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DIVERSITY, DOMESTICATION, AND PRESERVATION OF WILD MUSHROOMS IN BIODIVERSITY HOTSPOT OF THE VINDHYAN FOREST, NORTHERN INDIA

Pramod Kumar Singh*, Surabhi Srivastava and Shreyashi Srivastava

Department of Botany, Udai Pratap Autonomous College, Varanasi, U.P., India

*Corresponding author E-mail: guidepk75@gmail.com

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ABSTRACT

Wild mushrooms are abundant in the Vindhyan forest and Hathinala biodiversity hotspot; however, they are underutilized despite their relevance to staple diet. The Vindhyan forest is located in eastern Uttar Pradesh, and the Hathinala biodiversity hotspot is integral part of the Vindhyan forest. But the abundance of fungi in these woods is facing extinction because of human activity and pollution. The purpose of this study was to collect, identify, and cultivate mushrooms from the Hathinala biodiversity hotspot within the Vindhyan forest. We randomly collected, identified, dried, and stored wild mushroom samples to observe species richness. We compared the physical properties of edible and wild mushrooms to those described in the literature. In this investigation, we collected and examined 32 different mushroom species. The storage and preservation of edible mushrooms are critical aspects of ensuring their safety, quality, and nutritional value by using different preservation methods such as formalin solution, freezing, and drying. Culture preservation aims to maintain phenotypic, genotypic and physiological traits in mushroom cultures likes *Pleurotus* species for extended periods. Most short-term storage methods involve storing tubes at ambient temperature (28-35ºC) for 1-2 months or in a refrigerator (5-8ºC) for 3-4 months. Frequent sub-culturing is necessary for this method. Storage of mushroom cultures on wheat grain in a refrigerator at low temperatures (5-8ºC) was examined. Mushroom cultures could be kept at low temperatures on contamination-free wheat grain for over a year without changing their growth or shape.

***Key words*:** Mushroom diversity, preservation, *Pleurotus species*, culture media, and biodiversity hotspot.

Introduction

Fungi are one of the largest and most dominant kingdoms in the world, represented by unicellular to multicellular filamentous forms, ranging from simple mycelia to complex fruiting bodies, such as mushrooms, whose fruiting is apparent to the naked eye. These organisms represent approximately 10% of the total global fungal diversity. These fungi belong to two major phyla (Ascomycota and Basidiomycota) out of seven recognized phyla. Ongoing research is being conducted to improve our understanding of the species that live on Earth. Conservative estimates indicate that hundreds of thousands of species remain without formal scientific classification (Coastello *et al*., 2012). In this discussion, fungi are frequently disregarded; nonetheless, it has lately been suggested that they should be included in global conservation goals alongside plants and animals (Cao *et al*., 2021; Goncalves *et al*., 2021). There are approximately 135,000 species of these organisms that are now known (Hibbett *et al*., 2016), out of an estimated 2.2–6.0 million species (Taylor *et al*., 2014; Hawksworth and Lucking, 2017). This makes them one of the categories of organisms with the greatest amount of biodiversity that has not yet been investigated. In Uttar

