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Research Article
Proximate Analysis and Ecological Niche Modeling of Macrofungi in Ecologically Significant North-East India

[1]Pranaba N. Bhattacharyya and [2]Bhaskar Sarma

[1]Department of Botany, Nanda Nath Saikia College, Titabor 785630, Jorhat, Assam, India
[2]Department of Botany, Dhemaji College, Kulapather, Dhemaji 787057, Assam, India

Abstract

Background and Objective: Macrofungi are diverse in their occurrence and topography. They play an inevitable role in agriculture, forestry, pharmacology and other industries. Studies on the diversity, occurrence, frequency distribution and nutritive values of the putative macrofungal genetic resources in the pristine locations of N.E. India holds the potential to attract the global economy and human welfare. The present survey was carried out to explore the less-explored macrofungal species diversity in nine undisturbed reserved forests of Dhemaji district, Assam. **Materials and Methods:** Mushroom identification was made using the standard taxonomy of fungi identification. Macrofungal proximate analysis for diverse categories was made using standard protocols. Ecological niche modelling of *Hygrocybe splendidissima*, macrofungi, enlisted in the IUCN red list of threatened species is made to identify the unexplored topography of the species and equally to report their utility in future conservation. **Results:** A total of 40 macrofungal species, belongs to 32 genera and 24 families were identified. Proximate nutritive values showed differences in the various edible mushrooms studied. The results of the analysis showed protein content, ranging from 10.65±0.03-42.76±0.021 g/100 g and carbohydrate content ranged from 27.67±0.03-70.09±0.028 g/100 g. Further ecological niche modelling of *H. splendidissima*, identified various forest areas in Northeast India that have suitable climatic conditions for reinforcement and in-situ conservation of the fungus. **Conclusion:** The present approach holds promises in exploiting the less explored and putative mushroom species of ecologically biodiverse regions like Northeast India.

Key words: Ecological niche modelling, edibility, *Hygrocybe splendidissima*, macrofungi, proximate analysis, putative microbial resources, species diversity

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Corresponding Author: Bhaskar Sarma, Department of Botany, Dhemaji College, Kulapather, Dhemaji 787057, Assam, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

'Fungus' word is derived from the Latin word 'fungour' that means to flourish. There are approximately 1,44,000 known species of fungi, which includes yeasts, rusts, smuts, mildews, moulds and mushrooms. Although, the number of known fungi had significantly increased up to 1,00,000[1], however, it still accounts only for 7% of the world's total fungal population.

'Macrofungi' the term is usually applied to the members of the fungi belonging to the phylum Ascomycota and Basidiomycota[2], which are either epigeous or hypogeous and large enough to be seen by naked eyes and can be picked by hand. Rahi and Malik[3], while studying the diversity of mushrooms of nutraceutical and therapeutic significance, reported the worldwide distribution of Ascomycota and Basidiomycota that contains a species diversity of approximately 40,000 and 30,000, respectively.

Initially, the macrofungal forms, the mushrooms are considered fungus[4]. These are the fungal forms that are being characterized as non-chlorophyllous spore-bearing fruiting bodies (sporocarps). Mushrooms are native to diverse habitat conditions like soils, deserts, wooden logs and in association with trees and barks in the grasslands, or other foodstuffs. These are the large group of simple thallus-like plants that typically have a stem (stipe), a cap (pileus) and gills (lamellae)[5]. The chief characteristics of a typical mushroom include the cup fungi including the Agaricus, jelly and flask type fungal forms, chanterelles and corticoid fungi, earth stars, polypores, puffballs, stinkhorns, bird's nest fungi etc. According to Singh *et al.*[6] the terrestrial macrofungi are either saprophytic or symbiotic in their mood of the action.

Macrofungi are always been an object of awe, fascination and sources of growth, nutrition and medicine traditions for improving the human civilization and global economy, since time immemorial. Involvement of macrofungal associations has been reported in agriculture and forestry, pharmaceuticals and food industries and allied fields of research and interest[7]. Govorushko *et al.*[2] and Mohanan[8] reported the involvement of macrofungi in key ecological dynamics like degradation of lignocellulose and pectic compounds, litter decomposition, biogeochemical cycling and nutrient transport system. According to Lutzoni *et al.*[9] macrofungi are vital in improving the plant-microbe relationships as in certain instances they can act as an ectomycorrhizal (EM) fungus and thereby assist in plant symbiosis. Dimitrijevic *et al.*[10] defined the mushroom as "meat of forest" or the "meat of the poor" due to its nutritive and cost-effective value of it. Mushrooms are also known for their valuable contributions in the field of nutrition and therapeutics[11]. Lu *et al.*[4] evaluated the potential of macrofungal populations in the production and processing of diverse types of pharmaceuticals including the biologically active metabolites and other nutritive compounds and supplements. The edible mushrooms harbour significant proportions of nutritive components like carbohydrates, proteins, fats, diverse vitamins like riboflavin, niacin, pantothenic acid and minerals like selenium, copper, phosphorous, zinc, potassium etc. and thus, regarded as active and integral parts of balanced diet required for optimum growth and nutrition. Mushrooms are also known as the potent reservoir of diverse types of energy and crude fibres.

As there exist limited reports on relative abundance and species diversity of macrofungal genetic resources in the pristine locations like northeastern parts of India, the present investigation holds perspectives to explore the less explored macrofungal genetic resource diversity in certain pristine locations of N.E. India so that new mushroom species might get special interest and novelty due to their edibility or other economic perspectives in the integral fields like medicine, agriculture and industry.

