3 accessions of *L. squarrosulus* var. *squarrosulus*, a total of 76 amplicons were produced by 10 ISSR primers, out of which 54 were observed to be polymorphic with an average of 5.4 polymorphic bands per primer and 22 were found to be monomorphic bands. The average polymorphism was observed to be 73.05%. The average PIC value was found to be 0.51 per primer. The average *Rp* value was found to be 7.91 and the average MI was found to be 2.96. The number of observed alleles (Na) was 1.68 (±0.54) and the effective number of alleles (Ne) was 1.47 (±0.38). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.27 (±0.21) and 0.41(±0.31) respectively (**Table 3.3 and 3.4**).

Lentinus sajor-caju

A total of 57 amplicons were produced in 6 accessions of *L. sajor-caju* by 10 ISSR primers, out of which 35 were observed to be polymorphic with an average of 3.5 polymorphic bands per primer and 22 were found to be monomorphic bands. The average polymorphism was observed to be 61.4%. The average PIC value was found to be 0.41 per primer. The average *Rp* value was found to be 9.11 and the average MI was found to be 1.34. The number of observed alleles (Na) was 1.42 (±0.5) and the effective number of alleles (Ne) was 1.33 (±0.42). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.21 (±0.22) and 0.26 (±0.31) respectively (**Table 3.3 and 3.5**).

Table 3.6: Intra-specific diversity in *Lentinus tigrinus* species as revealed by ISSR, DAMD and RAPD markers

MARKER	TB	Average No. of Bands/Primer	Total No. of PB	Average No. of Polymorphic Bands/Primer	Total No. of MB	Average Polymorphism (%)	Average PIC	Average *Rp*	Average MI
ISSR	55	5.5	48	4.8	07	86.21	0.63	6.85	3.06
DAMD	40	4.0	30	3.0	10	76.15	0.47	8.3	1.51
RAPD	49	6.13	35	4.38	14	71.52	0.51	7.63	2.34
ISSR+DAMD+RAPD	144	5.14	113	4.04	31	78.42	0.54	7.59	2.3

Notes: TB = Total Band; PB = Polymorphic Band; MB = Monomorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index

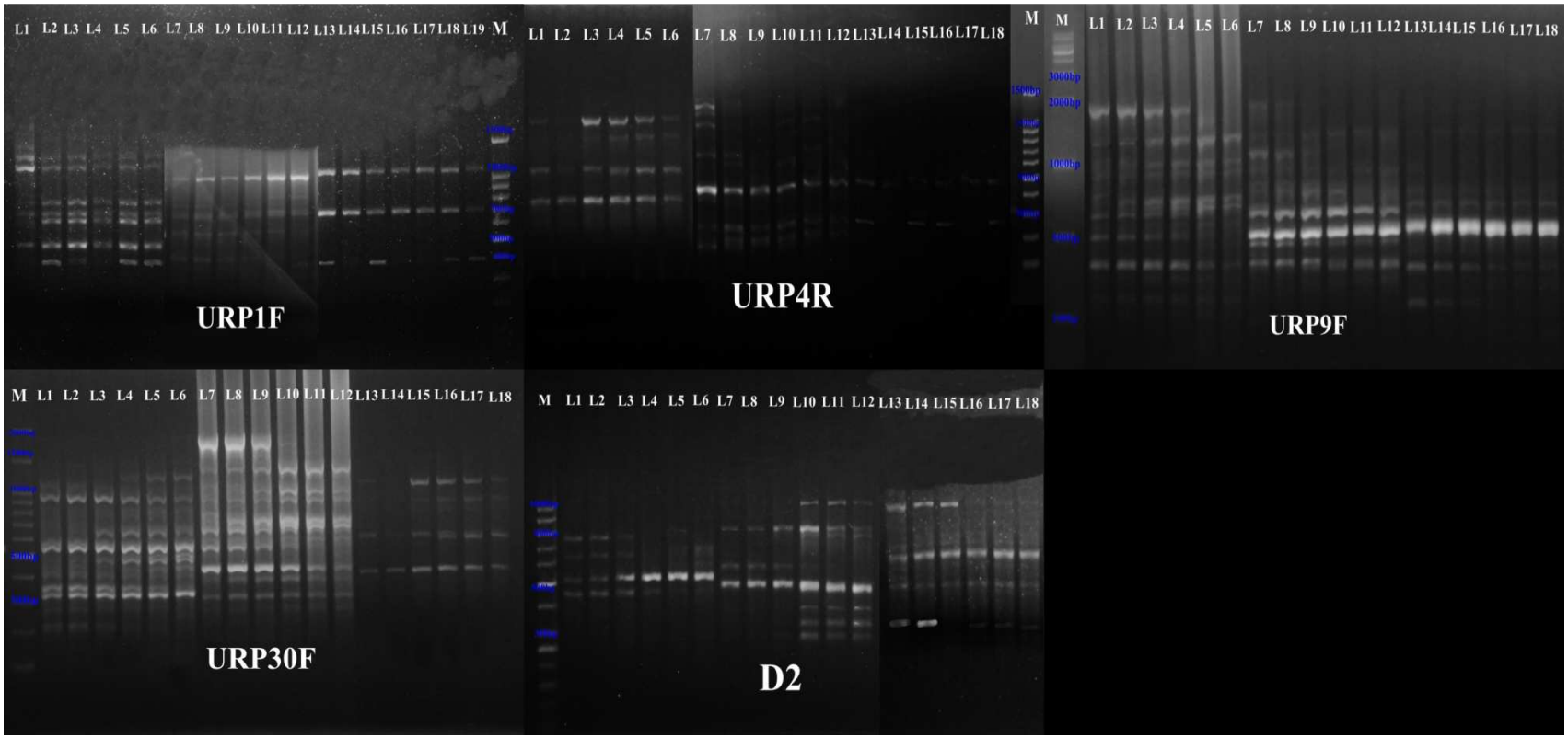


Figure 3.12 shows the representative of the banding pattern obtained for 18 accessions of *Lentinus* species with DAMD primers – URP1F, URP4R, URP9F, URP30F & D2.

Lentinus tigrinus

A total of 55 amplicons were produced in 6 accessions of *L. tigrinus* by 10 ISSR primers, out of which 48 were observed to be polymorphic with an average of 4.8 polymorphic bands per primer and 7 were found to be monomorphic bands. The average polymorphism was observed to be 86.21%. The average PIC value was found to be 0.63 per primer. The average *Rp* value was found to be 6.85 and the average MI was found to be 3.06. The number of observed alleles (Na) was 1.57 (±0.4) and the effective number of alleles (Ne) was 1.4 (±0.38). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.23 (±0.21) and 0.34(±0.3) respectively (**Table 3.3 and 3.6**).

DAMD Analysis

Inter-specific natural genetic variation in three *Lentinus* species as revealed by DAMD markers

Out of 25 primers screened, 10 primers resulted clear and polymorphic bands. **Figure 3.12** shows the representative of the banding pattern. A total of 85 amplicons were produced out of which 81 were observed to be polymorphic with an average of 8.1 polymorphic bands per primer and 4 were found to be monomorphic band. The percentage of polymorphic bands ranged from 85.7% to 100% with an average of 95.3% polymorphism among the 18 accessions of *Lentinus* species belonging to *L. sajor-caju*, *L. squarrosulus, L. squarrosulus* var. *squarrosulus* and *L. tigrinus* respectively. Primers URP30F and URP9F yielded the highest number of polymorphic bands which is 13, while primer D19 yielded the lowest number of polymorphic bands i.e. 5. The PIC value ranged from 0.61 to 0.94 with an average PIC value of 0.77 per primer. The *Rp* values ranged from 8 to 20.86 with an average of 14.78 per primer and the highest *Rp* value of 20.86 was observed in primer URP6R. The MI values ranged from 4.44 to 8.1 with an average of 6.27 per primer. The highest MI value was observed in primer D10. The number of observed alleles (Na) was 1.95 (±0.21) and the

effective number of alleles (Ne) was 1.52 (±0.32). The Nei's gene diversity (H) and Shannon's index (I) among all the populations were 0.31 (±0.15) and 0.48 (±0.19) respectively. The total gene diversity (Ht) and Heterozygosity (Hs) within the populations was found to be 0.31 (±0.02) and 0.15 (±0.01). The mean coefficient of genetic differentiation (Gst) was found to be 0.51 while the estimate of gene flow (Nm) was found to be 0.48 (**Table 3.7 and 3.8**).

Table 3.7: Data on polymorphism obtained by DAMD primers used to assess genetic diversity of *Lentinus* species

Sl. No.	**Marker**	**Primer Name**	**TB**	**PB**	**MB**	**PPB**	**PIC**	***Rp***	**MI**
1.	**DAMD**	D1	8	8	0	100	0.84	12.75	7.14
2.		D2	9	8	1	88.9	0.75	15.56	5.67
3.		D10	7	7	0	100	0.94	8	8.1
4.		D17	7	7	0	100	0.76	14.57	6.46
5.		D19	5	5	0	100	0.81	14	6.89
6.		URP30F	13	13	0	100	0.77	14.62	6.55
7.		URP6R	7	6	1	85.7	0.61	20.86	4.44
8.		URP9F	14	13	1	92.9	0.75	15	6.1
9.		URP1F	8	8	0	100	0.73	17.25	6.21
10.		URP4R	7	6	1	85.7	0.75	15.14	5.46
TOTAL			85	81	4				
AVERAGE/PRIMER			8.5	8.1	0.4	95.3	0.77	14.78	6.27

Notes: TB = Total Band; PB = Polymorphic Band; MB = Monomorphic Band; PPB = Percentage of Polymorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index

Table 3.8: Summary of the various genetic diversity indices analyzed in *Lentinus* species using DAMD marker

Species Name	N	PPB	Average PIC	Average *Rp*	Average MI	Na (Mean±SD)	Ne (Mean±SD)	H (Mean±SD)	I (Mean±SD)	Ht (Mean±SD)	Hs (Mean±SD)	Gst	Nm
Lentinus squarrosulus	6	59.39	0.41	8.63	1.81	1.45±0.5	1.3±0.38	0.17±0.2	0.25±0.29	0.31±0.02	0.15±0.01	0.51	0.48
Lentinus sajor-caju	6	70.94	0.48	7.94	1.74	1.38±0.49	1.24±0.35	0.14±0.19	0.21±0.28				
Lentinus tigrinus	6	76.15	0.47	8.3	1.51	1.35±0.48	1.26±0.38	0.15±0.21	0.21±0.29				
Lentinus squarrosulus* +*Lentinus sajor-caju*+ *Lentinus tigrinus	18	95.29	0.77	14.78	6.27	1.95±0.21	1.52±0.32	0.31±0.15	0.48±0.19				

Notes: N= No. of accessions; PPB = Percentage of Polymorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index; Na = Observed no. of alleles; Ne = Effective no. of alleles; H = Nei's gene diversity; I = Shannon's Index; Ht = Total gene diversity; Gst = Genetic differentiation; Nm = Gene flow

Intra-specific natural genetic variation in three *Lentinus* species as revealed by DAMD markers

Lentinus squarrosulus

For intra-specific variation, the 3 accessions of *L. squarrosulus* and 3 accessions of *L. squarrosulus* var. *squarrosulus* produced a total of 67 amplicons by 10 DAMD primers, out of which 38 were observed to be polymorphic with an average of 3.8 polymorphic bands per primer and 29 were found to be monomorphic bands. The average polymorphism was found to be 59.39%. The average PIC value was observed to be 0.41 per primer. The average *Rp* value was found to be 8.63 and the average MI was found to be 1.81. The number of observed alleles (Na) was 1.45 (±0.5) and the effective number of alleles (Ne) was 1.3 (±0.38). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.17 (±0.2) and 0.25(±0.29) respectively (**Table 3.4 and 3.8**).

Lentinus sajor-caju

The 6 accessions of *L. sajor-caju* produced a total of 46 amplicons by 10 DAMD primers, out of which 32 were observed to be polymorphic with an average of 3.2 polymorphic bands per primer and 14 were found to be monomorphic bands. The average polymorphism was found to be 70.94%. The average PIC value was observed to be 0.48 per primer. The average *Rp* value was found to be 7.94 and the average MI was found to be 1.74. The number of observed alleles (Na) was 1.38 (±0.49) and the effective number of alleles (Ne) was 1.24 (±0.35). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.14 (±0.19) and 0.21 (±0.28) respectively (**Table 3.5 and 3.8**).

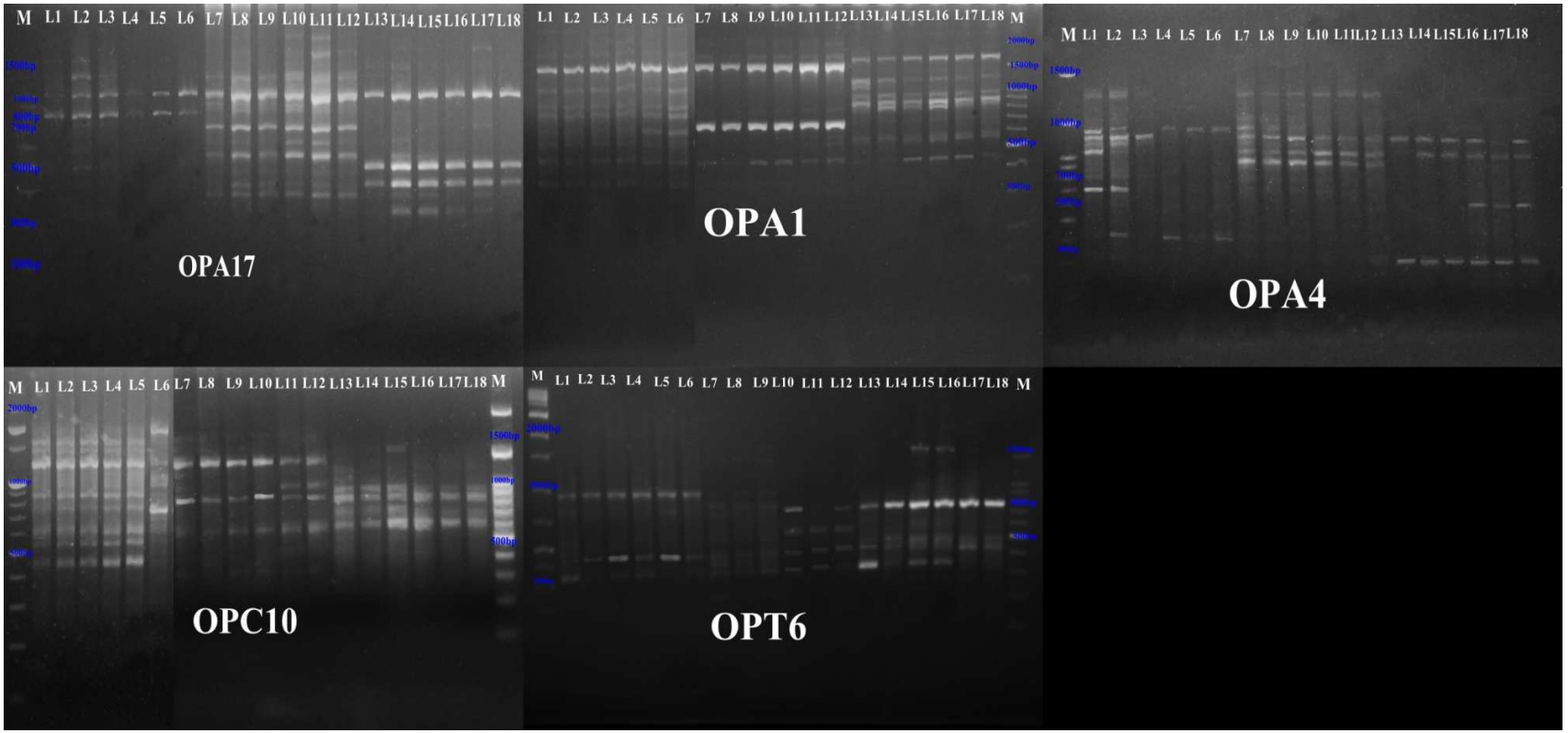


Figure 3.13 shows the representative of the banding pattern obtained for 18 accessions of *Lentinus* species with RAPD primers – OPA17, OPA1, OPA4, OPC10 & OPT6.

Lentinus tigrinus

A total of 40 amplicons were produced in 6 accessions of *L. tigrinus* by 10 DAMD primers, out of which 30 were observed to be polymorphic with an average of 3 polymorphic bands per primer and 10 were found to be monomorphic bands. The average polymorphism was found to be 76.15%. The average PIC value was observed to be 0.47 per primer. The average *Rp* value was found to be 8.3 and the average MI was found to be 1.51. The number of observed alleles (Na) was 1.35 (±0.48) and the effective number of alleles (Ne) was 1.26 (±0.38). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.15 (±0.21) and 0.21(±0.29) respectively (**Table 3.6 and 3.8**).

RAPD Analysis

Inter-specific natural genetic variation in three *Lentinus* species as revealed by RAPD markers

Out of 25 primers screened, 8 primers resulted clear and polymorphic bands. **Figure 3.13** shows the representative of the banding pattern. A total of 68 amplicons were produced out of which 67 were found to be polymorphic with an average of 8.38 polymorphic bands per primer and 1 was found to be monomorphic band. The percentage of polymorphic bands ranged from 85.71% to 100% with an average of 98.21% polymorphism among the 18 accessions of *Lentinus* species belonging to *L. sajor-caju, L. squarrosulus, L. squarrosulus* var. *squarrosulus* and *L. tigrinus* respectively. Primer OPA17 yielded the highest number of polymorphic bands which is 12, while primer OPT7 yielded the lowest number of polymorphic bands i.e. 5. The PIC value ranged from 0.61 to 0.84 with an average PIC value of 0.71 per primer. The *Rp* values ranged from 12.67 to 22.67 with an average of 18.01 per primer and the highest *Rp* value of 22.67 was observed in primer OPA1. The MI values

ranged from 4.88 to 7.14 with an average of 5.91 per primer. The highest MI value was observed in primer OPC10. The number of observed alleles (Na) was 1.99 (±0.12) and the effective number of alleles (Ne) was 1.66 (±0.29). The Nei's gene diversity (H) and Shannon's index (I) among all the populations were 0.38 (±0.12) and 0.56 (±0.15) respectively. The total gene diversity (Ht) and Heterozygosity (Hs) within the populations was found to be 0.38 (±0.02) and 0.22 (±0.02). The mean coefficient of genetic differentiation (Gst) was found to be 0.43 while the estimate of gene flow (Nm) was found to be 0.66 (**Table 3.9 and 3.10**).

Table 3.9: Data on polymorphism obtained by RAPD primers used to assess genetic diversity of *Lentinus* species

Sl. No.	**Marker**	**Primer Name**	**TB**	**PB**	**MB**	**PPB**	**PIC**	***Rp***	**MI**
1.	**RAPD**	OPA1	9	9	0	100	0.61	22.67	5.19
2.		OPT 6	7	6	1	85.71	0.67	19.43	4.88
3.		OPA4	9	9	0	100	0.69	19.33	5.87
4.		OPA17	12	12	0	100	0.77	15.5	6.55
5.		OPD18	9	9	0	100	0.64	20.44	5.44
6.		OPT7	5	5	0	100	0.62	20.8	5.27
7.		OPC10	9	9	0	100	0.84	12.67	7.14
8.		OPT5	8	8	0	100	0.82	13.25	6.97
TOTAL			68	67	1				
AVERAGE/PRIMER			8.5	8.38	0.13	98.21	0.71	18.01	5.91

Notes: TB = Total Band; PB = Polymorphic Band; MB = Monomorphic Band; PPB = Percentage of Polymorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index

Table 3.10: Summary of the various genetic diversity indices analyzed in *Lentinus* species using RAPD marker

Species Name	N	PPB	Average PIC	Average *Rp*	Average MI	Na (Mean±SD)	Ne (Mean±SD)	H (Mean±SD)	I (Mean±SD)	Ht (Mean±SD)	Hs (Mean±SD)	Gst	Nm
Lentinus squarrosulus	6	82.68	0.56	7.42	2.92	1.59±0.5	1.42±0.39	0.24±0.21	0.35±0.3	0.38±0.02	0.22±0.02	0.43	0.66
Lentinus sajor-caju	6	69.33	0.51	7.68	2.94	1.61±0.5	1.38±0.37	0.22±0.2	0.33±0.29				
Lentinus tigrinus	6	71.52	0.51	7.63	2.34	1.51±0.5	1.32±0.38	0.19±0.21	0.28±0.3				
Lentinus squarrosulus +Lentinus sajor-caju+ Lentinus tigrinus	18	98.21	0.71	18.01	5.91	1.99±0.12	1.66±0.29	0.38±0.12	0.56±0.15				

Notes: N= No. of accessions; PPB = Percentage of Polymorphic Band; PIC = Polymorphic Information Content; *Rp* = Resolving Power; MI = Marker Index; Na = Observed no. of alleles; Ne = Effective no. of alleles; H = Nei's gene diversity; I = Shannon's Index; Ht = Total gene diversity; Gst = Genetic differentiation; Nm = Gene flow

Intra-specific natural genetic variation in three *Lentinus* species as revealed by RAPD markers

Lentinus squarrosulus

At the intra-specific level, the 3 accessions of *L. squarrosulus* and 3 accessions of *L. squarrosulus* var. *squarrosulus* produced a total of 48 amplicons by 8 RAPD primers, out of which 40 were observed to be polymorphic with an average of 5 polymorphic bands per primer and 8 were found to be monomorphic bands. The average polymorphism was found to be 82.68%. The average PIC value was observed to be 0.56 per primer. The average *Rp* value was found to be 7.42 and the average MI was found to be 2.92. The number of observed alleles (Na) was 1.59 (±0.5) and the effective number of alleles (Ne) was 1.42 (±0.39). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.24 (±0.21) and 0.35(±0.3) respectively (**Table 3.4 and 3.10**).

Lentinus sajor-caju

A total of 61 amplicons were produced in 6 accessions of *L. sajor-caju* by 8 RAPD primers, out of which 40 were observed to be polymorphic with an average of 5 polymorphic bands per primer and 21 were found to be monomorphic bands. The average polymorphism was found to be 69.33%. The average PIC value was found to be 0.51 per primer. The average *Rp* value was found to be 7.68 and the average MI was found to be 2.94. The number of observed alleles (Na) was 1.61 (±0.5) and the effective number of alleles (Ne) was 1.38 (±0.37). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.22 (±0.2) and 0.33 (±0.29) respectively (**Table 3.5 and 3.10**).

Table 3.11: Summary of the various genetic diversity indices analyzed in *Lentinus* species using ISSR, DAMD and RAPD markers

Markers	N	PPB	Average PIC	Average *Rp*	Average MI	Na (Mean±SD)	Ne (Mean±SD)	H (Mean±SD)	I (Mean±SD)	Ht (Mean±SD)	Gst	Nm
ISSR	18	94.19	0.7	17.84	5.51	1.98±0.31	1.62±0.34	0.35±0.16	0.52±0.2	0.35±0.02	0.36	0.91
DAMD	18	95.3	0.77	14.78	6.27	1.95±0.21	1.52±0.32	0.31±0.15	0.48±0.19	0.31±0.02	0.51	0.48
RAPD	18	98.21	0.71	18.01	5.91	1.99±0.12	1.66±0.29	0.38±0.12	0.56±0.15	0.38±0.02	0.43	0.66
ISSR+DAMD+RAPD	18	95.78	0.72	16.79	5.89	1.97±0.22	1.61±0.32	0.35±0.15	0.52±0.19	0.35±0.02	0.43	0.66

Notes: Na = Observed no. of alleles; Ne = Effective no. of alleles; H = Nei's gene diversity; I = Shannon's Index; Ht = Total gene diversity; Gst = Genetic differentiation; Nm = Gene flow

Table 3.12: Genetic variations as revealed through combined RAPD +ISSR + DAMD markers among three *Lentinus* species

Population	N	PPB	Na (Mean±SD)	Ne (Mean±SD)	H (Mean±SD)	I (Mean±SD)	Ht (Mean±SD)	Gst	Nm
Lentinus squarrosulus	6	70.92	1.57±0.51	1.39±0.39	0.22±0.21	0.33±0.31	0.35±0.02	0.43	0.66
Lentinus sajor-caju	6	66.2	1.45±0.49	1.31±0.39	0.18±0.21	0.26±0.29			
Lentinus tigrinus	6	78.42	1.48±0.5	1.33±0.39	0.19±0.21	0.28±0.29			
Lentinus squarrosulus+ Lentinus sajor-caju+ Lentinus tigrinus	18	95.78	1.97±0.22	1.61±0.32	0.35±0.15	0.52±0.19			

Notes: N= No. of accessions; Na = Observed no. of alleles; Ne = Effective no. of alleles; H = Nei's gene diversity; I = Shannon's Index; Ht = Total gene diversity; Gst = Genetic differentiation; Nm = Gene flow

Lentinus tigrinus

A total of 49 amplicons were produced in 6 accessions of *L. tigrinus* by 8 RAPD primers, out of which 35 were found to be polymorphic with an average of 4.38 polymorphic bands per primer and 14 were found to be monomorphic bands. The average polymorphism was found to be 71.52%. The average PIC value was found to be 0.51 per primer. The average *Rp* value was found to be 7.63 and the average MI was found to be 2.34. The number of observed alleles (Na) was 1.51 (±0.5) and the effective number of alleles (Ne) was 1.32 (±0.38). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.19 (±0.21) and 0.28 (±0.3) respectively (**Table 3.6 and 3.10**).

Table 3.11 shows the summary of the various genetic diversity indices analyzed in *Lentinus* species using ISSR, DAMD and RAPD markers and the combination of all the three markers data (ISSR+DAMD+RAPD). The combined marker data shows average polymorphism of 95.78%. The average PIC value was observed to be 0.72 per primer. The average *Rp* value was found to be 16.79 and the average MI was found to be 5.89. The number of observed alleles (Na) was 1.97 (±0.22) and the effective number of alleles (Ne) was 1.61 (±0.32). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.35 (±0.15) and 0.52 (±0.19). The total gene diversity (Ht) was found to be 0.35 (±0.02), mean coefficient of genetic differentiation (Gst) was found to be 0.43 while the estimate of gene flow (Nm) was found to be 0.66 respectively.

Table 3.12 shows the genetic variations as revealed through combined RAPD +ISSR + DAMD markers data among the three *Lentinus* species. In the 3 accessions of *L. squarrosulus* and 3 accessions of *L. squarrosulus* var. *squarrosulus*, the combined markers data produced average mean polymorphism of 70.92%. The number of observed alleles (Na)

was 1.57 (±0.51) and the effective number of alleles (Ne) was 1.39 (±0.39). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.22 (±0.21) and 0.33 (±0.31). In the 6 accessions of *L. sajor-caju*, the combined markers data produced average mean polymorphism of 66.2%. The number of observed alleles (Na) was 1.45 (±0.49) and the effective number of alleles (Ne) was 1.31 (±0.39). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.18 (±0.21) and 0.26 (±0.29). In the 6 accessions of *L. tigrinus*, the combined markers data produced average mean polymorphism of 78.42%. The number of observed alleles (Na) was 1.48 (±0.5) and the effective number of alleles (Ne) was 1.33 (±0.39). The Nei's gene diversity (H) and Shannon's index (I) were found to be 0.19 (±0.21) and 0.28 (±0.29). The total gene diversity (Ht) was found to be 0.35 (±0.02), mean coefficient of genetic differentiation (Gst) was found to be 0.43 while the estimate of gene flow (Nm) was found to be 0.66 respectively.

Phylogenetic analysis

The UPGMA dendrogram constructed based on pair-wise similarity matrix was calculated by DICE similarity coefficient for all the three marker (ISSR, DAMD and RAPD) datasets. Similar tree topologies was observed for all the markers which resolved all the accessions of *Lentinus* species into two major clusters – Cluster I and Cluster II (**Figure 3.14, 3.15, 3.16**). Cluster I divided into two sub-clusters - A and B. *L. sajor-caju* and *L. tigrinus* formed the sub-clusters - A and B which included all the 12 accessions of *L. sajor-caju* and *L. tigrinus*. Cluster II included all the 6 accessions of *L. squarrosulus* and *L. squarrosulus* var. *squarrosulus* which also divided into two sub-clusters - C and D. *L. squarrosulus* and *L. squarrosulus* var. *squarrosulus* formed the sub-clusters.