Pradesh also particularly in Eastern U.P peoples indicated that a little interest and have a tiny background for mushroom farming and consumption. Mushrooms are particularly perishable food items that tend to lose their unique organoleptic qualities immediately after harvesting. Their short shelf-life is mainly explained by the substantial losses of water vapour that encourage dehydration, high respiration and microbial colonization by bacteria or fungi. Furthermore, enzymatic activity and biochemical modification lead to mushrooms quality losses (Simon, A.E. Gonzalez-Fandos, Tobar, V. 2005). Fungi represent a significantly potential but inadequately investigated category that necessitates thorough documentation. Nonetheless, habitat degradation, urban expansion, infrastructure development, mining activities, deforestation for agriculture, excessive grazing, contamination, and industrial growth pose significant dangers to them (Botanical Survey of India). A total of 32 species from 26 genera were recognized during the field survey. This collection comprised wild varieties of Pleurotus, Hexagonia, Ganoderma, Irpex, Trametes, Podoscypha,. Fomitopsis, Calvatia, Macrolepiota, Calocycbe, Coprinellus, Clitocybe, Termitomyces, Schizophyllum, Agaricus Marasmiellus, Marasmius, Mycena, Coprinopsis, Leucocoprinus, Coprinus, Micromphales, Lentinellus, Fuscoporia, Collybia and Dacrymyces. These species were predominantly located in the Hathinala biodiversity hotspot from the Vindhyan regions of eastern Uttar Pradesh. Despite their potential, fungi remain inadequately documented. The reduction of specific mushroom species is attributed to natural occurrences like wildfires, avalanches, landslides, the succession of host trees, and climate change. These extinct species might possess industrial, gastronomic, and medicinal applications that humanity currently does not recognize. Wild mushrooms are a synthetic yet beneficial collection of fungi whose fruiting bodies are visible to the unaided eye. Eastern Uttar Pradesh lies in the part of Uttar Pradesh (UP) between 23°51’ N and 28°30’ N and 81°31’ E to 84°39’ . Vindhya Range is located at 23°28’ 00” N, 79°44’ 25” E and is a 1,200 km long range of hills in central India, forms a natural boundary between North India and the Deccan Plateau, and is rich in geology and rivers. Winter temperatures often sit between 10°C and 25°C. Summers can climb beyond 40°C in exposed plateau regions. Hathinala is located at 24°17’59.76” N to 83°06’25.40” E in Sonabhadra District. It has an average elevation of 283 metres (931 feet) (UP Ecotourism, Government of Uttar Pradesh, n.d.). Preservation is the process of permanently preserving items in metabolically inactive states. For successful storage, the culture’s morphological, physiological, and genetic characteristics must remain unaltered, and the purity, viability, and genomic integrity of the culture must be preserved. Different methods have been developed over the years for the preservation of cultures. These include sterile distilled water, cryopreservation, freeze-drying, sub-culture and sterile mineral oil. These methods enable for storage of strains of different microorganisms for long periods under suitable conditions (Castro-Rios, K., and Bermeo-Escobar, L.P. 2021). Generative shifts and contamination complicate the preservation of civilizations (Chang & Miles, 2004; Latha *et al*., 2022). The primary goal of culture preservation is to maintain the viability and stability of cultures over long periods of time without compromising their phenotypic, genotypic, or physiological characteristics. Currently, the standard practice for short-term storage of mushroom cultures involves keeping the culture tubes at room temperature (28-35ºC) for 1-2 months or in the refrigerator (5-8ºC) for an average of 3-4 months (Veena, S.S., Meera P. 2010; Aguilar *et al.*, 2024). However, this method requires frequent sub-culturing, which can lead to contamination and degeneration. Therefore, the present study aimed to develop a simple, inexpensive, and straightforward method of preserving mushroom cultures. We investigated whether it would be possible to store different mushroom cultures on wheat grain in a refrigerator at low temperatures (5-8ºC) or whether mushroom culture required cryopreservation. Mushroom strains are more effectively kept on grain media compared to agar media during cryopreservation (Pathak, 2018). The results demonstrated that the mushroom cultures could be kept at low temperatures on contamination-free wheat grain for over a year without experiencing any changes in growth and morphology. Collection, identification, and preservation of the mushrooms, as well as their germplasm, are necessary to conserve them from extinction and for future studies (Matilda, D. *et al.*, 2023).

Materials and Methods

Study Area

The fruiting bodies were gathered from Hathinala Biodiversity hotspot of the Sonabhadra district of Eastern Uttar Pradesh (Fig. 1). The rainy season (July-September 2025) was the time for the survey. Sterile polyethylene bags were used to store the collected samples for future research. We combed through secondary forests, undisturbed woodlands, agricultural fields, bush fallows, and farmers’ trails to find places to collect. Using a digital camera, we documented and photographed the morphological features of macrofungi and their fruiting bodies in their native environment.

