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Macro-fungi diversity in Thulo Ban Community Forest of Arjam, Myagdi District, Nepal

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Abstract

Macro-fungi produce large fructifications that are visible without the help of a microscope. They play an important role in the conservation of forest ecosystems and biodiversity**.** The current study deals with the diversity of macro-fungi in the subtropical mixed forest of Arjam, Myagdi District. The study was conducted from June to September 2020, at a height of 1250 to 1450 meters above sea level. In three transects, 10 m×10 m quadrat was used and a total of 18 plots were made. A total of 70 macrofungal taxa were collected. Among them, 56 were identified at the species level, and 14 were generic levels belonging to 26 different families and 12 orders. The highest species-containing family was Russulaceae, with 16 species, whereas the densest species was *Mycena* sp., comprising 11.8 percent and *Cantharellus cibarius* was the most frequent species, consisting of 44.44 percent.

Keywords**:** *Cantharellus cibarius*, forest ecosystem**,** Russulaceae, subtropical mixed forest

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Introduction

Fungi are a distinct group of organisms more closely related to animals than plants (Keizer, 1998; Seen- Irlet *et al*., 2008). Macrofungi are Ascomycetes and Basidiomycetes members that produce mature spore-bearing and morphologicall distinct fruiting bodies that are visible to the naked eye (Arnolds 1992; Redhead and Berch, 1997). They grow either epigenous or hypogynous in nature (Acharya, 2020). The best-known example of macro-fungi is the mushroom. The most familiar type of macro-fungi is umbrella shaped while other species are in the form of gilled fungi, coral fungi, jelly fungi, bracket fungi, puffballs, and bird's nest fungi (Jha and Tripathi, 2012).

Macro-fungi can be found in a variety of habitats depending on the species of trees and other substrates (Parveen *et al*., 2017). They can be saprophytic, parasitic, or mycorrhizal in their ecology (Sharma, 2017). The occurrence of macrofungal fruiting bodies is dependent on humidity, nutritional substrate, and mild atmospheric temperature (Dickinson and Lucas, 1979).

Macro-fungi play key roles in the conservation of forest ecosystems and biodiversity through carbon and other nutrient recycling (Gates, 2009; Hawksworth, 1991; Molina *et al.*, 2001). They are an important source of food for forest animals, and also serve as a home for many soil insects and other small organisms that are part of the forest ecosystem (Teke *et al*., 2019).

There are an estimated 1.5 million fungal species worldwide, but only 74,000-120,000 have been described (Garibay-Orijel *et al*., 2009).

Approximately, 14,000 described species of the millions of fungi estimated to exist on the planet produced fruiting bodies large enough to be considered mushrooms. 7,000 of them are considered edible. Due to the wide variety of climatic conditions, such as tropical, sub-tropical, temperate, and alpine, Nepal is considered as the homeland for the mushroom's floral diversity (Aryal and Budhathoki, 2012; Poudel and Bajracharya, 2011). But, due to limited scientific research so far, in Nepal 1,291 mushroom species have been recorded among them 34 species of mushrooms have been described as endemic, 159 species are edible, 100 poisonous and 34 have medicinal value (Devkota and Aryal, 2020). Since most of Nepal's mushroom biodiversity is unexplored, it is essential to document the diversity, distribution, and abundance of these macrofungi in Nepal. And Mycological exploration and investigation is carried out more in Central Nepal in comparison to the eastern and western regions of Nepal (Adhikari, 1999, Adhikari, 2000; Adhakari and Bhattarai, 2014).

The main objective of this research work was to explore the diversity of macro-fungi and to assess patterns of species diversity and distribution along environmental variables.

Materials and Methods

Study Area

The study was carried out in the Thulo Ban Community Forest of Arjam, Beni Municipality 1, Myagdi District, Gandaki Province, Nepal (Fig. 1). Geographically, it is located in between 28° 19' 20" N 83° 34' 18" E and 28° 19' 26" N 83° 34' 42" E. The total area occupied by the forest is 114 hectares. The study area has a subtropical climate and consists of subtropical pine mixed forest dominated by tree species such as *Pinus roxburghii*, *Schima wallichii*, *Rhododendron arboreum*, *Egelhardia spicata*, and *Lyonia ovalifolia*.

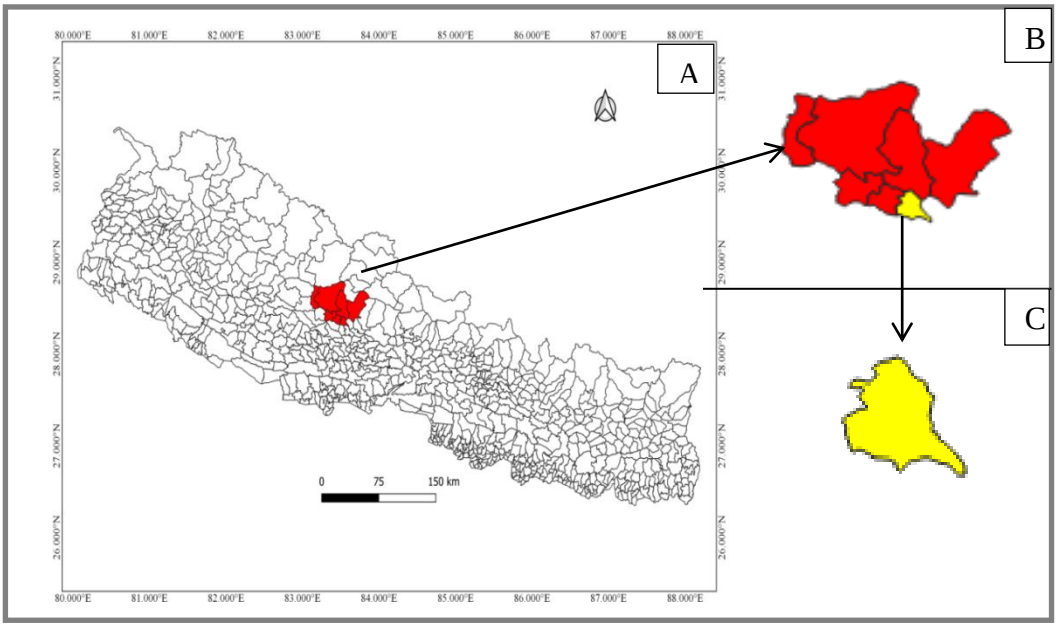

Figure 1. Map of study area

Sample collection

Mushroom samples were gathered from the end of June to the beginning of September 2020. Macrofungi were sampled using a systematic random procedure at altitudes ranging from 1250-1450 meters above sea level. The sampling was done by using a 10 m×10 m quadrats in three transects at the distance of 100 m, and a total of 18 plots were made. Distance between each plot was approximately 50 m. (Baral *et al*., 2015). Before collecting, specimens were photographed in their

natural habitat by using digital camera (Sony, DSC - W350) and their morphological characteristics such as size of fruiting body, cap color, cap surface, cap margin, scale, gill color, gill attachment, gill spacing, stipe length, width, color, shape, type of veil, annuls and volva, geographic location were noted (Srivastava and Bano, 2010). From the middle of each plot, ecological factors such as tree canopy (percentage) were estimated visually. After collecting the sample spore print were taken. To prevent the intermixing of spores and external infections, the spore print papers were labeled, stored in a Ziploc bag, and brought to the laboratory of the Central Department of Botany for identification.

