

Morphological characterization of *Pleurotus sapidus*

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ABSTRACT

Pleurotus mushrooms are not only known for their nutritional and medicinal benefits but also known for their economic significance, providing food and minerals, agricultural waste management and foreign exchange. *Pleurotus*, commonly known as the 'oyster mushroom,' is a delicate variety, highly nutritious, widely cultivated and consumed in India. Its morphological characteristics depend significantly on the quality of the substrate and on environmental conditions. This research is mainly focused on a detailed examination of the morphological, cultural characteristics and life cycle of *Pleurotus sapidus*. This research focuses on traits like pileus color, diameter, shape, margin, stipe color and length, gill attachment, mycelial growth (cm) spore print and different stages of lifecycle. Spore prints were prepared which were used to aid identification and characterization. Pure cultures were established using tissue culture technique on malt extract agar (MEA) medium. The pileus of this mushroom is whitish to gray color and fleshy in texture, while the stipe is cream-colored with a smooth surface. The spores are cylindrical to oblong shape, and the spore print is white. On MEA plates, the mushroom exhibits whitish mycelial growth.

Keywords: *Pleurotus sapidus*, morphology, spores, cultural characterization, life cycle

INTRODUCTION

Mushrooms are achlorophyllous, saprophytic macro-fungi with fleshy fruiting bodies that obtain nutrients from lignocellulosic materials such as dead wood stumps, saw dust and agricultural waste. Oyster mushroom is also known as 'Dhingri' in India belongs to class Agaricomycetes and family Pleurotaceae. It is an important edible diet food in India and naturally grows in the temperate and tropical forests. Among edible mushrooms, *Pleurotus* spp. is particularly popular, due to its excellent flavor, rapid mycelial growth, and excellent saprophytic colonization ability. Due to ease of cultivation, high production potential, compatibility with low-cost agricultural byproducts and easy marketability, oyster mushrooms have become a preferred choice for growers. In addition to their nutritional and culinary benefits, oyster mushrooms have gained attention for their potential in medicine, bioconversion, bioremediation, and bio pulping. It is widely cultivated, offering both great nutritional value and medicinal benefits (Correa *et al.*, 2016). The *Pleurotus* mushroom ranks as the second most widely consumed and third largest cultivated mushroom worldwide, with China leading its production (Royse *et al.*, 2017, Muzaffar *et al.*,

2023). The genus *Pleurotus* includes numerous species valued for their culinary versatility and ability to adapt to diverse agro-climatic conditions, which attracted the attention of researchers globally.

Oyster mushrooms grow either epigeously or hypogaeously and are large enough to be seen with naked eye and harvested by hand (Chang and Miles, 1992). Depending on the species, they display caps (pileus) in different shades of white, cream, gray, light brown, pink, or yellow, with gills (lamellae) running from the cap's edge to the stalk (stipe). Stipes are long or short and its position is central or lateral. The gills present underneath the caps bear the spores. The spores are smooth, cylindrical, and germinate readily within 48–96 hours on different mycological media. The pure white mycelium of *Pleurotus* exhibits robust growth on media (Chaudhary and John, 2017). Oyster mushrooms grow best at moderate temperatures ranging from 20°C to 30°C, with a humidity of 55–80%. They can be cultivated year-round with adequate humidity control, even during summer. The *Pleurotus* species is highly regarded for its nutritional profile, being rich in antioxidants, dietary proteins, fiber, vitamins, and minerals (Krishnapriya *et al.*, 2017). They are low in calories, sodium, fat, and cholesterol,

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while offering a rich source of protein, carbohydrates, fiber, vitamins, and minerals. These characteristics make them a valuable dietary addition with numerous health benefits, as supported by studies like those of Kues and Liu, 2000. The main objective of the current research is to characterize the morphological characteristics of *Pleurotus sapidus* and understand its life cycle. The morphological, cultural characteristics of *Pleurotus sapidus*, along with its life cycle, are critical parameters for identification and classification of *Pleurotus sapidus*.

MATERIALS AND METHODS

Location of the Study

The study was carried out in research laboratory, Botany Department, Govt. Nagarjuna P.G. College of Science, located in the Raipur capital of Chhattisgarh. This area experiences a tropical wet and dry climate and is characterized by temperatures that remain moderate throughout the year, except from March to June, which can be extremely hot.