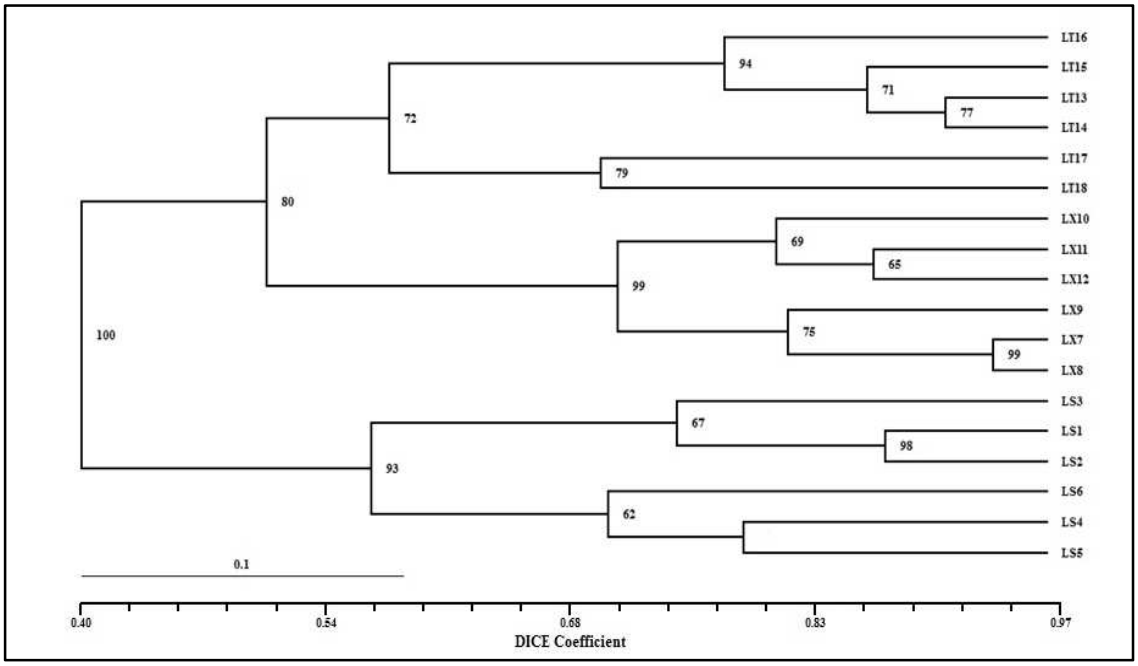


Figure 3.14: UPGMA dendrogram constructed based on ISSR data for all 18 accessions of *Lentinus* from Nagaland.

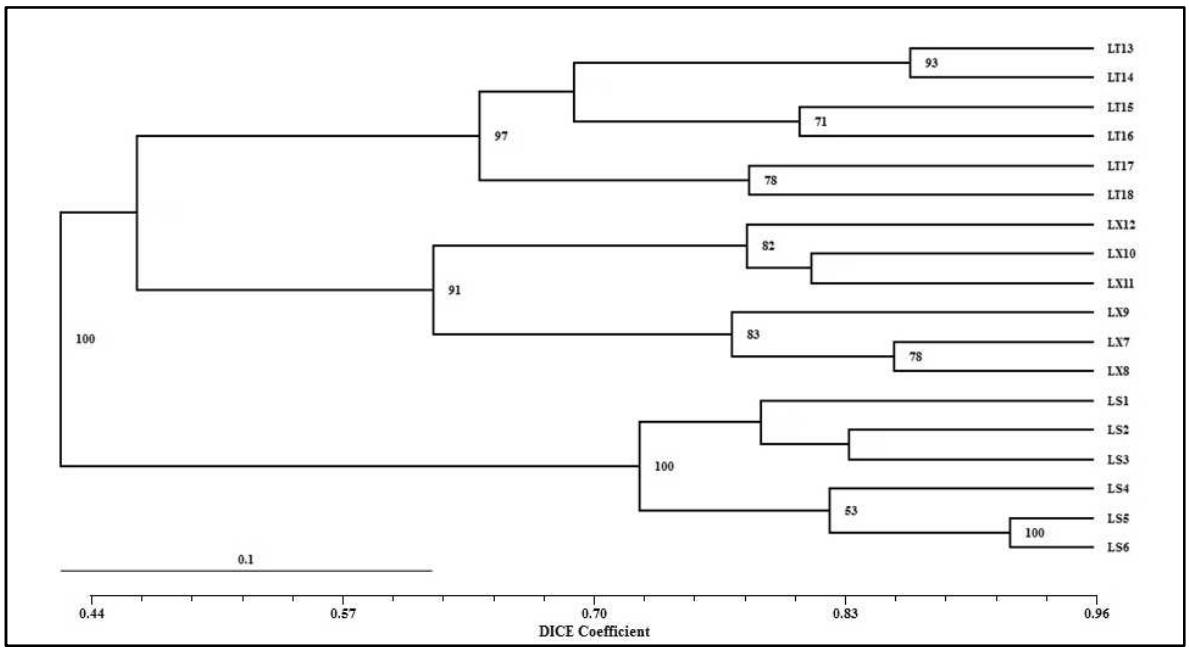


Figure 3.15: UPGMA dendrogram constructed based on DAMD data for all 18 accessions of *Lentinus* from Nagaland.

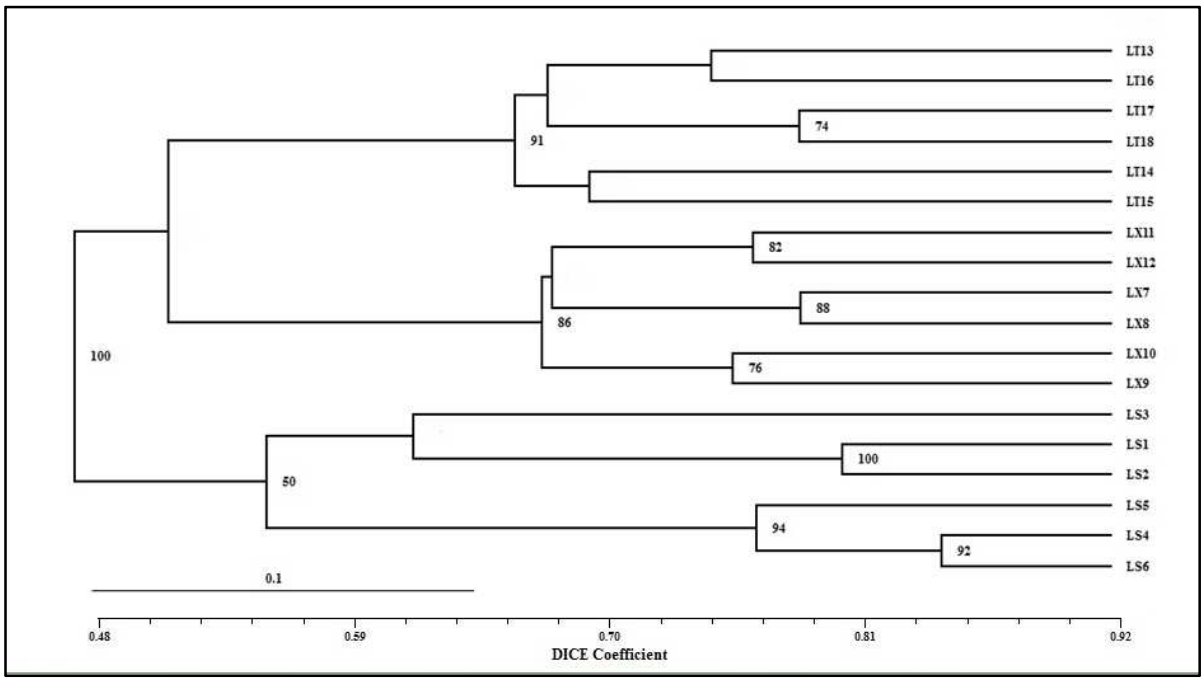


Figure 3.16: UPGMA dendrogram constructed based on RAPD data for all 18 accessions of *Lentinus* from Nagaland.

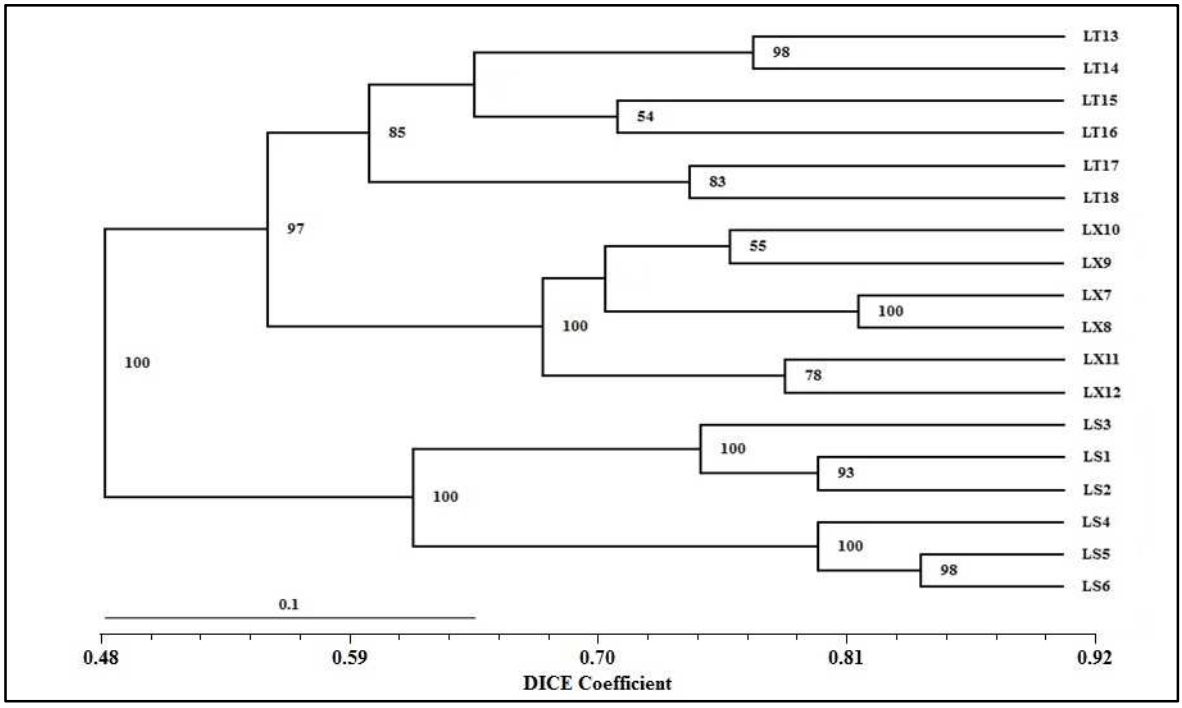


Figure 3.17: UPGMA dendrogram constructed based on pooled data (ISSR+DAMD+RAPD) for all 18 accessions of *Lentinus* from Nagaland.

A UPGMA dendrogram was also constructed based on pair-wise similarity matrix calculated by DICE similarity coefficient from pooled data of all the three markers (ISSR, DAMD and RAPD) i.e., concatenation. The dendrogram obtained showed similar tree topology given by ISSR, DAMD and RAPD markers. **Figure 3.17** shows the tree representation from the pooled data. The UPGMA dendrogram resolved all the accessions of *Lentinus* species into two major clusters from the pooled data – Cluster I and Cluster II. Cluster I divided into two sub-clusters of *L. sajor-caju* and *L. tigrinus* and Cluster II included all the 6 accessions of *L. squarrosulus* and *L. squarrosulus* var. *squarrosulus* which also divided into two sub-clusters.

For *L. squarrosulus* and *L. squarrosulus* var. *squarrosulus*, the dendrogram obtained by ISSR, DAMD and RAPD markers grouped the *L. squarrosulus* accessions (LS1-Mokokchung, LS2-Tuensang, LS3-Wokha) in one cluster and the accessions of *L. squarrosulus* var. *squarrosulus* (LS4-Zunheboto, LS5-Kohima, LS6-Longleng) in another cluster. For *L. tigrinus*, the dendrogram obtained by DAMD and RAPD markers formed three small sub-clusters while the dendrogram obtained by ISSR marker formed a slightly different tree topology. However, in all the analysis the collections of Longleng and Tuensang (LT17 and LT18) clustered together. For *L. sajor-caju*, the dendrogram obtained by ISSR and DAMD markers grouped the *L. sajor-caju* collections into two clusters. The collections from Longleng (LX7), Mokokchung (LX9) and Wokha (LX8) resolved into one sub-cluster and the collections from Kohima (LX10), Tuensang (LX11) and Zunheboto (LX12) resolved into the other sub-cluster. However, RAPD marker resolved the tree into three small sub-clusters, collections from Mokokchung (LX9) and Kohima (LX10) in one sub-cluster, collections from Longleng (LX7) and Wokha (LX8) formed one sub-cluster and

collections from Tuensang (LX11) and Zunheboto (LX12) formed the other sub-cluster. The clustering pattern obtained from all the three markers showed the extent of genetic diversity present in *Lentinus* species; however, it may or may not be related to geographical locations. Nevertheless, it can be observed that the genetic diversity or genetic similarity differed from species to species in different geographical locations.

Mantel's correlation (Mantel, 1967) of the similarity matrices for the marker systems was done by using the MXCOMP module of NTSYS version 2.21software to test the Goodness-of-Fit for individual marker system used. Rohlf (1988, 2000) included a Goodness-of-Fit test known as cophenetic correlation in order to examine the accuracy of the tree that was generated by the markers data whether it represented the original data well. The cophenetic correlation is executed between the genetic distance datasets and one that is generated from the tree itself i.e., a cophenetic matrix, then the two matrices are analyzed using the Mantel test. If the correlation is high, then that means the tree represents the original data well. If the correlation is low, then that means the tree does not represent the original data well. Given below are the Mantel's correlation (*r*) values of the three marker systems used in the present study to test the Goodness-of-Fit for each marker system. Mantel's correlation (*r*) values of individual marker system is observed to be high which points out that the tree generated represented the original data accurately.

Mantel's correlation (Mantel, 1967) for each marker system

Marker system	Mantel's correlation (*r*)	Goodness-of-Fit (Rohlf, 1988)
ISSR	0.88273	*r*≥0.9; very good fit *r*≥0.8; good fit *r*≥0.7; poor fit *r*<0.7; very poor fit
DAMD	0.92508	
RAPD	0.82677	

* P < 0.01

Mantel's correlation (Mantel, 1967) of the similarity matrices between each marker system was done to check the level of correlation between the marker systems. Given below are the Mantel's correlation (*r*) values between the three marker systems. Mantel's correlation (*r*) values displayed a significant difference in the degree of correlation which revealed the extent of genome coverage by the markers as each marker system targeted a specific region and are independent of each other.

Mantel's correlation (Mantel, 1967) between marker systems

Marker system	Mantel's correlation (*r*)	Goodness-of-Fit (Rohlf, 1988)
ISSR and DAMD	0.59659	*r*≥0.9; very good fit *r*≥0.8; good fit *r*≥0.7; poor fit *r*<0.7; very poor fit
DAMD and RAPD	0.60343	
RAPD and ISSR	0.44838	

* P < 0.01

Discussion

The morphological characters play a vital role in identification of mushrooms however, for some species, morphological characters are not enough to authenticate the species identity as mushrooms are polymorphic in nature. Thus, it becomes imperative to use molecular tools for correct identification of mushrooms. The molecular phylogenetic studies based on ITS, 18S and 28S *rRNA* genes revealed the inter-species relationships among the mushroom species. The genus *Lentinus* is known to be highly plastic and always appear in varying morphological characters (Abdullah, 2014). However, the utilization of molecular markers in the present study helped in reaffirming the identification of the WEM species generated by the morphological observations. Even the intra-specific relationship between the *Lentinus* species was deduced from the phylogenetic tree topologies. In the present study, amplification of ITS1-5.8S rDNA-ITS2 regions by universal primers ITS5 and ITS4 produced DNA fragment band between (500-800) bp which are in consistency with Gardes and Bruns (1993) and Maeta *et al*. (2008) who proposed the expected DNA fragment size to be between (600-800) bp for rDNA target region. Blaalid *et al.* (2013) also proposed similar explanations that the ITS region of fungi varies between (450-750) bp approx in length with few exceptions. The observation of this study is in agreement with past reports where it is concluded that ITS region is a reliable barcode for identifying mushrooms and other fungal species (Schoch *et al*., 2012). The ITS region have always been targeted for studying the genetic relationship because this region has the highest degree of variation as compared to other nuclear rDNA regions which allows discriminating between species or strains of mushrooms (Bruns *et al*., 1991; Gardes and Bruns, 1993; Martin *et al*., 2004; Begerow *et al*., 2010). This region has also been immensely used in fungal ecological studies for a broader

resolution of identity at and below the genus level (Begerow *et al*., 2010). The 18S *rRNA* phylogenetic trees were constructed using amplified sequences of 18S *rRNA* sequences and similar sequences retrieved from GenBank through NCBI blast. The nucleotide blasting of 18S *rRNA* sequences of *S. commune* AO-DEBCR-7 in NCBI showed 99% identity with *S. commune* and *S. radiatum* but for the rest of the WEM species, the nucleotide sequences did not show good identity results after blasting. This confirms and is in agreement with other reports that the data generated from 18S *rRNA* is limited for accurate identity of the mushroom species due to very less hyper variable domains in fungi for 18S *rRNA* genes which limits its effectiveness (Schoch *et al*., 2012). Therefore, the trees generated by 18S *rRNA* datasets could not resolve conclusively the species identity and phylogeny. The phylogenetic tree generated from 28S *rRNA* sequences showed similarity with the tree topology generated from ITS sequences and was quite useful in interpreting the phylogeny between the mushroom species presently studied. The 28S *rRNA* gene has variable regions with sequence divergency- D1, D2, and D3 which makes it sufficient to study the phylogenetic relationships between the fungal species (Hong *et al*., 2000; Wesselink *et al*., 2002). This observation is in agreement to Schoch *et al*. (2012) reports that 28S *rRNA* genes gives superior species resolution in some taxonomic groups but slightly inferior to the ITS and hence, for fungal identifications including mushrooms, a combination of two markers is best. The current molecular advances in multi-loci phylogeny gives stable and well defined phylogenetic analysis, but still there is much to be resolved. One of the major problems in species identity is that only a few mushroom species have been sequenced and deposited in public repositories like GenBank. Moreover, the target data for some mushroom species is totally absent in GenBank domains which lead to erroneous tree topology and inference.

Thus, it is advisable to use both morphological and phylogenetic species criterion together to define a species (Cai *et al.,* 2011). Nevertheless, it is always better to use two or more markers for identification and characterization of mushrooms and other fungi for authentication. In Nagaland, the WEM species are generally identified by conventional method which many a times results in wrong identification as many mushrooms show variations in their morphological characters like texture, color, size, etc depending on soil and climatic conditions in different regions. To popularize the consumption of WEM species, it is important to identify conclusively. In countries like Japan and China, WEM have already been domesticated and commercialized but in remote regions like Nagaland, it is still under-studied and unknown, as such WEM are available only during the mushrooming season to the people.

The present work is the first attempt from Nagaland to correctly identify some of the popular WEM species based on the data generated by the multi-gene molecular characterization along with their morphological traits. The utilization of molecular markers for mushroom identification is highly desirable and practical because it is reliable and quick. The molecular phylogenetic analysis also resolved successfully the WEM species with respect to their infrageneric groups. The high CI values of the mushrooms species indicated the low homoplasy nature. In this study, the ITS and 28S *rRNA* datasets were observed to be more informative then the 18S *rRNA* datasets. Moreover, the molecular barcodes produced for each of the mushroom specimens in the present analysis will help in future research and conservation of these popularly consumed WEM of the region. This work will create awareness to researchers working in the field of fungi to make more in-depth study on wild mushrooms of Nagaland. As said in previous chapters, there is very few works carried out on

mushroom diversity of Nagaland, so proper documentation, identification and characterization of the macrofungal population is the need of the hour to create a database of the rich mushroom wealth of the state.

Mushrooms are in high demand because of its therapeutic and pharmaceutical applications apart from being a popular nutritious food to humans. The information on the genetic diversity and variability of these mushrooms is necessary to enable their sustainable cultivation, utilization and conservation of the existing germplasm. ISSR and RAPD molecular markers are among the most widely used to examine the genetic diversity, genetic map construction, linkage analysis and DNA fingerprinting in plants (Darvasi *et al*., 1993; Neale and Savolainen, 2004). Mushrooms are healthy natural food which originates from an organic farming system (Moore and Chiu, 2001). For sustainable mushroom cultivation and production, novel strains with improved characters are essential. But, mushroom strains are not easy to discriminate due to lack of definite distinguishable characters which impedes strain improvement and protection problematic. These provide reliable information for strain protection and identification in mushrooms. Over the years, the method for detection and assessment of genetic diversity has extended from assessment of discrete morphological to biochemical and molecular traits. There are very few reports available on inter- and intra-specific genetic diversity of *Lentinus* using ISSR, RAPD and DAMD markers. Dwivedi *et al*. (2017) has studied the inter- and intra-specific genetic diversity of *Lentinus* sp. using RAPD marker system. The present study is the first of its kind to use RAPD, ISSR and DAMD molecular markers both individually and in combination to study the extent of genetic diversity and variability among the natural populations of *Lentinus sajor-caju*, *L. squarrosulus*, *L. squarrosulus* var. *squarrosulus* and *L. tigrinus* respectively from North-East

India. The main advantages of using these primers are that they are quick, reliable, highly polymorphic and randomly distributed throughout the genomes. As the markers are PCR-based, therefore, do not need any sequence information for primer construction and require very small amounts of template DNA. The use of these molecular markers has aided to evaluate genetic diversity by recognizing the polymorphic nucleotide sequences dispersed throughout the genome and to determine inter- and intra-species genetic relationship (Gostimsky *et al*., 2005; Karakas *et al*., 2010) among wild and cultivated taxa. A comparison of the degree of polymorphism and discriminatory efficiency of RAPD, ISSR and DAMD markers observed that each marker technique was able to detect genetic variation in the three populations of *Lentinus* species.

In the present study, 28 primers (10 ISSR+10 DAMD+8 RAPD) were used to examine the genetic diversity within and among the populations of the three *Lentinus* species. A total of 237 bands were scored in 18 accessions of three *Lentinus* species, out of which 227 bands were polymorphic with average polymorphism of 95.78%. The average PIC value was found to be 0.72 per primer and the average *Rp* and MI value was found to be 16.79 and 5.89 respectively (**Table 4.10**). The discriminatory power of a primer is a reliable indicator of the efficiency of that primer in genetic diversity assessment. The utility and efficiency of the molecular markers used in the present study was tested by comparing the PIC, *Rp* and MI values within and among the populations for their discriminatory power. The PIC is used to evaluate the overall utility of a marker system in discriminating the extent of polymorphism across the taxa (Powell *et al*., 1996). The PIC of each primer in all three marker systems was determined to assess the level of informativeness i.e., high PIC > 0.5, moderate 0.5 > PIC >0.25 and low PIC < 0.25 (Botstein *et al*., 1980; Weber *et al*., 2005;

Dwivedi *et al*., 2017). In the present study, the mean PIC values were observed to be high in all the three *Lentinus* species. The DAMD marker system showed a slightly higher mean PIC value (0.77) as compared to RAPD (0.71) and ISSR (0.7) across the 18 accessions of *Lentinus* species. The mean *Rp* value was higher in RAPD (18.01) as compared to ISSR (17.84) and DAMD (14.78) and the mean MI value was higher in RAPD (18.01) as compared to ISSR (17.84) and DAMD (14.78) (**Table 3.2, 3.7 and 3.9**). In *L. squarrosulus* species, high mean PIC value was observed in RAPD (0.56) as compared to ISSR (0.51) and DAMD (0.41). The mean *Rp* value was higher in DAMD (8.63) as compared to ISSR (7.91) and RAPD (7.42) and the mean MI value was higher in ISSR (2.96) as compared to RAPD (2.92) and DAMD (1.81) (**Table 3.4**). In *L. sajor-caju* species, high mean PIC value was observed in RAPD (0.51) as compared to DAMD (0.48) and ISSR (0.41). The mean *Rp* value was higher in ISSR (9.11) as compared to DAMD (7.94) and RAPD (7.68) and the mean MI value was higher in RAPD (2.94) as compared to DAMD (1.74) and ISSR (1.34) (**Table 3.5**). In *L. tigrinus* species, high mean PIC value was observed in ISSR (0.63) as compared to RAPD (0.51) and DAMD (0.47). The mean *Rp* value was higher in DAMD (8.3) as compared to RAPD (7.63) and ISSR (6.85) and the mean MI value was higher in ISSR (3.06) as compared to RAPD (2.34) and DAMD (1.51) (**Table 3.6**). The overall comparative assessment of all the molecular approaches (ISSR, DAMD and RAPD) revealed hyper variability in the *Lentinus* species. This shows the extent of genome coverage as ISSR markers targets the microsatellite regions, while DAMD markers target the core minisatellite regions and RAPD markers amplify randomly within the target genome.

To evaluate the genetic variation within and among the *Lentinus* species parameters like number of polymorphic bands (NPB), percentage of polymorphic loci (PPB), observed

number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (H), Shannon index (I), Total gene diversity (Ht) and Heterozygosity (Hs) within populations were calculated. Polymorphism is common in nature and occurs when various phenotypes exist in populations of a single species. It is the outcome of evolution, heritable and modified according to the laws of nature. Polymorphism relates to genetic variation, biodiversity and adaptation (Dobzhansky, 1970). The potential of species to survive environmental processes depends a lot on the degree of genetic variability it possess (Ayala and Kiger, 1984). The average polymorphism observed in *L. squarrosulus* populations by the three markers are 73.05% (ISSR), 59.39% (DAMD) and 82.68% (RAPD). In *L. sajor-caju* populations, it is observed as 61.4% (ISSR), 70.94% (DAMD) and 69.33% (RAPD) and in *L. tigrinus* populations, it is observed as 86.21% (ISSR), 76.15% (DAMD) and 71.52% (RAPD). Among the three *Lentinus* species, *L. tigrinus* showed the highest polymorphism as compared to *L. sajor-caju* and *L. squarrosulus* mushrooms. Even the pooled data (ISSR+DAMD+RAPD) showed that *L. tigrinus* exhibited the highest polymorphism (78.42%) as compared to *L. squarrosulus* (70.92%) and *L. sajor-caju* (66.2%) mushrooms. In *L. squarrosulus* species, ISSR marker showed maximum Na (1.68±0.54), Ne (1.47±0.38), H (0.27±0.21) and I (0.41±0.31) as compared to RAPD Na (1.59±0.5), Ne (1.42±0.39), H (0.24±0.21), I (0.35±0.3) and DAMD markers Na (1.45±0.5), Ne (1.3±0.38), H (0.17±0.2) and I (0.25±0.29). In *L. sajor-caju* species, RAPD marker showed maximum Na (1.61±0.5), Ne (1.38±0.37), H (0.22±0.2) and I (0.33±0.29) as compared to ISSR Na (1.42±0.5), Ne (1.33±0.42), H (0.21±0.22), I (0.26±0.31) and DAMD markers Na (1.38±0.49), Ne (1.24±0.35), H (0.14±0.19) and I (0.21±0.28). In *L. tigrinus* species, ISSR marker showed maximum Na (1.57±0.4), Ne (1.4±0.38), H (0.23±0.21), I (0.34±0.3) as compared to RAPD

Na (1.51±0.5), Ne (1.32±0.38), H (0.19±0.21), I (0.28±0.3) and DAMD markers Na (1.35±0.48), Ne (1.26±0.38), H (0.15±0.21) and I (0.21±0.29). The Heterozygosity (Hs) within the populations was observed to be 0.23±0.02 (ISSR), 0.22±0.02 (RAPD) and 0.15±0.01 (DAMD), and the total gene diversity (Ht) was almost similar for all the three marker systems i.e. 0.38±0.02 (RAPD), 0.35±0.02 (ISSR) and 0.31±0.02 (DAMD) between the mushroom species. The genetic variation among all 18 accessions of *Lentinus* species, the Na (1.98±0.31), Ne (1.62±0.34), H (0.35±0.16), I (0.52±0.2), Ht (0.35±0.02), Hs (0.23 ± 0.02), Gst (0.36) and Nm (0.91) was observed for ISSR marker. While for DAMD marker, the Na (1.95±0.21), Ne (1.52±0.32), H (0.31±0.15), I (0.48±0.19), Ht (0.31±0.02), Hs (0.15 ± 0.01), Gst (0.51) and Nm (0.48) was observed. For RAPD marker, the Na (1.99±0.12), Ne (1.66±0.29), H (0.38±0.12), I (0.56±0.15), Ht (0.38±0.02), Hs (0.22±0.02), Gst (0.43) and Nm (0.66) was observed. The pooled data from all the three markers showed the Na (1.97±0.22), Ne (1.61±0.32), H (0.35±0.15), I (0.52±0.19), Ht (0.35±0.02), Hs (0.21± 0.01), Gst (0.43) and Nm (0.66). All these data revealed that the three marker systems used were efficient in analyzing the genetic diversity of the *Lentinus* species.