Preservation of macrofungi

Collected mushrooms were preserved using both dry and liquid methods. Macrofungi were preserved in liquid using (25: 5: 70 ml of rectified alcohol, formalin and distilled water) (Hawksworth *et al.*, 1995). Air and sun drying were used for dry preservation.

Microscopic study and identification of macrofungi

For, microscopic examination the spore print papers were scratched with a needle and placed on a slide, stained with 1-2 drops of cotton blue and lactophenol, covered with a cover slip, and examined under a microscope to find the length and width of each species spore. In the case of small spores, immersion oil was used to magnify the spore. The preserved specimens were identified using various books and standard literature (Phillips, 1981; Corner, 1970; Adhikari, 2000; Watling, 1973); consult with mushroom field guide as well as websites (www.mycoweb.com; www.mushroomexpert.com). Macro-fungi were also distinguished by spore print color and morphological characteristics.

Evaluation Diversity index, Frequency and Density

Diversity indices like the Shannon-Wiener diversity index (H') and Simpson diversity index (D) was calculated by following formula (Magurran, 2004). $H' = -\Sigma\ Pi\ \ln Pi$ (Where, H'=Diversity index, Pi= ratio of individuals of species i divided by all individuals, n= number of species, N of all species). Simpson Diversity Index (D) $= \frac{\Sigma n\,(n-1)}{N(N-1)}$ (Where, D=Simpson's index, N=Total number of individuals of all species, n=Total number of organisms of a particular species). Similarly, the density and frequency of macrofungal species were determined as per (Daubenmire, 1959).

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

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

A regression analysis was performed using R-Studio to determine the impact of environmental variables on macro-fungal species richness.

Soil sampling and analysis

To measure soil pH and moisture, soil samples were collected at a depth of 15 cm from four corners and the middle of each plot using a digger. These samples were well mixed before being placed in a Zipper bag containing approximately 200 g of soil for laboratory analysis. Soil pH was determined by using a pH meter (model-HM-1003) in a 1:2 ratio of the soil-water mixture. Before taking the measurement, the pH meter was calibrated with a buffer solution of known pH (pH 4 and pH 7). Following that, 50 ml of distilled water was poured into 25 g of soil. A magnetic stirrer was used to stir the mixture for up to 30 minutes before allowing it to settle for 5 minutes. The electrode was dipped into the mixture and the result (pH) value was recorded. Triplicate readings were taken from each soil sample. Soil moisture content was determined by using the formula (Zobel *et al.*, 1987). For the calculation of the moisture content in the soil, clean and dry crucibles were taken. A 10 g fresh soil sample from each sample was heated in a hot air oven at 105˚C for 48 hours. Then the crucible was cooled thoroughly and weighted again.

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

Results

Macrofungal diversity

In total, 70 macrofungal taxa were collected. Among them, 56 were identified at the species level and 14 were identified at the generic level, of which 3 were ascomycota and 67 were basidiomycota. These fungi belong to 26 different families and 12 orders (Fig. 2). The Russulaceae family (16 species) was found to be the most dominant family, followed by Amanitaceae (10 species), Boletaceae (9 species) and so on. Among the collected macrofungal species 8 were new to Nepal (Table 1).