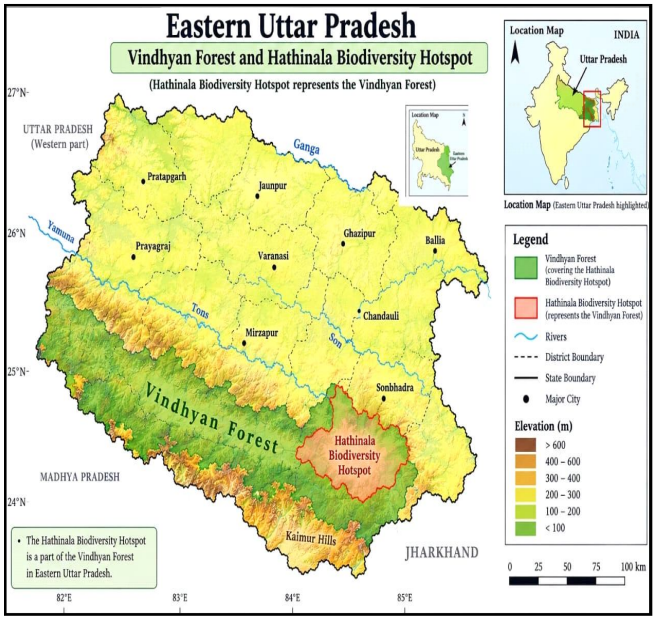


Fig. 1: Hathinala Biodiversity Hotspot represent the Vindhyan forest of Northern India: Various wild mushrooms depicted on the map represent the richness of mushroom species. **Source**: SRTM DEM, Survey of India, FSI, BSI.)

Physical and photographic characterization of edible and wild mushrooms

The survey examined the physical features of edible and wild mushroom species from study site of the Vindhyan forest. Samples were sorted by principal taxa and dried in a homemade field dryer. They went into a herbarium. The mushrooms were photographed with a GPS camera. Location, veil remnants, detachable scales, fibrils, volva, and diagnostic features were also recorded. Different edible and wild mushroom species identified from survey investigations and the locations were examined for physical qualities such as cap form, gills or holes, volva, etc. The genus was discovered by comparing descriptions to literature.

Laboratory studies on mushroom features

A hand lens and light microscope were used to look at the following features of mushrooms, such as the shape, edge, and size of the cap in both longitudinal and vertical part, as well as color and decoration. Hymenophore (gills or pores): shape from below surface, gill edges, color, and spore print. Insertion, consistency, size, shape, color, and decoration of the weave. What the flesh is made of, its color, smell, and taste.

Mushroom preservation techniques

The vibrant, pure, and viable morphology of many species requires complementary and unique alternative methods for color, texture, and flavor. By following preservation techniques, cultures can be kept viable and stable for extended periods of time without compromising their physiological, phenotypic, or genotypic characteristics.

Preservation in formalin (40 %) solution

Thirty samples of macrofungi were taken to the lab. Samples were cleaned with 0.1% $HgCl_2$ for 40-45 seconds, washed with distilled water, and then soaked in 70% ethanol for a few seconds after proper cutting. Samples were stored at 28-35ºC in culture tubes or phyto-jars with 40% formalin solution.

Cryopreservation (freezing at -20°C and storage in liquid nitrogen [-196°C])

The macrofungal specimens were sectioned using a surgical scalpel. Following appropriate trimming and sterilization, they were preserved using cryopreservation (liquid nitrogen at -196°C) and low-temperature storage (+4°C and 20°C).

Drying technique: medical blade or a chisel and hammer were used to cut out the macrofungal samples of mushrooms species. After the right amount of trimming, samples were sent to a gas laboratory oven (LO) for drying and the sun to dry (SD).