Gene flow is the movement of genes from one population to another. The extent of gene flow within the same population or between the related species determines their potential for genetic differentiation (Slatkin, 1985; McDermott and McDonald, 1993). Kumar *et al*. (2014) classified the estimate of gene flow (Nm) in nature as Nm < 1 – Low, Nm > 1 – Moderate and Nm > 4 – Extensive. Low or moderate Nm values between populations indicate high levels of genetic diversity. In the present study, low gene flow was observed for all marker systems viz. 0.91 (ISSR), 0.48 (DAMD) and 0.66 (RAPD) and also in combination (ISSR+DAMD+RAPD), the gene flow was low (0.66). On the other hand,

the relative genetic differentiation (Gst) was observed to be high *viz*. 0.36 (ISSR), 0.51 (DAMD), 0.43 (RAPD) and 0.43 (ISSR+DAMD+RAPD). This revealed that the populations of three *Lentinus* species show low gene flow having higher genetic differentiation (Gst) indicating high genetic diversity between them. The analysis of gene flow is an essential factor for genetic diversity analysis. To ensure genetic sustainability, a high range of genetic variation and diversity within populations is beneficial as adaptability and genetic diversity always correlates to each other, which is crucial for conservation in nature (Gregory *et al*., 2006). The UPGMA dendrogram revealed the genetic relationship among all the 18 collections of *Lentinus* species. The DICE coefficient of genetic similarity ranged from 0.40 to 0.97 (ISSR marker), 0.44 to 0.96 (DAMD marker), 0.48 to 0.92 (RAPD marker) and 0.48 to 0.92 (pooled data) (**Figure 3.14, 3.15, 3.16, 3.17**) and the cophenetic correlation (Mantel's test) for all the marker systems corresponded to a good fit i.e., ISSR – 0.88, DAMD – 0.93 and RAPD – 0.83. The genetic similarity or dissimilarity varies from place to place and between species to species respectively. The UPGMA clustering was also confirmed by PCoA analysis which revealed the genetic distinctiveness of the *Lentinus* populations studied (**Figure 3.18, 3.19, 3.20, 3.21**). Swingland (2001) has defined the genetic diversity as the presence of heritable variation in populations of same species and these heritable characters may probably express on its own as altered morphology, anatomy, physiological behavior or biochemical features. The genetic diversity and variability of any species is crucial because this forms the basis of the evolutionary potential of that species to adapt to the ever changing environment, whereas the loss of genetic variation reduces the ability of any species to cope up (Lande, 1988; Frankham, 1996; Shah *et al*., 2008; Hu *et al*., 2010). The presence of genetic variability is crucial because they are the building blocks of genetic diversity and the

foundation for sustainability. For conservation of germplasm, the genetic diversity will be the determining factor; as such the need arises to characterize the genetic resources available for their constructive utilization in breeding programmes (Bhandari *et al*., 2017). The present study significantly revealed the existence of high genetic diversity among and within the *Lentinus* species of Nagaland which remains under-utilized. The study demonstrated that the use of different markers targeting various regions or parts of the genome is a useful tool in assessing and examining the genetic diversity and variability of any organism including mushrooms. Thus, the integration of the three different markers – ISSR, DAMD and RAPD, targeting different amplification regions in the present study, was observed to be effective in detecting genetic variability and diversity of the mushroom resource. Hence, the marker systems used were highly efficient and informative in discriminating the mushroom species. This study may be useful in selection of unique gene pools for biotechnological exploitation and innovation of *Lentinus* species for the improvement of society at large. This result may therefore be used to widen the genetic bases in order to improve the quality of cultivated strains. Therefore, understanding the genetic variations and diversity of any crop plant including edible mushrooms will play a beneficial role in food security.

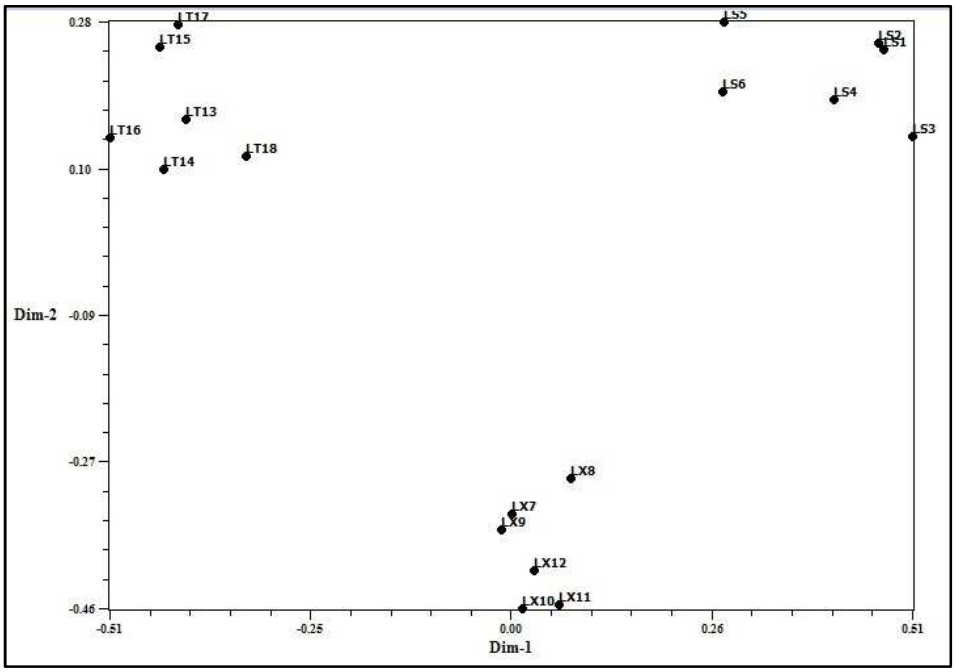


Figure 3.18: Scatter plot of the 18 accessions of *Lentinus* species using principle coordinate analysis (PCoA) based on ISSR data.

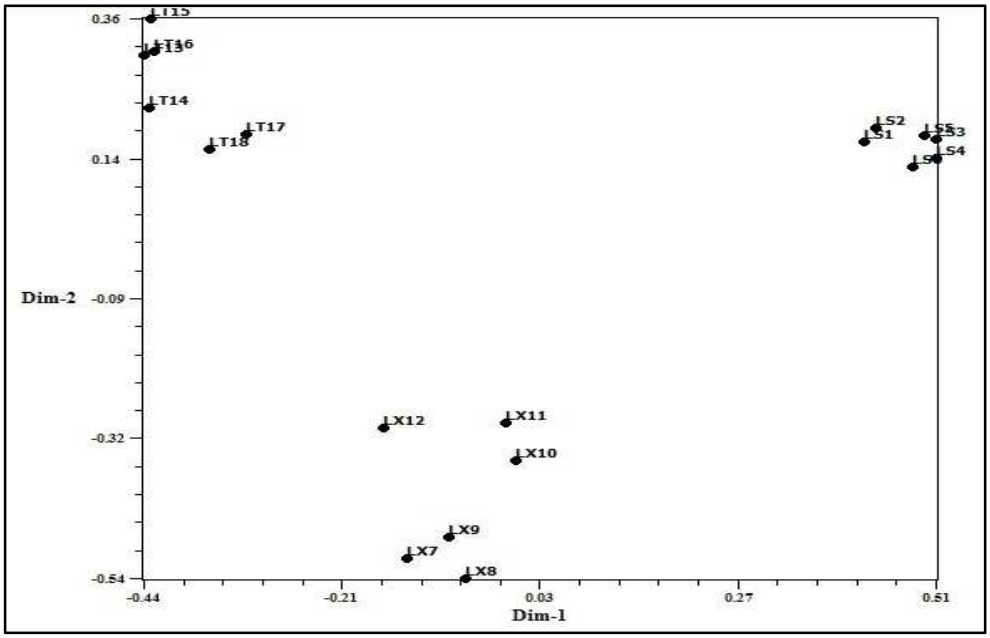


Figure 3.19: Scatter plot of the 18 accessions of *Lentinus* species using principle coordinate analysis (PCoA) based on DAMD data.

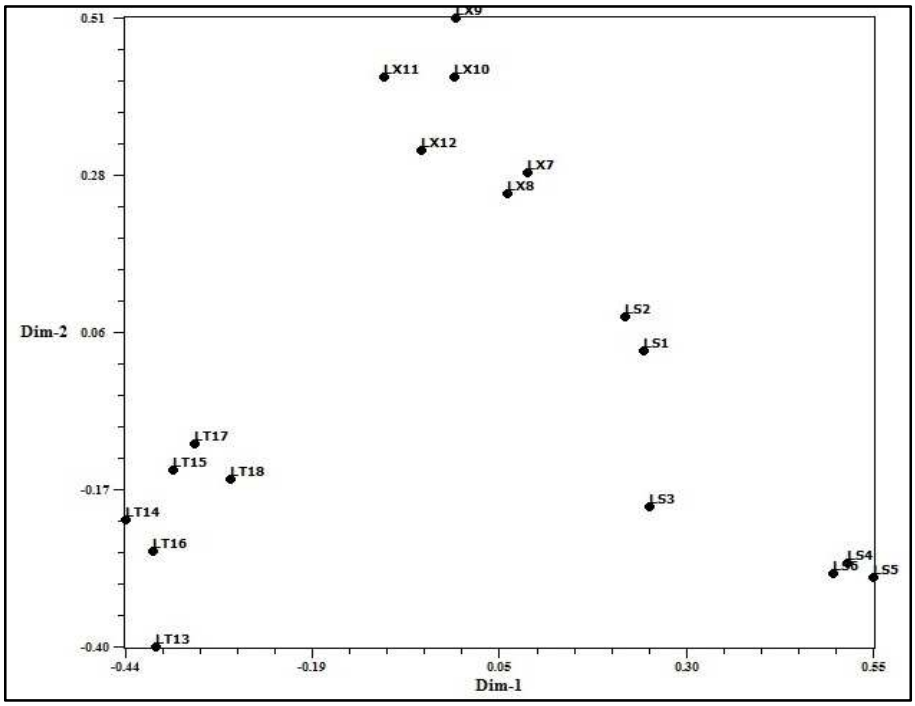


Figure 3.20: Scatter plot of the 18 accessions of *Lentinus* species using principle coordinate analysis (PCoA) based on RAPD data.

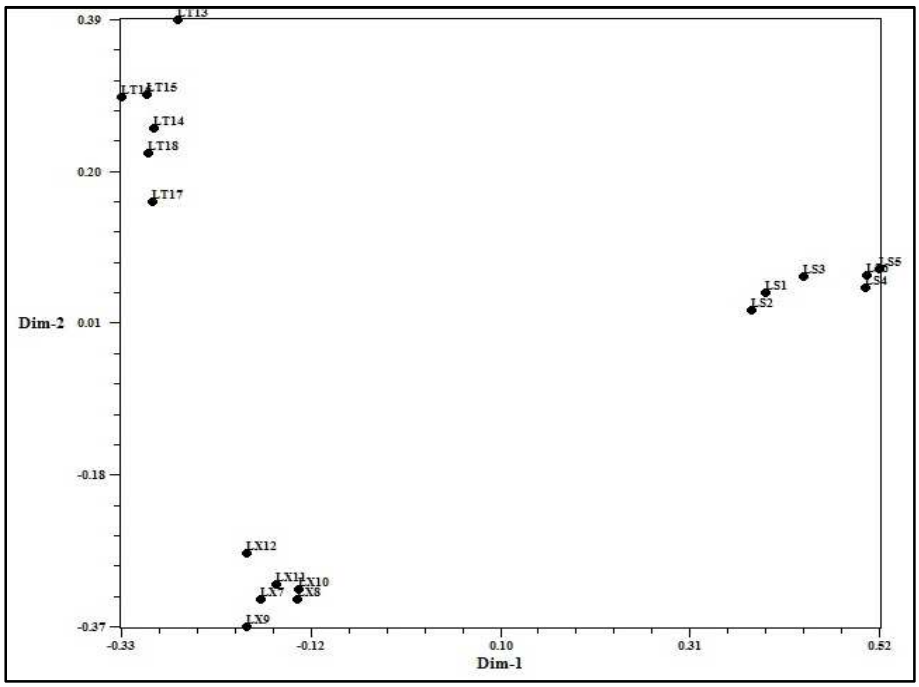


Figure 3.21: Scatter plot of the 18 accessions of *Lentinus* species using principle coordinate analysis (PCoA) based on pooled data of the three marker systems.

Summary and Conclusion

Nagaland is home to many popular wild edible mushroom species like *Lentinula edodes*, *Lentinus squarrosulus*, *L. sajor-caju*, *L. tigrinus*, *Schizophyllum commune*, *Termitomyces heimii*, *Auricularia auricula-judae*, *A. polytricha*, *Pleurotus* spp. etc. This chapter deals with the identification and molecular phylogenetic studies of six WEM species and one variety of *L. squarrosulus* based on morphological characteristics and molecular markers (ITS, 18S *rRNA* and 28S *rRNA* genes) data. The combined results of morphology and molecular analysis authenticated the identity of all the WEM species used in the present study. The ITS markers complemented 100% to morphological identity indicating that ITS marker can be regarded as universal DNA barcode marker for identifying mushrooms. The use of 28S *rRNA* markers also helped in identification and characterization of the mushroom species. However, the 18S *rRNA* markers could not resolve species identity for some of the species studied. The application of molecular markers for identification of mushrooms is highly desirable and practical because it is reliable and quick. The molecular phylogenetic analysis could also resolve successfully the six WEM species with respect to their infrageneric groups. The high CI values of the mushrooms species indicated the low homoplasy nature. The ITS and 28S *rRNA* datasets were found to be more informative then the 18S *rRNA* datasets. Moreover, the molecular barcodes generated for each individual mushroom specimen in the present investigation will help in future research and conservation of these popularly consumed WEM of the region.

The use of three different markers (ISSR, DAMD and RAPD) both individually and in combination provided a convenient method to assess the genetic variation and diversity in the 18 accessions of the three *Lentinus* species. The UPGMA dendrogram also revealed the

extent of genetic relationship among all the accessions of *Lentinus* species which was confirmed by PCoA analysis. Genetic variability and diversity assessment at intra- and inter-specific levels are vital for crop improvement because the presence of natural genetic variation is crucial to have opportunities for development of improved traits in crop production. The present study revealed the existence of high genetic diversity within the *Lentinus* species of Nagaland which remains under-utilized. All the marker systems used were observed to be highly efficient and informative in discriminating the mushroom species. Thus, this study throws light on the potential and importance of mushrooms especially the edible mushrooms as an economically valuable crop. Understanding the genetic variations and diversity of any crop plant including edible mushrooms will play a beneficial role in food security.

Chapter - 4

Nutritional Analysis

Introduction

Since the early ages, mushrooms have been regarded around the world as the most delectable and succulent of foods because of its unique taste and flavor. The utilization of mushrooms as food is probably old like the human civilization and mushrooms have been regarded as an important source of nutritious food to humans (Chang and Miles, 1992). Though around 2000 fungal species are reported to be edible, but only less than 25 mushroom species are accepted as food globally and very few have achieved the level of commercial item (Bonatti *et al*., 2004). *Agaricus bisporus*, *Auricularia* species, *Pleurotus* species, *Lentinula edodes* and *Volvariella volvacea* are some of the most accepted edible mushrooms globally. Wild Edible Mushrooms (WEMs) are popularly used as food and medicines in many African and Asian countries and is appreciated for its nutritional compositions (Boa, 2004). They are known for nutritional and medicinal values and considered to be nutraceuticals due to its antioxidant, antitumor and antimicrobial properties

(Barros *et al*., 2007; Ferreira *et al*., 2007). Further, edible mushrooms are considered as healthy food as they are rich in proteins, fibers, minerals, vitamins, low/no calories and cholesterol lowering properties apart from the flavor they impart (Wani *et al*., 2010). Fresh mushrooms are considered to have protein content twice to that of vegetable protein content and possess all the 9 amino acids essential for the health of humans (Sumbali, 2005) and the food value of mushrooms lies between vegetables and meat (Bano, 1976).

The recent demand of mushrooms as a source of bioactive compounds is due to its hypocholesterolemic, antioxidant, immuno-potentiating, anticancer, hepatoprotective and antiviral agents. This new compounds is termed as 'nutriceuticals' which is extractable from the mushroom mycelium or the fruiting body and represents a major component of the rising biotechnology industry of mushroom (Chang and Buswell, 1996). Mushrooms are regarded as functional foods because they possess therapeutic properties and at present, around 270 mushroom species are reported to have therapeutic properties (Ooi and Liu, 1999). Secondary metabolites like polyphenols, terpenes, steroids and polyketides which have antioxidant properties and pharmacological applications in mushrooms plays vital role in boosting the health of consumers (Cheung *et al*., 2003; Barros *et al*., 2007). These secondary metabolites like flavonoids and anthocyanins show pharmacological activities like antimicrobial, immuno-modulatory, antioxidant, antifungal, anti-inflammatory, anticancer etc (Ramesh and Pattar, 2010; Keles *et al*., 2011).

It has been reported that mushrooms play important role as topoisomerase inhibitor, reactive oxygen species inducer, anti-mitotic, mitotic kinase inhibitor, angiogenesis inhibitor, leading to apoptosis and also checking cancer proliferation and act as anti-cancer compounds (Patel and Goyal, 2013). Mushrooms are also known to cut cholesterol, fight against skin

diseases, diabetes, stress, insomnia, asthma and allergies (Bahl, 1983). Most organisms possess repair systems and antioxidant defense systems for protection against free radical damage by oxidative enzymes like superoxide dismutase (SOD) and catalase (CAT) and by chemical compounds like ascorbic acid, carotenoids, a-tocopherol, glutathione and polyphenolic compounds (Mau *et al*., 2001), yet these mechanisms are not sufficient. Naturally available antioxidants are studied extensively due to their properties which help cells from damage caused by oxidative stress in organisms (Cazzi *et al*., 1997). Mau *et al*. (2004) reported mushrooms as rich resources of naturally available antioxidants.

Globally, mushrooms are one of the most abundant unused sources of enzyme complex and can flourish successfully on varieties of inexpensive substrates such as cellulose, pectin, hemicelluloses, lignin and industrial wastes not suitable even for animals (Bano *et al*., 1963). International Society for Mushroom Science (ISMS) has initiated the campaign to popularize mushroom consumption throughout the world. Wild edible mushrooms are non-wood forest resource commonly used by mycophilic societies which has been documented by workers around the world in many countries (Jones and Whalley, 1994; Roberto *et al*., 2005). Tibuhwa (2013) documented and studied the necessity of WEM as a means of income for the poor rural dwellers and as healthy food in Tanzania.

India has suitable agro-climatic habitats which favor the growth of diverse variety of fungi. In India, information about the food and nutritional value of mushrooms has been provided by many workers (Hira *et al*., 1990; Murugkar and Subbulakshmi, 2005). In Nagaland, WEMs are considered as traditionally important nutritious food which forms an important part of the food culture of all the tribes in the state. A total of 52 WEM have been reported in this study. The local markets were surveyed to gather information on the wild

edible varieties sold by the locals. During the mushrooming season, WEM species like *Auricularia auricula-judae*, *A. polytricha*, *Lentinula edodes*, *Lentinus squarrosulus*, *L. sajor-caju*, *Schizophyllum commune*, *Termitomyces heimii* etc are sold at local markets (Ao *et al*., 2016a; Ao *et al*., 2016b). The WEM species available in Nagaland are under-studied and under-exploited; as such it becomes imperative to thoroughly screen and explore the potential mushrooms available in the state. This chapter deals with the biochemical and antioxidant analysis of ten popular WEMs from Nagaland, India.

Materials and Methods

Sample Collection and Extraction

The mushroom samples were collected from local markets and forest areas during the season of availability from different areas of Nagaland. The samples were either sun dried or oven dried at 70-75^{o}C for 6-12 h. The samples were extracted according to the procedure and methods followed for each estimation.

Biochemical Analysis

Moisture content

For calculation of moisture content, pre-weighed mushrooms were maintained in oven (100±5°C) for 12-48 hrs and weighed till a constant weight was achieved. The moisture content was estimated as given below:

$$\text{Moisture Content (\%)} = \frac{\text{Loss in Weight}}{\text{Weight of Sample}} \times 100$$

Dry matter content

Dry matter content was taken as the final weight obtained after the sample have been dried in the oven at 100±5°C for 12-48 hrs and calculated using the following formula:

$$\text{Dry Matter Content (\%)} = \frac{\text{Final Weight}}{\text{Weight of Sample}} \times 100$$

Total protein

Lowry's protocol (Lowry *et al*., 1951) was used for estimation of total protein content. Bovin Serum Albumin (BSA) used as the standard for calculating the protein in the samples. About 0.5 g of mushroom sample was used to get extract using 10 ml of 0.1 M phosphate buffer (*p*H 7.4). Then 5 ml of the reagent mixture which is prepared by mixing 2%, w/v sodium carbonate (prepared in 0.1 N sodium hydroxide solution) and 1%, w/v copper sulphate (prepared in 1%, w/v potassium sodium tartarate solution) was added to 0.1 ml of the sample. The solution was then incubated for 10 min at room temperature followed by addition of 0.5 ml of 1 N Folin- Ciocalteau reagent and incubation in the dark for 20-30 min. The absorbance of the resulting solution was measured at 660 nm using UV-Vis spectrophotometer (ELICO Double Beam SL 210).

Total carbohydrate

Phenol Sulphuric Acid method (Dubois *et al*., 1956) was followed for quantification of total carbohydrate. To 0.1 ml of the sample, 1 ml phenol solution (5%, v/v) and 5 ml of H_2SO_4 (96%, v/v) were added. The volume of the test sample was made up to 10 ml with pure water and mixed well followed by incubation for 20 min at 25-30^{o}C in water bath. The absorbance of the final test solution was measured at 490 nm against glucose as standard.

Reducing Sugar

Reducing sugar was determined following DNS method (Miller, 1972). To 0.1 ml of sample extract, 3 ml of DNS reagent (Take 1 g NaOH, 19.2 g Sodium Potassium Tartarate, 1.0 g DNS powder, 0.05 g Sodium Sulphite and 0.2 g Phenol crystals for preparing 100 ml of

DNS reagent) was added and the resulting mixture was kept for 5 min in a boiling water bath. Rochelle salt solution (1 ml, 40%) was added to the mixture and the volume of the test sample was made up to 10 ml with pure water. After cooling, the absorbance of the final solution was measured at 510 nm against glucose as standard. The amount of non-reducing sugar was determined as the difference between total carbohydrate and reducing sugar and expressed as g/100g of dry weight.

Crude fiber

The amount of crude fiber was estimated by the procedure of Maynard (1970) and AOAC (2000). The samples were treated with petroleum ether to remove fat and boiled with 200 ml of 0.255 N sulphuric acid for 30 min. The solution was filtered through muslin cloth and boiled with 200 ml of 0.313 N sodium hydroxide solution for 30 min followed by filtration through muslin cloth and the residue washed with 25 ml of boiling 1.25% sulphuric acid, 50 ml of water and 25 ml of alcohol. The residue was transferred to ashing dish (pre-weighed, W1) and dried for 2 hrs at 130±2^{o}C, then cooled the dish in a dessicator and weighed (W2). The dish was heated at 660±15^{o}C for 30 min and cooled and weighed again. The difference in weights represents the amount of crude fiber. The amount of crude fiber calculated by using the formula:

$$\text{Crude Fiber (\%)} = \frac{\text{Loss in weight}}{\text{Weight of Sample}} \times 100$$

Ash content

The powdered mushroom sample (about 1.0 g) was ashed in a muffle furnace in previously ignited and cooled crucible of known weight at 550±5°C for 1 hr. The crucible

and its contents were then cooled in desiccators and reweighed. The rate of the incombustible residue accounts for ash content (AOAC, 2000).

$$\text{Ash Content (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100$$

Determination of Antioxidant Activity, Total Phenolic Content, Total Flavonoid Content

Preparation of Methanol Extract

Methanolic extraction procedure was used for sample extraction. About 10 g of dried mushroom was grinded in liquid nitrogen using mortar and pestle. The powder was mixed with 100 ml of methanol and incubated for 24 hrs at 25^{o}C under continuous stirring at 150 rpm and the process repeated until the extraction solvent became colorless. The extract was then filtered on filter paper Whatman No. 4 and the filtrate was used directly for antioxidant analysis.

2, 2-Diphenyl-1-picrylhydazyl (DPPH) radical scavenging assay

The DPPH stable free radical method was used to determine the antioxidant activity (Aoshima *et al*., 2004). To 0.1 ml of mushroom methanol extract at different extract concentrations (10 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml), 2.9 ml of DPPH (0.1 mM DPPH prepared in methanol) was added and vortexed vigorously. Incubation of the reaction mixture for 30 min was done in the dark at 30^{o}C and the absorbance of the mixture was measured at 517 nm. Trolox and Ascorbic acid was used as the standard for the evaluation of antioxidant activity. Inhibition of free radicals by DPPH was calculated by the following equation:

DPPH scavenging effect (%) = [(A_o-A_1/A_o) X 100] where A_o is the absorbance of control reaction and A_1 is the absorbance in the presence of sample.

Total phenolic content

The presence of polyphenols was determined by Folin-Ciocalteu method (Singleton and Rossi, 1965). To 0.1 ml of the filtered extract, 0.9 ml of sterile water and 1 ml of the Folin-Ciocalteu reagent were added. The mixture was incubated for 5 min followed by 2 ml of saturated sodium carbonate (75 g L^{-1}) was added to the mixture. The final volume of the test sample was made to 10 ml with pure water. The reaction mixture was incubated in the dark for 90 min. The resulting absorbance of the blue colored solution was measured at 765 nm. Quantification was done based on gallic acid standard in methanol (80%, v/v) and the total phenolic content of the mushroom samples were expressed as mg of gallic acid equivalents (GAE) per g of the sample extract.

Total flavonoid content

The presence of flavonoids was estimated by colorimetric method of Sahreen *et al*. (2010). To 0.3 ml of extract, 3.4 ml of methanol (30%), 0.15 ml of sodium nitrite (0.5 M) and 0.15 ml of aluminium chloride hexahydrate (0.3 M) were added. After 5 min of incubation at room temperature, 1 ml of 1 M NaOH was added to the solution. The absorbance of the solution was measured at 510 nm. Quercetin was used as the standard and the results expressed as mg of quercetin equivalents (QE) per g of the sample extract.

Statistical Analysis

All the experiments were repeated thrice and the results represent the mean of three replicates. Data expressed as Mean ± Standard Deviation.

Results

Surveys were conducted in the local markets to gather information on the WEM varieties sold by the locals. The WEM species like *A. auricula-judae*, *A. polytricha*, *L. edodes*, *L. squarrosulus*, *L. tigrinus*, *L. sajor-caju*, *S. commune*, *T. heimii* and *Pleurotus* sp. are the most common mushrooms sold in the local markets during the mushrooming season. **Figure 4.1** shows the ten WEM species used for the nutritional analysis and the biochemical composition of the ten WEM species is presented in **Table 4.1**. The macronutrient profile shows that the mushrooms are nutritionally rich and possess bioactive properties like antioxidant ability due to the presence of phenols and flavonoids.

The moisture content was observed to be high in all the studied WEM species which varied from 88.9% in *A. auricula-judae* to 87.3% in *L. squarrosulus*, 86.2% in *L. squarrosulus* var. *squarrosulus*, 85.1% in *L. sajor-caju*, 82.8% in *L. edodes*, 82.01% in *A. polytricha*, 81.1% in *T. heimii*, 80.03% in *L. piperatus*, 73.7% in *L. tigrinus*, 69.8% in *S. commune* and 49.8% in *L. sulphureus*. The presence of moisture provides flavor and texture to the mushrooms.

The dry matter content was determined for all the studied WEM species. The dry matter content ranged from 50.2% in *L. sulphureus* to 30.2% in *S. commune*, 26.3% in *L. tigrinus*, 19.97% in *L. piperatus*, 18.9% in *T. heimii*, 17.9% in *A. polytricha*, 17.2% in *L. edodes*, 14.9% in *L. sajor-caju*, 13.8% in *L. squarrosulus* var. *squarrosulus*, 12.7% in *L. squarrosulus* and 11.1% in *A. auricula-judae*.

Figure 4.1 shows the ten WEM species used for nutritional analysis- 1. *Auricularia auricula-judae*, 2. *A. polytricha*, 3. *Laetiporus sulphureus*, 4. *Lentinula edodes*, 5. *Lentinus squarrosulus*, 6. *L. squarrosulus* var. *squarrosulus*, 7. *L. sajor-caju*, 8. *L. tigrinus*, 9. *Lactifluus piperatus*, 10. *Schizophyllum commune*, 11. *Termitomyces heimii*.