MATERIALS AND METHODS

Description of the study area, climate and topography: Assam is considered one of the richest biodiverse regions in N. E. India. Dhemaji district in Assam (94° 12' 18'' E and 95°41' 32'' E longitudes and 27° 05' 27'' N and 27° 57' 16'' N latitudes) represents key treasures of Integration of Traditional Knowledge (ITK) and conservation strategies for natural flora and fauna. The various tribes inhabiting the region has maintained several traditional health care practices that are solely dependent on biological floras like plants or macrofungal resources. Geographically the area is situated by Arunachal hills on the North and the East and stretches to the Brahmaputra river with Subansiri at one side and the Siang river on the other.

The present investigation was undertaken to explore the less explored macrofungal species diversity in nine undisturbed reserve forests, namely, Jiadhal, Subansiri, Sissi, Simen, Archiac, Jamjing, Senga, Gali and Pova of Dhemaji district, Assam, N.E. India during January, 2019-December, 2020. As the major part of the study area is located near the foothills of Arunachal Pradesh, it exhibits differences in climatological parameters like temperature, rainfall, humidity, wind etc. The climate is per-humid, characterized by high rainfall, mild summer and winter and falls under cool to warm

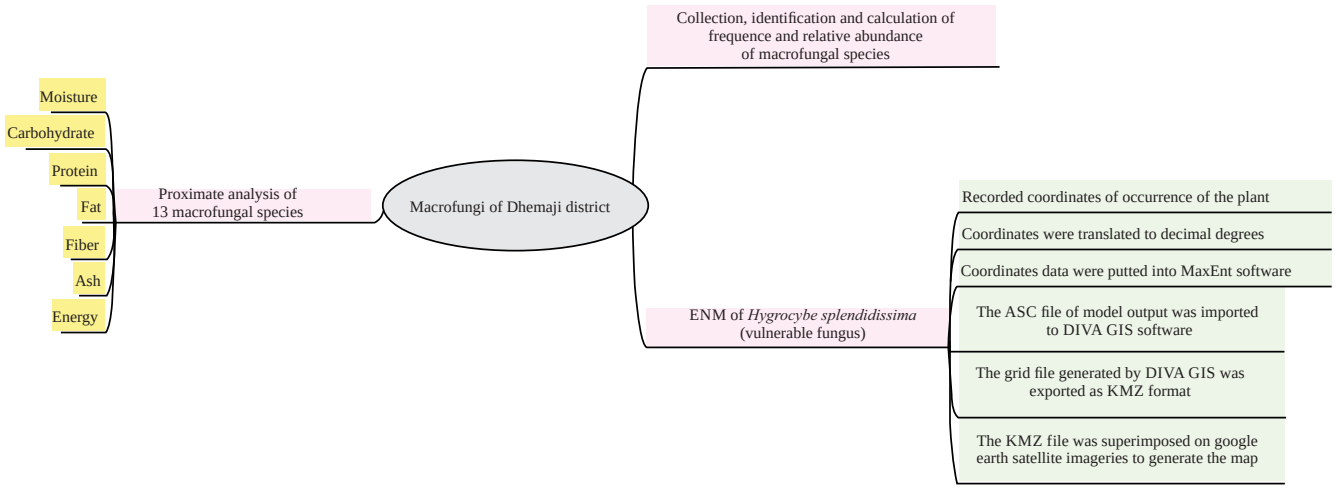

Fig. 1: Flow chart of the methodology used during the entire course of an investigation

per-humid thermic-agroecological subzone. The annual rainfall of the district ranges from 2600-3200 mm and the relative humidity varies from 90-73%. The temperature also varies between 39.9°C in summer and 5.9°C in winter.

Collection of the macrofungi and identification of the species: The survey for the mushroom collection was made throughout the year in four seasons, respectively. The information and knowledge on macrofungi occurrence and distribution patterns were acquired through questionnaires and discussions with local people, village headmen, medical practitioners and other cultivators and planters in the area. Hand collection of the macrofungi was made after observing and recording the habitat and thorough consultation with the village experts and photography capturing (Nikon D5600/ DSLR/EN-EL 14A Lithium-Ion, Thailand) in the field. The samples were collected with extreme care to avoid damage using knives or forceps and transported to the laboratory in sterilized polypropylene bags. Microbial accession numbers were given to each sample collected for data recording. Macrofungal species details like its host/substratum, colour, smell (if any) of the sporocarps and other visible features like size, shape and appearances were recorded during their collection in the field. Macrofungi identification was made using various taxonomic monographs[12-16]. The methodology flowchart mentioning different events starting from field survey, sample collection up to data analysis is represented in Fig. 1. The laboratory investigations related to specimen identification and related research was conducted in the Department of Botany, Dhemaji College and NNS College, after field survey and specimen collection.

Proximate analysis of edible macrofungal for estimated food value

Moisture content estimation: Estimation of moisture content was made using[17]. For this, twenty grams of fresh mushroom was weighed into a weighed moisture box (A and D company Ltd., N 92, P1011656, Japan) and dried in a hot air oven at 100~105°C and cooled in a desiccator. The process of heating and cooling was repeated till the constant weight was achieved[18].

The moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight } (W_1) - \text{Final weight } (W_2)}{\text{Initial weight } (W_1)} \times 100$$

Determination of total protein: Total protein content was measured according to Lowry *et al.*[19]. For this, 5 g of ground mushroom was taken with 50 mL of 0.1 N NaOH and boiled for 30 min. The solution was cooled at room temperature (RT) and centrifuged at 1000 rpm using a DSC-200T tabletop centrifuge machine (Digisystem Laboratory Instruments, Taipei, Taiwan). The supernatant, so collected was measured and analyzed for total protein content. For the determination of protein content from a fresh mushroom species, 5.0 g of the sample was taken with 50 mL phosphate buffer and homogenized with a tissue homogenizer (Polytron, Lucerne, Switzerland). About 5.0 mL of the homogenization was taken with 50 mL of 0.1 N NaOH and protein content was determined as mentioned above.

Total lipid estimation: Total lipid was estimated using a modified methodology of Folch *et al.*[20]. For this, 5.0 g of

ground mushroom was suspended in 50 mL of chloroform: Methanol (2: 1 v/v) mixture. The mixer was shaken thoroughly and allowed to stand for 3 days. The solution was filtered and centrifuged at 1000 rpm using a centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by repeated heating. The remaining extract was obtained as the crude lipid. For the determination of total lipid from fresh mushroom, 5.0 g of the sample was mixed with 50 mL phosphate buffer and homogenized with a tissue homogenizer. About 5.0 mL of homogenization was taken with 50 mL of chloroform: Methanol (2:1 v/v) mixture and lipid content was determined as mentioned in the procedure above.

Determination of crude fibre: The crude fibre content of the mushroom sample was determined using the protocol as mentioned by Raghuramulu *et al.*[17]. For this, 10 g of moisture and the fat-free mushroom sample was taken in a beaker and 200 mL of boiling 0.255 N H_2SO_4 was added to it. Keeping the volume constant by adding sterile distilled water (SDW) to it, the mixture was boiled for 30 min. The mixture was then filtered through a muslin cloth and the residue was washed with hot water till it is found to be free from acid incorporation. The material was transferred to the same beaker and 200 mL of boiling 0.313 N NaOH was added to it. After boiling for 30 min, the mixture was filtered through a muslin cloth and the residue was washed with hot water till it is found as free from alkali, followed by extra washing with some alcohol and ether. It was then transferred to a crucible and dried overnight at 80~100 and weighed (We) in an electric balance. The crucible was heated in a muffle furnace at 600 for 5~6 hrs, cooled and weighed again (Wa).

The difference in the weights (We-Wa) represents the weight of the crude fibre, so obtained[18]:

$$\text{Crude fiber (g/100 g sample)} = \frac{100 - (\text{moisture+fat})] \times (\text{We} - \text{Wa})}{\text{Wt of sample}}$$

Determination of total ash: About 1.0 g of the mushroom sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangle and heated over a low flame till the entire material was completely charred. This is followed by heating it in a muffle furnace for about 5~6 hrs at 600°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing, the crucible was heated in the muffle furnace for 1 hr, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or greyish-white in colour. Total ash content was calculated as follows[18]:

$$\text{Ash content (g/100 g sample)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Total carbohydrate estimation: The available carbohydrate in the edible species was determined following Raghuramulu *et al.*[17] and Alam *et al.*[18], using the following formula:

$$\text{Available carbohydrate (g/100 g sample)} = 100 - [(\text{moisture+fat+protein+ash+crude fiber}) \text{ g/100 g}]$$

Energy estimation: The available energy in the edible mushroom was calculated following Leal *et al.*[21] and Sharif *et al.*[22], using the following formula:

$$\text{Available energy (kcal)} = 4 \times (\text{protein (g)} + \text{carbohydrate (g)}) + 9 \times \text{fat (g)}$$

Data analysis: The frequency and relative abundance of macrofungi (%) were calculated using the following formula:

$$\text{Frequency (\%)} = \frac{\text{Number of sites in which the species is present}}{\text{Total number of sites examined}} \times 100$$

$$\text{Relative abundance} = \frac{\text{Total number of individual species}}{\text{Total number of species}} \times 100$$

Experimental values are considered as Means±Standard Deviation (SD). Three replicates were maintained in each case. Data were analyzed statistically using SPSS 16.0 software (IBM Corporation SPSS, North America).

Ecological niche modelling of *Hygrocybe splendidissima*: From field surveys, collection and observations, *Hygrocybe splendidissima*, a macro fungus was recorded in the primary locations. As the species was enlisted in the IUCN Red List of threatened species, ecological niche modelling is made to identify the unexplored geographical locations of the species that would find its utility in habitat conservation. For this, a Global Positioning System (GPS) of accuracy up to 10-40 m was used to record the coordinates of occurrence points of *H. splendidissima*. Habitat distribution modelling software as suggested by Adhikari *et al.*[23] was used to translate the coordinates into decimal degrees. For habitat modelling of *H. splendidissima*, the normalized difference vegetation index