Culture Preparation: Potato dextrose agar was used to culture several mushrooms *in vitro*. Each mushroom's mycelium disks, which were 1 cm in diameter, were aseptically inserted in Petri dishes with 20 mL of culture medium. The dishes were incubated in the dark at 28°C. Mycelium diameter was measured every two days for eight days. Previous sources say these mushroom spawns were grown on wheat. Several mushroom species were grown on Potato Dextrose Agar (PDA) in 26°C–30°C incubators. The spawn was kept in cells at 26-28°C and 60-65% relative humidity.

Spawn Preparation: As with seeds of crop plants, mushroom cultivation starts from the vegetative hyphae as seed material known as "spawn" (Kligman 1950). In the mushroom industry, both the vegetative body (mycelium) and planting material (substrate) are called spawn. Mushroom spawn was prepared using an altered method of spawn preparation (Hassan & Ibrahim, 2023). They were fastened together with rubber bands. The bottled grains were sterilized in an autoclave at 121°C for 1 h at a pressure of 15 psi.

Agro-industrial waste as a substrate for mushroom cultivation

The most common agro-industrial waste used as a substrate is wheat straw, rice straw, sawdust, sugarcane bagasse, corn residue, cotton seed waste, etc. (Jandaik and Kapoor 1975).

Table 1: Phylogenetic representation of mushrooms in Vindhyan forest: Different species of mushroom was taxonomically classified up to the level of orders.

Class	Order
Agaricomycete	**Polyporales**
Pleurotus florida, P.ostreatus, P.pulmonarius, P;sajor-caju, Hexagonia tenuis, Ganoderma polychronum, Irpex lacteus, Trametes versicolor, Podoscypha petalodes,. Fomitopsis spp., Trametes elegans,. Calvatia gigantean	
Macrolepiota procera, Calocycbe indica, Coprinellus micaceus, Clitocybe robusta , Termitomyces heimii , Schizophyllum commune, Agaricus bisporus Marasmiellus candidus, Marasmius siccus, Mycena galericulata, Agaricus arevensis, Coprinopsis lagopus,. Leucocoprinus cepestipes, Leucocoprinus cretaceus , Coprinus comatus,	**Agaricales**
Micromphales foetidum	**Auriculariales**
Lentinellus sp.	**Russulales**
Fuscoporia gilva	**Hymenochaetales**
Basidiomycetes *Collybia confluens*	**Agaricales**
Dacrymycetes *Dacrymyces spathularia*	**Dacrymycetales**

Substrates Preparation: The study utilized four isolates of *Pleurotus ostreatus, P.pulmonarius, P. sajor-caju, P. florida* (edible oyster mushrooms). After boiling the wheat grains for two hours in a grain boiler, washing them with enough water came next. 4% chalk powder (calcium carbonate) was added to cooked grains (4g/ 100g grain) to absorb moisture, remove starch stickiness, and make the grain free-flowing. The grains were carefully placed into 15ml Borosil screw-cap vials until two-thirds full. The vials were autoclaved at 121ºC for two hours at 15 lb pressure. We cooled the vials after autoclaving. In order to extract agar (PDA medium) from 90 mm diameter sterile culture plates, a 5 mm diameter mycelial plug was placed in the middle of each plate. Plates were stored at 30 and 35ºC in the dark. A sterile cork borer removed 5 mm mycelial disks off the developing cultures' margins when they had developed enough. Four disks were aseptically transferred to cooled jars. Inoculated jars were stored in a BOD incubator at the correct temperature. Growth was monitored daily, and the tubes were refrigerated after 7–10 days of grain colonization

Table 2: Growth temperature regimes for selected mushroom species assessed *in vitro*.

Isolates	Temparature range	Optimum temp-arature range
Pleurotus ostreatus	**25-35**	**30**
Pleurotus pulmonarius	**25-35**	**30**
Pleurotus florida	**25-35**	**30**
Pleurotus sajor-kaju	**25-35**	**30**

(Graca *et al*., 2015). Analysis of recovered growth in PDA culture medium. Two months after refrigeration, the grains were restored to room temperature (25-30°C). Each isolate grain was added to media. Regrowth of vegetative hyphae on PDA plates determined the isolates' viability before and after preservation. Calculated and compared growth rates.