Table 4.1: Biochemical composition of ten popular wild edible mushrooms of Nagaland, India

Mushroom species	Moisture (%)	Dry matter (%)	Total Protein (g/100g DW)	Total Carbohydrate (g/100g DW)	Reducing sugar (g/100g DW)	Non-reducing sugar (g/100g DW)	Crude fiber (%)	Ash (%)
Auricularia auricula-judae	88.9±.02	11.1±.02	56.92±.01	18.67±.01	2.51±.01	16.16±.01	11.1±.08	3.15±.3
Auricularia polytricha	82.01±.04	17.9±.04	42±.02	16.03±.02	4.82±.02	11.21±.02	10.45±.3	8.44±.8
Lactifluus piperatus	80.03±.02	19.97±.02	19.33±.02	9.2±.07	3.12±.02	6.08±.05	5.09±.1	5.38±.6
Laetiporus sulphureus	49.8±.02	50.2±.02	22.73±.01	7.65±.01	4.32±.01	3.33±.01	6.09±.08	4.81±.5
Lentinula edodes	82.8±.01	17.2±.01	43.81±.02	38.44±.01	6.19±.06	32.25±.07	3.6±.07	5.59±.3
Lentinus sajor-caju	85.1±.02	14.9±.02	62.27±.02	6.81±.01	5.08±.03	1.73±.02	7.02±.05	8.41±.2
Lentinus squarrosulus	87.3±.02	12.7±.02	27.86±.01	9.32±.01	6.05±.02	3.27±.02	1.71±.2	10.66±.4
Lentinus squarrosulus var. ***squarrosulus***	86.2±.01	13.8±.01	18.77±.02	19.14±.01	5.39±.08	13.75±.06	6.1±.1	3.12±.2
Lentinus tigrinus	73.7±.04	26.3±.04	31.85±.03	16.09±.3	5.76±.02	10.33±.2	7.09±.08	3.41±.2
Schizophyllum commune	69.8±.02	30.2±.02	24.42±.02	5.31±.01	2.33±.02	2.98±.02	11.01±.08	6.02±.6
Termitomyces heimii	81.1±.02	18.9±.02	60.53±.01	22.74±.01	7.81±.04	14.93±.03	8.11±.04	5.66±.02

The total protein content was observed to be high in all the studied mushrooms. The highest protein content was observed in *L. sajor-caju* (62.27 g/100g, DW) which is followed by *T. heimii* (60.53 g/100g, DW), *A. auricula-judae* (56.92 g/100g, DW), *L. edodes* (43.81 g/100g, DW), *A. polytricha* (42 g/100g, DW), *L. tigrinus* (31.85 g/100g, DW), *L. squarrosulus* (27.86 g/100g, DW), *S. commune* (24.42 g/100g, DW), *Laetiporus sulphureus* (22.73 g/100g, DW), *Lactifluus piperatus* (19.33 g/100g, DW) and *L. squarrosulus* var. *squarrosulus* (18.77 g/100g, DW).

In the present study, the highest total carbohydrate content was observed in *L. edodes* (38.44 g/100g, DW) which is followed by *T. heimii* (22.74 g/100g, DW), *L. squarrosulus* var. *squarrosulus* (19.14 g/100g, DW), *A. auricula-judae* (18.67 g/100g, DW), *L. tigrinus* (16.09 g/100g, DW), *A. polytricha* (16.03 g/100g DW), *L. squarrosulus* (9.32 g/100g, DW), *L. piperatus* (9.2 g/100g, DW), *L. sulphureus* (7.65 g/100g, DW), *L. sajor-caju* (6.81 g/100g, DW) and *S. commune* (5.31 g/100g, DW).

The amount of reducing sugar was also determined which ranged from 7.81 g/100g, DW in *T. heimii* to 6.19 g/100g, DW in *L. edodes*, 6.05 g/100g, DW in *L. squarrosulus*, 5.76 g/100g, DW in *L. tigrinus*, 5.39 g/100g, DW in *L. squarrosulus* var. *squarrosulus*, 5.08 g/100g, DW in *L. sajor-caju*, 4.82 g/100g, DW in *A. polytricha*, 4.32 g/100g, DW in *L. sulphureus*, 3.12 g/100g, DW in *L. piperatus*, 2.51 g/100g, DW in *A. auricula-judae* and 2.33 g/100g, DW in *S. commune*. The amount of non-reducing sugar was determined as the difference between total carbohydrate and reducing sugar and ranged from 32.25 g/100g, DW in *L. edodes* to 1.73 g/100g, DW in *L. sajor-caju*.

Fiber is also a part of carbohydrate and the crude fiber content was determined for the WEM species. The amount of crude fiber ranged from 11.1% in *A. auricula-judae* to 11.01%

in *S. commune*, 10.45% in *A. polytricha*, 8.11% in *T. heimii*, 7.02% in *L. sajor-caju*, 6.1% in *L. squarrosulus* var. *squarrosulus*, 6.09% in *L. sulphureus*, 5.09% in *L. piperatus*, 3.6% in *L. edodes* and 1.71% in *L. squarrosulus*.

The ash content in the studied mushrooms ranged from 10.66% in *L. squarrosulus* to 8.44% in *A. polytricha*, 8.41% in *L. sajor-caju*, 6.02% in *S. commune*, 5.66% in *T. heimii*, 5.59% in *L. edodes*, 5.38% in *L. piperatus*, 4.81% in *L. sulphureus*, 3.41% in *L. tigrinus*, 3.15% in *A. auricula-judae* and 3.12% in *L. squarrosulus* var. *squarrosulus*. Determination of ash content is a vital part of nutritional analysis as it is the first step in the preparation of a sample for mineral elements analysis.

The total phenolic and flavonoid content was also determined during the study and the results are shown in **Figure 4.2**. The presence of phenolic compounds like phenolic acids and flavonoids indicates antioxidant activity of the compound. In the present study, the phenolics ranged from 18.7g GAE/100g, DW in *L. squarrosulus* to 17.4g GAE/100g, DW in *T. heimii*, 17.2g GAE/100g, DW in *L. sajor-caju*, 16.4g GAE/100g, DW in *S. commune*, 16.1g GAE/100g, DW in *L. sulphureus*, 10.2g GAE/100g, DW in *L. edodes*, 10.1g GAE/100g, DW in *L. tigrinus*, 9.9g GAE/100g, DW in *A. polytricha*, 8.1g GAE/100g, DW in *L. piperatus*, 7.7g GAE/100g, DW in *L. squarrosulus* var. *squarrosulus* and 7.3g GAE/100g, DW in *A. auricula-judae*.

The flavonoid content were lower as compared to phenolics and ranged from 9.3g QE/100g, DW in *L. sulphureus* to 6.6g QE/100g, DW in *L. sajor-caju*, 5.6g QE/100g, DW in *S. commune*, 4.7g QE/100g, DW in *T. heimii*, 4.5g QE/100g, DW in *L. squarrosulus*, 3.7g QE/100g, DW in *L. tigrinus*, 3.2g QE/100g, DW in *L. edodes*, 2.1g QE/100g, DW in *L. piperatus*, 1.8g QE/100g, DW in *A. polytricha*, 1.7g QE/100g, DW in *L. squarrosulus* var. *squarrosulus* and 1.5g QE/100g, DW in *A. auricula-judae*.

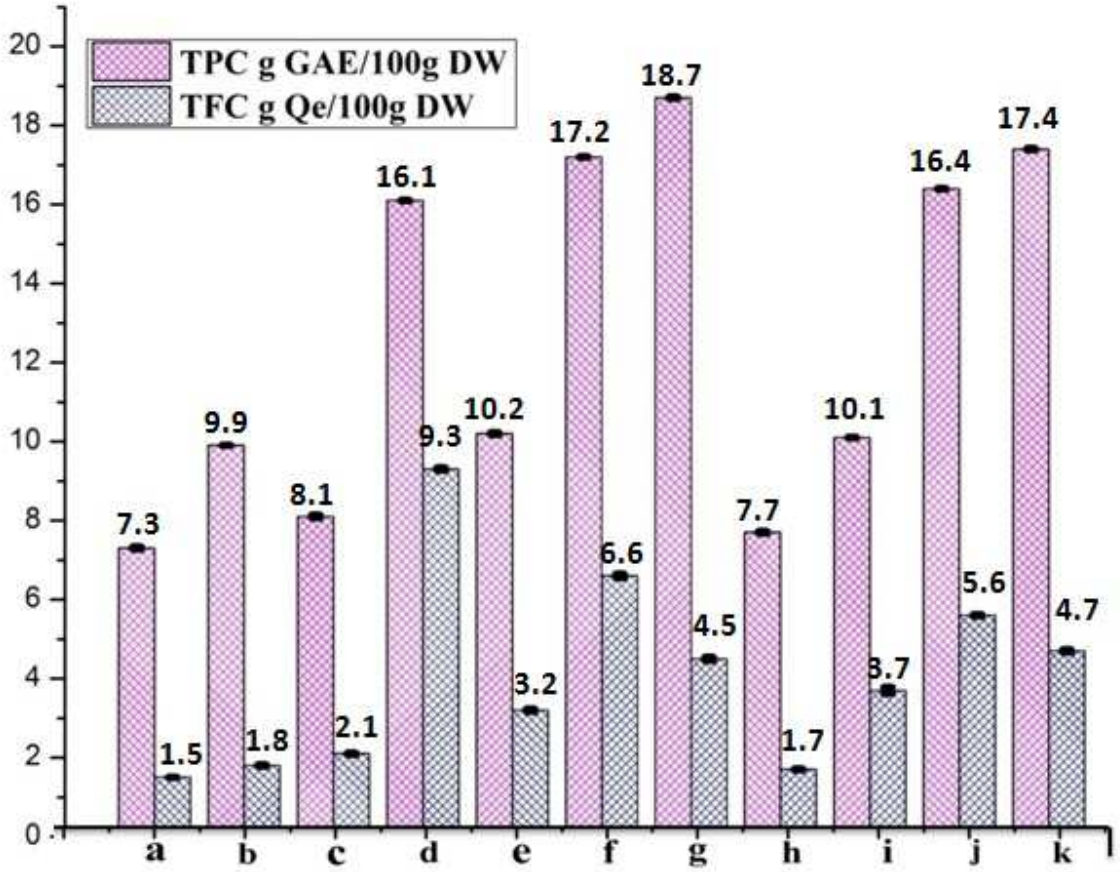


Figure 4.2: Total phenolic (TPC g GAE/100g DW) and flavonoid (TFC g QE/100g DW) content of the ten popular wild edible mushrooms- a. *Auricularia auricula-judae*, b. *A. polytricha*, c. *Lactifluus piperatus*, d. *Laetiporus sulphureus*, e. *Lentinula edodes*, f. *Lentinus sajor-caju*, g. *L. squarrosulus*, h. *L. squarrosulus* var. *squarrosulus*, i. *L. tigrinus*, j. *Schizophyllum commune*, k. *Termitomyces heimii*.

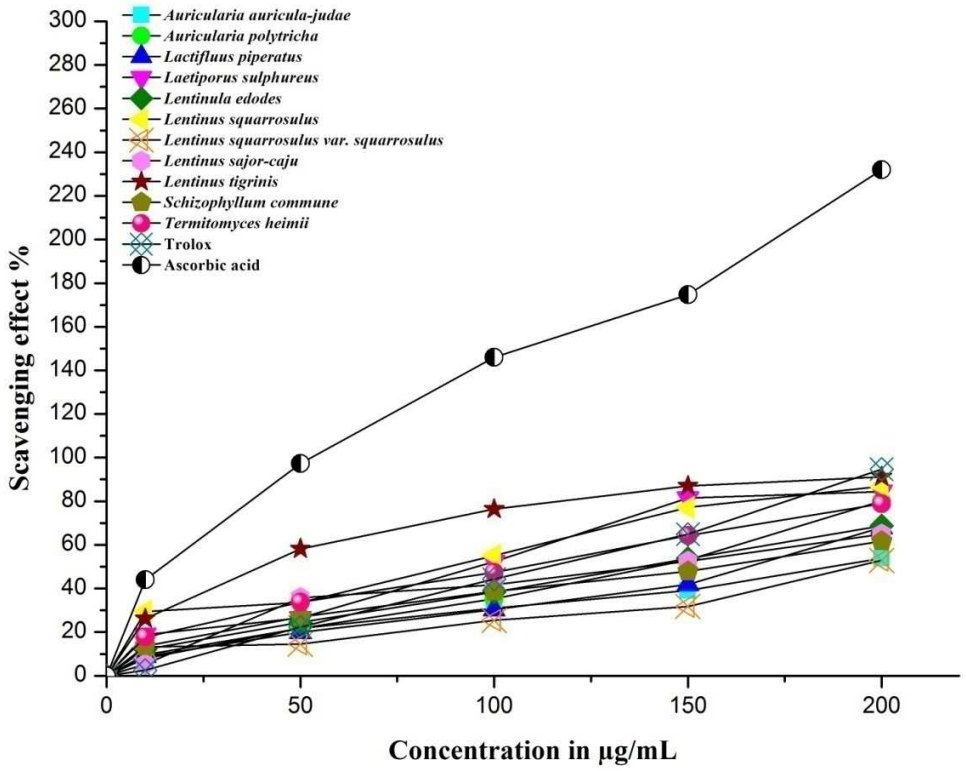


Figure 4.3: The scavenging effect (%) of the ten wild edible mushrooms at different extract concentrations against Trolox and Ascorbic acid on DPPH radicals.

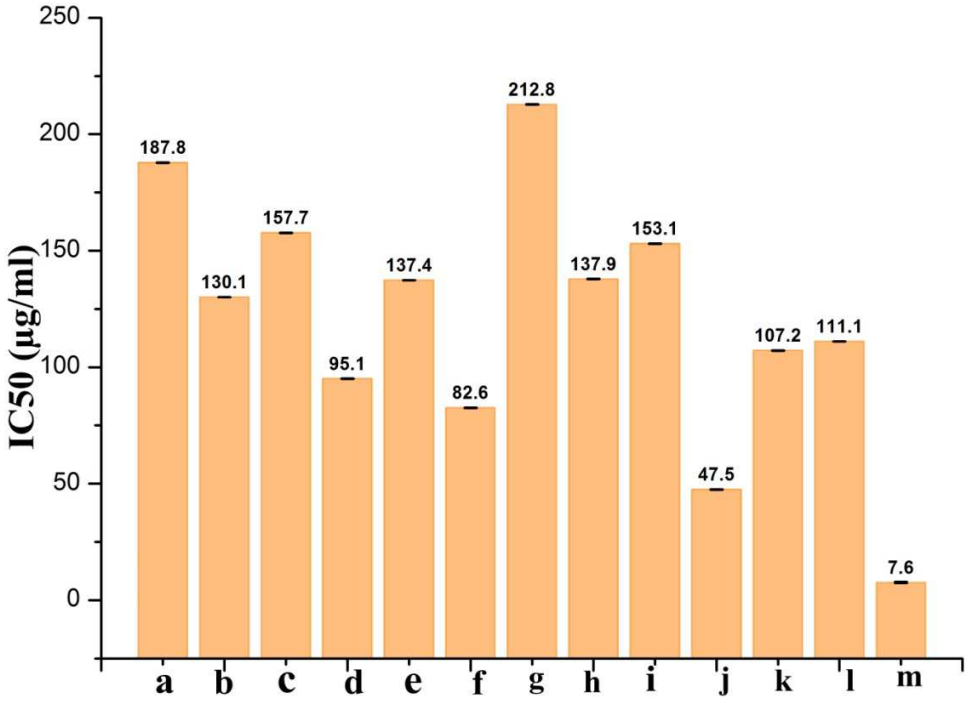


Figure 4.4: The comparison of the IC50 values between the ten wild edible mushrooms, ascorbic acid and trolox - a. *Auricularia auricula-judae*, b. *A. polytricha*, c. *Lactifluus piperatus*, d. *Laetiporus sulphureus*, e. *Lentinula edodes*, f. *Lentinus squarrosulus*, g. *L. squarrosulus* var. *squarrosulus*, h. *L. sajor-caju*, i. *Schizophyllum commune*, j. *L. tigrinus*, k. *Termitomyces heimii*, l. Trolox, m. Ascorbic acid.

The antioxidant capacity of the WEM species was determined by DPPH scavenging assay. Lower absorbance of the samples during the reaction indicated higher free radical scavenging activity. The IC50 value of the mushroom samples was calculated to study the inhibition ability of each mushroom against DPPH free radicals. IC50 is the quantity of sample required to scavenge 50% of the DPPH radicals. The lower the IC50 value higher is the capacity of the samples to inhibit the free radicals. The IC50 value of the mushroom species ranged from 47.5 μg/ml in *L. tigrinus* to 82.6 μg/ml in *L. squarrosulus*, 95.1μg/ml in *L. sulphureus*, 107.2 μg/ml in *T. heimii*, 130.1 μg/ml in *A. polytricha*, 137.4 μg/ml in *L. edodes*, 137.9 μg/ml in *L. sajor-caju*, 153.1 μg/ml in *S. commune*, 157.7 μg/ml in *L. piperatus*, 187.8 μg/ml in *A. auricula-judae* and 212.8 μg/ml in *L. squarrosulus* var. *squarrosulus* against Trolox (111.1 μg/ml) and Ascorbic acid (7.6 μg/ml) as standard. The scavenging percentage (%) of the ten WEM species at different extract concentrations against trolox and ascorbic acid is shown in **Figure 4.3**. The comparison of the IC50 values between the ten wild edible mushrooms, ascorbic acid and trolox is shown in **Figure 4.4**.

Discussion

It was observed that all the studied WEM species are nutritionally rich. The present study revealed the nutritional compositions of these species, but there are wide variations in the macronutrient profiles even within the species. In mushroom, this phenomenon is common. In the past, similar reports have been published by many workers in different mushroom species (Rudawska and Leski, 2005; Kalac, 2009; Pushpa and Purushothama, 2010). The differences in the nutritional compositions of mushrooms is related with factors like different species, mycelium and fructifications, developmental stage, storage conditions, climatic conditions in different regions of the world, soil conditions, level of pollution, metal

accumulations, processing and cooking conditions etc which overall affects the nutritional composition of mushrooms (Vetter, 1993; Barros *et al*., 2007; Kalac, 2009). During the present investigation, *L. squarrosulus* and *L. squarrosulus* var. *squarrosulus* collected from different locations showed variations in their macronutrient compositions and the remaining mushrooms also showed variations in their nutritional profiles when compared to data reported from different workers which supports the findings of other workers that soil conditions and environmental factors play a vital role in the nutritional composition of mushrooms (Beelman and Edwards, 1989; Vetter, 1993; Rudawska and Leski, 2005; Barros *et al*., 2007; Kalac, 2009). During their study, Pushpa and Purushothama (2010) and Manikandan (2011) have reported variations in the nutritional compositions of *A. bisporus*.

It has been observed that the nutritional values of the mushrooms are directly related to moisture content and environmental conditions (Colak *et al*., 2009; Kalac, 2009). Moisture content was found to be high for most of the studied WEM species. This high moisture content makes the mushrooms easily perishable and susceptible to microbes and enzyme activity apart from the taste and flavor they impart. The very high moisture content of fresh mushrooms makes them not suitable to be kept for long duration as high water activity enhances the microbial growth (Brock *et al*., 1986; Heleno *et al*., 2009). Because of the unique flavor of mushrooms, it stimulates the human appetite. Young mushrooms contain higher moisture content then as compared to fully matured ones. The dry matter content was also determined from the final weight obtained after the WEM samples have been oven dried at 100±5°C for 12-48 hrs. The dry matter content ranged from 50.2% in *L. sulphureus* to 11.1% in *A. auricula-judae*.

Protein is an essential component of dry matter of mushrooms. In this study, the protein content of dried mushrooms was observed to be richer than carbohydrate which is similar to previous reports (Manzi *et al*., 1999; Manzi, *et al*., 2004). Edible mushrooms collected from the wild are considered nutritionally richer i.e., richer source of proteins and lower fat content than commercial mushrooms (Barros *et al*., 2008). Some workers have reported that the protein content depends on the genetic makeup of the mushrooms and the chemical and physical differences during growth (Sanmee *et al*., 2003). The protein content of mushrooms is generally regarded to be higher than green vegetables (Jonathan, 2002). Verma *et al*. (1987) reported that mushrooms are important for vegetarians because they contain some essential amino acids which are found only in animal proteins. Bioactive proteins also known as fungal immuno-modulatory proteins is a new class of bioactive compounds isolated from mushrooms which has shown potential application in metastasis and suppressing tumor invasion (Lin *et al*., 2010). Because of high protein content in mushrooms, the FAO of the UN has emphasized to adopt mushroom as an ideal food to combat protein deficiency in the world.

In the present study, carbohydrate is found as another abundant macronutrient. Studies have reported edible mushrooms to be rich source of carbohydrates which range from 28.38% to 82.8% (DW) (Ragunathan *et al*., 1996; Thatoi and Singdevsachan, 2014). A considerable part of carbohydrates occur in the form of polysaccharides represented by starch, glycogen and fibers which is necessary for proper functioning of the alimentary tract (Manzi *et al*., 2001). Polysaccharides are the most potent mushroom derived substances and the main polysaccharides present in mushrooms are β-glucans which constitutes around half of the fungal cell wall mass. β-glucans are responsible for antioxidant, anti-tumor, anti-

cancer, anti-cholesterolemic, immuno-modulating and neuroprotective activities of many mushrooms (Zhang *et al*., 2007; Khan *et al*., 2013). *Lentinula edodes* mushroom showed very high carbohydrate content (38.44g/100g, DW) when compared to the remaining mushroom species. From this particular mushroom, an anti-cancer compound named *Lentinan* is extracted and is in high demand due to its pharmaceutical applications. *A. polytricha* and *A. auricula-judae* are also medicinal mushrooms that are popularly known for their anti-tumor, anti-coagulant, hypoglycemic properties, etc (Wasser 2002; Reza *et al*., 2012; Choi *et al*., 2018).

Fiber is also a part of carbohydrate and very significant amounts of crude fiber was observed during the study for all the WEM species. The presence of fibers is an important characteristic in mushrooms as fibers are necessary in maintaining healthy and balanced diet in humans. Cheung (1998) reported that edible mushrooms are ideal choice of food for the treatment of atherosclerosis due to fiber content. He also concluded that the addition of edible mushrooms into the human diet had a hypocholesterolemic effect due to dietary fibers such as glucans which can increase intestinal motility, reduce bile and cholesterol absorption.

Ash is defined as the inorganic residue or substance resulting from the incineration of dry matter or oxidation of the organic matter in the powdered sample. Even though minerals represent only a very small proportion of dry matter, sometimes less than 7% of the total, they play a crucial role from a physicochemical and nutritional point of view. In the present study, the highest ash value was observed in *L. squarrosulus* (10.66%) and ranged from 10.66% to 3.12%. Similar ash values in the studied WEM species have been reported by some previous workers (Ayaz *et al*., 2011; Johnsy *et al*., 2011; Usha and Suguna, 2014; Enas *et al*., 2016)**.** The value of ash content is important because it determines the minerals present

in the food. The minerals like K, P, Na, Ca, Mg represent the major constituents and Cu, Zn, Fe, Mo, Cd represent the minor constituents in mushrooms (Bano *et al*., 1981; Bano and Rajarathanum, 1982; Chang, 1982). It is reported that K, P, Na and Mg comprise about 56 to 70% of the total ash content in mushrooms while potassium alone forms 45% of the total ash (Li and Chang, 1982). The mineral content of mushrooms varies from species to species, age and diameter of fruiting body and also depends on the type of the substratum (Demirbas, 2001). It is reported that the mineral content of WEM is higher than cultivated mushrooms (Mattilla *et al*., 2001; Rudawska and Leski, 2005).

Generally, the presence of phenols and flavonoids indicates mushrooms as biologically active compounds which exhibit antioxidant, anticancer, anti-inflammatory, immuno-modulatory, anti-tumor, anti-viral, hypocholesterolemic and hypoglycemic properties and plays a significant role in pharmacology. The highest phenolic content was observed in *L. squarrosulus* (18.7g/100g, DW) and the lowest in *A. auricula-judae* (7.3 g/100g, DW). The flavonoid content was found to be lower as compared to phenolic content in the present study which ranged from 9.3 g/100g in *L. sulphureus* to 1.5 g/100g in *A. auricula-judae*. In mushrooms, the antioxidants present are mainly phenolic compounds than tocopherols, ascorbic acid and carotenoids, and are thus regarded to possess antioxidant activity. Therefore, the results showed that the presence of phenolics and flavonoids is a useful indicator for possessing antioxidant activity in mushrooms and also that phenolic compounds play a vital role in the maintenance of human health.

The antioxidant activity of the ten WEM species was tested using DPPH scavenging assay. The methanolic extracts of the mushrooms at different extract concentrations showed significant antioxidant activity and hence, are sources of rich natural antioxidants. Trolox

(111.1 µg/ml) and Ascorbic acid (7.6 µg/ml) was used as reference antioxidant compound during the experiment. The IC50 values of *L. tigrinus*, *L. squarrosulus*, *L. sulphureus* and *T. heimii* were lower than that of trolox (111.1 µg/ml); however, the IC50 value of ascorbic acid (7.6 µg/ml) was lower than all the mushroom species studied. Nevertheless, the mushroom species studied were able to scavenge the DPPH radicals showing strong antioxidant activity. Among the 10 WEM species, *L. tigrinus* (47.5 µg/ml, IC50) exhibited the highest scavenger of DPPH radicals followed by *L. squarrosulus* (82.6µg/ml, IC50). Oxidation is a necessary mechanism for living organisms to produce energy; however oxygen-centered free radicals produced *in vivo* continuously, result in tissue damage and cell death. Free radicals cause oxidative damage which leads to aging, cirrhosis, diabetes, atherosclerosis and cancer (Halliwell and Gutteridge, 1984). The current interest in natural antioxidant substances is due to restriction in the applications of synthetic antioxidants like BHA (2-tert-butyl-4-methoxyphenol), BHT (butylated hydroxytoluene) etc. (Branen, 1975). Antioxidants play a significant role in maintaining human health due to their ability to scavenge free radicals in the bodies reducing oxidative damage (Elmastas *et al*., 2007; Ferreira *et al*., 2007) and mushrooms are rich sources of antioxidants (Puttaraju *et al*., 2006; Oyetayo, 2007). The antioxidant potential, phenolic and flavonoid content of various mushrooms have been reported by many workers (Ramesh and Pattar, 2010; Keles *et al*., 2011; Boonsong *et al*., 2016; Sanchez, 2017) and it is said that the antioxidant potential of mushrooms is higher than most vegetables and fruits (Sanchez, 2017). Thus, antioxidant supplements or foods containing antioxidants may be utilized to help the human body fight against oxidative damage.

There has been tremendous progress in mushroom cultivation and research because of its immense nutritional and medicinal properties. Mushrooms are known to man since time immemorial. The health benefits of consuming mushrooms and mushroom products have been reported by many workers around the world (Chang and Miles, 2004; Ferreira *et al*., 2007). These WEM are healthy food supplements required to maintain balanced diets as it contains macro as well as micro nutrients and functional minerals. Recently, mushrooms are popularly advocated as a low caloric diet food especially for cancer, diabetic and cardiac patients. Mushrooms are also gaining importance as a protein supplementary food especially for the people living in under developed countries. Present day world is facing human nutritional problems especially protein deficiency, and the State of Food Security and Nutrition in the World (SOFI) has called upon all countries and stakeholders to come together to fight hunger and check all forms of malnutrition by 2030 as the present scenario is that the population of undernourished people is increasing alarmingly in the world. Moreover, the world is in demand for cheaper and abundant source of natural antioxidants which can be provided by mushrooms. Therefore, mushrooms should be promoted as healthy food supplements i.e. as neutraceuticals worldwide to overcome malnutrition and provide immunity to the body.

Summary and Conclusion

This chapter reports the biochemical and antioxidant analysis of ten popular WEM from Nagaland. In this study, biochemical analysis including phenolics, flavonoids and antioxidant activity was done for the ten mushroom species. High moisture content was observed during the study which ranged from 88.9% in *A. auricula-judae* to 49.8% in *L. sulphureus* and the content of dry matter ranged from 50.2% in *L. sulphureus* to 11.1% in *A.*

auricula-judae. Total protein content was observed to be high in all the studied mushrooms and ranged from 62.27 g/100g (*L. sajor-caju*) to 18.77 g/100g (*L. squarrosulus* var. *squarrosulus*); the total carbohydrate content ranged from 38.44 g/100g (*L. edodes*) to 5.31 g/100g (*S. commune*); reducing sugar content ranged from 7.81 g/100g (*T. heimii*) to 2.33 g/100g (*S. commune*). The crude fiber content ranged from 11.1% in *A. auricula-judae* to 1.71% in *L. squarrosulus* and the ash content ranged from 10.66% in *L. squarrosulus* to 3.12% in *L. squarrosulus* var. *squarrosulus*. The highest phenolic content was recorded in *L. squarrosulus* (18.7 g/100g) and lowest in *A. auricula-judae* (7.3 g/100g). The flavonoids ranged from 9.3 g/100g in *L. sulphureus* to 1.5 g/100g in *A. auricula-judae*. All the studied mushroom species exhibited significant antioxidant activity against DPPH free radicals and the highest antioxidant activity was observed in *L. tigrinus* (47.5 µg/ml, IC50). The study concludes that mushrooms can be advocated as healthy food supplements to enhance the immune system and as defensive mechanism against diseases.

The findings of present study reflect that all the WEMs are nutritionally very rich exhibiting significant antioxidant activity. As WEM are easily accessible source of rich natural antioxidants and promising nutritious food, this study brings to light the scope of mushroom cultivation and production which is eco-friendly and cost-effective. This can be a means of income generation especially to the rural poor. Training and awareness campaigns should be organized for the rural communities to promote the mushroom industry. This will help in alleviating poverty which will indirectly play a role in achieving food and nutrition security. Moreover, a thorough screening and exploration of the wild mushrooms is required to study its medicinal properties and bioactivity. The present work also create awareness to conserve and manage this natural resource as the present environmental crisis like climate

change and global warming adversely affects the survivability of mushrooms which require specific micro-climatic conditions for its growth. It is necessary to remember that the nutritional and medicinal value of mushrooms is most likely to be vanished at this rate if the wild mushroom species are not documented and studied. Even the unsystematic collection of mushrooms in forest areas by the mushroom hunters and deforestation has led to decline in the macrofungal population. Therefore, measures must be taken to conserve this precious natural resource which has immense health and medicinal benefits.