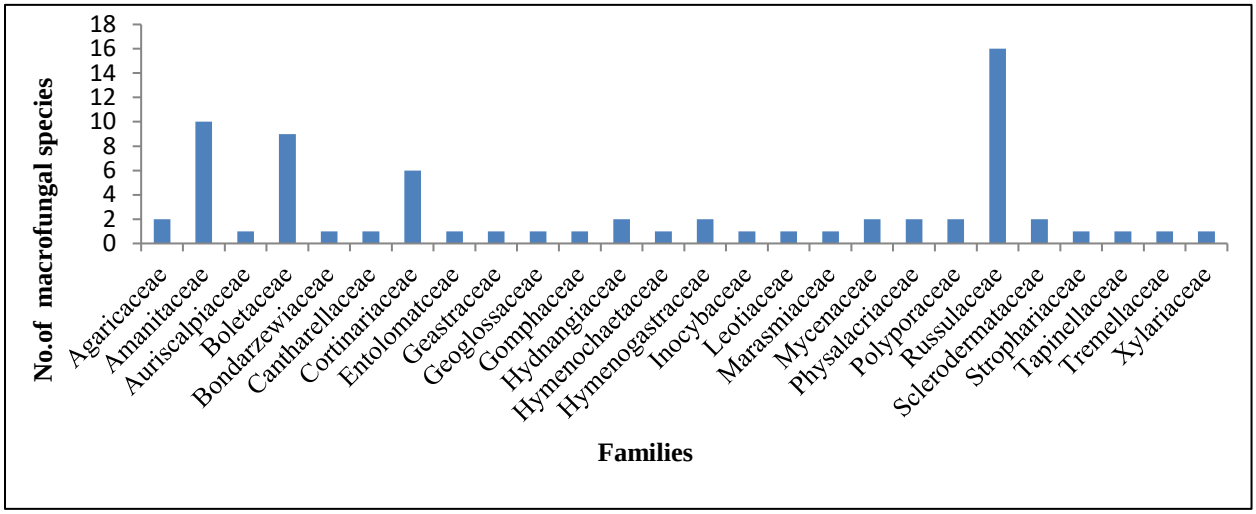

Figure 2. Distribution of macro-fungi according to families

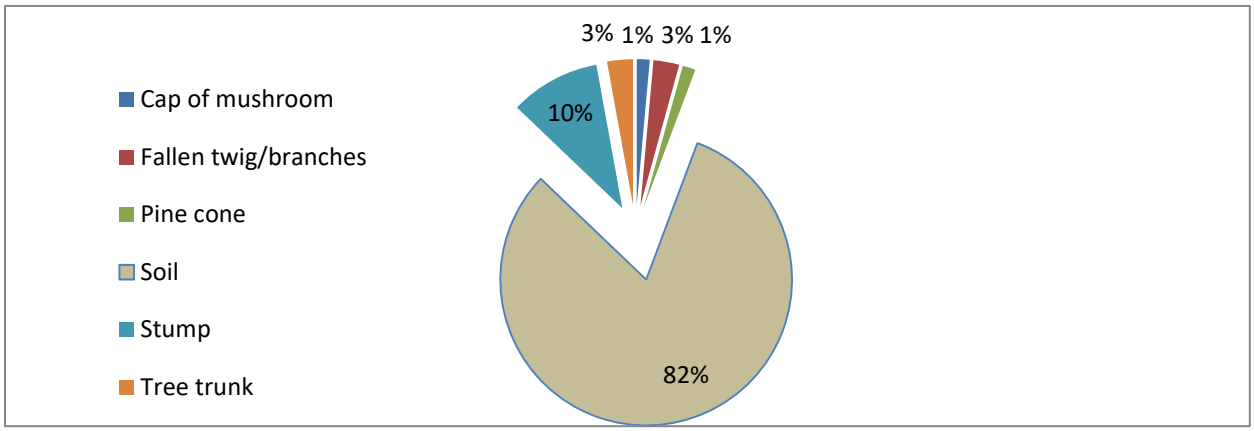

Figure 3. Distribution of macrofungi according to habitat

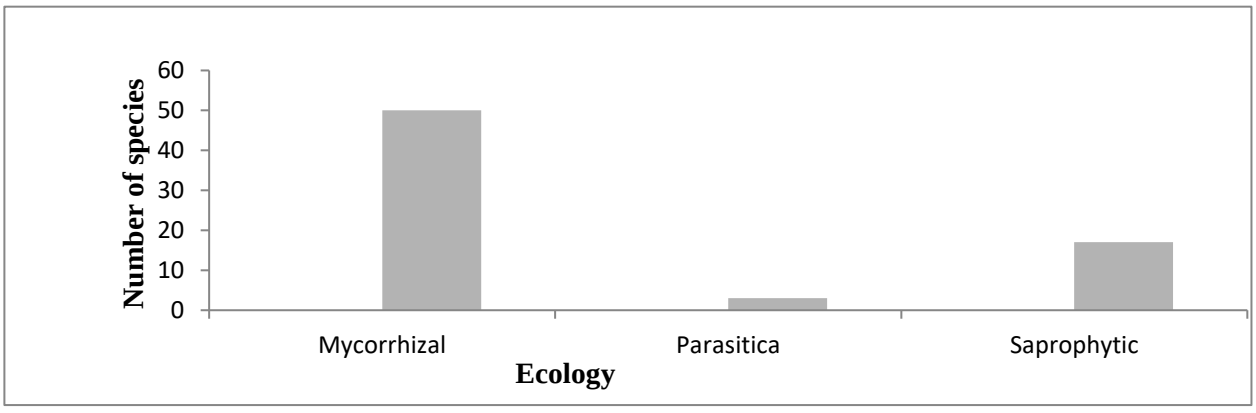

Figure 4. Distribution of macrofungi according to ecology

Relationships between macrofungal species richness and environmental variables

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

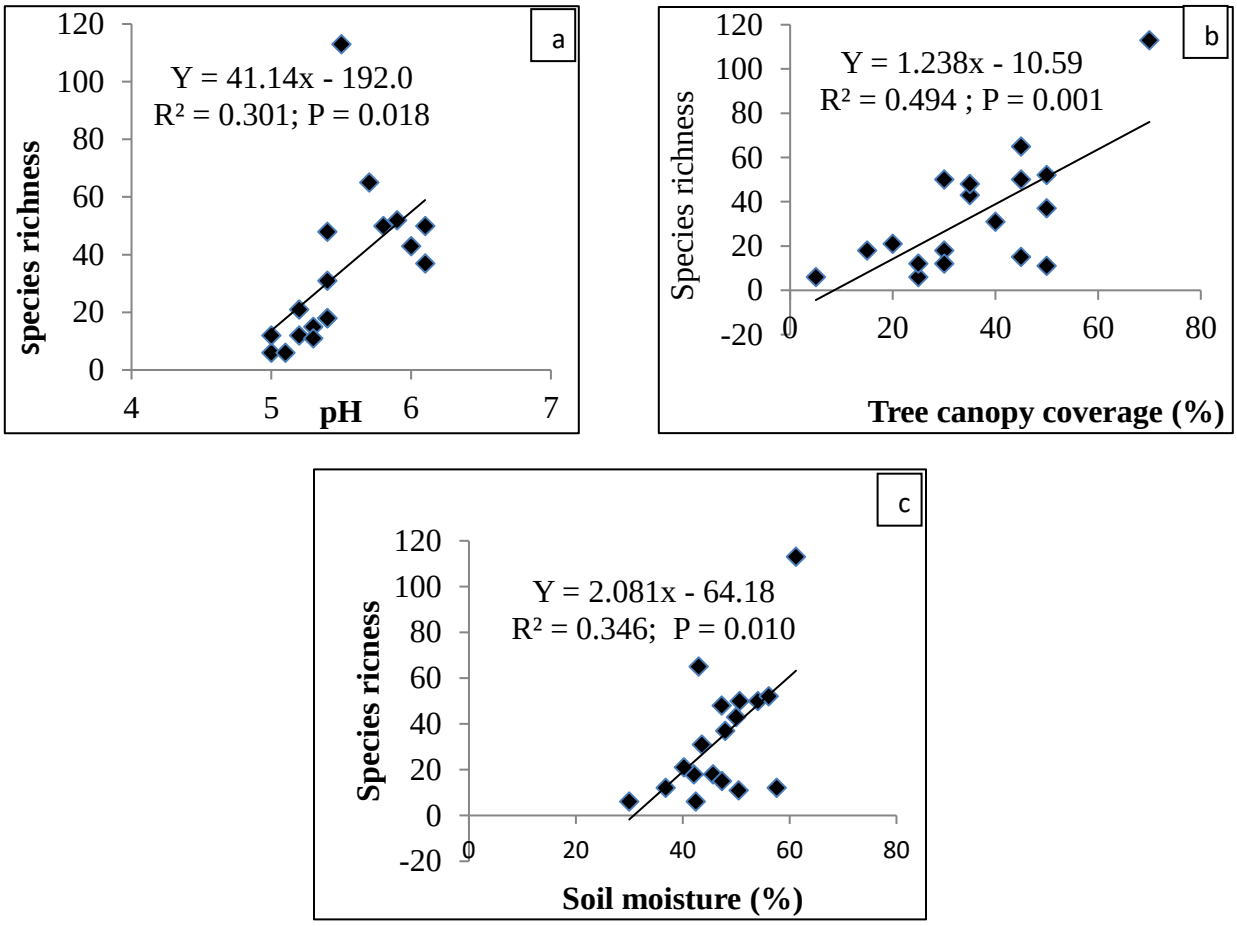

Figure 5. (a) Relationships between macrofungal species richness and soil pH; (b) Relationships between macrofungal species richness and tree canopy coverage; (c) Relationships between macrofungal species richness and soil moisture.

Discussion

Macrofungal diversity

Among the 70 species of macrofungi collected, Russulaceae was found to be the most dominant family, which is consistent with the results provided by (Shrestha *et al*., 2021). Macrofungi were found to grow in different habitats and soil was found to be the main habitat for mushroom growth. The maximum number of macrofungi was found in the soil and the remaining all were found in other habitats. The variation in mushroom species occurrences observed in different habitats might be due to their distinctive modes of nutrition (Parveen *et al*., 2017). Certain species of macrofungi are associated with specific type of trees and plants (Hawkworth, 2001). The Pinus has a strong association with ectomycorrhizal

fungi, which might be the reason the study found high mycorrhizal species. The Shannon diversity index and Simpson index were 3.49 and 0.95. These values indicate a high diversity of macrofungal species in the study area which might be because of the study area had favorable climatic conditions for macrofungal growth and development.