Results and Discussion

Mushroom diversity in Hathinala biodiversity hotspots of the Vindhyan forest

Hathinala biodiversity hotspot was the site of mushroom sample collection. Thirty-two mushrooms were collected from woodlands such as forests and plantations, as well as farmlands and grasslands of the Vindhyan forest. Images of the samples illustrating their morphological features are shown in Fig. 2. Table 1 shows the phylogenetic representation of 32 mushroom species, which belonged to the following classes: Agaricomycetes, Basidiomycetes, and Dacrymycetes. Order: Polyporales, Agaricales, Auriculariales, and Hymenochaetales . The most dominant family are Agaricaceae having 7 species, followed by Pleurotacea 4 species, Polyporaceae 3 species; Psathyrellaceae, Lyophyllaceae, Clitocybaceae and Omphalotaceae having 2 species; Dacrymycetaceae, Fomitopsidaceae, Irpicaceae, Ganodermataceae, Podoscyphaceae, Schizophyllaceae, Marasmiaceae, Mycenaceae, Auriscalpiaceae and Hymenochaetaceae having 1 species.

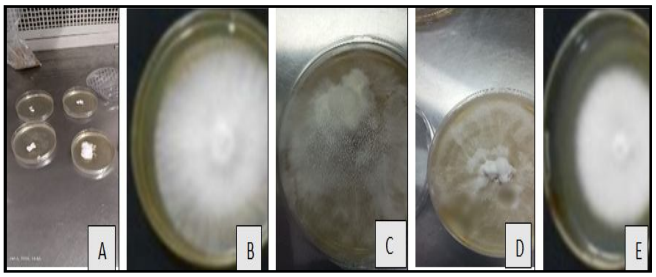


Plate 1: Restoration of mycelial growth of *Pleurotus* species. (A) Freshly inoculated plates of four *Pleurotus species*. (B), *Pleurotus ostreatus*, (C). *P. pulmonarius*, (D). *P.florida* (E). *P. sajor-caju*: Growth patterns varied from cotton like fluffy (*P. ostreatus* and *P. florida*) to spiral (*Pleurotus pulmonarius* and *Pleurotus sajor-caju*).

Growth properties of strains on mycological media

Four edible mushrooms were selected for proper culture and growth on different temperature. These species were grown on Potato Dextrose Agar (PDA) on 26°C, 28°C, and 30°C. Tropical mycelial isolates generally grew well under optimal growth conditions.

Various temperature regimes for selected mushroom species

Four edible mushroom strains were isolated from the Vindhyan forests and incubated at 26 °C, 28 °C and 30°C. Plates of all four strains of mushrooms newly inoculated on mycological media and cultured at 26°C. The experiment was carried out in triplicates. Plate 1 shows that the four mushroom strains completely colonized the media in an average of 6 days. The growth pattern spanned from fluffy growth which was observed in different *Pleurotus* species.

Mycelia growth on PDA plates at 26°C: The *Pleurotus ostreatus* strain covered the mycological media in five days with an average of growth rate of 2.73 cm per day at 26°C. It was succeeded by *Pleurotus pulmonarius* strain, which colonized the media in six days with an average daily growth rate of 2.63 cm. *Pleurotus sajor-caju* strain covered the medium in six days with an average growth rate of 2.66 cm. The strain *Pleurotus florida* had an average daily growth rate of 2.75 cm and covered the media in six days.

Mycelia growth on PDA plates at 28°C: *Pleurotus ostreatus* and *P.pulmonarius* strains covered mycological media in five days, with daily average mycelial growth rates of 2.81 cm and 2.72 cm. That makes them the fastest-growing strains at that temperature.

Pleurotus sajor-caju strain (2.94 cm) and *Pleurotus florida* strain (2.80 cm) covered mycological media in six days.