Mushrooms have the solution to some extent on many of the present day problems like food quality, unemployment, environmental pollutions and pharmaceutical applications. Mushrooms as food and medicines are solutions to many health problems. Thus, mushrooms are a boon to mankind and should be exploited judiciously for the improvement of society.

Chapter - 5

Summary

Nagaland has a forest cover of 12,489 sq. km approx. which is 75.33% of the state's geographical area. The state has a rich biodiversity of wild mushrooms but is under studied. There is the need to identify the rich mushroom biodiversity of the state which will help in creating strategies for management and conservation as various anthropogenic factors like deforestation and unsystematic collection in forest areas by the mushroom hunters, and climate change has affected the survivability of mushrooms in nature.

During the present research work, surveys were undertaken in different districts of Nagaland. The wild mushrooms were collected between the altitudinal ranges of 150 m ASL to 3000 m ASL. The mushroom populations reduced in number with the increase of altitude and the highest number of mushrooms was collected between the altitudinal range of 900 m and 1400 m ASL. A total of 141 mushroom species belonging to 80 genera under 44 families have been identified correctly. The Basidiomycetes mushrooms dominated over Ascomycetes mushrooms. A total of 52 mushroom species were identified as edible, 10

mushrooms were identified to be poisonous and the remaining 79 mushrooms were found to be inedible. The collected mushroom species are predominantly found to be parasitic, saprophytic and ecto-mycorrhizal in habitat. Though mushrooms are found round the year, the peak season of collection is April to July. Mushrooms are mostly found in the wet season than the dry season and during the present research work, most mushroom species were collected during the wet season. The dry season is dominated mostly by polypores and bracket fungi like *Trametes gibbosa, T. versicolor, Pycnoporus cinnabarinus, Microporus xanthoporus, Daldinia concentrica, Ganoderma lucidum* and *Schizophyllum commune* etc. During the study, it was observed that the population of wild mushrooms decreased in the collection areas over time which is a clear indication that mushrooms are very valuable biological indicator to assess the ecosystem damage. The presence of both micro- and macro-fungi in the nature is significant because of the role they play in recycling of nutrients, biodegradation; and as food to humans and animals and also to pharmaceutical industries.

The market surveys during the research work revealed that WEM are highly coveted food resource and forms a vital part of the food culture of all the tribes of Nagaland. The WEM species like *Lentinula edodes, Lentinus squarrosulus, L. sajor-caju, L. tigrinus, Lactifluus volemus, S. commune, Termitomyces heimii, T. microcarpus, T. eurhizus, Auricularia auricula-judae*, etc. are the popular wild edible varieties sold at local markets. The tribal population throughout the state collects the mushrooms from the wild and sells in the market during the season of availability. Besides, the number of medicinally important mushrooms available in the state is also high. For sustainable and commercial production, the WEM species need to be domesticated and popularized as nutritious food in order to have market acceptability which will indirectly help the ethnic people to earn their livelihoods.

The morphological characters play a significant role in the identification of mushrooms however, for some species, morphological characters are not sufficient as mushrooms are polymorphic in nature. Thus, it becomes imperative to use molecular tools for correct identification of mushrooms. During the present study, identification and molecular phylogenetic studies based on ITS, 18S and 28S *rRNA* genes were carried out for six popular WEM species *viz*. *L. tigrinus*, *L. squarrosulus*, *L. squarrosulus* var. *squarrosulus*, *L. sajor-caju*, *L. edodes*, *S. commune* and *T. heimii*. The combined results of morphology and molecular analysis authenticated the identity of all the WEM species used in the study. The ITS markers complemented 100% to morphological identity indicating that ITS marker can be regarded as universal DNA barcode marker for identifying mushrooms which is in agreement to past reports. The use of 28S *rRNA* markers also helped in identification and characterization of the mushroom species. However, the 18S *rRNA* markers could not resolve species identity for some of the species studied. The present work is the first attempt from Nagaland to correctly identify some of the popular WEM species based on the data generated by the multi-gene molecular characterization along with their morphological traits. The molecular phylogenetic studies also resolved successfully the studied WEM species with respect to their infrageneric groups. The high Consistency Index (CI) values of the mushroom species indicated the low homoplasy nature. The ITS and 28S *rRNA* datasets were observed to be more informative than the 18S *rRNA* datasets. The current molecular advances in multi-loci phylogeny gives stable and well defined phylogenetic analysis, but still there is much to be resolved. One of the major problems in species identity is that only a few mushrooms have been sequenced and deposited in public repositories like GenBank. Moreover, the target data for some mushroom species is totally absent in GenBank domains

which lead to erroneous tree topology and inference. Therefore, it is always better to use two or more markers for identification and characterization of mushrooms for authentication. Moreover, the molecular barcodes generated for each individual mushroom specimen in the present investigation will help in future research and conservation of these popularly consumed WEM of the region.

Mushrooms are in high demand because of its therapeutic and pharmaceutical applications apart from being a popular nutritious food to humans. The information on the genetic diversity and variability of these mushrooms is necessary to enable their sustainable cultivation, utilization and conservation of the existing germplasm. The present study is the first of its kind to use RAPD, ISSR and DAMD molecular markers both individually and in combination to study the degree of genetic variability present among the natural populations of *Lentinus sajor-caju*, *L. squarrosulus*, *L. squarrosulus* var. *squarrosulus* and *L. tigrinus* respectively from Nagaland. A comparison of the degree of polymorphism and discriminatory efficiency of RAPD, ISSR and DAMD showed that each marker technique was able to detect genetic variation in the three populations of *Lentinus* species. In the present study, 28 primers (10 ISSR+10 DAMD+8 RAPD) were utilized to evaluate the genetic diversity within and among the populations of the three *Lentinus* species. A total of 237 bands were scored in 18 accessions of three *Lentinus* species, out of which 227 bands were polymorphic with average polymorphism of 95.78%. The average PIC value was observed to be 0.72 per primer and the average *Rp* and MI value was observed to be 16.79 and 5.89 respectively. The discriminatory power of a primer is a reliable indicator of the usefulness of that primer in genetic variation and diversity analysis. The utility and efficiency of each molecular markers used in the present study was tested by comparing the PIC, *Rp*

and MI values within and among the populations for their discriminatory power. The overall comparative assessment of all the molecular approaches (ISSR, DAMD and RAPD) revealed hyper variability in the *Lentinus* species. This shows the extent of genome coverage as ISSR markers targets the microsatellite regions, while DAMD markers target the core minisatellite regions and RAPD markers amplify randomly in the targeted genome. The average polymorphism observed in *L. squarrosulus* populations by the three markers are 73.05% (ISSR), 59.39% (DAMD) and 82.68% (RAPD). In *L. sajor-caju* populations, it is observed as 61.4% (ISSR), 70.94% (DAMD) and 69.33% (RAPD) and in *L. tigrinus* populations, it is observed as 86.21% (ISSR), 76.15% (DAMD) and 71.52% (RAPD). Among the three *Lentinus* species, *L. tigrinus* showed the highest polymorphism as compared to *L. sajor-caju* and *L. squarrosulus* mushrooms. Even the pooled data (ISSR+DAMD+RAPD) showed that *L. tigrinus* exhibited the highest polymorphism (78.42%) as compared to L. squarrosulus (70.92%) and *L. sajor-caju* (66.2%) mushrooms. In *L. squarrosulus* species, ISSR marker showed maximum Na (1.68±0.54), Ne (1.47±0.38), H (0.27±0.21) and I (0.41±0.31) as compared to RAPD Na (1.59±0.5), Ne (1.42±0.39), H (0.24±0.21), I (0.35±0.3) and DAMD markers Na (1.45±0.5), Ne (1.3±0.38), H (0.17±0.2) and I (0.25±0.29). In *L. sajor-caju* species, RAPD marker showed maximum Na (1.61±0.5), Ne (1.38±0.37), H (0.22±0.2) and I (0.33±0.29) than ISSR Na (1.42±0.5), Ne (1.33±0.42), H (0.21±0.22), I (0.26±0.31) and DAMD markers Na (1.38±0.49), Ne (1.24±0.35), H (0.14±0.19) and I (0.21±0.28). In *L. tigrinus* species, ISSR marker showed maximum Na (1.57±0.4), Ne (1.4±0.38), H (0.23±0.21), I (0.34±0.3) as compared to RAPD Na (1.51±0.5), Ne (1.32±0.38), H (0.19±0.21), I (0.28±0.3) and DAMD markers Na (1.35±0.48), Ne (1.26±0.38), H (0.15±0.21) and I (0.21±0.29). The Heterozygosity (Hs) within the populations was observed

to be 0.23±0.02 (ISSR), 0.22±0.02 (RAPD) and 0.15±0.01 (DAMD), and the total gene diversity (Ht) was almost similar for all the three marker systems i.e. 0.38±0.02 (RAPD), 0.35±0.02 (ISSR) and 0.31±0.02 (DAMD) between the mushroom species. The genetic variation among all 18 accessions of *Lentinus* species, the Na (1.98±0.31), Ne (1.62±0.34), H (0.35±0.16), I (0.52±0.2), Ht (0.35±0.02), Hs (0.23 ± 0.02), Gst (0.36) and Nm (0.91) was observed for ISSR marker. While for DAMD marker, the Na (1.95±0.21), Ne (1.52±0.32), H (0.31±0.15), I (0.48±0.19), Ht (0.31±0.02), Hs (0.15±0.01), Gst (0.51) and Nm (0.48) was observed. For RAPD marker, the Na (1.99±0.12), Ne (1.66±0.29), H (0.38±0.12), I (0.56±0.15), Ht (0.38±0.02), Hs (0.22±0.02), Gst (0.43) and Nm (0.66) was observed. The pooled data from all the three markers showed the Na (1.97±0.22), Ne (1.61±0.32), H (0.35±0.15), I (0.52±0.19), Ht (0.35±0.02), Hs (0.21±0.01), Gst (0.43) and Nm (0.66). All these data revealed that the three marker systems used were efficient in assessing the genetic variation and diversity of the *Lentinus* species. In the present study, low gene flow was observed for all marker systems *viz.* 0.91 (ISSR), 0.48 (DAMD) and 0.66 (RAPD) and also in combination (ISSR+DAMD+RAPD), the gene flow was low (0.66). On the other hand, the relative genetic differentiation (Gst) was observed to be high *viz*. 0.36 (ISSR), 0.51 (DAMD), 0.43 (RAPD) and 0.43 (ISSR+DAMD+RAPD). This revealed that the populations of three *Lentinus* species show low gene flow having higher genetic differentiation (Gst) indicating high genetic diversity between them. The assessment of gene flow is a crucial factor for genetic diversity analysis. The UPGMA dendrogram also revealed the degree of genetic relationship among all the 18 accessions of *Lentinus* species. The genetic similarity or dissimilarity varies from one place to the other and between species to species. The use of three different markers (ISSR, DAMD and RAPD) both individually and in combination

provided a convenient method to assess the genetic variation and diversity in the 18 accessions of the three *Lentinus* species. Genetic variability and diversity assessment at intra- and inter- specific levels are vital for crop improvement because the presence of natural genetic variation is crucial to have opportunities for development of improved traits in crop production. This study reveals the presence of high genetic diversity within the *Lentinus* species of Nagaland which remains under-utilized. The study demonstrated that the use of different markers targeting various regions of the genome is a useful tool in assessing and examining the genetic diversity and variability of any organism including mushrooms. Hence, the integration of the three different markers – ISSR, DAMD and RAPD, targeting different amplification regions in this study was observed to be effective in detecting genetic variability and diversity of the mushroom resource. Thus, all the marker systems used were highly efficient and informative in discriminating the mushroom species. This study may be useful in selection of unique gene pools for biotechnological exploitation and innovation of *Lentinus* species for the improvement of society at large. This result may also be utilized to widen the genetic bases in order to improve the quality of cultivated strains.

Nutrition is the most important reason for all the societies of the world. During the present study, nutritional analysis was performed for ten popular WEM species of Nagaland. The macronutrient profile showed that the mushrooms are nutritionally very rich and possess bioactive properties like antioxidant ability due to the presence of phenols and flavonoids. The moisture content was high in all the studied WEM species which ranged from 88.9% in *A. auricula-judae* to 49.8% in *L. sulphureus*. The presence of moisture provides flavor and texture to the mushrooms. The dry matter content ranged from 50.2% in *L. sulphureus* to 11.1% in *A. auricula-judae*. Young mushrooms contain higher moisture content then as

compared to fully matured ones. Protein is an essential component of dry matter of mushrooms. In this study, the protein content of dried mushrooms was richer than carbohydrate. The highest protein content was observed in *L. sajor-caju* with 62.27 g/100g and lowest in *L. squarrosulus* var. *squarrosulus* (18.77 g/100g, DW). In the present study, the total carbohydrate content ranged from 38.44 g/100g, DW in *L. edodes* to 5.31 g/100g DW in *S. commune*. The content of reducing sugar ranged from 7.81 g/100g, DW in *T. heimii* to 2.33 g/100g, DW in *S. commune* and the crude fiber ranged from 11.1% in *A. auricula-judae* to 1.71% in *L. squarrosulus*. The presence of fibers is an important characteristic in mushrooms as fibers are necessary in maintaining healthy and balanced diet in humans. The ash value ranged from 10.66% in *L. squarrosulus* to 3.12% in *L. squarrosulus* var. *squarrosulus*. The highest phenolic content was observed in *L. squarrosulus* (18.7 g/100g, DW) and the lowest in *A. auricula-judae* (7.28g GAE/100g, DW). The highest flavonoid content was observed in *L. sulphureus* (9.32 g/100g, DW) and the lowest in 1.46 g QE/100g, DW in *A. auricula-judae*. The antioxidant ability of the ten WEM species was determined by DPPH scavenging assay. The methanolic extracts of the mushrooms at different extract concentrations showed significant antioxidant activity and hence, are sources of rich natural antioxidants. *L. tigrinus* (47.5 µg/ml, IC50) and *L. squarrosulus* (82.6 µg/ml, IC50), exhibited the highest inhibition ability of DPPH radicals when compared to the remaining mushroom species studied. The results showed that the presence of phenolics and flavonoids is a useful indicator for possessing antioxidant activity in mushrooms. Recently, natural antioxidant supplements are gaining popularity due to restriction in the utilization of synthetic antioxidants. As WEM are easily accessible source of rich natural antioxidants and promising nutritious food, this study brings to light the scope

of mushroom cultivation and production which is eco-friendly and cost-effective. At present, wild mushrooms represent an abundant and precious non-wood forest resource globally. Moreover, the world is in demand for cheaper and abundant source of natural antioxidants which can be provided by mushrooms. The FAO (Food and Agriculture Organization) has emphasized the adoption of mushrooms as an ideal food to combat protein deficiency in the world. Because of the alarming food crisis faced in the world today, the State of Food Security and Nutrition in the World (SOFI) has called upon all countries and stakeholders to come together to end hunger and fight all forms of malnutrition by 2030.

The WEM available in Nagaland are under-studied and under-exploited so the time has arrived to identify and explore the wild mushroom biodiversity. It is essential to thoroughly screen and explore the potential mushrooms available as sustainable development of mushroom industry in Nagaland is only possible when systematic research and work is initiated in this field with a proper vision to improve the socio-economic status of the people. The present study demonstrates the potential and importance of mushrooms especially the edible mushrooms as an economically valuable crop. The findings of this study may be regarded as a database of wild mushrooms of the state and will be helpful to future researchers. This study also promotes awareness to utilize this underutilized local resource, which will not only provide nutritious food but employment opportunities especially to the disadvantaged groups (i.e. unemployed and old people). Efforts need to be made to conserve and promote this valuable natural bio-resource as the present environmental crisis like climate change and global warming adversely affects the survivability of mushrooms which require specific micro-climatic conditions for its growth. It is necessary to remember that the nutritional and medicinal value of mushrooms is most likely to be vanished at this rate if the

wild mushroom species are not documented and studied. Therefore, measures must be taken to conserve this precious natural resource which has immense health and medicinal benefits. Understanding the genetic variations and diversity of any crop plant including edible mushrooms will play a beneficial role in food security.

Mushrooms have the solution to some extent on many of the present day problems like food quality, unemployment, environmental pollutions and pharmaceutical applications. Mushrooms as food and medicines are solutions to many health problems. Thus, mushrooms are a boon to mankind and should be exploited judiciously for the improvement of society.

References

Abdullah S (2014) Diversity of Malaysian *Lentinus* Fr. and chemical analysis of anti-oxidative extract from mycelia of *Lentinus Squarrosulus* Mont., Ph.D. Thesis, Faculty of Science University of Malaya, Kuala Lumpur.

Abou-Heilah AN, Kasionalsim MY, Khaliel AS (1987) Chemical composition of the fruiting bodies of *Agaricus bisporus*. Intl J Exp Bot 47: 64-68.

Achenbach LA, Patrick J, L. Gray L (1996) Use of RAPD markers as a diagnostic tool for the identification of *Fusarium solani* isolates that cause soybean sudden death syndrome. Plant Dis 80: 1228-1232.

Adair S, Kim SH, Brueil C (2002) A molecular approach for early monitoring of decay basidiomycetes in wood chips. FEMS Microbiol Lett 211: 117-122.

Agarwal K, Prasad MP, Rindhe G (2013) Genomic discrimination of eleven commercial mushrooms by DNA fingerprinting using RAPD marker. Intl Res J Biol Sci 2(10): 1-5.

Ajith TA, Janardhanan KK (2007) Indian medicinal mushrooms as a source of antioxidant and antitumor agents. J Clin Biochem Nutr 40(3): 157-162.

Akbar F, Rabbani MA, Masood MS, Shinwari ZK (2011) Genetic diversity of sesame (*Sesamum indicum* L.) germplasm from Pakistan Using RAPD Markers. Pak J Bot 43(4): 2153-2160.

Aletor VA (1995) Compositional studies on edible tropical species of mushrooms. Food Chem 54: 265-268.

Ammirati J, Ammirati S, Norvell L, O'Dell T, Puccio M, Seidl M, Walker G (1994) The Puget Sound Mycological Society, S. Readhead, J. Grins, H. Burdsall, T. Volk, and K. Nakosone, Preliminary Report on the Fungi of Barlow Pass, Washington. McIlvania 11(2): 10-33.

Andrew EE, Kinge TR, Tabi EM, Thiobal N, Mih AM (2013) Diversity and distribution of macrofungi (mushrooms) in the Mount Cameroon Region. J Env Microbiol 3: 318-334.

Angami A, Gajurel PR, Rethy P, Singh B, Kalita SK (2006) Status and Potential of wild edible plants of Arunachal Pradesh. Indian J Trad Know 5(4): 541-550.

AOAC (2000) Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

Ao T, Deb, CR, Khrumo N (2016a) Wild edible mushrooms of Nagaland, India: a potential food resource. J Exp Biol Agricult Sci 4(1): 59-65. http://dx.doi.org/10.18006/2015.4(1).59.65.

Ao T, Seb J, Ajungla T, Deb CR (2016b) Diversity of wild mushrooms in Nagaland, India. Open J For 6: 404-419.

Aoshima H, Tsunoue H, Koda H, Kiso Y (2004) Ageing of whiskey increases 1,1-diphenyl-2-picryl hydrozyl radical scavenging activity. J Agricult Food Chem 52(16): 5240-5244.

Appiah T, Agyare C, Luo Y (2017) Molecular identification of some Ghanaian mushrooms using Internal Transcribed Spacer Regions. Mol Biol 6(3): 1-5. http://doi: 10.4172/2168-9547.1000191.

Apshahana K, Sharma AK (2018) Wild edible mushrooms traded by ethnic communities of Senapati and Kangpokpi district of Manipur, India. J Pharmacog Phytochem 7(1): 2303-2306.

Atkinson CF (1961) Studies of American Fungi Mushrooms Edible, Poisonous, Hafner publishing Co, New York. Pp. 322.

Atri NS, Saini SS, Saini MK (1997) Studies on genus *Russula* Pers. from North Western Himalayas. Mushroom Res 6(1): 1-6.

Ayala FJ, Kiger JA (1984) Modern Genetics. The Benjamin/Cummings Publishing Company, Inc. California, 3:1-923.

Ayaz FA, Torun H, Ozel A, Col M, Duran C, Sesli E, Colak A (2011) Nutritional value of some wild edible mushrooms from the Black Sea region (Turkey), Turk J Biochem 36(4): 384-393.

Bahl N (1983) Medicinal value of edible fungi. In: Proceeding of the International Conference on Science and Cultivation Technology of Edible Fungi. Indian Mushroom Science II. Pp. 203-209.

Bano Z (1976) Nutritive value of Indian mushrooms and medicinal practices. Eco Bot 31: 367-371.

Bano Z, Bhagya S, Srinivasan KS (1981) Essential amino acid composition and proximate analysis of the mushrooms, *Pleurotus eous* and *P. florida*. Mushroom Newsletter for the Tropics 1: 6-10.

Bano Z, Rajarathanam S (1982) *Pleurotus* mushrooms as a nutritious food. In: Tropical mushrooms –Biological Nature and cultivation methods (Chang ST, Quimio, TH, eds.) The Chinese University press, Hongkong. Pp. 363-382.

Bano Z, Srinivasan KS, Srivastava HC (1963) Amino acid composition of the protein from a mushroom (*Pleurotus flabellatus*). Appl Microbiol 11: 184-187.

Barros L, Baptista P, Correia DM, Casal S, Oliveira B, Ferreira ICFR (2007) Fatty acid and sugar compositions and nutritional value of five wild edible mushrooms from Northeast Portugal. Food Chem 105: 140-145.

Barros L, Cruz T, Baptista P, Estevinho ML, Ferreira CFRI (2008) Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol 46:2743-2747.

Baruah P (2008) Pre-seminar draft on formulation of a broad base policy for sustainable development of mushroom industry, Mushroom Development Foundation, Guwahati. Pp. 6-18.

Baruah HK, Sing DK, Islam M (1971) On the distribution of higher Basidiomycetes in the Sibsagar district, Assam. Bull Bot Surv India 13(3 & 4): 285-289.

Basumatary M, Gogoi M (2016) Uses of wild edible macro fungi by Bodo community of Kokrajhar district, Assam, India. Tropical Plant Res 3(1): 176-181.

Beelman RB, Edwards CG (1989) Variability in the composition and nutritional value of the cultivated mushroom *Agaricus bisporus*. Mushroom News 37(17): 20-26.

Begerow D, Nilsson H, Unterseher M, Maier W (2010) Current state and perspectives of fungal DNA barcoding and rapid identification procedures. Appl Microbiol Biotech 87(1): 99-108.

Berger KJ, Guss DA (2005) Mycotoxins revisited: part II. J Emerg Med 28(2): 175-183.

Bhandari HR, Bhanu AN, Srivastava K, Singh MN, Shreya et al. (2017) Assessment of genetic diversity in crop plants - an overview. Adv Plants Agric Res 7(3): 279-286. doi: 10.15406/apar.2017.07.00255.

Bhattacharjee J, Bhattacharjee D, Paul T, Kumar A, Chowdhury S (2015) Diversity of mushrooms in Indo-Bangladesh region of North-East India. J Andaman Sci Association. 19(1): 75-82.

Bian YB, Song XY (2005) Novel molecular markers and their application to edible mushroom research. Acta Edulis Fungi 13(1): 82-87.

Blaalid R, Kumar S, Nilsson R, Abarenkov K, Kirk P, Kauserud H (2013) ITS1versus ITS2 as DNA metabarcodes for fungi. Mol Ecol Res 13: 218-224.

Blackwell M (2011) The Fungi: 1, 2, 3 ... 5.1 million species? Ame J Bot 98: 426-438.

Boa E (2004) Wild edible fungi: a global overview of their use and importance to people. Non-wood forest products 17, Rome: Food and Agricultural Organization Publications.

Bonatti M, Karnopp P, Soares HM, Furlan SA (2004) Evaluation of *Pleurotus ostreatus* and *Pleurotus sajor-caju* nutritional characteristics when cultivated in different lignocellulosic wastes. Food Chem 88(3): 425-428.

Boonsong S, Klaypradit W, Wilaipuna P (2016) Antioxidant activities of extracts from five edible mushrooms using different extractants. Agriculture and Natural Resources 50: 89-97.

Borkar P, Doshi A, Navathe S (2015) Mushroom diversity of Konkan region of Maharashtra, India. J Threatened Taxa 7(10): 7625-7640.

Borneman J, Hartin RJ (2000) PCR primers that amplify fungal *rRNA* genes from environmental samples. Appl. Environ. Microbiol. 66: 4356-4360.

Bostein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using Restriction Fragment Length Polymorphisms. Ame J Human Genet. 32: 314-331.

Branen AL (1975) Toxicology and biochemistry of butylated hydroxyanisol and butylated hydroxytoluene. J Ame Oil Chem Socy 5: 59- 63.

Breene WM (1990) Nutritional and medicinal value of speciality mushrooms. J Food Protect 53: 883-894.

Brock OT, Thomas K, Brock M, David MW (1986) Basic Microbiology with Applications. Antibiotics and other Chemotherapeutic Agents, 3rd Edn. Pp 144-155.

Bruns T D, White T J, Taylor JW (1991) Fungal molecular systematics. Annual Rev Ecol Systematics 22: 525-564.

Butler AHR (1915) The fungus lore of the Greeks and Romans. Trans Br Mycol Soc 5: 21-26.

Cai L, Giraud T, Zhang N, Begerow D, Cai G, Shivas RG (2011) The evolution of species concepts and species recognition criteria in plant pathogenic fungi. Fungal diversity 50(1):121-133. doi: 10.1007/s13225-011-0127-8.

Carvalho AOR, Vieira LGE (2001) Determinação das Condições Ótimas para Análises de PCR-RAPD em Atta sexdens rubropilosa Forel (Hymenoptera: Formicidae). Neotrop Entomol 30: 593-600.

Castle AJ, Horgen PA, Anderson JB (1987) Restriction fragment length polymorphisms in the mushrooms *Agaricus brunnescens* and *Agaricus bitorquis*. Appl Environ Microbiol 53: 816-822.

Cazzi R, Ricardy R, Aglitti T, Gatta V, Petricone P, De SR (1997) Ascorbic acid and b-carotene as modulators of oxidative damage. Carcinogenesis 18: 223-228.

Chang ST (1982) Prospects for mushroom protein in developing countries. In: Tropical Mushroom - Biological Nature and Cultivation Methods (Chang ST, Quimio TH. eds.), Chinese University Press, Hong Kong. Pp. 463-473.

Chang ST (1996) Mushroom research and development-Equality and mutual benefit. Mushroom Biology and Mushroom Prods., Royse (ed). Penn State Univ. Pp. 1-10.

Chang ST, Buswell JA (1996) Mushroom Nutriceuticals. World J Microbiol Biotechnol 12: 473-476.

Chang ST, Miles PG (1992) Mushroom biology – A new discipline. Mycologist 6: 64-65.

Chang ST, Miles PG (2004) Mushrooms cultivation, nutritional value, medicinal effect and environmental impact. Mycologist 6: 64-65.

Chillali M, Hakima II, Guillaumin JJ, Mohammed C, Escarmant BL, Botton B (1998) Variation in the ITS and IGS regions of ribosomal DNA among the biological species of European Armillaria. Mycol Res 102(5): 533-540.

Chen YQ, Hu B, Xu F, Zhang W, Zhou H, Qu LH (2004) Genetic variation of *Cordyceps sinensis*, a fruit-body-producing entomopathogenic species from different geographical regions in China. FEMS Microbiol Lett 230: 153-158.

Cheung LM, Cheung CK, Ooi VEC (2003) Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem 81: 249-255.

Cheung PCK (1998) Plasma and hepatic cholesterol levels and feacal neutral sterol excretion are altered in hamsters fed straw mushroom diet. J Nutrition 128: 1512-1516.

Chihara G, Maeda Y, Hamuro J, Sasaki T, Fukuoka F (1969) Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing. Nature 222: 687-688.

Choi YJ, Park IS, Kim MH, Kwon B, Choo YM, Jeong S, Yu KY, Choe CH, Kim J (2018) The medicinal mushroom *Auricularia auricula-judae* (Bull.) extract has antioxidant activity and promotes procollagen biosynthesis in HaCaT cells. Natural Product Research, Short Communication: 1-4. https://doi.org/10.1080/14786419.2018.1468332.

Christensen CM (1943) Common Edible Mushrooms. The University of Minnesota Press. Minneapolis.

Crawshay R (1930) The spore ornamentation of the Russulales. Baillière, Tindall & Cox. Cornell University.

Crisan EW, Sands (1978) A Nutritional value. In: The biology and cultivation of edible mushrooms (Chang ST and Hayes WA, eds.). Academic Press, New York. Pp. 172-189.

Colak A, Faiz O, Sesli E (2009) Nutritional composition of some wild edible mushrooms. Turk J Biochem 34: 25-31.

Cowan PW (2001) Fungi- Life supports for ecosystem. Essential ARB 4: 1-5.

Crous PW, Isabella HR, Alan W, Seonju L, Hugh G, Wilhelm B, Bernard S, Wilhelm ZB, Michael JW, David LH (2006) How many species of Fungi are there in tip of Africa? Studies Mycol 55: 13-33.

Das K (2009) Mushrooms of Sikkim I: Barsey Rhododendron Sanctuary. Sikkim State Biodiversity Board and Botanical Survey of India.