Relationship between the diversity of macrofungal species and environmental variables

Moisture content and soil pH are two important abiotic factors that influence fungal growth. Appearances of macrofungal fruiting bodies are highly dependent on these factors. The majorities of mushroom grow and thrive well at pH levels that are close to neutral or slightly basic (Khan *et al*., 2013). This study found pH of that community forest was 5 – 6 and most of the macrofungal species were ectomycorrhizal. (Yamanaka, 2013) found that pH values of 5 or 6 were optimal for the majority of ectomycorrhizal species. Similarly, (Zhang *et al*., 2010) found the highest macrofungal diversity in shaded forests than in more exposed/sunny forest slopes. In the present research work soil moisture was found to have positive effect on macrofungal diversity. (Trudell and Edmonds, 2004; Bhandari and Jha, 2017; Shah *et al*., 2020) also prove that fungal growth increases as soil moisture increases. Tree canopy is an important factor in habitat formation (Nakamura *et al*., 2017). Higher canopy cover provides shade and reduces moisture loss, and increased canopy cover resulted in more litter on the forest floor, which provides additional habitat for fungal growth (Gabel and Gabel, 2007). The result showed that the richness of macrofungal species increases with increased canopy cover; this finding is consistent with the findings of (Santos-Silva *et al*., 2011).

Conclusion

The Thulo Ban Community Forest of Arjam Myagdi District was rich in macrofungal species. Macrofungi species richness had positive relationship with environmental variables such as Soil pH, soil moisture and tree canopy cover. *Cantharellus cibarius* had the highest frequency of 44.44 % whereas *Mycena* sp. had the highest density of 11.88 %. Macrofungi play a crucial role in the forest ecosystem by decomposing organic matter, cycling nutrients, and forming mutualistic relationships with other plants therefore their exploration as well as conservation is needed.

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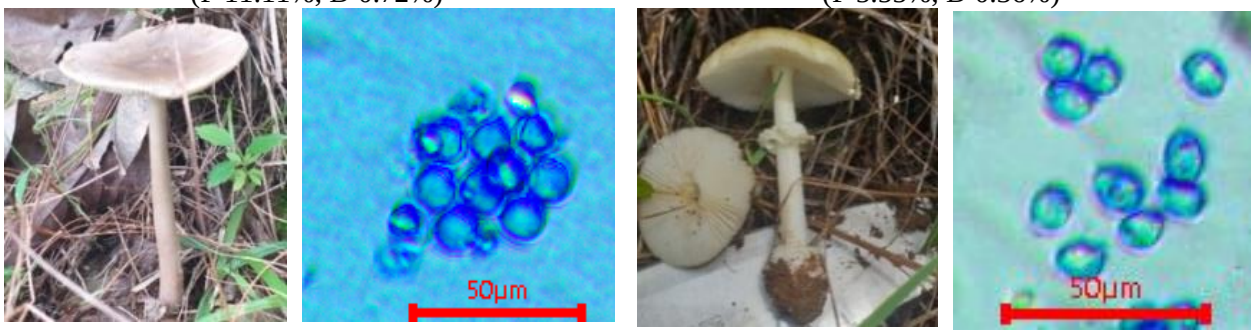
Appendix 1. List of the macrofungi with their spore frequency (F) and density (D)

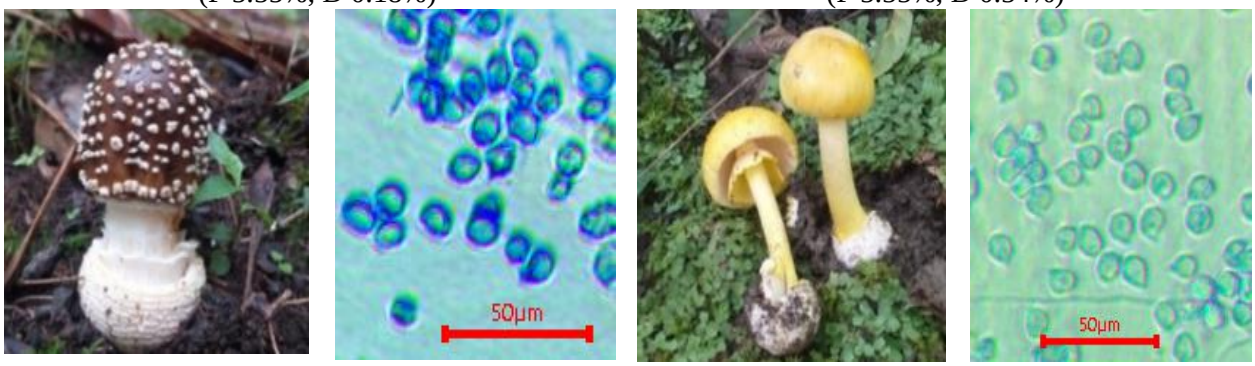
1. *Amanita abrupta* Peck
(F 11.11%, D 0.72%)

2. *Amanita caesarea* (Scop.) Pers.
(F 5.55%, D 0.36%)



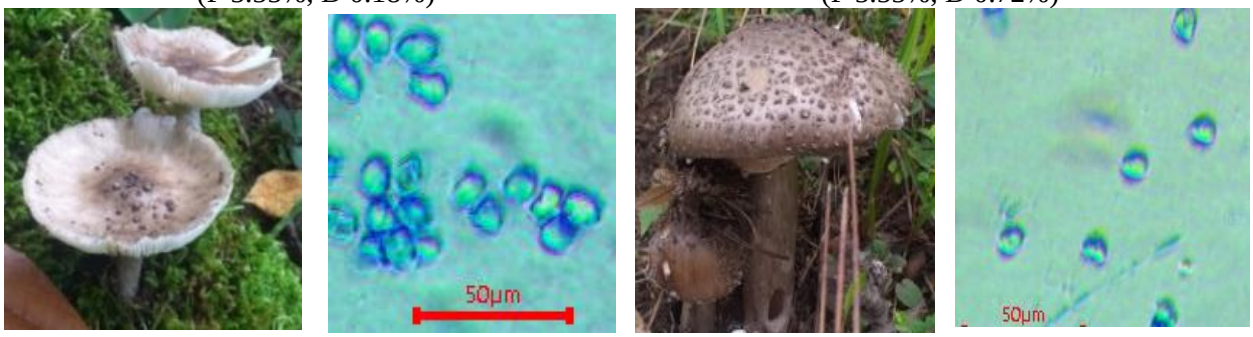
3. *Amanita fulva* Fr.
(F 5.55%, D 0.18%)

4. *Amanita multisquamosa* Peck
(F 5.55%, D 0.54%)