Mycelial growth on PDA plates at 30°C**:** *Pleurotus sajor-caju* strain exhibited the most rapid coverage of the media by the fourth day, achieving an average daily growth rate of 2.84 cm. Subsequently, the *Pleurotus ostreatus* strain and the *Pleurotus pulmonarius* strain



Plate 2: Stored Spawn with wheat grain of different *pluerotus species after one year*: (E). *P. ostreatus* (F), *P. pulmonarius* (G), *P.florida* (H). *P. sajor-kaju.*



Fig. 2: Mushroom biodiversity and their documentation in Vindhyan forest of Eastern Uttar Pradesh. [1. *Schizophyllum commune*,2. *Pleurotus florida*, 3. *Irpex lacteus*, 4. *Macrolepiota procera*, 5. *Calocycbe indica* 6. *Trametes versicolor*,7. *Fuscoporia gilva*, 8. *Micromphales foetidum* 9. *coprinellus micaceus*, 10. *Clitocybe robusta* 11. *Podoscypha petalodes*,12. *Termitomyces heimii* 13. *Ganoderma polychronum*, 14. *Pleurotus ostreatus* 15. *Hexagonia tenuis*, 16. *Agaricus bisporus* 17. *Pleurotus pulmonarius* 18. *Pleurotus sajor-caju*, 19. *Marasmiellus candidus*, 20. *Marasmius siccus*, 21. *Fomitopsis spp*. 22. *Mycena galericulata*, 23. *Agaricus arevensis* 24.*Trametes elegans*, 25. *Coprinopsis lagopus*, 26.*Leucocoprinus cepestipes*, 27.*Leucocoprinus cretaceus* 28. *Lentinellus sp*. 29. *Dacryopinax spathularia*, 30. *Coprinus comatus*, *31. Collybia confluens, 32.Clavita gigantea*].

Table 3: Mycelial growth of several mushroom isolates *in vitro* both before and after storage: All examined species were successfully recovered in a viable state using this technique, even after a year of storage.

Isolate	Growth (mm/day) before storage	Growth (mm/day) after 1 year
Pleurotus ostreatus	14.4	14.7
Pleurotus pulmonarius	14.0	14.3
Pleurotus florida	13.6	13.7
Pleurotus sajor-caju	12.0	12.7

occupied the medium within five days, exhibiting average growth rates of 2.78 cm and 2.86 cm, respectively. The *Pleurotus florida* strain required six days to fully colonize the medium, exhibiting a daily growth rate of 2.97 cm.

Mycelium from tropical isolates grew well on artificial media with optimal temperature, aeration, and humidity conditions. All of the cultures used in this study were tropical, with maximum mycelial development occurring between 26 and 30° Celsius. The probable reason for the temperature-based growth of mushroom mycelium on wheat grain may be either because of protection given by media or the innate quality of the grains.

Mycelial growth restoration of *Pleurotus* species:

A year of storage did not affect the average mycelial growth of the species that were studied (Plate 2). Bacterial contamination was not found in any of the spawn bags.

As with newly grown mycelium, cultures may be easily revived, and mycelial development might begin from the wheat grain within one to two days of inoculation (Plate 1).

The mycelial characteristics of these samples did not change. Various strains have various patterns and densities of mycelia development. The growth pattern ranged from fluffy growth as in *P. sajor-caju* and *P. florida* to spiral growth as in *Pleurotus ostreatus* strain and *Pleurotus pulmonarius* strain (although that of *Pleurotus ostreatus* strain was denser) to cotton-like as in *Pleurotus pulmonarius* strain and leathery growth as in the *Pleurotus florida* strain (Plate 1).