Das K (2010) Diversity and conservation of wild mushrooms in Sikkim with special reference to Barsey Rhododendron Sanctuary. NeBIO, 1(2): 1-13.

Das K, Sharma JR (2005) *Russulaceae* of Kumaon Himalaya. BSI and Ministry of Environment and Forests, Govt. of India.

Das P, Gautom T, Barooah M, Boro RC (2017) A Study on the Diversity and Habitat Specificity of Macrofungi of Assam, India. Intl J Curr Microbiol App Sci 6(12): 275-297. https://doi.org/10.20546/ijcmas.2017.612.034.

Das RA, Saha AK, Joshi SR, Das P (2017) Wild edible macrofungi consumed by ethnic tribes of Tripura in Northeast India with special reference to antibacterial activity of *Pleurotus djamor* (Rumph. ex Fr.) Boedijn. Int Food Res J 24(2): 834-838.

Darvasi A, Weintreb V, Minke S, Weller M, Soller (1993) Detecting Marker-QTL Linkage and Estimating QTL Gene Effect and Map Location Using a Saturated Genetic Map. Genetics 134: 943-951.

Davidson S, Johnsy G, Samuel SA, Kaviyarsan V (2012) Mushrooms in the food culture of the Kanni tribe of Kanyakumari district. Indian J Trad Know 11(1): 150-153.

Demirbas A (2001) Concentrations of 21 metals in 18 species of mushrooms growing in the East Black Sea region. Food Chem 75: 453-457.

Deshmukh SK (2004) Biodiversity of tropical basidiomycetes as sources of novel secondary metabolites. In: Microbiology and Biotechnology for Sustainable Development (Jain PC, ed.), CBS Publishers and Distributors, New Delhi. Pp 121-140.

Deshmukh SK (2006) Poisonous and Hallucinogenic Mushrooms of India. Intl J Med Mushrooms 8(3): 251-262.

Diez JM, James TY, Mcmunn M, Ibanez I (2013) Predicting species-specific responses of fungi to climatic variation using historical records. Global Change Biol 19: 3145-3154.

Dobzhansky T (1970) Genetics of Evolutionary Process. New York: Columbia University Press.

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: 11-15.

Dubois MKA, Gilles JK, Hamilton PA, Rebers, Smith F (1956) Colorimetric method for determination of sugars and related substances. Analytic Chem 28: 350-356.

Du P, Cui BK, Dai YC (2011) High genetic diversity in wild culinary-medicinal wood ear mushroom, *Auricularia polytricha* (Mont.) Sacc., in tropical China revealed by ISSR analysis. Intl J Med Mushrooms 13: 289-298.

Du P, Cui BK, Zhang CF, Dai YC (2013) Genetic diversity of wild *Auricularia auricula-judae* revealed by ISSR analysis. Biochem Systematics Ecol 48: 199-205.

Dwivedi S, Singh S, Chauhan UK, Tiwari MK (2017) Inter- and intra-specific genetic diversity (RAPD) among three most frequent species of macrofungi (*Ganoderma lucidum, Leucoagricus* sp. and *Lentinus* sp.) of Tropical forest of Central India. J Genet Eng Biotechnol 16(1): 133-141. https://doi.org/10.1016/j.jgeb.2017.11.008.

Elmastas M, Isıldak O, Turkekul I, Temur N (2007) Determination of antioxidant activity and compounds in wild edible mushrooms. J Food Comp Analysis 20(3-4): 337-345.

Enas AE, Sabahelkhier MK, Malaz MM (2016) Nutritional Composition and Minerals content of five Species of Wild Edible Mushroom, Brought from UAE: Mushroom considered as protein source. Intl J Adv Res 4(2): 1108-1112.

Fan L, Pan H, Soccol AT, Pandey A, Soccol CR (2006) Advances in Mushroom Research in the Last Decade. Food Technol Biotechnol 44(3): 303-311.

Fangkrathok N, Junlatat J, Sripanidkulchai B (2013) *In vivo* and *in vitro* anti-inflammatory activity of *Lentinus polychrous* extract. J Ethnopharmacol 147: 631-637.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

Feng B, Xu J, Wu G, Zeng NK, Li YC, Tolgor B, Kost GW, Yang ZL (2012) DNA sequence analyses reveal abundant diversity, endemism and evidence for Asian origin of the Porcini mushrooms. Plos One 7(5): 1-12.

Ferreira ICFR, Baptista P, Boas VM, Barros L (2007) Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal. Food Chem 100: 1511-1516.

Ferreira ME, Grattapaglia D (1996) Introdução ao Uso de Marcadores Moleculares em Análise Genética. Brasília: Embrapa Cenargen. Pp. 220.

Ferriol M, Pioo B, Nuez F (2003) Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theor Appl Genet 107: 271-282.

Fonseca GG, Gandra EA, Sclowitz LF, Correa APA, Costa JAV, Levy JA (2008) Oyster mushrooms species differentiation through molecular markers RAPD. Intl J Pl Breed Gen 2: 13-18.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conserv Biol 10: 1500-1508.

Fu LZ, Zhang HY, Wu XQ, Li HB, Wei HL, Wu QQ, Wang LA (2010) Evaluation of genetic diversity in *Lentinula edodes* strains using RAPD, ISSR and SRAP markers. World J Microbiol Biotechnol 26: 709-716.

Galor WS, Benzie IFF, Tomlinson B, Buswell JA (2003) Lingzhi (*Ganoderma lucidum*): molecular aspects of health effects. In Herbal Medicines, [L Packer, B Halliwell and CN Ong, editors]. New York: Marcel Dekker Inc.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Mol Ecol 2: 113-118.

Gostimsky SA, Kokaeva ZG, Konovalov FA (2005) Studying plant genome variation using molecular markers. Russ J Genet 41(4): 378-388. https://doi.org/10.1007/s11177-005-0101-1.

Gogoi G, Parkash V (2015) A checklist of gilled mushrooms (Basidiomycota: Agaricomycetes) with diversity analysis in Hollongapar Gibbon Wildlife Sanctuary, Assam, India. J Threatened Taxa 7(15): 8272-8287.

Gregory A, Burke T, Ferris R, Robson J, Smithers R, Whitlock R (2006) The conservation of genetic diversity: Science and policy needs in a changing world Gregory. JNCC Report no: 383. Pp. 1-87.

Gruen VEC, Wong HX (1982) Immunodulatory and Antitumour activities of a polysaccharide-peptide complex from a mycelial culture of *Trichoderma* sp. Sciences 57: 269-281.

Guan XJ, Xu L, Shao YC, Wang ZR, Chen FS, Luo XC (2008) Differentiation of commercial strains of *Agaricus* species in China with inter-simple sequence repeat marker. World J Microbiol Biotechnol 24: 1617-1622.

Guglielmo F, Gonthier P, Garbelotto M, Nicolotti G (2008) PCR-based method for the identification of important wood rotting fungal taxa within *Ganoderma*, *Inonotus* s.l. and *Phellinuss*.l. FEMS Microbiol Lett 282: 228-237.

Gurtner SC, Pinar G, Lubitz W, Rolleke S (2001) Analysis of fungal communities on historical church window glass by denaturing gradient gel electrophoresis and phylogenetic 18S rDNA sequence analysis. J Microbiol Methods 47: 345-354.

Halliwell B, Gutteridge JMC (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 219: 1-4.

Hamada H, Kakunaga T (1982) Potential Z-DNA forming sequences are highly dispersed in the human genome. Nature 298: 396-398.

Harsh NSK, Joshi K (2008) Mushrooms: The vegetable of the future. India Science and Technology. Available at SSRN: https://ssrn.com/abstract=1474175.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ, Abaci O, Aime C, Asan A, Bai FY, Beer ZW, Begerow D, Berikten D, Boekhout T, Buchanan PK, Burgess T, Buzina W, Cai L, Cannon PF, Crane JL, Damm U, Daniel HM, Diepeningen AD, Druzhinina I, Dyer PS, Eberhardt U, Fell WF, Frisvad JC, Geiser DM, Geml J, Glienke C, Gräfenhan T, Groenewald JZ, Groenewald M, Gruyter J, Kellermann EG, Guo LD, Hibbett DS, Hong SB, Hoog GS, Houbraken J, Huhndorf SM, Hyde KD, Ismail A, Johnston PR, Kadaifciler DG, Kirk PM, Kõljalg U, Kurtzman CP, Lagneau PE, Lévesque CA, Liu X, Lombard L, Meyer W, Miller A, Minter DW, Najafzadeh MJ, Norvell L, Ozerskaya SM, Ozic R, Pennycook SR, Peterson SW, Pettersson OV, Quaedvlieg W, Robert VA, Ruibal C, Schnürer J, Schroers HF, Shivas R, Slippers B, Spierenburg H, Takashima M, Taşkın

E, Thines M, Thrane U, Uztan AH, Raak MV, Varga J, Vasco A, Verkley G, Videira SIR, Vries RP, Weir BS, Yilmaz Y, Yurkov A, Zhang N (2011) The Amsterdam declaration on fungal nomenclature. IMA Fungus 2(1): 105-112.

Heath DD, Iwama GK, Devlin RH (1993) PCR primed with VNTR core sequence yields species specific patterns and hyper variable probes. Nucleic Acids Res. 21: 5782-5785.

Heleno SA, Barros L, Sousa MJ, Martins A, Ferreira ICFR (2009) Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. Microchem J 93: 195-199.

Hillis D, Dixon M (1991) Ribosomal DNA: Molecular evolution and phylogenetic inference. Q Rev Biol 66: 411-453.

Hira CK, Vadhera S, Mann APS (1990) Biochemical changes in sporophores of two mushroom species before and after opening of caps. Annals Biol 6: 95-99.

Hofstetter V, Clemencon H, Vilgalys R, Moncalvo JM (2002) Phylogenetic analyses of the Lyophylleae (Agaricales, Basidiomycota) based on nuclear and mitochondrial rDNA sequences. Mycological Res 106: 1043-1059.

Hopple Jr, JS, Vilgalys R (1994) Phylogenetic relationship among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. Mycologia 86: 96-107.

Hong SG, Chun J, Nam JS, Park YD, Bae KS (2000) Phylogenetic analysis of genus Sporobolomyces based on partial sequences of 26S rDNA. J Microbiol Biotechnol 10: 363-366.

Hu YP, Wang L, Xie XL, Yang J, Li Y, Zhang HG (2010) Genetic diversity of wild populations of *Rheum tanguticum* endemic to China as revealed by ISSR analysis. Biochem Sys Ecol. 38: 264-274.

Hur I, Chun J (2004) A method for comparing multiple bacterial community structures from 16S rDNA clone library sequences. J Microbiol: 42: 9-13.

Ikekawa T, Uehara N, Maeda Y, Nakanishi M, Fukuoka F (1969) Anti-tumor activity of aqueous extracts of edible mushrooms. Cancer Res 29: 734-175.

Isikhuemhen OS, Mikiashvili NA, Adenipekun CO, Ohimain EI, Shahbazi G (2012) The tropical white rot fungus, *Lentinus squarrosulus* Mont.: lignocellulolytic enzymes activities and sugar release from cornstalks under solid state fermentation. World J Microbiol Biotechnol 28: 1961-1966.

Jasalavich C, Ostrotsky A, Jellison J (2000) Detection and identification of decay fungi in spruce wood by restriction fragment length polymorphism analysis of amplified genes encoding *rRNA*. Appl Environ Microbiol 66: 4725-4734.

Jeffreys AJ, Wilson V, Thein SL (1985) Hypervariable 'minisatellite' regions in human DNA. Nature 314: 67-73.

Johnsy G, Sargunam DS, Dinesh MG, Kaviyarasan V (2011) Nutritive value of edible wild mushrooms collected from the Western Ghats of Kanyakumari District. Botany Res Intl 4(4): 69-74.

Jonathan SG (2002) Vegetable growth requirements and antimicrobial activities of some higher fungi in Nigeria Ph.D. thesis, University of Ibadan.

Jong SC, Birmingham JM (1991) Medicinal benefits of the mushroom *Ganoderma*. Adv Appl Microbiol 37: 101-134.

Jones S, Janardhanan KK (2000) Antioxidant and antitumor activity of *Ganoderma lucidum* (curt ex Fr.). P. Karst-Reshi (Aphyllophoromycetieae) from south India. Intl J Med Mushrooms 2: 195-200.

Jones EB, Whalley J (1994) A fungus foray to Chiang Mai market in northern Thailand. The Mycologist 8: 87-90.

Kalac P (2009) Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chem 113: 9-16.

Kalac P, Svoboda L (2000) A review of trace element concentrations in edible mushrooms. Food Chem 69: 273-281.

Kalita K, Bezbaroa RN, Kumar R, Pandey S (2016) Documentation of wild edible mushrooms from Meghalaya, Northeast, India. Curr Res Environ Appl Mycol J Fungal Biol 6(4): 238-247.

Karaca M, Ince AG (2008) Minisatellites as DNA markers to classify bermuda grasses (*Cynodon* spp.): confirmation of minisatellite in amplified products. J Genet 87: 83-86.

Karaca M, Saha S, Zipf A, Jenkins JN, Lang DJ (2002) Genetic diversity among forage bermudagrass (*Cynodon* spp.): evidence from chloroplast and nuclear DNA fingerprinting. Crop Sci 42: 2118-2127.

Karakas O, Gurel F, Uncuoglu AA (2010) Exploiting a wheat EST database to assess genetic diversity. Genet Mol Biol 33: 719-730.

Keles A, Koca I, Genccelep H (2011) Antioxidant properties of wild edible mushrooms. J Food Processing Technol 2: 130-136.

Khan MA, Tania M, Liu R, Rahman MM (2013) *Hericium erinaceus*: an edible mushroom with medicinal values. J Complement Integr Med 10(1): 1-6.

Khaund P and Joshi SR (2013) Wild edible macrofungal species consumed by the Khasi tribe of Meghalaya, India. Indian J Natural Products Res 4: 197-204.

KimuraM, Crow J (1964) The number of alleles that can be maintained in a finite population. Genetics 49: 725-738.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111-120.

Kim KS, Lee YS (2000) Rapid accurate species-specific detection of *Phytophthora infestans* through analysis of ITS regions in its rDNA. J Microbiol Biotechnol 10: 652-655.

Kindermann J, El-Ayouti Y, Samuels G J, Kubicek CP (1998) Phylogeny of the Genus *Trichoderma* Based on Sequence Analysis of the Internal Transcribed Spacer Region 1 of the rDNA Cluster. Fungal Genet Biol 24: 298-309.

King TA (1993) Mushrooms, the ultimate health food but little research in US to prove it. Mushroom News 41: 29-46.

Kluge AG, J. S. Farris JS (1969). Quantitative phyletics and the evolution of anurans. Syst Zool 18: 1-32.

Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Palme JB, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Duenas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lucking R, Martin MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Poldmaa K, Saag L, Saar I, SchublerA, Scott JA, Senes C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson KH (2013) Towards

a unified paradigm for sequence-based identification of fungi. Mol Ecol 22: 5271-5277.

Kosztarb M (1983) Editorial. A Biological Survey of the United States, Science.

Kumar R, Pandey S, Rishi R, Giri K, Mishra G (2015) Unrecorded macrofungi from the Narpuh Reserve Forest of Meghalaya, India. Intl J Curr Life Sci 1: 118-123.

Kumar A, Mishra P, Singh SC, Sundaresan V (2014) Efficiency of ISSR and RAPD markers in genetic divergence analysis and conservation management of *Justicia adhatoda* L., a medicinal plant. Plant Syst Evol 300: 1409-1420.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35: 1547-1549.

Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Keeuwenhoek 73: 331-371.

Lalrinawm H, Vabeikhokhei JMC, Zothanzama; Zohmangaiha (2017) Edible mushrooms of Mizoram. Sci Vis 17(3): 172-181.

Lakhanpal TN, Rana M (2005) Medicinal and nutraceutical genetic resources of mushrooms. Plant Genetic Resources: Characterization and Utilization 3(2): 288-303.

Lambert EB (1938) Principles and problems of mushroom culture. Bot Rev 4: 397-426.

Lande R (1988) Genetics and demography in biological. Conserv Sci 241: 1455-1460.

Lan J, Xu JT, Wang QY (1998) Electrophoretic studies on esterase and peroxidase isozymes in *Ganoderma* spp. Chinese Pharm J 33: 12-14.

Lee YM, Choi YK, Min BR (2000) PCR-RFLP and sequence analysis of the rDNA ITS region in the *Fusarium* spp. J Microbiol 38: 66-73.

Lee JS, Lim MO, Cho KY, Cho JH, Chang SY, Nam DH (2006) Identification of medicinal mushroom species based on nuclear large subunit rDNA sequences. J Microbiol 44: 29-34.

Lewontin RC (1972) The apportionment of human diversity. Evol Biol 6: 381-398.

Li GSF, Chang ST (1982) Nutritive value of *Volvariella volvacea*, In: Tropical mushrooms-Biological nature and cultivation methods (Chang ST, Quimio TH, eds.) Chinese University Press, Hong Kong. Pp. 199-219.

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet 103: 455-461.

Lian B, Zang JP, Hou WG, Yuan S, Smith DL (2008) PCR-based sensitive detection of the edible fungus *Boletus edulis* from rDNA ITS sequences. Electronic J Biotechol 11: 102-109.

Lim Y, WS A, Lee A, Kim SB, Yong HY, Yeon YK, Park DW, Jeong, Park JS (2005) Diversity of denitrifying bacteria isolated from Daejon sewage treatment plant. J Microbiol 43: 383-390.

Lin CH, Sheu GT, Lin YW, Yeh CS, Huang YH, Lai YC, Chang JG, Ko JL (2010) A new immunomodulatory protein from *Ganoderma microsporum* inhibits epidermal growth factor mediated migration and invasion in A549 lung cancer cells. Process Biochem 45(9): 1537-1542.

Liu GT (1999) Recent advances in research of pharmacology and clinical application of *Ganoderma* (P. Karst) species (Aphyllophoromycetideae) in China. Int J Med Mushrooms 1: 63-67.

Liu J, Wang ZR, Li C, Bian YB, Xiao Y (2015) Evaluating genetic diversity and constructing core collections of Chinese *Lentinula edodes* cultivars using ISSR and SRAP markers. J Basic Microbiol 55: 749-760.

Liu XB, Jing Li J, Yang ZL (2018) Genetic diversity and structure of core collection of winter mushroom (*Flammulina velutipes*) developed by genomic SSR markers. Hereditas 155: 1-8. https://doi 10.1186/s41065-017-0038-0.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275.

Lynch M, Milligan BG (1994) Analysis of population structure with RAPD markers. Mol Ecol 3: 91-99.

Ma FY, Luo XC (2002) PCR-based restriction analysis of internal transcribed spacers of nuclear ribosomal DNA in the genus *Pleurotus*. Mycosystema 21: 356-362.

Maeta K, Ochi T, Tokimoto K, Shimomura N, Maekawa N, Kawaguchi N (2008) Rapid species identification of cooked poisonous mushrooms by using real-time PCR. Applied Environ Microbiol 74: 3306-3309.

Malekzadeh K, Shahri BJM, Mohsenifard E (2011) Use of ISSR markers for strain identification in the button mushroom, *Agaricus bisporus*. In: Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7), October 4-7, 2011, World Society of Mushroom Biology and Mushroom Products. Pp 1-5.

Manikandan K (2011) Nutritional and medicinal values of mushrooms. In: Mushrooms Cultivation, Marketing and Consumption (Singh M, Vijay B, Kamal S, Wakchaure GC, eds). Directorate of Mushroom Research, Solan, India. Pp. 11-14.

Manoharachary C, Sridhar K, Singh R, Adholeya, Suryanarayanan TS, Rawat S, Johri BN (2005) Fungal Biodiversity: Distribution, Conservation and Prospecting of Fungi from India. Curr Sci 89(1): 58-71.

Mantel M (1967) The detection of disease clustering and generalized regression approach. Cancer Res 27: 209-220.

Manzi P, Aguzzi A, Pizzoferrato L (2001) Nutritional value of mushrooms widely consumed in Italy. Food Chem 73: 321-325.

Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L (1999) Nutrients in edible mushrooms: an inter-species comparative study. Food Chem 65: 477-482.

Manzi P, Marconi S, Aguzzi A, Pizzoferrato, L (2004) Commercial mushrooms: Nutritional quality and effect of cooking. Food Chem 84: 201-206.

Maric S, Bolaric S, Martincic J, Pejic I, Kozumplik V (2004) Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. Plant Breed 123: 366-369.

Martin P, Muruke M, Hosea K, Kivaisi AK, Zerwas N, Bauerle C (2004) A rapid PCR-RFLP method for monitoring genetic variation among commercial mushroom species. Biochem Mol Biol Edu 32: 390-394.

Mattila PH, Piironen VI, Uusi R, Koivistoinen PE (1994) Vitamin D contents in edible mush. J Agr Food Chem 42: 2449-2453.

Mattila P, Konko K, Eurola M, Pihlawa JM, Astola J, Lietaniemi VV, Kumpulainen J, Valtonen M, Piironen V (2001) Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. J Agric Food Chem 49: 2343-2348.

Mau JL, Chao GR, Wu KT (2001) Antioxidant properties of methanolic extracts from several ear mushrooms. J Agric Food Chem 49: 5461-5467.

Mau CN, Huang SJ, Chen CC (2004). Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. Food Chem 87: 111-118.

Maynard AJ (1970) Methods in Food Analysis. Academic press. New York. Pp. 176.

Mc Connel, JE, Esselen, WB (1947) Carbohydrate in cultivated mushrooms. Food Res 12: 118-121.

Miller GL (1972) Use of Dinitrosalicyclic acid reagent for determination of reducing sugar. Analytic Chem 31(3): 426-428.

Mizuno T, Wang G, Zhang J, Kawagishi H, Nishitoba T, Li J (1995) Reishi, *Ganoderma lucidum* and *G tsugae*: Bioactive substances and medicinal effects. Food Reviews Int 11: 151-166.

McDermott JM, McDonald BA (1993) Gene flow in plant pathosystems. Ann Rev Phytopathol 31: 353-373.

Meyer W, Mitchell TG, Freedman EZ, Vilgays R (1993) Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. J Clin Microbiol 31: 2274-2280.

Mizuno T (1999) Bioactive substances in *Hericium erinaceus* (Bull.: Fr.) Pers. (Yamabushitake), and its medicinal utilization. Int J Med Mushrooms 1: 105-119.

Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B (2011) How many species are there on Earth and in the ocean? PLoS Biol 9(8): 1-8.

Moreau PA, Peintner U, Gardes M (2006) Phylogeny of the ectomycorrhizal mushroom genus *Alnicola* (Basidiomycota, Cortinariaceae) based on rDNA sequences with special emphasis on host specificity and morphological characters. Mol Phylogen Evol 38: 794-807.

Mortimer PE, Xu J, Karunarathna SC, Hyde KD (2014) Mushrooms for Trees and People. A Field Guide to Useful Mushrooms of the Mekong Region. World Agroforestry Centre, East and Central Asia, Kunming, 650201 PR China.

Moore AJ, Challen MP, Warner PJ, Elliott TJ (2001) RAPD discrimination of *Agaricus bisporus* mushroom cultivars. Appl Microbiol Biotechnol 55(6): 742-749.

Moore D, Chiu SW (2001) Filamentous fungi as food. In: Exploitation of Filamentous Fungi (Pointing SB and Hyde D, eds.), Hong Kong: Fungal Diversity Press.

Muruke M, Kivaisi AK, Magingo FSS, Danell E (2002) Identification of mushroom mycelia using DNA techniques. Tanzania J Sci 28: 115-128.

Murugkar DA, Subbulakshmi G (2005) Nutritional value of edible wild mushrooms collected from the Khasi hills, Meghalaya. Food Chem 89(4): 599-603.

Nazrul MI, Bing BY (2010) ISSR as new markers for identification of homokaryotic Protoclones of *Agaricus bisporus*. Curr Microbiol 60: 92-98. http://doi10.1007/s00284-009-9506-8.

Neale DB, Savolainen O (2004) Association genetics of complex traits in conifers. Trends Plant Sci 9(7): 325-330.

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70: 3321-3323.

Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Nishida FH, Sundberg WJ, Menge JA, States JS, Tulloss RE, Blanco CJ (1992) Studies in the Mycoflora of the Chiricahua Mountains, Cochise County, Arizona, USA. I. Preliminary report on species distributions, ecology, and biogeographic affinities, Chiricahua Mountains Research Symposium Proceedings (AM Barton and SA Sloane) March 16-17. Pp. 35-38.

Ooi VEC, Liu F (1999) A review of pharmacological activities of mushroom polysaccharides. Intl J Med Mushrooms 1: 195-206.

Oyetayo FL (2007) Potential antioxidant properties of Nigerian edible mushrooms. Agrofood Industry Hi-tech. 18: 44-45.

Ozturk C, Kasik G, Dogan HH , Aktas S (2003) Macrofungi of Alanya district. Turkish J Botany 27: 303-312.

Pandey VN, Srivastava AK (1994) Fleshy fungi of ethno-botanical food used in North Eastern Tarai region of Uttar Pradesh. In: Proceedings of National Symposium on Mushroom, NRCM-Solan, 3.

Park DS, Ryu YJ (1996) The Genetic Relationship Analysis of *Ganoderma* spp. Using the PCR-RFLP and RAPD. J Agric Sci Biotechnol 38: 251-260.

Park HG, Go HG, Kim SH, Park WM (2004) Molecular identification of Asian isolates of medicinal mushroom *Hericum erinaceum* by phylogenetic analysis of nuclear ITS rDNA. J Microbiol Biotechnol 14: 816-821.

Parveen A, Khataniar L, Goswami G, Hazarika DJ, Das P, Gautom T, Barooah M, Boro RC (2017) A study on the diversity and habitat specificity of Macrofungi of Assam,

India. Intl J Curr Microbiol App Sci 6(12): 275-297. doi.org/10.20546/ijcmas.2017.612.034.

Patel S, Goyal A (2013) Recent developments in mushrooms as anti-cancer therapeutics. J Biotechnol 2: 1-15.

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv Agron 46: 39-90.

Pegler DN (1983) The genus *Lentinus* 'A World Monograph'. Kew Bulletin Additional Series X. Royal Botanic Garden Kew.

Philips R (2006) Mushrooms. Pan Macmillan, London.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, And TS, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2: 225-238.

Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor App Genet 98:107-112.

Purkayastha RP, Chandra A (1985) Manual of Indian edible mushrooms. Jagendra Book Agency, New Delhi, India. Pp. 267-270.

Pushpa H, Purushothoma KB (2010) Nutritional analysis of wild and cultivated edible medicinal mushrooms. World J Dairy Food Sci 5(2): 140-144.

Puttaraju NG, Venkateshaiah SU, Dharmesh SM, Urs SM, Somasundaram R (2006) Antioxidant Activity of Indigenous Edible Mushrooms. J Agric Food Chem 54: 9764-9772.

Qi JJ, Ma RC, Chen XD, Lan J (2003) Analysis of genetic variation in *Ganoderma lucidum* after Space Flight. Adv Space Res 31: 1617-1622.

Qin LH, Song CY, Tan Q, Chen MJ, Pan YJ (2006) Use of ITS and ISSR markers to identify cultivated strains for *Lentinula edodes* (Chinese). Mycosystema 25: 94-100.

Rai RD (1997) Medicinal mushrooms. In: Advances in Mushroom Biology and Production (Rai, RD, Dhar BL, Verma RN, eds.), Mushroom Society of India. NRCM, Solan, Himachal Pradesh. Pp. 355-368.

Ragunathan R, Gurusamy R, Palaniswamy M, Swaminathan K (1996) Cultivation of *Pleurotus* spp. on various agro-residues. Food Chem 55: 139-144.

Ramesh C, Pattar MG (2010) Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of Western Ghats of Karnataka, India. Pharmacog Res 2(2): 107-112.

Ravash R, Hiran BS, Alavi A, Zarvagis Z (2009) Evaluation of genetic diversity in oyster mushroom (*Pleurotus eryngii*) isolates using RAPD marker. J Sci Technol Agri Natural Res 13(47): 729-739.

Reddy MP, Sarla N, Siddiq EA (2002) Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128: 9-17.

Rehman H, Rabbani MA, Shinwari ZK, Akbar F (2015) RAPD markers based genetic diversity of safflower (*Carthamus tinctorius* L.) germplasm. Pak J Bot 47(SI): 199-204.

Reza MA, Jo WS, Park SC (2012) Comparative antitumor activity of jelly ear culinary-medicinal mushroom, *Auricularia auricula-judae* (Bull.) J. Schrot. (higher basidiomycetes) extracts against tumor cells *in vitro*. Int J Med Mushrooms 14(4): 403-409. DOI: 10.1615/IntJMedMushr.v14.i4.80.

Roberto GO, Cifuentes J, Torres AE, Caballero J (2006) People using macro-fungal diversity in Oaxaca, Mexico. Fungal Biodiversity 21: 41-67.

Roldan-Ruiz I, Dendauw J, Van Bockstaele E, Depicker A, De Loose M (2000) AFLP markers reveal high polymorphic rates in rye grasses (*Lolium* spp.). Mol Breed 6:125-34. http://dx.doi.org/10.1023/A:1009680614564.

Rohlf FJ (1998) NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.02e. Applied Biostatistics Inc., Exeter Software, Setauket.