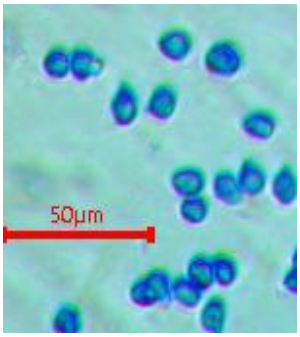
5. *Amanita pantherina* (DC.) Krombh.
(F 5.55%, D 0.18%)

6. *Amanita phalloides* (Vaill. ex Fr.) Link
(F 5.55%, D 0.72%)

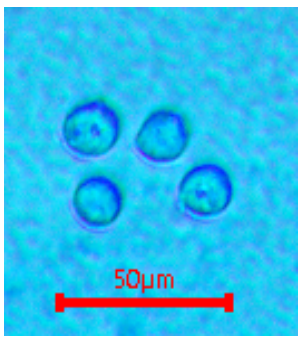


7. *Amanita porphyria* Alb. &Schwein.
(F 5.55%, D 0.18%)

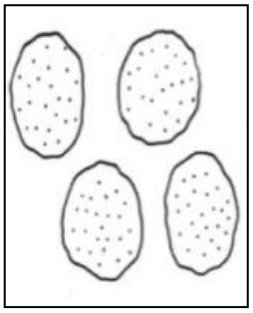
8. *Amanita rubescens* Pers.
(F 5.55%, D 0.54%)

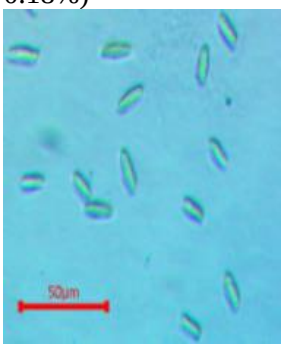
9. *Amanita spissacea* S. Imai
(F 5.55%, D 0.36%)

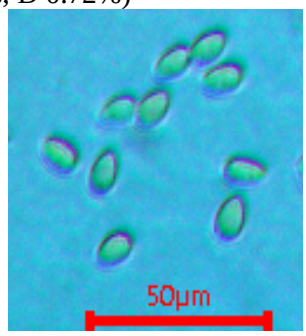
10. *Amanita vaginata* (Bull.) Lam.
(F 5.55%, D 0.18%)

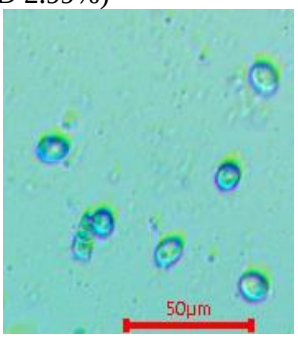
11. *Auriscalpium vulgare* Gray
(F 11.11%, D 0.72%)

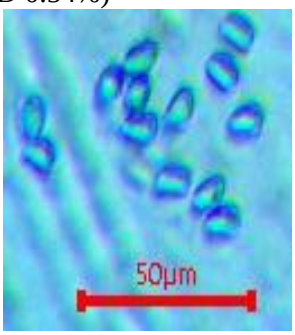
12. *Boletus edulis* Bull.
(F 27.77%, D 2.99%)

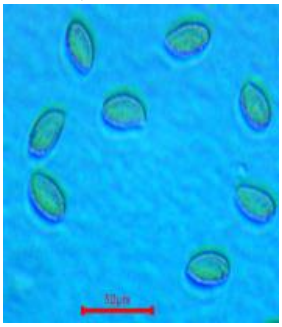
13. *Boletus eximius* Peck
(F 5.55%, D 0.54%)

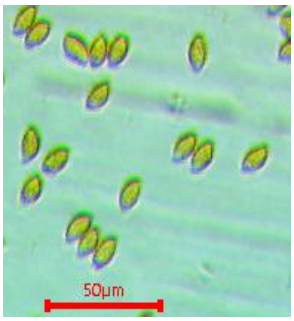
14. *Cantharellus cibarius* Fr.
(F 44.44%, D 7.92%)

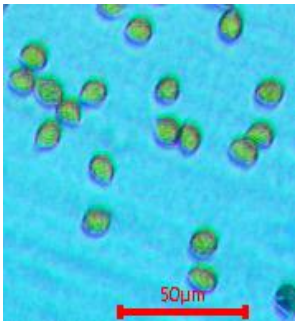
15. *Coltricia cinnamomea* (Jacq.) Murrill
(F 5.55%, D 0.18%)

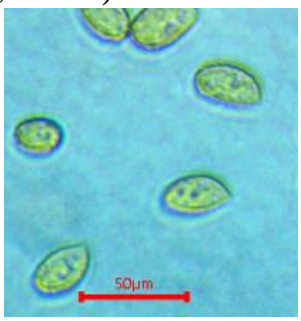
16. *Cortinarius callisteus* (Fr.) Fr.
(F 5.55%, D 0.18%)

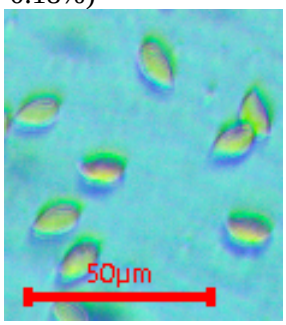
17. *Cortinarius purpurascens* Fr
(F 5.55%, D 0.9%)

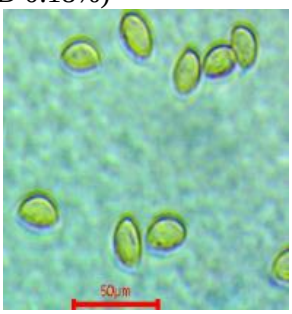
18. *Cortinarius rubellus* Cooke
(F 5.55%, D 0.18%)

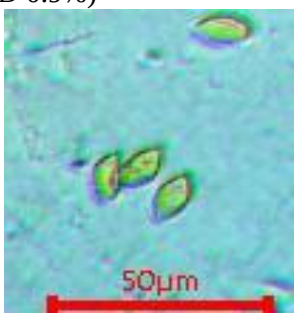
19. *Cortinarius* sp.
(F 5.55%, D 0.18%)

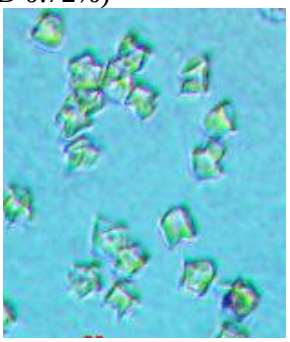
20. *Cortinarius* sp.
(F 5.55%, D 0.9%)

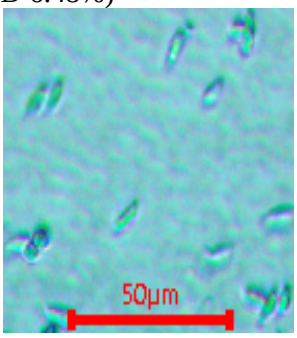
21. *Cortinarius* sp.
(F 5.55%, D 0.72%)