Fig. 3: Drying Technique of Mushrooms. A: *Pleurotus species.* B: *Ganoderma species*. C: *Hexagonia* species. D: *Trametes pubescens.*

Wild and Laboratory growth of *Pleurotus* strain:

Plate 3A. and 3a. show flushes of *Pleurotus ostreatus* strain growing in the wild and on wheat straw respectively. This strain's fruit body stipe is short, and the cap edge turns brownish with age. 3b. of flushes of the *Pleurotus sajor-caju* strain cultivated on wheat straw. A notable characteristic of this strain was the curved tips of the fruiting body cap and its faint gray hue. 3B. depicts flushes of the *Pleurotus sajor-caju* strain thriving in their natural habitat. Plate 3C. *Pleurotus florida* strain growing in the wild.(3c): Flushes of *Pleurotus florida* strain fruiting on wheat straw.



Fig. 4: Preservation of mushrooms in a formalin (40%) solution. **[A.** *Trametes versicolor* **B.** *Hexagonia tenuis* **C.** *Agaricus comtulus* **D.** *Dacryopinax spathularia* **E.** *Schizophyllum commune* **F.***P.sajor-caju***, G. H.** *Macrolepiota procera,* **I.***Deconica coprophila* **J.** *P.pulmonarius* **K.** *P.florida* **L.***Coprinellus micaceus* **M.***Calocybe indica,* **N.** *Termitomyces heimii* **O.***Podoscypha petaloides,* **P.** *Agaricus arevensis* **Q.***Irpex lacteus* **R.** *P.ostreatus* **S.***Clitocybe robusta,***T.***Marasmius siccus*].

Table 4: Rate of mycelial growth of four different types of mushrooms in composted bags.

Period of colonization (Weeks)	*Pleurotus ostreatus*	*Pleurotus pulmonarius*	*Pleurotus florida*	*Pleurotus sajor-caju*
1	8.01±0.70	9.12±0.86	10.02±0.12	8.26±0.62
2	7.48±0.68	8.2±1.08	7.80±1.03	5.28±0.42
3	3.52±0.26	2.65±0.90	6.56±0.15	3.82±0.42
4	NA	NA	2.64±0.49	2.35±0.92
Growth Rate/day	1.02	0.97	0.93	0.74

Plate3D. *Pleurotus sajor-caju* strain growing in the wild. (3d): Flushes of *Pleurotus sajor-caju* strain fruiting on wheat straw.

Excluding the *P.ostreatus* and *P. pulmonarius* strains that occupied the bags in three weeks, the *P. sajor-caju* and *P.florida* strains fully colonized the bags in four weeks under identical environmental circumstances. At the conclusion of first week the *P.ostreatus* strain, which occupied 10.2 cm of the bag in the initial week, had the most rapid growth, succeeded by the *P.pulmonarius* strain, *P.florida* strain, and *P. sajor-caju* strain.

The growth rate for all strains diminished progressively as they transitioned into the second week (Table 3). A subsequent decline occurred throughout the third and fourth weeks as well (T.H. Quimio *et al.*, 1990; Dada, 2018). This is probably a result of the mycelia deteriorating the substrates, thus resulting in the formation of mushroom fruit bodies. This is collaborated by reports from others indicating that the mycelial growth rate diminished as the strains occupied the substrates (R. Upadhyay *et al.*, 2002).

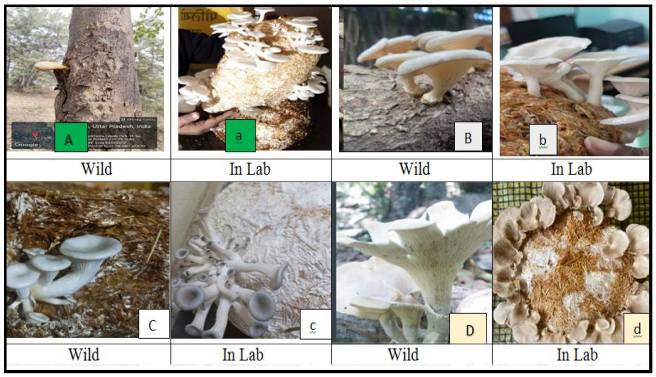


Plate 3: A. *Pleurotus ostreatus* strain growing in the wild. a: Flushes of *Pleurotus ostreatus* strain fruiting on wheat straw after six weeks of incubation. B. *Pleurotus pulmonarius* strain growing in the wild. b: Flushes of *Pleurotus pulmonarius* strain fruiting on wheat straw after six weeks of incubation. C: *Pleurotus florida* strain growing in the wild. c: Flushes of *Pleurotus florida* strain fruiting on wheatstraw after six weeks of incubation. D: *Pleurotus sajor-caju* strain growing in the wild. d: Flushes of *Pleurotus sajor-caju* strain fruiting on wheat straw after six weeks of incubation.