Rohlf FJ (2000) NTSYS-PC numerical taxonomy and multivariate analysis system, version 2.10. Exeter Software, New York.

Ro H, Kim SS, Ryu JS, Jeon C, Lee TS, Lee H (2007) Comparative Studies on the Diversity of the Edible Mushroom *Pleurotus eringii*: ITS Sequence Analysis, RAPD Fingerprinting, and Physiological Characteristics. Mycol Res 111: 710-715.

Rolim LN, Cavalcante MAQ, Urben AF, Buso GSC (2011) Use of RAPD Molecular Markers on Differentiation of Brazilian and Chinese *Ganoderma lucidum* strains. Braz Archives Biol Technol 54(2): 273-281.

Rosendahl S (2008) Communities, populations and individuals of arbuscular mycorrhizal fungi. New Phytol 178: 253-266.

Roy A, De AB (1996) Polyporaceae of India. International Book Distributors, Dehradun, India.

Rudawska M, Leski T (2005) Macro- and microelement contents in fruiting bodies of wild mushrooms from the Notecka forest in west-central Poland. Food Chem 92: 499-506.

Rzhetsky A, Nei M (1992) A simple method for estimating and testing minimum evolution trees. Mol Biol Evol 9: 945-967.

Sachan SKS, Patra JK, Thatoi HN (2013) Indigenous knowledge of ethnic tribes for utilization of wild mushrooms as food and medicine in Similipal Biosphere Reserve, Odisha, India. J Agricult Tech 9(2): 403-416.

Sahoo AK, Mohapatra KB, Behera BB, Jadhao KR, Rout GR (2014) Phylogenetic Analysis of high yielding strain of paddy straw Mushroom (*Volvariella volvacea*) by using ISSR markers. Middle-East J Scientific Res 21(8): 1197-1202.

Sahreen S, Khan M, Khan RA (2010) Evaluation of antioxidant activities of various solvent extracts of *Carisa apaca* fruits. Food Chem 122: 1205-1211.

Salazar O, Schneider JHM, Julian MC, Keijer J, Rubio V (1999) Phylogenetic sub grouping of *Rhizoctonia solani* AG2 isolates based on ribosomal ITS sequences. Mycologia 91: 459-467.

Sanchez C (2017) Reactive oxygen species and antioxidant properties from mushrooms. Synth Syst Biotechnol 2:13-22.

Sanmee R, Dell B, Lumyomg P, Izumori K, Lumyong S (2003) Nutritive value of popular wild edible mushrooms from northern Thailand. Food Chem 82: 527-532.

Seifert KA (2009) Progress towards DNA barcoding of Fungi. Mol Ecol Res 9(S1): 83–89.

Slatkin M (1985) Gene flow in natural populations. Ann Rev Syst. 16: 393-430. https://doi.org/10.1146/annurev.es.16.110185.002141.

Sing NI, Sing SM (1993) Edible Fleshy Fungal Flora of Manipur. Bioveel 4(2): 153-158.

Sing, Sing NI, Sing SM, TC (2002) Fleshy fungi of Manipur. In: Plant Genetic Diversity: Exploration, Evaluation, Conservation (Vijay SP, Kondo K, Sharma ML and Gupta A, eds.). Afficiated East West Press Pvt. Ltd. New Delhi. Pp. 9-13.

Singer R (1961) Mushrooms and Truffles, Leonard Hill Books Ltd, London. Pp. 1-272.

Singh TC, Nivedita L, Singh NI (2007) Endemic Bio-resources of India-Conservation and Sustainable development with special reference to N.E India In: Endemic Bioresources of India (Singh NI, Singh B, Singh MP, eds.), Dehradun, India. Pp. 67-94.

Singh HB, Adhikary RK, Sarma RK, Sharma TC, Rao PG (2008) Cultivation of Shiitake mushroom- a potential agro-industry for hilly areas of North East India. Natural Product Radiance 7(1): 74-78.

Singleton VL, Rossi JA (1965) Colorimerty of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Ame J Enol Viticult 16: 144-158.

Simioniuc D, Uptmoor R, Friedt W, Ordon F (2002) Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs. Plant Breed 121: 429-435.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. PNAS 109(16): 6241-6246.

Shah A, Li DZ, Gao LM, Li HT, Möller M (2008) Genetic diversity within and among populations of the endangered species *Taxus fauna* (Taxaceae) from Pakistan and implications for its conservation. Biochem Syst Ecol 36: 183-193.

Shao YC, Xu L, Chen FS (2011) Genetic diversity analysis of *Monascus* strains using SRAP and ISSR markers. Mycoscience 52: 224-233.

Shinwari ZK, Terauchi R, Kawano S (1994a) Molecular Systematics of Liliaceae-Asparagoideae-Polygonatae. 1. RFLP analysis of cpDNA in several species of Asiatic *Disporum* species. Plant Species Bio 9: 11-18.

Shinwari ZK, Terauchi R,Utech FH, Kawano S (1994b) Recognition of the New World *Disporum* Section Prosartes as Prosartes (Liliaceae) based on the sequence data of the rbcL gene. Taxon 43(3): 353-366.

Singh SK, Yadav MC, Upadhyay RC, Kamal S, Rai RD, Tewari RP (2003) Molecular characterization of specialty mushroom germplasm of the National Mushroom Repository. Mushroom Res 12: 67-78.

Smith AH (1963) The Mushroom hunter's field guide. University of Michigan press, Annarbor. Pp. 67.

Schmidt O, Low KW (2005) Differentiation of indoor wood decay fungi with MADLI-TOF mass spectrometry. Holzforschung 59: 374-377.

Staniaszek M, Marczewski W, Szudyga K, Maszkiewicz J, Czaplicki A, Guo Q (2002) Genetic relationship between Polish and Chinese strains of the mushroom *Agaricus bisporus* (Lange) Sing., determined by the RAPD method. J Appl Genet 43(1): 43-47.

Sumbali G (2005) The fungi. Narosa publishing house, New Delhi. Pp. 220.

Sun SJ, Gao W, Lin SQ, Zhu J, Xie BG, Lin ZB (2006) Analysis of genetic diversity in *Ganoderma* population with a novel molecular marker SRAP. Appl Microbiol Biotechnol 72(3): 537-543.

Swapana S, Syed A, Krishnappa M (2008) Diversity of macro fungi in semi evergreen and moist deciduous Forests of Shimoga District-Karnatka, India. J Mycol Plant Pathol 38(1): 21-26.

Swingland IR (2001) Biodiversity, Definition of. Encyclopedia of Biodiversity 1: 377-390.

Sysouphanthong P, Thongkantha S, Zhao RL, Soytong K, Hyde KD (2010) Mushroom diversity in sustainable shade tea forest and the effect of fire damage. Biodiversity Conserv. 19: 1401-1415.

Talukdar NC (2009) Scientific management of Bio-resources of Manipur. Yojana 53: 23-27.

Tamura K (1992) Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Mol Biol Evol 9: 678-687.

Tang LH, Xiao Y, Li L, Guo Q, Bian YB (2010) Analysis of genetic diversity among Chinese *Auricularia auricula* cultivars using combined ISSR and SRAP markers. Curr Microbiol 61: 132-140.

Tao PF, Xu XH, Liu HJ (2011) Genetic diversity analysis of wild *Auricularia auricula* from Heilongjiang Province. Chinese Agr Sci Bull 27: 182-186.

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 12: 4127-4138.

Thatoi H, Singdevsachan SK (2014) Diversity, nutritional composition and medicinal potential of Indian mushrooms: A review. Afr J Biotechnol 13(4): 523-545.

Tibuhwa DD (2012) Folk taxonomy and use of mushrooms in communities around Ngorongoro and Serengeti National Park, Tanzania. J Ethnobiol Ethnomed 8: 36-45.

Tibuhwa DD (2013) Wild mushroom-an underutilized healthy food resource and income generator: experience from Tanzania rural areas. J Ethnobiol Ethnomed 9: 49-62. doi:10.1186/1746-4269-9-49.

Thekkuttuparambil AA, Kainoor KJ (2007) Indian medicinal mushrooms as a source of antioxidant and anti-tumor agents. J Clin Biochm Nutr 40(3): 157-162.

Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: Improving the sensitivity of progressive multiple alignment through sequence weighting. Nucleic Acids Res 22: 4673-4680.

Tsumura Y, Ohba K, Strauss SH (1996) Diversity and inheritance of inter-simple sequence repeat polymorphism in Douglas-fir (*Pseudotsuga menziesit*) and sugi (*Cryptomeria japonica*). Theor Appl Genet 93: 40-45.

Usha S, Suguna V (2014) Investigation on the nutritional value of edible mushrooms *viz*., *Auricularia polytricha* and *Pleurotus ostreatus*. Asian J Sci Technol 5(8): 497-500.

Varshney RK, Chabane K, Hendre PS, Aggarwal RK, Graner A (2007) Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. Plant Sci 173(6): 638-649.

Verma RN, Singh GB, Bilgrami KS (1987) Fleshy fungal flora of N. E.H. India- I. Manipur and Meghalaya. Indian Mush Sci 2: 414-421.

Verma RN, Singh GB, Singh MS (1995) Mushroom flora of North Eastern Hills, In: Advances in Horticulture (Chanda KL and Sharma SR, eds.), Vol. 13- Mushrooms. Malhotra Publishing House, New Delhi, India. Pp. 329-349.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol 172: 4239-4246.

Vijayan K (2005) Inter Simple Sequence Repeat (ISSR) Polymorphism and Its Application in Mulberry Genome Analysis. Intl J Industrial Entomol 10(2): 79-86.

Vishwakarma MP, Bhatt RP, Gairola S (2011) Some medicinal mushrooms of Garhwal Himalaya, Uttarakhand, India. Intl J Med Arom Plants 1(1): 33-40.

Vetter J (1993) Toxic elements in certain higher fungi. Food Chem 48: 207-208.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new concept for DNA fingerprinting. Nucleic Acids Res 21: 4407-4414.

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18(24): 7213-7218.

Wang SZ, Bai C, Fan J, Gao Y, Yang JF, Yang YJ (2003) A Study on the RAPD Analysis of *Ganoderma lucidum* and *Pleurotus ostreatus*. Protoplast Fusant Genome. Acta Edulis Fungi 10: 1-5.

Wani BA, Bodha RH, Wani AH (2010) Nutritional and medicinal importance of mushrooms. J Med Pl Res 4(24): 2598-2604.

Wasser S (2002) Medicinal mushrooms as a source of anti-tumor and immuno-modulating polysaccharides. Appl Microbiol Biotech 60(3): 258-274.

Watling R (1993) Comparison of the macromycete biotas in selected tropical areas of Africa and Australia. In: Aspects of Tropical Mycology (Isaac S, Frankland J, Watling R and Whalley AJS, eds.), Cambridge, UK: Cambridge University Press. Pp. 171-189.

Weber A, Karst J, Gilbert B, Kimmins JP (2005) *Thuja plicata* exclusion in ectomycorrhiza-dominated forests: testing the role of inoculum potential of arbuscular mycorrhizal fungi. Oecologia 143: 148-56.

Wesselink JJ, Iglesia B, James SA, Dicks JL, Roberts IN, Smith RVJ (2002) Determining a unique defining DNA sequence for yeast species using hashing techniques. Bioinformatics 18: 1004-1010.

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications (Innis MA, Gelfand DH, Sninsky JJ and White TJ, eds.), Academic Press Inc, San Diego, California. Pp. 315-322.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531-6535.

Wongchalee P, Pukahute C (2012) Diversity of mushrooms in dry Dipterocarp forest at Phuphan National Park, Sakon Nakhon Province. Natural Sci 4(12): 1153-1160.

Yan PS, Luo XC, Qi Z (2004) RAPD molecular differentiation of the cultivated strains of the jelly mushrooms, *Auricularia auricula* and *A. polytricha*. World J Microbiol Biotechnol 20: 795-799.

Yang ZL (2011) Molecular techniques revolutionize knowledge of basidiomycete evolution. Fungal Diversity 50: 47-58. Doi: 10.1007/s13225-011-0121-1.

Yang Z, Rannala B (2012) Molecular phylogenetics, principles and practice. Nature Reviews Genetics 13: 303-314.

Yang QY, Hu YJ, Li XY, Yang SX, Liu JX, Liu TF, Xu GM, Liao LM (1993) A New Biological Response Modifier - PSP. In: Mushroom Biology and Mushroom Products (Chang ST, Buswell JA, Chiu SW, eds.). The Chinese University Press, Hong Kong. Pp. 247-259.

Yeh FC, Young RC, Boyle T (1999) Popgene version 1.31: Microsoft window-based freeware for population genetic analysis. University of Alberta, Alberta. Pp. 1-228.

Yu MY, Ma B, Luo X, Zheng LY, Xu XY, Yang ZR (2008) Molecular diversity of *Auricularia polytricha* revealed by inter simple sequence repeat and sequence-related amplified polymorphism markers. Curr Microbiol 56: 240-245.

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeats (SSR)-anchored PCR amplification. Genomics 20: 176-183.

Zhang H, Gong F, Feng Y, Zhang C (1999) Flammulin purified from the fruit bodies of *Flammulina velutipes* (Curt.: Fr.) P. Karst. Int J Med Mushrooms 1: 89-92.

Zhang JC, Ma QF, Zhang PQ, Dai XD, Han ZH, Kong XH (2007) Identification of cultivated strains of *Auricularia auricula* from northeastern China by ISSR marker (Chinese). Mycosystema 26(4): 534-538.

Zhang DW, Zhao L, Wu TX (2007) Optimization of *Auricularia auricula* exopolysaccharide fermentation medium by orthogonal experiment design. J Guizhou Univ Technol. (Nat Sci Ed) 36: 40-43.

Zhang JX, Huang CY, Ng TB, Wang, HX (2006) Genetic polymorphism of ferula mushroom growing on *Ferula sinkiangensis*. Appl Microbiol Biotechnol 71(3): 304-309. http://dx.doi.org/10.1007/s00253-005-0139-y.

Zhou Z, Bebeli PJ, Somers DJ, Gustafson JP (1997) Direct amplification of minisatellite-region DNA with VNTR core sequences in the genus *Oryza*. Theor Appl Genet 95: 942-949.

Zhu S, Mori M (1993) The research on *Ganoderma lucidum* (part-1). Shanghai Medical University Press.

Zrodlowski Z (1995) The influence of washing and peeling of mushrooms *Agaricus bisporus* on the level of heavy metal contaminations. Pol J Food Nutr Sci 4: 23-33.

Annexure - I

GPS Coordinates from Collection Sites

LOCATION	LATITUDE (N)	LONGITUDE (E)	ELEVATION
Mokokchung	N 26°16.010'	E 094°28.350'	1180 m
	N 26°16.004'	E 094°28.371'	1148 m
	N 26°16.007'	E 094°28.359'	1152 m
	N 26°16.017'	E 094°28.387'	1150 m
	N 26°13.562'	E 094°28.636'	913 m
	N 26°15.091'	E 094°28.323'	1014 m
	N 26°15.111'	E 094°28.326'	1011 m
	N 26°15.018'	E 094°28.296'	1019 m
	N 26°15.399'	E 094°24.447'	1566 m
	N 26°14.991'	E 094°28.282'	1080 m
	N 26°14.860'	E 094°28.242'	856 m
	N 26°15.018'	E 094°28.260'	873 m
	N 26°12.448'	E 094°28.327'	966 m
	N 26°24.021'	E 094°31.176'	1044 m
	N 26°23.218'	E 094°32.560'	1114 m
	N 26°07.463'	E 094°30.114'	1300 m
	N 26°19.193'	E 094°30.484'	1320 m
Tuensang	N 26°11.957'	E 094°44.628'	2109 m
	N 26°11.965'	E 094°44.625'	2106 m
	N 26°11.956'	E 094°44.731'	2155 m

	N 26°11.962'	E 094°44.667'	2103 m
	N 26°11.018'	E 094°44.714'	2099 m
	N 26°11.173'	E 094°44.621'	2084 m
	N 26°12.609'	E 094°45.661'	1951 m
	N 26°12.609'	E 094°45.794'	2014 m
	N 26°12.518'	E 094°45.794'	2034 m
	N 26°12.459'	E 094°55.298'	2037 m
	N 26°12.359'	E 094°56.067'	1559 m
	N 26°12.532'	E 094°56.435'	1463 m
	N 26°12.123'	E 094°44.960'	2179 m
	N 26°12.122'	E 094°44.944'	2179 m
	N 26°12.110'	E 094°44.949'	2184 m
	N 26°12.096'	E 094°44.935'	2181 m
	N 26°12.087'	E 094°44.907'	2191 m
	N 26°12.098'	E 094°44.875'	2175 m
	N 26°12.450'	E 094°55.290'	2054 m
	N 26°12.350'	E 094°56.060'	1626 m
Longleng	N 26°28.340'	E 094°48.541'	1093 m
	N 26°29.247'	E 094°49.107'	1305 m
	N 26°29.560'	E 094°49.485'	1138 m
	N 26°28.900'	E 094°48.179'	1155 m
	N 26°52.230'	E 094°19.194'	532 m
	N 25°59.421'	E 094°21.384'	389 m

Zunheboto	N 26°59.244'	E 094°20.373'	634 m
	N 26°16.020'	E 094°28.386'	1165 m
	N 26°12.214'	E 094°28.477'	920 m
	N 26°12.439'	E 094°28.311'	966 m
	N 26°12.364'	E 094°28.371'	1021 m
	N 26°15.126'	E 094°28.557'	976 m
	N 26°15.034'	E 094°29.151'	941 m
	N 26°15.043'	E 094°29.178'	934 m
	N 26°15.165'	E 094°28.471'	1041 m
	N 26°11.568'	E 094°29.201'	1496 m
	N 26°06.584'	E 094°23.414'	466 m
	N 26°12.437'	E 094°25.211'	424 m
	N 26°06.573'	E 094°28.517'	1108 m
	N 26°06.584'	E 094°23.326'	461 m
Kohima	N 25°39.309'	E 094°06.191'	1444 m
	N 25°59.557'	E 094°15.517'	1103 m
	N 25°36.296'	E 094°02.129'	2659 m
	N 25º36.448'	E 094º00.034'	2680 m
	N 25º38.517'	E 094º04.233'	2255 m
Wokha	N 26°06.062'	E 094°16.393'	1685 m
	N 26°15.060'	E 094°05.230'	250 m
	N 26°05.277'	E 094°15.323'	1374 m
	N 26°05.582'	E 094°17.349'	1959 m

Dimapur	N 25°51.467'	E 093°45.131'	165 m
	N 25°53.549'	E 093°41.202'	175 m
	N 25°41.377'	E 093°31.583'	199 m
Phek	N 25°41.375'	E 094°25.200'	1828 m
	N 25°32.360'	E 094°20.113'	1792 m
	N 25°34.274'	E 094°14.161'	1726 m
	N 25°35.461'	E 094°17.585'	2237 m

Annexure - II

CTAB DNA Extraction Protocol (Doyle and Doyle, 1987)

Chemical reagents:

CTAB reagent	Chloroform (C)
β-Mercaptoethanol	Isoamyl alcohol (I)
NaCl	Isopropanol
EDTA.Na_2	Ethanol
Tris-Phenol (P)	RN*ase*
Tris-HCl	TE Buffer
Agarose	Ethidium bromide
TAE Buffer	TBE Buffer

Preparation of CTAB DNA Extraction Buffer for 100 ml

1.4 M NaCl	Dissolve 8.18 g NaCl or take 28 ml from 5 M NaCl solution
100 mM Tris-HCl	Take 5 ml from 2 M Tris-HCl/ pH 8.0
20 mM EDTA.Na_2	Take 4 ml from 0.5 M EDTA.Na_2/ pH 8.0
2% CTAB	Add 2.0 g of CTAB reagent to the buffer solution after it is autoclaved
0.2% β-Mercaptoethanol	Add 20 µl of β-Mercaptoethanol to 10 ml of CTAB buffer solution before addition of the sample

DNA Extraction Procedure:

1. Weigh 0.5 g of dried mushroom sample and then grind the sample into a fine powder in liquid nitrogen using a mortar and pestle. Then add the powdered sample in pre-warmed 2% CTAB buffer (10ml).
2. The sample solution is incubated in a water bath at 60^{o}C for 1 hr with timely mixing by inversion in between.
3. After incubation, the sample is centrifuged at 10,000 rpm for 10 mins.
4. The resulting supernatant is collected in fresh centrifuge tubes and RN*ase* (20 mg/ml) treatment is performed (6-10µl of RN*ase* to each centrifuge tube and incubation at 37^{o}C in water bath for 45 minutes).
5. Then the supernatant is treated with equal volumes of C: I (24:1). The sample mixture is mixed for 10 mins after which centrifugation is performed at 10,000 rpm for 10 mins.
6. After centrifugation, the upper aqueous phase is collected in fresh centrifuge tubes and treated with equal volumes of P: C: I (25: 24: 1) and mixed for 10 mins.
7. The mixture is then centrifuged at 10,000 rpm for 10 minutes.
8. After centrifugation, the upper aqueous phase is again collected in fresh centrifuge tubes and Step 5 is repeated if the supernatant is not clear.
9. Then equal volumes of chilled isopropanol is added to the supernatant to precipitate DNA and incubated at -20^{o}C for 5 mins.
10. Then centrifugation is performed at 14,000 rpm for 10 minutes.
11. After centrifugation, the supernatant is decanted slowly without disturbing the DNA pellet formed at the base of the centrifuge tube.

12. Then the DNA pellet is washed in 500-1000 µl of 70% chilled ethanol and centrifuged again at 14,000 rpm for 5 mins.
13. After that, the ethanol solution is decanted slowly and the DNA pellet is air dried for 20-30 mins to remove ethanol but the pellet should not be over dried.
14. Then 50-200 µl of TE buffer is added to resuspend the DNA pellet.
15. Finally, the DNA sample is tested qualitatively on 0.8% agarose gel electrophoresis and quantified by Thermo Scientific Multiskan Go Spectrophotometer.

Annexure – III

Primers Used in the Study

PRIMER NAME	SEQUENCE	BASES	REGION
ITS1F	CTT GGT CAT TTA GAG GAA GTA A	22	ITS
ITS4B	CAG GAG ACT TGT ACA CGG TCC AG	23	
ITS5	GGA AGT AAA AGT CGT AAC AAG G	22	
ITS4	TCC TCC GCT TAT TGA TAT GC	20	
NS1	GTA GTC ATA TGC TTG TCT C	19	18S *rRNA* gene
NS2	GGC TGC TGG CAC CAG ACT TGC	21	
LR0R	ACC CGC TGA ACT TAA GC	17	28S *rRNA* gene
LR5	TCC TGA GGG AAA CTT CG	17	

Annexure - IV

PCR Protocol Used for Amplification of ITS, 18S and 28S *rRNA* gene

PCR master mix composition

COMPONENTS	INITIAL CONCENTRATION	25 µl	FINAL CONCENTRATION
PCR buffer with 25 mM $MgCl_2$	10 X	2.5 µl	1 X
dNTPs	10 mM	0.5 µl	0.2 mM
Forward primer	10 mM	0.5 µl	0.2 mM
Reverse primer	10 mM	0.5 µl	0.2 mM
Template DNA	>1000 ng	2 µl	50-100 ng
Taq polymerase	3 U	0.2 µl	≤1 U
Pure water		18.8 µl	

Formula to calculate:

Initial concentration X Initial volume = Final concentration X Final volume

$$N_1 \text{ X } V_1 = N_2 \text{ X } V_2$$

PCR cycle conditions:

1. Initial Denaturation - 95°C, 5’
2. Final Denaturation - 95°C, 1”
3. Primer Annealing - 55°C for ITS
   - 54°C for 18S
   - 52°C for 28S

   50”
4. Primer Extension - 72° C, 1’

   35 cycles
5. Final Extension - 72° C, 10’
6. 4° C ∞

Annexure - V

Internal Accession Nos., Genbank Accession Nos. and Sequences of the Gene

GenBank Accession Number	Organism Name & Accession No	Nucleotide Sequence
KU343186	***Lentinula edodes* strain AO-DEBCR-1** internal transcribed spacer1, partial sequence; 5.8S ribosomal RNA gene, complete sequence and internal transcribed spacer 2, partial sequence. (**NUBOT-TA-LE-06**)	GCGGAGGGTACTGCGGAGGACATTATTGATTTTTTGGTGGTGGATTGTTGCTGGCCTTTGG GTATGTGCACATCCTCCTCTGATTCTATTCATCCACCTGTGCACTTTTTGTAGGAGTTCTTT CATCAGGTTTTGAACAGGTGCTCATTACGAGTTAACTTGGGAAGGACTAGTTGAAAAGAC TTCTATGTTCTTATAAACTATTGAAGTATGTTATAGAATGATTTTGTTATTGGGACTTTATT GACCCTTTAAACTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGA ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCAAATCTTTG AACGCACCTTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATTAAATTCT CAACTTTATAAGTTTTTGCTTATTAAAGCTTGGATATTGGAGGTTTGCAGGGGTTTTTTCA CCCCTTCTTAATTTGTTAGTGGAACCCTGGTTTGGTGGTTTATCACCCTGGGGTTGATACT AATTAGCGTTTTGGATGAAGAATTACAAAAAAAGAGAGACA (**590 bp**)
KT207468	***Lentinus squarrosulus* strain AO-DEBCR-2** internal transcribed spacer1, partial sequence; 5.8S ribosomal RNA gene, complete sequence and internal transcribed spacer2, partial sequence (**NUBOT-TA-LS-40**)	CCGGTAAGCTACTGCGGAGACATTATCGAGTTTTGAAACGGGTTGTAGCTGGCCTTCCGA GGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCAGGAGC TTCGAAAGCGAGAAAAGGGCCTTCACGGGCTTTTTCCTGGCTAATTTTTACTGGGCCTAAC TTTTCATAACAACCCCTTTATAAATTATCAAATGGTGTATGGCAATGTACCCCACCTATAT ACACTTTTCACCACCGAACCCCTGGCTCCCCCCACCAATAAAAAACCCACCAAATGGCAA AAATAATGGTGATTTGCAAAATTCATGGAACCTCCAAACCTTTGAACCCCCCTTGCCCTCC TGGGAATTCCAAGAACCAGGCCGGTTTAATGGCCTGGAATTTCCCACCCAACCGGGTTCT TAAGGGAACTTGCTTAGGCTTGGACTTGGAGGTTCTTGTCGGCTTGCTTCAATGTCAAGTC GGCTCCTCTTAAATGCATTAGCTTGGTTCCTGTGCGGATCGGCTCACGGTGTGATAATTGT CTACGCCGCGACCGTTGAAGCGTTTTTAAGCCAACCTCCAATCCTCCTTTTCCAGACCAAA ACCTCCAACTCCGGCCTCAATCAAGGAAGGCTACCCGCTGAACTTAAACAAACAATAAGC GGAAGGAA (**675 bp**)
KT207470	***Lentinus squarrosulus* strain AO-DEBCR-3** internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2,	CCGTCAGCTACTGCGGACGACATTATCGAGTTTTGAACGGGTTGTAGCTGGCCTTCCGAG GCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCAGGAGCT TCGAAAGCGAGAAAGGGGCCTTCACGGGCTTTTTTCTTGCCTAGTTGTTACTGGGCCTACG TTTCACTACAAACACTTATAAAGTATCAGAATGTGTATTGCGATGTAACGCATCTATATAC AACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAAAACGCAGCGAAATGCGATA AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTT

	partial sequence (**NUBOT-TA-LS-40**)	GGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATTCTCAACCTAACGGGTTCTTAA CGGGACTTGCTTTAGGCTTGGACTTGGAGGTTCTTGTCGGCTTGCTTCAATGTCAAGTCGG CTCCTCTTAAATGCATTAGCTTGGTTCCTGTGCGGATCGGCTCACGGTGTGATAATTGTCT ACGCCGCGACCGTTGAAGCGTTTTTATAGGCCAGCTTCTAGTCGTCTCTTTACGAGACAAT AATCATCGAACTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAAT AAGCGGAGGAA (**679 bp**)
KX342920	***Lentinus sajor-caju* strain AO-DEBCR-4** internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence (**NUBOT-TA-LS-87**)	TTTGGGGACTGCGGAGGACATTATCGAGTTATTGAAACGGGTTGTAGCTGGCCTTACGAG GCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCAGGAGCT TCGAAAGCGGAGGGCCTTTGCGGGCTTTTCGTTATTAGTTGTGACTGGGCTCATGTCCACT ACAAACTCTTATAAAGTAACAGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTC AGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTC CGAGGAGCATGCCTGTTTGAGTGTCATGAAATTCTCAACCTGACGGGTTCTTAACGGAGC TTGGTTCAGGCTTGGACTTGGAGGCTTGTCGGCTTGCTTTGTCGAGTCGGCTCCTCTCAAA TGCGTTAGCTTGGTTCTTTGCGGATCGGCTCACGGTGTGATAATTGTCTACGCCGCGACCG TTGAAGCGTTTGAATGGGCCAGCTTATAGTCGTCTCCATCGCGAGACAACATTTCATCGA ACTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCCGGAG GAAGAAAAC (**678 bp**)
MG462731	***Lentinus tigrinus* strain AO-DEBCR-5** internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2 region (**NUBOT-TA-LT-69**)	GGAAAGGGGTCGGAGGCGCGGGAAGGATCATTATCGAGTTTTGAAACGGGTTGTAGCTG GCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGT TTCAGGAGCTTCTATAGCGTTTCTTACGCTGGAGTTGTGACTGGGCCCACGTTTACTACAA ACTCTTACAAGTATCAGAATGTGTATTGCGATGTAACGCATCTCTATACAACTTTCAGCAA CGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATCATCGAATCTTTG AACGCACCTTGCGCTCCTTGGTACATGCCTGTTTGAGTGTCATGAAATTTAATGGGACTTG CTTAAGGCTTGGACTTGGAGGCTTTTGTCGGCTTGCTTCTCCTCTCAAATGCATTAGCCTT GGTTCTTTGCGGAGGCTCACGGTGTGATAATTAGCGTTTTATTCTAATCGTCTCCTTGCGA GACAAGCATTCATCGAACTCTGACCTCAAATA (**517 bp**)
KT459337	***Termitomyces heimii* strain AO-DEBCR-6** 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (**NUBOT-TA-TH-64**)	TGGCTACCGGAAGGATCATTATTGAATTTAAACCCTGGTTGGGTTGTTGCTGGCCTCTAGG GGCATGTGCACGCCTGCCACCGTTTTCAACCACCTGTGCACCTTTTGTAGACTTTGGATAT ATACCGTTCGAGGGTCAAACCCCCTCCTCGGTTTTGAGGGCTTGCTGTGCTGCAAAGTTCG GCTTCCCTTGCATTCCCAGTCTATGCATCTTCTTATACCCCGTAATGAATGTATTAGAATGT TTTTTTATTGGCCTTTTTAGTGCCTTTAATCAAATACAACTTTCAGCAACGGATCTCTTGGC TCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT GAATCCTCCAATCTTTGAACGCACCTTGCGCTCCTTGGTATTACGAGGAGCATGCCTGTTT GAGTGTCATTAAATTCTCAACCTTTACCAGCTTTTGCGAGTTGGTTTAGGCTTGGATGTGG GGGTTTTTGCAGGCTTCTTAAGAAATCAGCTCCCCTTAAATGCATTAGTGGAAACCTTTGT TGGCCTGTTCCTGGTGTGATAATTATCTACATCGTGCGCAGTCAACTTTATTCTAATGGGG TTTTCTGCTTCTAACTCGTAATTCCTCTTTGTTGAGGAAGACGCTTTTTACCATTTTACCTC