(NDVI) and the maximum entropy modelling (MEM) was used to develop the model[23]. The NDVI was obtained from the Global Land Cover Facility (GLCF, University of Maryland). All the analysis were conducted at the spatial resolution of 250 m. MaxEnt employs presence-only data to estimate a species' geographic location[24,25]. Calibration was made as and when found to be necessary. For the calibration, the presence and background data locations were used where 75% of the records were utilized for training the model and 25% for the test[23]. Up to 20 replicated model runs were conducted and the replicated run types were cross-validated with a 10-percentile threshold rule of training, presence to validate the model robustness[23,26]. Since the program is already calibrated, therefore, the other parameters were set as default[23]. The replicated runs were generated as average, maximum, minimum, median and Standard Deviation (SD). The model's quality was determined using the Area Under the Curve (AUC) value and it was graded as very excellent (0.95<AUC<1.0), good (0.9<AUC<0.95), fair (0.8<AUC<0.9) and low (AUC<0.8)[27]. There was an analysis for the habitat type in the occurrence areas of the species as well as the predicted potential areas through repeated field surveys and investigations. To identify the actual habitat of the species, ASC (Action Script Communication) file of the model output to Diva GIS ver. 7.3 was imported and the Grid file as KMZ (Keyhole Markup Language Zipped) format for display in Google Earth was exported[23,26,28,29]. This was then followed by superimposing the exported KMZ files on Google Earth pro satellite imageries to determine the actual habitat condition of the areas of occurrence and areas that prevailing the same habitat for the reintroduction of the species[23,26-30].

RESULTS AND DISCUSSION

Diversity of macrofungal species populations and taxonomy: A total of 40 macrofungal species populations that belongs to 32 genera, 24 families and 9 orders were collected throughout different study seasons in Dhemaji district, Assam, N.E. India. Out of the collected samples, Basidiomycota represents 7 orders, namely Agaricales (20 species) followed by Polyporales (12 species), Russulales (02 species), Cantharellales (01 species), Hymenochaetales (01 species), Phallales (01 species) and Tremellales (01 species) and Ascomycota represents only 2 order, viz. Helotiales and Xylariales, respectively (Fig. 2). A complete list of identified macrofungal populations along with their scientific and common names and families has been represented in Table 1. Macrofungal species diversity and analysis of the edaphic factors influencing the occurrence of fungal communities of Church forests in dry Afromontane areas of Northern Ethiopia have been made by Alem *et al.*[31] and thereby indicated the influence of variations in climatology and study locations as prime factors in maintaining fungal diversity and distribution. Polyporaceae is recorded as the dominant family of all the collected mushroom populations with seven identified species during the present investigation followed by Agaricaceae (4), Psathyrellaceae (3), Fomitopsidaceae (2), Ganodermataceae (2), Hymenogastraceae (2), Mycenaceae (2), Russulaceae (2), Bolbitiaceae (1), Cyphellaceae (1), Clavulinaceae (1), Helotiaceae (1), Hygrophoraceae (1), Lyophyllaceae (1), Marasmiaceae (1), Meruliaceae (1), Nidulariaceae (1), Phallaceae (1), Physalacriaceae (1), Pleurotaceae (1), Repetobasidiaceae (1), Schizophyllaceae (1), Tremellaceae (1)

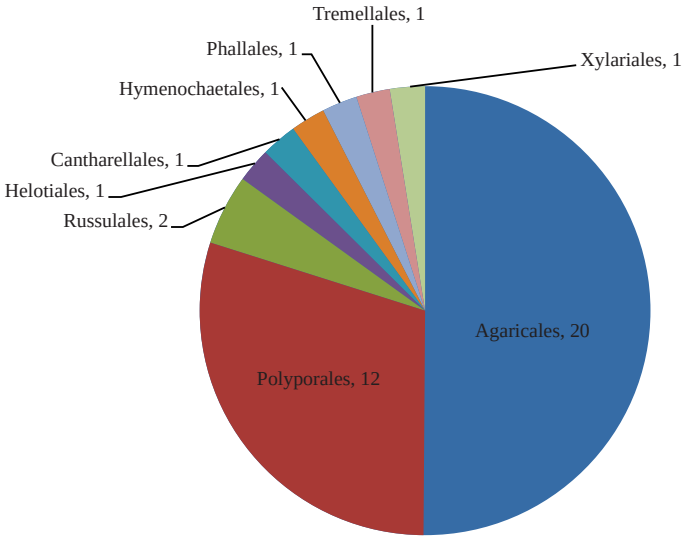

Fig. 2: Order-wise distribution of macrofungal population numbers

Table 1: List of macrofungal species populations isolated from the study locations along with their edibility and pharmaceutical importance