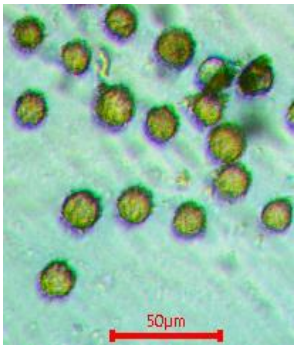
22. *Cyathus olla* (Batsch) Pers.
(F 11.11%, D 6.48%)

23. *Entoloma* sp. (F 5.55%, D 0.36%)

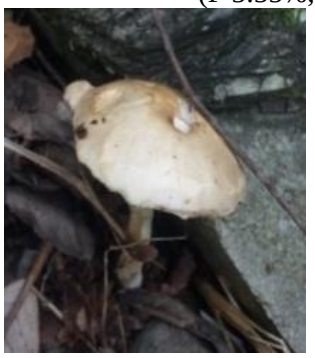
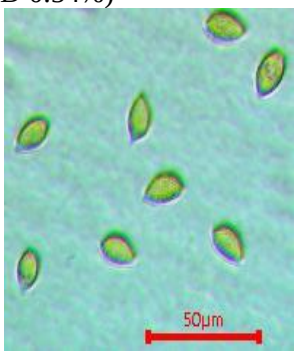



24. *Favolus* sp. (F 5.55%, D 5.76%)

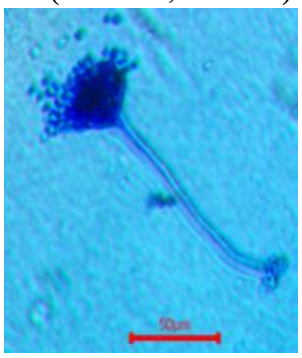



25. *Geastrum* sp.
(F 5.55%, D 0.54%)

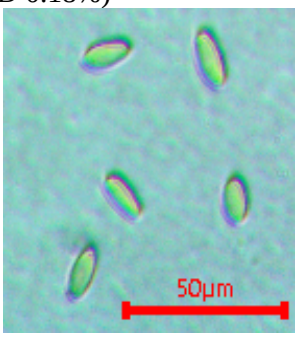

26. *Glutinoglossum glutinosum* (Pers.) Hustad, A.N. Mill., Dentinger & P.F. Cannon (F 11.11%, D 2.34%)

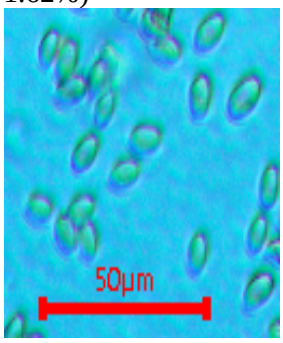
27. *Hebeloma* sp.
(F 5.55%, D 0.18%)

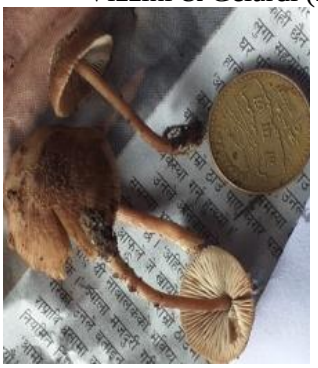
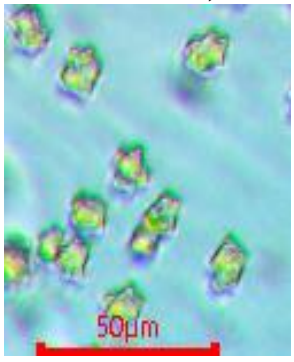
28. *Heterobasidion annosum* (Fr.) Bref.
(F 5.55%, D 1.62%)

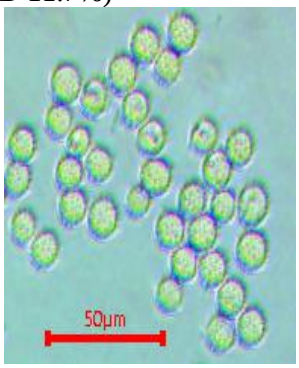
29. *Hortiboletus rubellus* (Krombh.) Simonini, Vizzini & Gelardi (F 5.55%, D 0.72%)

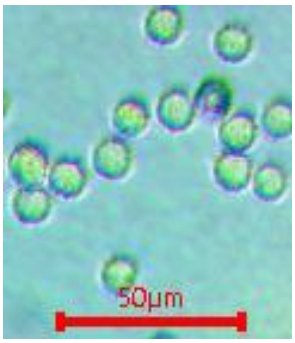
30. *Hypholoma fasciculare* (Huds.) P. Kumm.
(F 11.11%, D 11.7%)

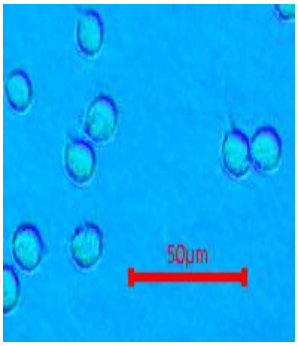
31. *Inocybe* sp.
(F 5.55%, D 0.54%)

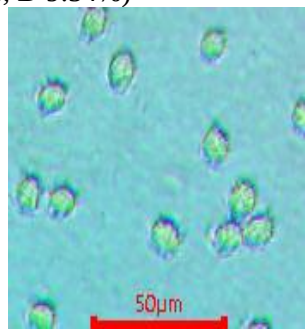
32. *Laccaria amethystina* Cooke
(F 11.11%, D 0.9%)

33. *Laccaria laccata* (Scop.) Cooke (F 38.88%, D 9.54%)

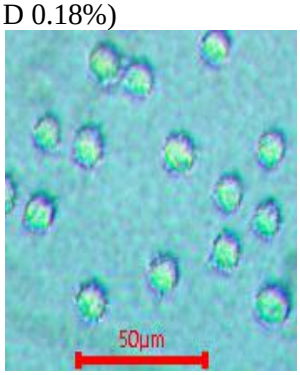



34. *Lactarius cinereobrunneus* D. Stubbe &Verbeken (F 5.55%, D 1.62%)

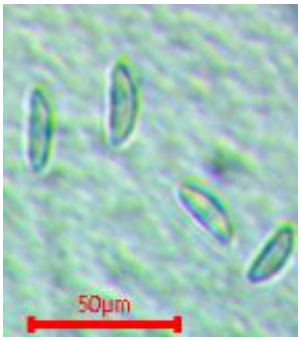



35. *Lactarius deterrimus* Gröger (F 5.55%, D 0.18%)

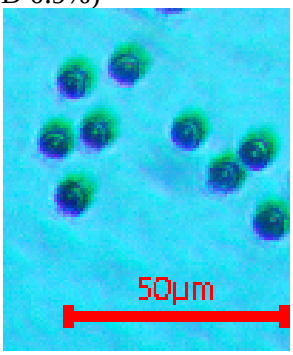

36. *Lactarius piperatus* (L.) Pers. (F 5.55%, D 1.44%)