There was a wide variation in the biological efficiencies recorded for the different mushroom strains as shown in Table 3.

Hathinala biodiversity hotspot from the Vindhyan forest has a large collection of wild edible and medicinal mushrooms. Mycelium grown on wheat grains was more genetically stable than mycelial disks on synthetic media after cryopreservation (Gupta *et al.*, 2019). These forests need to be conserved for conservation of uncommon wild mushrooms to maintain culture media of alternative species of *Pleurotus*, such as *P. sajor-caju* strain, *P. pulmonarius* and *P. florida* strains. All *Pleurotus* species have excellent replacements in situations when *Pleurotus ostreatus* strain is not available. Lab culture explained that growth various mushrooms strain was positive on grains media than agar media. Mycelium multiplied on wheat grains was genetically more stable in cultivated mode. Farmers will have the opportunity to grow multiple *Pleurotus* strains, increasing their options. The key weakness of the study was the minimal volume of samples gathered for each strain. The better survival of mushroom mycelium on wheat grain may be because the soft and tender mycelium is hidden inside the grain and thus could have endured cooling (Franks 1981, Grout & Morris 1987, Singh *et al.*, 2004, Ru & P., 2026), or it may be that the inherent quality of the grain is responsible for better colonization of the mushroom mycelium and thus better survival.

Preservation of different wild mushroom

Drying techniques preservation of some mushroom includes *Pleurotus* species, *Ganoderma* species, *Hexagonia* species, and *Trametes species* (Fig. 3). Preservation of mushrooms in a formalin (40%) solution includes *Trametes versicolor , Hexagonia tenuis, Agaricus comtulus, Dacryopinax spathularia, Schizophyllum commune, P.sajor-caju, Macrolepiota procera, Deconica coprophila, P.pulmonarius , P.florida , Coprinellus micaceus, Calocybe indica, Termitomyces heimii, Podoscypha petaloides, Agaricus arevensis, Irpex lacteus, P.ostreatus, Clitocybe robusta, Marasmius siccus* (Fig. 4). Due to

generative changes, preservation of cultures become a difficult task (Chang and Miles, 2004; Latha *et al.*, 2022)

Conclusion

Proper culture preservation is an integral part of successful mushroom cultivation. The present study demonstrates that the Vindhyan forests are rich in many species of wild mushrooms growing on different soils in the wild. To prevent the extinction of mushrooms and facilitate future research, it is crucial to have an active role in collecting, identification, laboratory cultivation, preservation, and germplasm collection. In the Vindhyan region, some edible mushrooms are not readily available and are considered a delicacy due to the little information on whether they can be domesticated or cultivated. The better survival of mushroom mycelium on wheat grain may be because the soft and tender mycelium is hidden inside the grain and thus could have endured cooling, or it may be that the inherent quality of the grain is responsible for better colonization of the mushroom mycelium and thus better survival.

Mycelial preservation on wheat grains at low temperatures eliminates the need for frequent subculturing, which prevents contamination and quality degradation. This method is also cost-effective, space-efficient, and maintenance-free, making it ideal for mushroom culture preservation. However, to fully utilize this method's potential, testing beyond a year is necessary.

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Author Contribution

Surabhi Srivastava performed fieldwork, photographed the mushrooms, and wrote the manuscript; Miss Shreyashi Srivastava assisted in the laboratory; and Professor Pramod Kumar Singh assisted with manuscript writing.

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