Botanical names	Common names	Families	Edibility and other uses
Agaricus arvensis Schaeff ex Seer	Horse mushroom	Agaricaceae	Edible
Agaricus bisporus Quél	Button mushroom	Agaricaceae	Edible
Ascocoryne sarcoides (Jacq.) J.W. Groves and D.E. Wilson	Jelly drops	Helotiales	Inedible
Chondrostereum purpureum (Pers.) Pouzar	Silver leaf	Cyphellaceae	Inedible
Clavulina cristata (Holmsk) J. Schröt	Coral fungi	Clavariaceae	Edible
Coprinellus disseminatus (Pers.) J.E. Lange	Fairy ink-cap	Psathyrellaceae	Unknown
Coprinellus micaceus (Bull.) Vilgalys, Hopple and Jacq. Johnson	Glistening ink cap	Psathyrellaceae	Edible
Cotylidia undulata (Fr.) P. Karst.	Trumpet skin	Repetobasidiaceae	Not known yet
Cyathus striatus (Huds.) Willd	Fluted bird's nest	Nidulariaceae	Inedible
Daedalea quercina (L.) Pers.	Maze-gill fungus	Fomitopsidaceae	Inedible
Flammulina velutipes (Curtis) Singer	Velvet shank	Physalacriaceae	Edible
Ganoderma sessile Murrill	NA	Ganodermataceae	Medicinal
Ganoderma applanatum (Pers.) Pat	Artist's fungus	Ganodermataceae	Medicinal
Hygrocybe splendidissima (Peter D. Orton)	Splendid waxcap	Hygrophoraceae	Edible
Lactarius piperatus (Scop. Ex. Fr.) S.F. Gray	Peppery milk-cap	Russulaceae	Edible, medicinal
Lactarius trivialis (Fr.) Fr.	NA	Russulaceae	Inedible
Laetiporus speciosus Battarra ex Murrill	Chicken of woods	Fomitopsidaceae	Edible
Lentinus levis (Berk. and M.A. Curtis) Murrill	Giant pannus	Polyporaceae	Edible but tough
Lenzites betulina (L.) Fr.	Gilled polypore	Polyporaceae	Inedible, medicinal
Leucocoprinus cepistipes (Sowerby) Pat.	NA	Agaricaceae	Edible but not very palatable
Lycoperdon perlatum Pers.	Common puffball	Lycoperdaceae	Edible when young, medicinal
Marasmius haematocephalus (Mont) Fr	Purple pinwheel mushroom	Marasmiaceae	Inedible
Microporus xanthopus (Fr.) Kuntze	Yellow footed polypore	Polyporaceae	Inedible, medicinal
Mycena acicula (Schaeff.) P. Kumm.	Orange bonnet	Mycenaceae	Inedible
Mycena haematopus (Pers.) P. Kumm.	Bleeding fairy helmet	Mycenaceae	Unknown
Panaeolus antillarum (Fr.) Dennis	NA	Bolbitiaceae	Edible but not commonly eaten
Parasola auricoma (Martinez)	Golden-haired inkcap mushroom	Psathyrellaceae	Not known
Phallus indusiatus Vent	Long net stinkhorn	Phallaceae	Inedible, medicinal
Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm	Oyster mushroom	Pleurotaceae	Edible
Podoscypha petalodes (Berk.) Pat.	Wine glass fungus	Meruliaceae	Inedible
Psilocybe cyanescens (Elsie Wakefield)	Wavy cap	Hymenogastraceae	Used for psychoactive
Psilocybe weraroa (G.H Cunningham)	NA	Hymenogastraceae	Used for psychoactive
Pycnoporus sanguineus (L.) Murrill	Cinnabar bracket	Polyporaceae	Inedible, medicinal
Schizophyllum commune Fries	Split gill mushroom	Schizophyllaceae	Edible, medicinal
Termitomyces schimperi (Pat.) R. Heim	Ejova (Singular) Omajowa (Plural)	Lyophyllaceae	Edible
Tremella fuciformis Berk.	White jelly mushrooms	Tremellaceae	Edible and also used in cosmetic beauty products
Trametes hirsuta (Wulfen) Pilát	Hairy bracket fungus	Polyporaceae	Inedible, medicinal
Trametes pubescens (Schumach.) Pilát	Bracket fungus	Polyporaceae	Inedible
Trametes versicolor (L.) Lloyd	Turkey tails	Polyporaceae	Inedible, decrease immune system depression
Xylaria hypoxylon (L.) Grev	Candlestick fungus	Xylariaceae	Inedible

and Xylariaceae (1) respectively. The photographs of the identified macrofungal species have been shown in Fig. 3. A similar study on exploring the unexplored diversity of wild mushroom species in Nagaland, India has been made by Ao *et al*.[32] and thereby mentioned the favourable agro-climatic conditions of N.E. India in influencing species diversity and distribution patterns. Macrofungal species diversity and distribution in the forest areas of Tripura, N.E. India has been made by Debnath *et al*.[33]. During the investigation, the workers have collected the wild mushroom species followed by sample identification using the assistance of key taxonomic monographs and photographs and thereby could able to construct a reference database of wild mushroom species of the state.

Season-wise distribution of macrofungal population numbers citing frequency of occurrence and relative abundance has been represented in Table 2. Mushroom species, *Coprinellus disseminates* and *Pleurotus ostreatus* exhibited the highest frequency of occurrence (up to 90%) while the lowest frequency of occurrence (%) was recorded for *Cyathus striatus*. Similarly, maximum values of relative abundance amongst the mushroom species throughout the study seasons were exhibited by *Trametes versicolor* (4.3%), while it was minimum for *Cyathus striatus* (0.3%).

Mushroom edibility and proximate analysis: Out of all the collected macrofungal populations, certain mushroom species are found to be edible while others are known as

Fig. 3: Photo plate showing the morphology of the isolated macrofungal species populations

poisonous. The edibility and pharmaceutical importance of mushroom species has been represented in Table 1. The edible mushroom populations (a total of thirteen species) show diverse nutritive values (Table 3). *Lycoperdon perlatum* measured the highest concentration of protein (42.76±0.021 g/100 g), while *Tremella fuciformis* recorded the least value of 10.65±0.03 g/100 g. For results related to moisture content, *Lactarius piperatus* recorded the highest value (13.77±0.026 g/100 g) and it was lowest in *Coprinellus micaceus* (5.16±0.02 g/100 g). Maximum carbohydrate content was estimated in *Schizophyllum commune* (70.09±0.028 g/100 g) while *Lycoperdon perlatum* was with least carbohydrate value (27.67±0.03 g/100 g). Similarly, the highest fibre content was measured in *Flammulina velutipes* (28.98±0.033) while least in *Schizophyllum commune* (1.56±0.028 g/100 g). The fat content was highest in *Coprinellus micaceus* (4.72±0.02 g/100 g) whereas least in *Lactarius piperatus* (1.04±0.028 g/100 g). *Pleurotus ostreatus* measured the highest values of ash content (8.76±0.022 g/100 g) while *Coprinellus micaceus* showed the least value (3.06±0.036 g/100 g). The edible and pharmaceutically important mushroom populations exhibited energy content