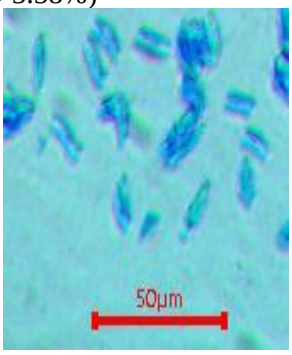
37. *Lactifluus volemus* (Fr.) Kuntze (F 5.55%, D 0.9%)

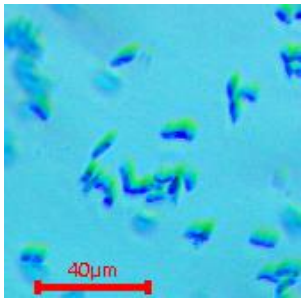
38. *Leotia lubrica* (Scop.) Pers. (F 5.55%, D 5.58%)

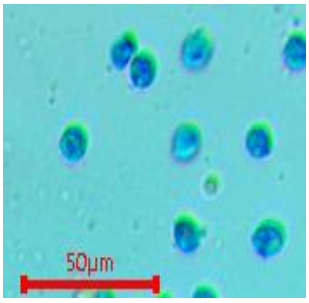
39. *Lycoperdon molle* Pers. (F 5.55%, D 0.18%)

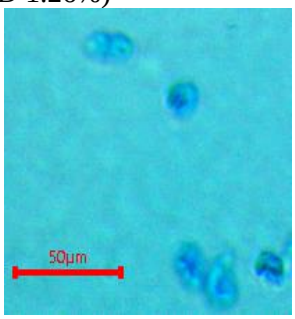
40. *Marasmius* sp. (F 5.55%, D 0.72%)

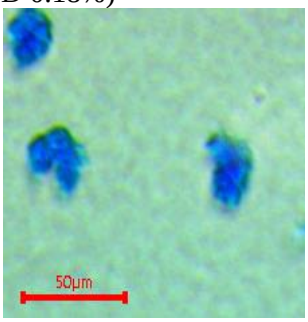
41. *Microporus xanthopus* (Fr.) Kuntze
(F 5.55%, D 1.26%)

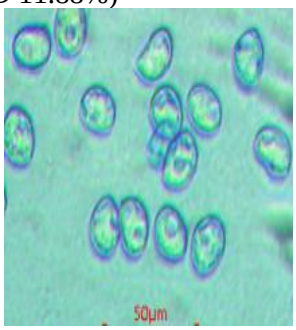
42. *Mycena* sp.
(F 5.55%, D 0.18%)

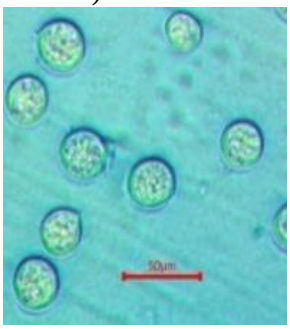
43. *Mycena* sp.
(F 5.55%, D 11.88%)

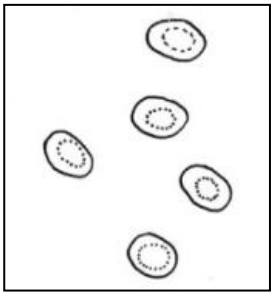
44. *Nyctalis agaricoides* (Fr.) Bon & Courtec.
(F 11.11%, D 1.08%)

45. *Oudemansiella radicata* (Relhan) Singer
(F 27.77%, D 1.26%)

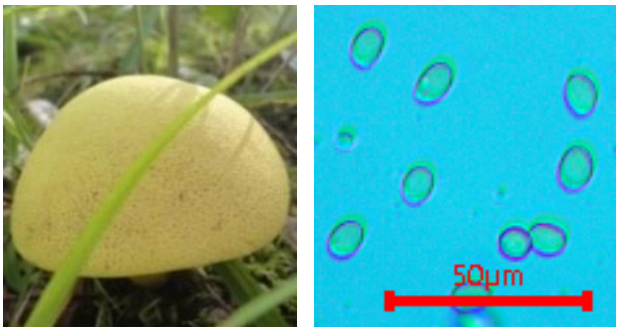



46. *Oudemansiella* sp. (Jungh.) Höhn.
(F 5.55%, D 0.54%)

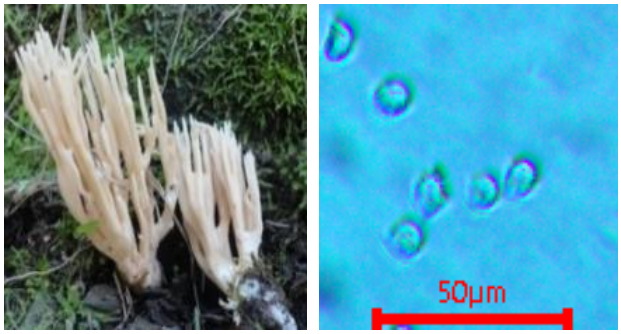



47. *Phellinus tremulae* (Bondartsev) Bondartsev & P.N.B (F 5.55%, D 0.54%)


48. *Phylloporus rhodoxanthus* (Schwein.) Bres.
(F 5.55%, D 0.36%)

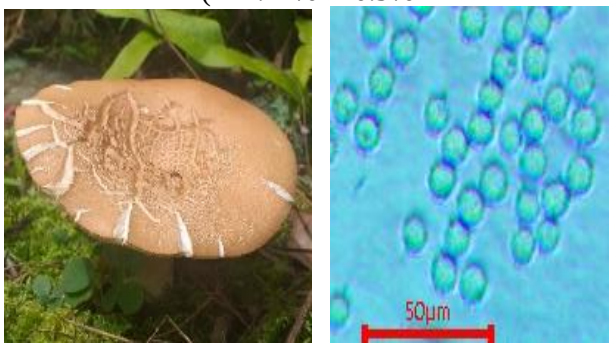


49. *Pulveroboletus ravenelii* (Berk. & M.A. Curtis) Murrill (F5.55% D0.54%

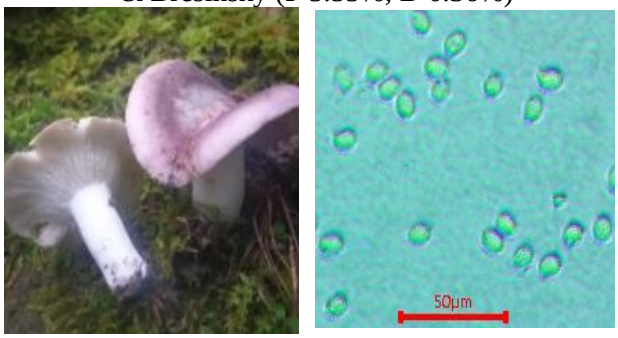


50. *Ramaria stricta* (Pers.) Quél. (F11.11% D0.9%

51. *Retiboletus nigerrimus* (R. Heim) Manfr. Binder & Bresinsky (F 5.55%, D 0.36%)