		AAATCAGGTAGGACTACCCGCTGAAATTAAAATATCTTATGCGGCAGGAAAAGGACCAC AAGGGTTTTCCCGTTTCCCTTTTACTTTAAACCCCCGGGGGG (**779 bp**)
KT229567	***Schizophyllum commune* strain AO-DEBCR-7** internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (**NUBOT-TA-SC-09**)	CCGTCAGGAGACTGCGGACGACATTACGAATCAACAAGTTCATCTTGTTCTGATCCTGTG CACCTTATGTAGTCCCAAAGCCTTCACGGGCGGCGGTTGACTACGTCTACCTCACACCTTA AAGTATGTTAACGAATGTAATCATGGTCTTGACAGACCCTAAAAAGTTAATACAACTTTC GACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTC CGAGGGGCATGCCTGTTTGAGTGTCATTAAATACCATCAACCCTCTTTTGACTTCGGTCTC GAGAGTGGCTTGGAAGTGGAGGTCTGCTGGAGCCTAACGGAGCCAGCTCCTCTTAAATGT ATTAGCGGATTTCCCTTGCGGGATCGCGTCTCCGATGTGATAATTTCTACGTCGTTGACCA TCTCGGGGCTGACCTAGTCAGTTTCAATAGGAGTCTGCTTCCAACCGTCTCTTGACCGAGA CTAGCGACTTGTGCGCTAACTTTTGACTTGACCTCAAATCAGGTAGGACTACCCGCTGAAC TTAAGCATATCAATAAGCCGGAGGAAAC (**634 bp**)
KT207467	***Lentinula edodes* strain AO-DEBCR-1** 18S ribosomal RNA gene, partial sequence (**NUBOT-TA-LE-06**)	AGGGCGCAGATCTAGTATAAACAATTTGTACTGTGAAACTGCGAATGGCTCATTAAATCA GTTATAGTTTATTTGATGATACCTTGCTACATGGATAACTGTGGTAATTCTAGAGCTAATA CATGCATTCAAGCCCCAACTTCTGGAAGGGGTGTATTTATTAGATAAAAAACCAACGCGG CTCGCCGCTCACTTGGTGATTCATAATAACTTCTCGAATCGCATGGCCTTGTGCCGGCGAT GCTTCATTCAAATATCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTTT CAACGGGTAACGGGGAATAAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACC ACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA CAATAAATAACAATATAGGGCTCTTTCGGGTCTTATAATTGGAATGAGTACAATTTAAAT CCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCCA (**528bp**)
KT207469	***Lentinus squarrosulus* strain AO-DEBCR-2** 18S ribosomal RNA gene, partial sequence (**NUBOT-TA-LS-40**)	GGGTCGAAGTCTAGTATAGCAAGTTTGTACTGTGAACTGCGAATGGCTCATTAAATCAGT TATAGTTTATTTGATGGTACCTTGCTACATGGATAACTGTGGTAATTCTAGAGCTAATACA TGCAATCAAGCCCCGACTTCCGGGAGGGGTGTATTTATTAGATAAAAAACCAACGCGGTT CGCCGCTCCATTGGTGATTCATAATAACTTCTCGAATCGCATGGCCTTGCGCCGGCGATGC TTCATTCAAATATCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTTTCA ACGGGTAACGGGGAATAAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCAC ATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACA ATAAATAACAATATGGGGCTCTTTCGGGTCTCATAATTGGAATGAGTACAATTTAAATCTC TTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCCA (**526 bp**)
KT207471	***Lentinus squarrosulus* strain AO-DEBCR-3** 18S ribosomal RNA gene, partial sequence (**NUBOT-TA-LS-40**)	GGGTCGCCTGTCTAGTATAACAGTTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGT TATAGTTTATTTGATGGTACCTTGCTACATGGATAACTGTGGTAATTCTAGAGCTAATACA TGCAATCAAGCCCCGACTTCCGGGAGGGGTGTATTTATTAGATAAAAAACCAACGCGGTT CGCCGCTCCATTGGTGATTCATAATAACTTCTCGAATCGCATGGCCTTGCGCCGGCGATGC TTCATTCAAATATCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTTTCA ACGGGTAACGGGGAATAAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCAC ATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACA

		ATAAATAACAATATGGGGCTCTTTCGGGTCTCATAATTGGAATGAGTACAATTTAAATCTC TTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCCA (**527 bp**)
KX342921	***Lentinus sajor-caju* strain AO-DEBCR-4** 18S ribosomal RNA gene, partial sequence (**NUBOT-TA-LS-87**)	GAAAAACATGTCTAGTATAACAGTTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAG TTATAGTTTATTTGATGGTACCTTGCTACATGGATAACTGTGGTAATTCTAGAGCTAATAC ATGCAATCAAGCCCCGACTTCCGGGAGGGGTGTATTTATTAGATAAAAAACCAACGCGGT TCGCCGCTCCATTGGTGATTCATAATAACTTCTCGAATCGCATGGCCTTGCGCCGGCGATG CTTCATTCAAATATCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTTTC AACGGGTAACGGGGAATAAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCA CATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGAC AATAAATAACAATATGGGGCTCTTTCGGGTCTCATAATTGGAATGAGTACAATTTAAATC TCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCAAAGTTTA (**533 bp**)
MG722855	***Lentinus tigrinus* strain AO-DEBCR-5** 18S ribosomal RNA gene, partial sequence (**NUBOT-TA-LT-69**)	TGGGAATGGCTCATAAATCAGTTATAGTTTTATTTGATGGTACCTTGCTACATGGGTAACT GTGGTATTTCTAGAGCTAATACATGCAATCAAGCCCCGACTTCCGGGAGGGGTGCTTTTAT AGATAAAAAAACCAACGCGGTTCGCCGCTCCATTGGTGATTCATAATAACTTCTCGAATC GCATGGCCTTGCGCCGGCGATGCTTCATTCAAATATCTGCCCTATCAACTTTCGATGGTAG GATAGAGGCCTACCATGGTTTCAACGGGTAACGGGGAATAAGGGTTCGATTCCGGAGAG GGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAAT CCCGACACGGGGAGGTAGTGACAATAAATAACAATATGGGGCTCTTTCGGGTCTCATAAT TGGAATGAGTACAATTTAA (**440bp**)
KT459336	***Termitomyces heimii* strain AO-DEBCR-6** 18S ribosomal RNA gene, partial sequence (**NUBOT-TA-TH-64**)	AAGTCTAGTATAACAAATTTGTACTGTGAACTGCGAATGGCTCATTAAATCAGTTATAGTT TATTTGATGGTACCTTGCTACATGGATAACTGTGGTAATTCTAGAGCTAATACATGCAATC AAGCCCCGACTTCCGGAAGGGGTGTATTTATTAGATAAAAAACCAACGCGGCTCGCCGCT CCCTTGGTGATTCATAATAACTTCTCGAATCGCATGGCCTTGCGCCGGCGATGCTTCATTC AAATATCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTTTCAACGGGT AACGGGGAATAAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAA GGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAATAAAT AACAATATAGGGCTCTTTCGGGTCTTATAATTGGAATGAGTACAATTTAAATCCCTTAACG AGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCAAA (**521 bp**)
KT253193	***Schizophyllum commune* strain AO-DEBCR-7** 18S ribosomal RNA gene, partial sequence (**NUBOT-TA-SC-09**)	TGTAGTCATTAGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTATAAACAAGTTTGTA CTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAATTTATTTGATGATACCTTGCTACA TGGATAACTGTGGTAATTCTAGAGCTAATACATGCAATCAAGCCCCGACTTCTGGAAGGG GTGTATTTATTAGATAAAAAACCAACGCGGCTCGCCGCTCACTTGGTGATTCATAATAACT TCTCGAATCGCATGGCCTTGCGCCGGCGATGCTTCATTCAAATATCTGCCCTATCAACTTT CGATGGTAGGATAGAGGCCTACCATGGTTTCAACGGGTAACGGGGAATAAGGGTTCGATT CCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAAT TACCCAATCCCGACACGGGGAGGTAGTGACAATAAATAACAATATAGGGCTCTTTCGGGT CCTATAATTGGAATGAGTACAATTAATACCTACGAGTTATGG (**525 bp**)
KT459341	***Lentinula edodes* strain AO-**	GNANNTNAGGTAAGACTAACAGGATTCCCCTAGTAACTGCGAGTGAAGAGGGAAAAGCT

	DEBCR-1 28S large subunit ribosomal RNA gene, partial sequence (**NUBOT-TA-LE-06**)	CAAATTTAAAATCTGGCAGTCTCTGATTGTCCGAGTTGTAATTTAGAGAAGTGTTACCCGT GTTGGACCGTGTACAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCCCGTCTTT GATACGGATCCCAATGCATTGTGGTACACTCTCGAAGAGTCGAGTTGTTTGGGAATGCAG CTCTAAATGGGTGGTAAATTCCATCTAAAGCTAAATATTGGGGAGAGACCGATAGCGAAC AAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAA TTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTGGTCAGGGATCAGCCTTCCTTATGG TTGGTGCATTTCCTGATTAACGGGTCAACATCAGTTTTGATCAGTGGATAAAAGCTTGAGG GATGTGGCATCTTCGGGTGTGTTATAACCTCTTGTTATATACATTGATTGGGACTGAGGAA CTCAGCACGCCGCAAGGCCGGGTTTTTAACCACGTTCGTGCTTAGGATGTTGGCATAATG GCTTTAATCGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATGCCTGCGAGTGTTTG GGTGGAAAACCCGAGCGCGTAATGAAAGTGAAAGTTAGGATCTCTGTCGTGGAGAGCAC TGACGCCCGGCCCAGACCTTTTGTGACGGTGCCGCGGTTGAGCATGTATGTTGGGACCCG AAAGATGGTGAACTATGCCTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTCG TAGCGATTCTGACGTGCAAATCGATCGTCGAATTTGGGTATAGGGGCGAAAGACTAATCG AACCATCTAGTAGCTGGTTCCTGCCGAATTTCCCCTCAGGAAA (**942 bp**)
KU170488	***Lentinus squarrosulus* strain AO-DEBCR-2** 28S ribosomal RNA gene, partial sequence (**NUBOT-TA-LS-40**)	TAGTAGAACTAACAGGATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTTA AAATCTGGCGGTCTTTGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCCGCGCTGGACC GTGTATAAGTCTCTTGGAACAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGACACGGA CTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAA TGGGTGGTGAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACC GTGAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGA AAGGGAAACGCTTGAAGTCAGTCGCGTCTGCCGGAACTCAGCCTTCCTTTTGGTTGGTGC ACTTTCTGGTAGACGGGCCAGCATCGATTTTGACCGTAGGATAAGGGCTGGAGAAATGTG GCACCTTCGGGTGTGTTATAGTCTTCAGTCGCATACTACGGTTGGGATCGAGGACCGCAG CGCGCCGCAAGGCAGGGGTTCGCCCACTTTCGCGCTTAGGATGCTGGCATAATGGCTTTA AACGACCCGTCTTGAAACACGGACCAAGGAGTCTAACAAACCTGCGAGTGTTTGGGTGGA AAACCCGAGCGCGTAATGAAAGTGAAAGTTGAGACCTCTGTCGTGGAGGGCATCGACGC CCGGACCTGACGTTCTCTGACGGATCCGCGGTAGAGCATGTTTGTTGGGACCCGAAAGAT GGTGAACTATGCCTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTCGTAGCGA TTCTGACGTGCAAATCGATCGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCAT CTAGTAGCTGGTTCCTGCCGAATTTCCCCTTCAGGAAA (**936 bp**)
KT459340	***Lentinus squarrosulus* strain AO-DEBCR-3** 28S large subunit ribosomal RNA gene, partial sequence (**NUBOT-TA-LS-40**)	GTGTTGTTACCCGCTCGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAG GATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTTAAAATCTGGCGGTCTT TGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCCGCGCTGGACCGTGTATAAGTCTCTT GGAACAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTGCTTTGT GATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCC ATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATG AAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTG

		AAGTCAGTCGCGTCTGCCGGAACTCAGCCTTCCTTTTGGTTGGTGCACTTTCTGGTAGACG GGCCAGCATCGATTTTGACCGTAGGATAAGGGCTGGAGAAATGTGGCACCTTCGGGTGTG TTATAGTCTTCAGTCGCATACTACGGTTGGGATCGAGGACCGCAGCGCGCCGCAAGGCAG GGGTTCGCCCACTTTCGCGCTTAGGATGCTGGCATAATGGCTTTAAACGACCCGTCTTGAA ACACGGACCAAGGAGTCTAACAAACCTGCGAGTGTTTGGGTGGAAAACCCGAGCGCGTA ATGAAAGTGAAAGTTGAGACCTCTGTCGTGGAGGGCATCGACGCCCGGACCTGACGTTCT CTGACGGATCCGCGGTAGAGCATGTTTGTTGGGACCCGAAAGATGGTGAACTATGCCTGA ATAGGGTGAAGCCAGAGGAAACTTGGTGGAGGCTCGTAGCGATTCTGACGTGCAAATCG ATCGTCAAATTTGGGTATAGTAGCGCGAGAACGACGCGCGATNANTCC (**947 bp**)
KX342922	***Lentinus sajor-caju* strain AO-DEBCR-4** 28S large subunit ribosomal RNA gene, partial sequence (**NUBOT-TA-LS-87**)	TTAACGGGAAAGAACTACAGGATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCA AATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCCGCGC TGGACCGTGTATAAGTCTCTTGGAACAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGA CACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGC TCAAAATGGGTGGTGAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACA AGTACCGTGAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAAT TGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCTGCCGGAACTCAGCCTTCCTCTCGGT TGGTGCATTTCTGGAGACGGGCCAGCATCGATTTTGACCGTCGGAAAAGGGCTGGGGAAA TGTGGCACCTTTTGGTGTGTTATAGTCTTCAGTCGCATACGGCGGTCGGGATCGAGGAAC GCAGCGCGCCTCAAGGCAGGGGTTCGCCCCTTTCGCGCTTATGATGCTGGCATAATGGCT TTAAACGACCCGTCTTGAAACACGACCAAGGTGTCTAAAAACCTGCTAGTGTTCCGTGTA AACCCGAGCGCGAATAAAGTGACAGTGAGACCTCTTCGTGAGGCATCGACCCCGGACAG ACGTTTCTGACGATCCGCACAGAGCTGCTTGTGAGACCCCAAGATGGGAAGTATACTCAT AGGTGAAGCACAGGAACTCTGTGGAGCCCGTATTATTCTGAGTGCAATCCTCACCAATTT GGTATGGGGCGAAGATAATTTACCTCTAGCTTGGTTCTGCTTATTTTCTTTAAAAAAATTT TTTTATATTTTATTTTTAATTTTTTCTTT (**928 bp**)
MG462732	***Lentinus tigrinus* strain AO-DEBCR-5** 28S ribosomal RNA gene, partial sequence (**NUBOT-TA-LT-69**)	AGAGGATCAAAACGTGATGGATGGCTCGATTAGTCTTTCGCACTATACCCAAATTTGACG ATCGATTTGCACGTCAGAATCGCTACGAGCCTCCACCAGAGTTTCCTCTGGCTTCACCCTA TTCAGGCATAGTTCACCATCTTTCGGGTCCCAACAAACATGCTCTACCGCGGATCCGTCAG AGAACGTCAGGTCCGGGCGTCGATGCCCTCCACGACAGAGGTCTCGACTTTCACTTTCATT AGCGGCTCGGGTTGCCAGACCGAGGACTCTTGGTTTATTAAACACCTTGACGCGTGTTGG AGGCGGTGCCTTGGGGCAATCGTACAAACAGGGAAACAGGGGAGTGGGGCAATCGATTA GGGGTAAATGGCACAACTGATTTCGGAGAGAA (**394 bp**)
KT459338	***Termitomyces heimii* strain AO-DEBCR-6** 28S large subunit ribosomal RNA gene, partial sequence (**NUBOT-TA-TH-64**)	GAAACTAACAAGGATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTTAAA ATCTGGTAGTCTCTGGCTGCCCGAGTTGTAATCTAGAGAAGCATTATCCGCGCTGGACCGT GTATAAGTGTCCTGGAATGGACCATCATAGAGGGTGAGAATCCCGTCTTTGACACGGACT CCCAGGGCTTTGTGATGTGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATG GGTGGTAAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGT GAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAA

		GGGAAACGCTTGAAGTCAGTCGCGTTAGCTGGGGATCAACCTTGCTTTTCTGCTTGGCTTA CTTCCCAGTTGACGGGTCAGCATCAGTTTTGACCAATGGATAAAGGCTAGGGGAATGTGG CACCTCCGGGTGTGTTATAGCCCTTGGTCATATACATTGGTTGGGACTGAGGAACTCAGC ACTTGTGCTTAGGATGCTGGCATAATGGCTTTAAGCGACCCGTCTTGAAACACGGACCAA GGAGTCTAACATGCCCGCGAGTGTTTGGGTGGAAAACCCGAGCGCGTAATGAAAGTGAA AAGTTGAGATCCCTGTCGTGGGGAGCATCGACGCCCGGACCAGACCTTTTGTGACGGATC CGCGGTAGAGCGTGTATGTTGGGACCCGAAAGATGGTGAACTATGCCTGAATAGGGTGA AGCCAGAGGAAACTCTGGTGGAGGCTCGTAGCGATTCTGACTTGCAAATCGATCGTCGAA TTTGGGGATAGGGGCGAAAGATTAATCGAACCTTCTAATAGCTGGTTCCTGCCGAAGTTT TCCC (**915 bp**)
KT459339	***Schizophyllum commune* strain AO-DEBCR-7** 28S large subunit ribosomal RNA gene, partial sequence (**NUBOT-TA-SC-09**)	ANGTGGATAAGACTAACAGGATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAA ATTTAAAATCTGGCGGTCTCCGGCCGTCCGAGTTGTAATTTAGAGAAGCGTTATCCGTGCT GGACCGTGTATAAGTCTCCTGGAATGGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGAC ACGGACTACCAGTGCATTGTGATGCGCTCTCGACGAGTCGAGTTGTTTGGGAATGCAGCT CAAAATGGGTGGTAAATGCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACA AGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAAT TGTCGAAAGGGAAACGGTTGCAGTCAGTCGCGTCCGCCGGGGATCAACCTTGCTTTTGCT TGGCGTATTTCCCGGTGGATGGGTCAGCATCAGTTTTGACCGCAGTTGAAAGGCTGGAGG AATGTGGCACCTTCGGGTGTGTTATAGCCTCCTGTCATATGCTGCGGTTGGGACTGAGGA ACTCAGCACGCCGCAAGGCCGGGATTCGTCCACGTTCGTGCTTAGGATGCTGGCATAATG TCTGTAACCGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATGCACGCGAGTGTTTG GGTGGCAAACCCGAGCGCGTAATGAAAGTGAAAGTTGAGACCTCTGTCGTGGAGGGCAT CGACGCCCGGACCAGAACTTTTGGGACGGATCCGCGGTAGAGCGTGTATGTTGGGACCCG AAAGATGGTGAACTATGCCTGAATAGGGCGAAGCCAGAGGAAACTCTGGTGGAGGCTCG TAGCGATTCTGACGTGCAAATCGATCGTCGAATTTGGGTATAGGGGCGAAAGACTAATCG AACCATCTAGTAGCTGGTTCCTGCCGAATTTCCCCTTCAGGAAA (**940 bp**)

Annexure - VI

Sequence Information of ISSR Primers Used in the Study

PRIMER NAME	SEQUENCE	BASES
ISSR1	$(CTC)_4$ SC	14
ISSR2	BDB $(ACA)_5$	15
ISSR13	GAG AGA GAG AGA GAG AC	17
ISSR11	AGA GAG AGA GAG AGA GGT	18
ISSR18	VHV GTG TGT GTG TGT GTG T	16
807	AGA GAG AGA GAG AGA GT	17
810	$(GA)_8$ T	17
841	$(GA)_8$ YG	18
842	$(GA)_8$ YC	18
834	AGA GAG AGA GAG AGA GYT	18

Annexure - VII

Sequence Information of DAMD Primers Used in the Study

PRIMER NAME	SEQUENCE	BASES
D1	ATC CAA GGT CCG AGA CAA CC	20
D2	GTG TGC GAT CAG TTG CTG GG	20
D10	GGA CAA GAA GAG GAT GTG GA	20
D17	CTC TGG GTG TCG TGC	15
D19	CCC GTG GGG CCG CCG	15
URP30F	GGA CAA GAA GAG GAT GTG GA	20
URP6R	GGC AAG CTG GTG GGA GGT AC	20
URP9F	ATG TGT GCG ATC AGT TGC TG	20
URP1F	ATC CAA GGT CCG AGA CAA CC	20
URP4R	AGG ACT CGA TAA CAG GCT CC	20

Annexure - VIII

Sequence Information of RAPD Primers Used in the Study

PRIMER NAME	SEQUENCE	BASES
OPA-1	CAG GCC CTT C	10
OPA-4	AAT CGG GCT G	10
OPA-17	GAC CGC TTG T	10
OPT-6	CAA GGG CAG A	10
OPT-7	GGC AGG CTG T	10
OPT-5	GGG TTT GGC A	10
OPD-18	GAG AGC CAA C	10
OPC-10	TGT CTG GGT G	10

Annexure - IX

PCR Protocol Used for ISSR, RAPD and DAMD Markers

PCR master mix composition

COMPONENTS	INITIAL CONCENTRATION	20 µl	FINAL CONCENTRATION
PCR buffer	10 X	2 µl	1 X
$MgCl_2$	50 mM	0.7 µl	1.75 mM
dNTPs	10 mM	0.8 µl	0.4 mM
Forward primer	10 mM	1 µl	0.5 mM
Template DNA	>1000ng	2 µl	50-200 ng
Taq polymerase	3 U	0.2 µl	≤1 U
Pure water		13.3 µl	

Formula to calculate:

Initial concentration X Initial volume = Final concentration X Final volume

$$N_1 \text{ X } V_1 = N_2 \text{ X } V_2$$

PCR cycles were performed in the following conditions:

1. Initial Denaturation - 94^oC, 4’
2. Final Denaturation - 94^oC, 1’
3. Primer Annealing - $(30\text{-}55)^oC$, 50”
4. Primer Extension - 72^o C, 1’

35 cycles

5. Final Extension - 72^o C, 7’
6. 4^o C ∞

List of Publications

1. Toshinungla Ao, Chitta Ranjan Deb and Neilazonuo Khruomo (2016) Wild Edible Mushrooms of Nagaland, India: A Potential Food Resource. Journal of Experimental Biology and Agricultural Sciences, 4(1): 59-65. DOI: http://dx.doi.org/10.18006/2015.
2. Toshinungla Ao, Jichule Seb, T Ajungla, Chitta Ranjan Deb (2016) Diversity of Wild Mushrooms in Nagaland, India. Open Journal of Forestry, 6: 404-419. DOI: http://dx.doi.org/10.4236/ojf.2016.65032.
3. Kikoleho Richa, Toshinungla Ao, Chitta Ranjan Deb (2018) Phylogenetic based studies on medicinal mushrooms found in Nagaland, India reveal closely related species. International Journal of Advanced Scientific Research, 3 (5): 63-65.

List of Seminar/Symposium, Conferences Attended and Presented Papers

1. National Workshop on Scientific Writing, Research Communication & IPR Issues, Department of Botany, Nagaland University, Lumami from August 28-29, 2014.
2. National Workshop on Database Designing for Biologists, BIF, Nagaland University, Lumami from September 9-11, 2014.
3. National Workshop on Applications of Biotechnology tools and Bioinformatics, jointly organized by Institutional Biotech Hub, Department of Botany & and BIF, Nagaland University, Lumami from March 30-April 04, 2015.
4. Presented at the National Seminar 'Globalization, Development and Environment with Special reference to North-East India' on the topic 'Wild Edible Mushrooms- A Potential Food Resource for Rural Livelihood' at Nagaland University, Lumami, March 19-20, 2015.
5. National Workshop on Computational Drug Designing-I, BIF, Nagaland University, Lumami from October 5-6, 2015.
6. Presented at the National Seminar **'**Inventory, Sustainable Utilization and Conservation of Bioresources**'** on the topic "Identification of four Wild Edible Mushrooms from Nagaland based on ITS, 18S and 28S ribosomal RNA sequences" organized by Department of Botany and Institutional Biotech Hub, Nagaland University, Lumami on February 27, 2016.
7. Presented a poster at the National Seminar **'**Inventory, Sustainable Utilization and Conservation of Bioresources**'** on the topic "Diversity of Wild Edible Mushrooms in Nagaland" organized by Department of Botany and Institutional Biotech Hub, Nagaland University, Lumami on February 27, 2016.
8. Hands on Training on Molecular Profiling and Genome Analysis jointly organized by Institutional Biotech Hub, Department of Botany and BIF, Nagaland University, Lumami from March 14-19, 2016.
9. Presented a poster at the National Seminar 'Advances in Biological Science Research' on the topic 'Wild Mushroom Diversity of Nagaland and Nutritive Value

of Certain Wild Edible Species' at Department of Botany, Nagaland University, Lumami, February 28 - March 01, 2017.

10. Presented a paper at the International Conference on 'Natural Resources Management and Technology Trends' at Department of Life Sciences, Manipur University, Imphal-795003, India, March 27-29, 2017.
11. National Seminar on 'Chemistry in Interdisciplinary Research' at Department of Chemistry, Nagaland University, Lumami from March 16-17, 2017.
12. Presented a paper on the topic 'Wild Edible Mushrooms of Nagaland, India' at the National Symposium on Mushrooms: Trends and Innovations in Mushroom Science from 27-28 April 2017 at ICAR-Directorate of Mushroom Research, Solan.
13. Resource person in workshop program on 'Mushroom Cultivation', organized by Department of Botany, Fazl Ali College, Mokokchung, June 10, 2017.
14. Short-Term Skill Development Training Programme in Biotechnology for Students of North-East India on 'Orchid Propagation' organized by DBT Sponsored Institutional Biotech Hub & Department of Botany, Nagaland University, Lumami jointly sponsored by Biotech Park, Lucknow, UP & Institutional Biotech Hub, Nagaland University, Lumami, November 16- December 15, 2017.
15. Hands on training on 'Genomics and Gene Expression Analysis, held at Department of Botany, Nagaland University, Lumami, July 18-23, 2018.
16. Skill and Entrepreneurial Development of the Tribal Youth under the theme 'Value-additions to Rich Bio-Resources with Special Reference to Medicinal and Aromatic Plants'organized by Adavance Level Institutional Biotech Hub, Department of Botany, Nagaland University, Lumami in collaboration with Biotech Park, Lucknow, UP sponsored by the National Academy of Sciences, India, July 25-28, 2018.

****