Table 2: Frequency of occurrence and relative abundance (%) of macrofungal species populations throughout different study seasons

Fungi	Seasons				Total	Frequency of occurrence (%)	Relative abundance (%)
	Spring	Summer	Autumn	Winter			
Agaricus arvensis	6	12	8	3	29	30	2.7
A. bisporus	9	8	4	3	24	25	2.3
Ascocoryne sarcoides	10	15	12	7	44	80	4.2
Chondrostereum purpureum	10	11	9	6	36	60	3.4
Clavulina cristata	11	12	6	3	32	25	3
Coprinellus disseminates	9	15	12	6	42	90	3.1
Coprinellus micaceus	7	9	7	5	28	30	2.6
Cotylidia undulata	6	10	11	6	33	40	3.1
Cyathus striatus	1	2	1	0	4	6	0.3
Daedalea quercina	5	9	7	2	23	40	2.2
Flammulina velutipes	4	10	7	3	24	40	2.3
Ganoderma sessile	7	13	10	5	35	70	3.3
G. applanatum	3	7	7	3	20	40	1.9
Hygrocybe splendidissima	2	6	3	3	14	30	1.3
Lactarius piperatus	4	7	6	3	20	40	1.9
L. trivialis	3	5	5	2	15	20	1.4
Laetiporus speciosus	5	7	6	4	22	50	2
Lentinus levis	1	4	4	0	9	10	0.8
Lenzites betulina	4	9	7	2	22	30	2
Leucocoprinus cepistipes	4	11	9	3	27	70	2.5
Lycoperdon perlatum	3	10	9	3	25	45	2.4
Marasmius haematocephalus	3	5	5	2	15	20	1.4
Microporus xanthopus	4	9	7	3	23	40	2.2
Mycena acicula	3	5	4	2	14	20	1.3
Mycena haematopus	6	10	9	4	29	50	2.7
Panaeolus antillarum	7	12	8	4	31	60	2.9
Parasola auricoma	4	9	6	3	22	30	2
Phallus indusiatus	3	6	4	2	15	25	1.4
Pleurotus ostreatus	13	14	11	5	43	90	4
Podoscypha petalodes	5	9	7	3	24	30	2.3
Psilocybe cyanescens	10	13	11	4	38	50	3.6
Psilocybe weraroa	2	5	2	0	9	10	0.8
Pycnoporus sanguineus	12	13	10	6	41	70	3.9
Schizophyllum commune	9	11	8	5	33	40	3.1
Termitomyces schimperi	7	13	9	5	34	50	3.2
Tremella fuciformis	2	4	0	2	8	10	0.7
Trametes hirsuta	7	13	10	4	34	40	3.2
Trametes pubescens	12	15	11	4	42	60	3.1
Trametes versicolor	13	16	10	7	46	70	4.3
Xylaria hypoxylon	5	11	9	6	31	30	2.9
Total	241	385	291	143	1060	-	-

Table 3: Moisture content and proximate analysis of edible mushroom species

Name of macrofungi	Moisture (g/100 g)	Carbohydrate (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Fiber (g/100 g)	Ash (g/100 g)	Energy (g/100 g)
Agaricus arvensis	8.10±0.02	32.74±0.021	33.21±0.028	4.16±0.034	16.12±0.024	5.67±0.033	301.24±0.023
A. bisporus	8.01±0.023	29.16±0.034	35.14±0.024	4.12±0.028	18.12±0.024	5.45±0.039	294.28±0.026
Clavulina cristata	8.20±0.03	50.45±0.021	23.23±0.021	3.98±0.028	10.24±0.028	4.80±0.033	330.54±0.026
Coprinellus micaceus	5.16±0.02	59.32±0.02	15.43±0.02	4.72±0.02	12.31±0.036	3.06±0.036	341.48±0.021
Flammulina velutipes	11.80±0.021	36.24±0.02	14.01±0.021	1.32±0.021	28.98±0.033	7.65±0.023	212.88±0.028
Hygrocybe splendidissima	8.45±0.02	37.62±0.02	26.71±0.024	2.64±0.021	19.45±0.028	5.13±0.021	281.08±0.031
Lactarius piperatus	13.77±0.026	60.94±0.03	13.65±0.028	1.04±0.028	6.79±0.021	3.81±0.021	307.72±0.033
Laetiporus speciosus	7.45±0.031	61.77±0.02	15.67±0.028	1.13±0.02	6.55±0.033	7.43±0.028	319.93±0.033
Lycoperdon perlatum	9.43±0.026	27.67±0.03	42.76±0.021	8.76±0.03	7.76±0.035	3.62±0.028	360.56±0.038
Pleurotus ostreatus	8.34±0.02	42.91±0.021	26.23±0.02	2.09±0.03	11.69±0.021	8.76±0.022	295.37±0.028
Schizophyllum commune	9.08±0.02	70.09±0.028	10.67±0.03	1.06±0.034	1.56±0.028	7.54±0.021	332.58±0.038
Termitomyces schimperi	8.66±0.014	62.26±0.028	13.20±0.034	1.24±0.028	7.76±0.024	6.88±0.026	312.28±0.033
Tremella fuciformis	11.02± 0.021	64.47±0.021	10.65±0.03	3.08±0.021	6.76±0.024	4.02±0.022	328.20±0.039