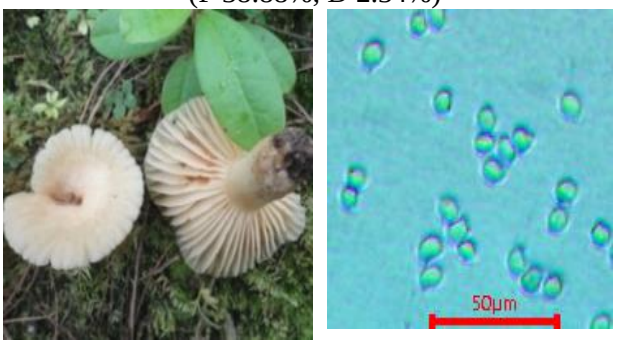


52. *Russula compacta* Frost (F 11.11%, D 0.72%)



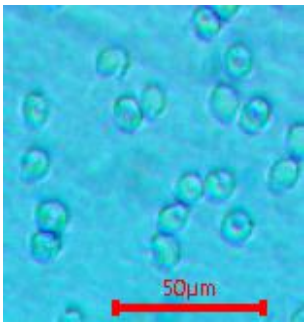
53. *Russula cyanoxantha* (Schaeff.) Fr. (F 38.88%, D 2.34%)

54. *Russula densifolia* Secr. ex Gillet (F 5.55%, D 0.36%)



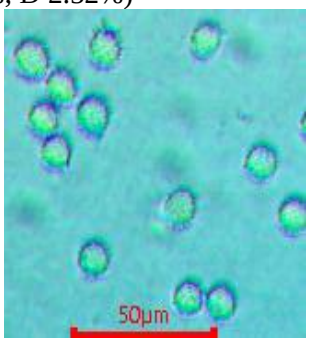
55. *Russula earlei* Peck (F 5.55%, D 0.72%)

56. *Russula emetica* (Schaeff.) Pers. (F 5.55%, D 0.36%)

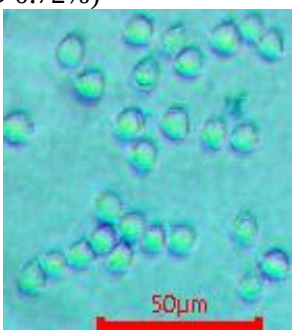



57. *Russula nigricans* Fr.
(F 27.77%, D 2.52%)

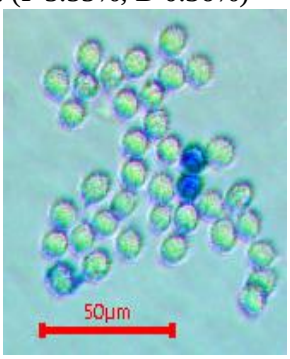

58. *Russula ochricompacta* Bills & O.K. Mill.
(F 5.55%, D 0.72%)

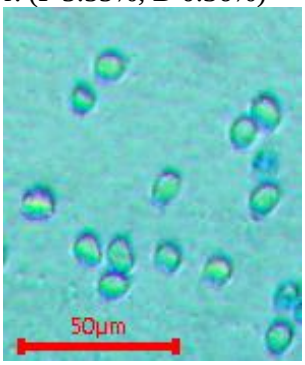
59. *Russula rosea* Pers. (F 5.55%, D 0.36%)

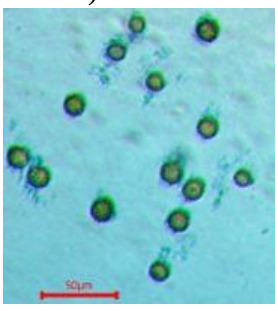
60. *Russula sanguinea* Fr. (F 5.55%, D 0.36%)

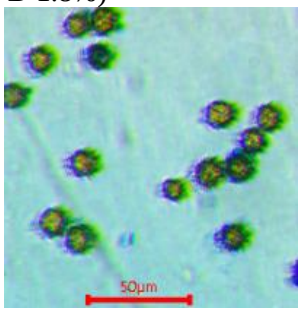
61. *Russula* sp.
(F 11.11%, D 0.9%)

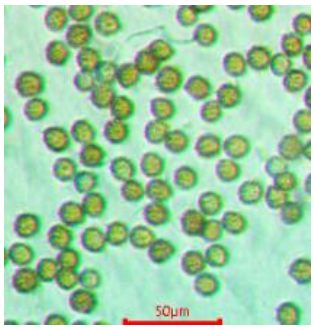
62. *Russula virescens* (Schaeff.) Fr.
(F 33.33%, D 1.8%)

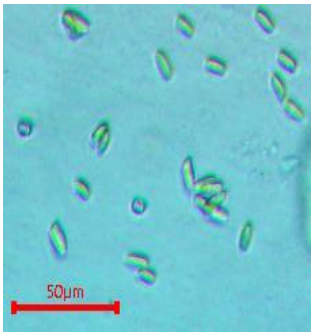
63. *Scleroderma cepa* Pers.
(F 27.77%, D 1.8%)

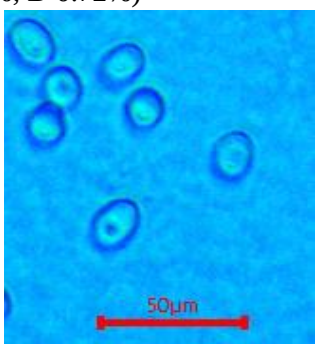
64. *Scleroderma verrucosum* (Bull.) Pers.
(F 5.55%, D 0.18%)

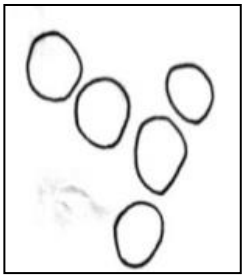
65. *Strobilomyces strobilaceus* (Scop.) Berk.
(F 11.11%, D 0.72%)

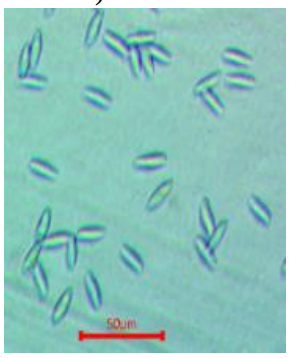
66. *Suillus granulatus* (L.) Roussel
(F 11.11%, D 1.08%)

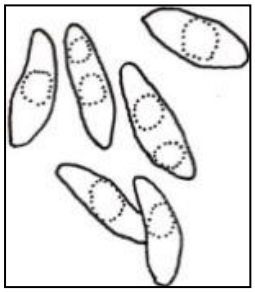
67. *Tapinella panuoides* (Fr.) E.-J. Gilbert
(F 5.55%, D 2.34%)

68. *Tremella mesenterica* Retz.
(F 5.55%, D 1.08%)

69. *Tylopilus* sp.
(F 5.55%, D 1.08%)

70. *Xylaria polymorpha* (Pers.) Grev.
(F 5.55%, D 0.54%)