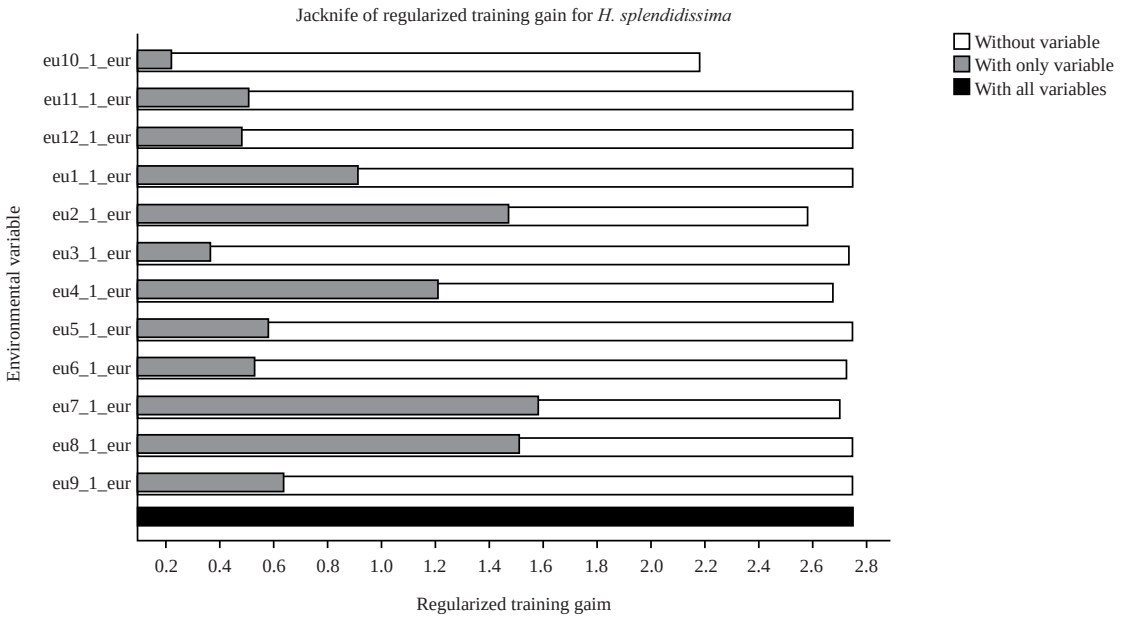

Fig. 4: Jackknife test of variable importance for *H. splendidissima*

Individual variable contribution (blue bar), contribution when a given variable is excluded (green bar), the whole set of variables (red bar)

Table 4: List of NDVI and variable contributions used in the ecological niche modelling of *H. splendidissima*

Variables	Description of the variables	Contribution (%)	Permutation importance
eu7	NDVI July	40.7	13.9
eu2	NDVI February	21.9	42.9
eu10	NDVI October	20.3	34.7
eu4	NDVI April	7.1	2.2
eu8	NDVI August	5.5	0.4
eu6	NDVI June	4.4	4.6
eu12	NDVI December	0.1	1
eu3	NDVI March	NIL	0.1
eu5	NDVI May	NIL	NIL
eu1	NDVI January	NIL	0.3
eu11	NDVI November	NIL	NIL
eu9	NDVI September	NIL	NIL

levels ranging from 212.88±0.028 Kcal/100 g in *Flammulina velutipes* to 360.56±0.038 Kcal/100 g in *Lycoperdon perlatum*. Proximate analysis of edible wild and cultivated mushroom species collected from Northeast Thailand has been made by Srikram and Supapvanich[34] and thereby indicated the significance of mushrooms as potent sources of macronutrients and biologically active compounds.

Ecological niche modeling of *H. splendidissima*: One of the identified macrofungus, *H. splendidissima* is a vulnerable fungus, according to the IUCN Red list, thus the species is at high risk of extinction. Although, the fungus is edible due to its rarity it should not be collected other than for research purposes. The model calibration test for *H. splendidissima* yields satisfactory results (AUCtest = 0.95±0.005). The Table 4 indicates the estimated values of relative contributions of the environmental variables to the Maxent model. The Fig. 4 represents the jackknife test results of variable importance. The environmental variable with the highest gain was observed when used in isolation as eu2_1_eur, which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most when it is omitted as eu10_1_eur, which therefore appears to have the most information that isn't present in the other variables. Values shown, here are the mean over replicate runs. The superimposition of predicted potential habitat distribution map on Google Earth Pro imageries identified in different forest areas of N.E. India viz., Assam (Mariani, Amguri, Bokakhat, Kohora, Boko), Nagaland (Mokokchung, Mangkolemba, Longleng, Merangkong, Tuli),

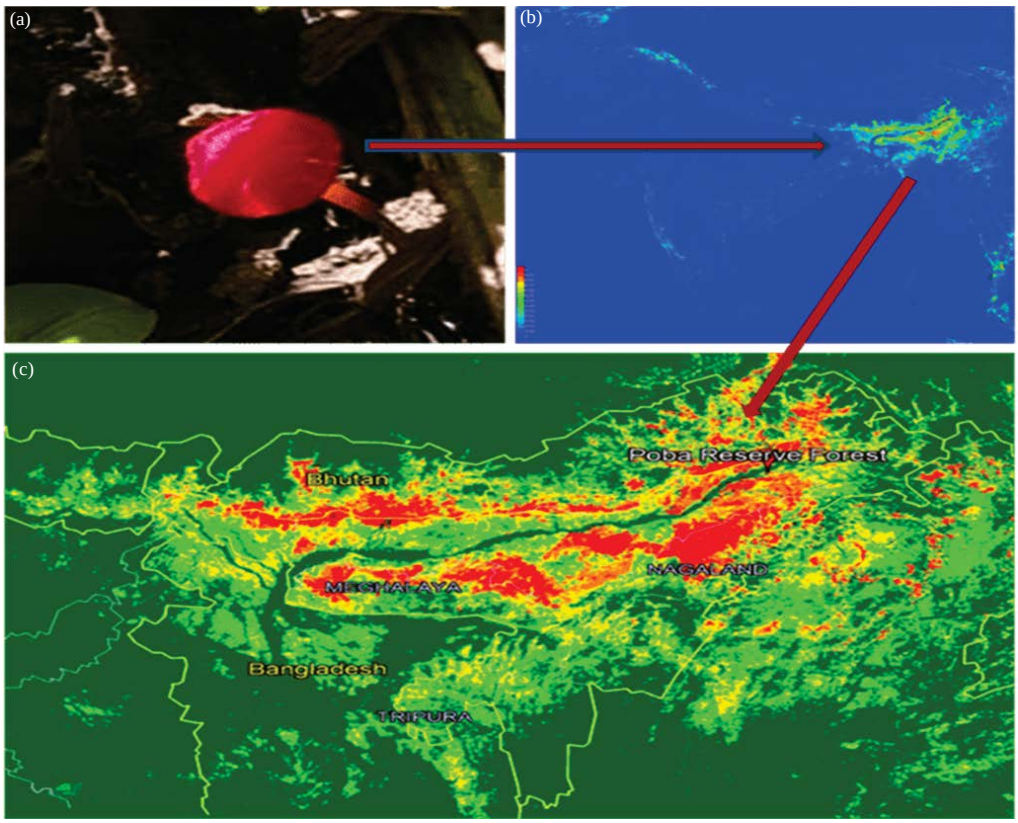

Fig. 5(a-c): Photographs showing *H. splendidissima* and ecological niche modelling, (a) Morphology of *H. splendidissima*, (b) India map and (c) Map showing potential habitat distribution of *H. splendidissima* in N.E. India
Red patches in the map indicate suitable habitat conditions of the economically important species for species conservation and regeneration activities

Meghalaya (Nongpoh, Umiam, Tura), Arunachal Pradesh (Itanagar, Naharlagun, Doimukh, Khellong, Chopal) is represented in Fig. 5a-c. The areas of red patches in the map indicate suitable habitat conditions of this economically important mushroom species, *H. splendidissima* that could be used for *in situ* conservation and regeneration programmes. Habitat degradation is considered as one of the threat factors in inhibiting the relative occurrence and frequency distribution of macrofungal populations and diversity[35]. The present approach, thus, predicts the habitat and topographic distribution of economically important edible mushroom species in addition to exploring the natural biodiversity of macrofungal populations in the hitherto-less explored regions like undisturbed reserve forests of N.E. India and thereby provides new lights to improving the knowledge and understandings on ecological niche prediction and related research. A similar ecological niche modelling approach to predict the disease biogeography has been made by Escobar and Craft[36] and they suggested the benefits of this ecological approach as one of the advanced tools in biological research and development. Although niche modelling is considered an effective tool in predicting the habitat, distribution and topographic mapping of biological materials for future plant conservation programmes, the study is, often considered robust and climate-dependent. There are also limitations related to the influence of biological interactions[37] on ecological niche modelling approach and habitat manipulation studies. Further, more study models need to be emphasized to correctly design and interpret the ecological niche models for better prediction of habitat analysis and topographic distribution mapping of economically significant biological substances.

CONCLUSION

As mushrooms are potent sources of several macro and micro-nutrients like carbohydrates, proteins, lipids and diverse types and nature of vitamins and minerals and biologically active metabolites, the studies related to exploring the less explored wild mushroom diversity and distribution from the pristine locations like undisturbed reserve forests hold fabulous perspectives. The edible and commercially viable

mushroom populations might serve the global economy and future implications in medicine, agriculture and industry.

SIGNIFICANCE STATEMENT

Wild mushrooms of nutritive values and commercial feasibilities have always attracted the interest of scientists due to their immense applicability not only as nutritive supplements for mankind but also their utility as potent non-chemical biological resources in medicine and drug design and discoveries, agriculture and other industrial applications. Undisturbed reserve forests of Assam, N.E. India harbours potent sources of economically significant fungal resources including the highly putative mushroom populations. The findings of the present survey and analysis are sure to assist the mycologists, environmental biologists, young mushroom cultivars and local entrepreneurs of the region to contribute more towards sustainability in understanding the less explored novel macrofungal resources in the ecologically significant biodiverse region.

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