Procurement and Maintenance of Culture

The pure cultures of *Pleurotus sapidus* used in this study was obtained from the Directorate of Mushroom Research, Chambaghat, Solan and Mushroom research laboratory, Indira Gandhi Krishi Vishwavidyalaya (IGKV) Raipur, Chhattisgarh. The cultures were preserved on Malt Extract Agar (MEA) medium and were periodically sub-cultured every three weeks to ensure the viability. These pure cultures were then used for spawn preparation.

Media Preparation

Malt Extract Agar (MEA) was used for culturing *Pleurotus sapidus*. The media composition included 20g malt extract powder, 20g dextrose, 20g agar-agar, and distilled water to make up a final volume of one liter and maintained 6.5 pH. The media was sterilized at 121°C for 40 minutes at 15 psi, cooled, and used for culturing *P. sapidus*.

Pure Culture Establishment of Mushrooms

Fresh, healthy, medium-sized sporocarps of *P. sapidus* were harvested and first sterilized externally with 70% ethanol. They were then cut longitudinally, and tissues from the stipe's upper portion were taken using a scalpel with a

sterilized blade. These tissues were placed on the surface of MEA media with the help of sterilized inoculation needles and sealed, labeled, and incubated at $24 \pm 2^\circ\text{C}$. Mycelial growth was monitored over 7–10 days, and actively growing mycelial culture were studied for their cultural characteristics.

Spawn preparation

For spawn preparation, the wheat grains were boiled in water for 1–2 hours until they became soft but not mushy. Excess water was drained, and the grains were allowed to surface dry. The dried grains were then filled into autoclavable bags or bottles and sterilized at 121°C for 1.5–2 hours. After cooling, the sterile grains were inoculated with actively growing mycelium of *P. sapidus* under aseptic conditions. The inoculated grains were stored at 22–25°C in a dark environment for 10–15 days until full colonization occurred. Once fully colonized, the spawn was ready for use or could be stored at 4°C for short-term preservation.

Mushroom Cultivation

Paddy straw was used as the substrate for *P. sapidus* cultivation. The paddy straw was soaked in lime water for 24 hours, followed by draining excess moisture by spreading it on an inclined surface. This prepared substrate was packed into polypropylene bags at a rate of 1 kg per bag and sterilized. After this spawn were introduced in substrate and rest it for mycelium run and fruiting body development. Mushroom flushes were then observed on the prepared substrate.

Evaluation of Macro-Morphological Characteristics

The *P. sapidus* exhibited variations in their macro-morphological characteristics such as stipe length and thickness, pileus size and shape, pileus surface, fruiting body color and texture, and the position of the stipe were assessed and documented with photographs following the method of Senthilarasu and Kumaresan, 2018.

Microscopic Examination of Mushroom Spores

The spores of *Pleurotus sapidus* were examined under a light microscope at 100x magnification using slides stained with cotton blue dye.

Study of life cycle

Each developmental stage was observed and recorded for study of *Pleurotus sapidus* life cycle.

RESULTS AND DISCUSSION

Pleurotus sapidus was characterized based on its distinct morphological characteristics. These features were assessed through measurements of the pileus and stipe, spore prints, spore shape, and mycelial growth on MEA plates. The morphological and cultural characteristics are detailed below:

Cultural Characteristics

The pure culture of *P. sapidus* was obtained after seven days of incubation at $24 \pm 2^\circ\text{C}$. Mycelial growth was white, radial and cottony, forming a matted texture that fully covered the plate within 10 days. Mature mycelium shows concentric ring as a part of its growth characteristics (Fig. 1).



Fig. 1: Pure culture of *Pleurotus sapidus*

Cap (Pileus)

The pileus ranged from 4.2 to 11.4 cm in diameter and exhibited colors varying from whitish to creamish-white and grey. Initially convex, the cap matured into a fan-shaped or shell-shaped structure, with a smooth, velvety, soft upper surface (Fig. 2). At maturity, it became nearly flat with a central depression and thick flesh. The cap margins were curled inward towards top during the early stages, later becoming wavy and mild slight similar to anise-like odor.

Stipe

The stipe measured 2.1–4.9 cm in length and 0.7–2.3 cm in diameter, with a slightly creamish white-colored, smooth surface (Fig. 2 and 3). It was laterally positioned, small to medium, and typically formed imbricated clusters. When fresh, the stipe was short, thick, and fleshy. When dried, it became compact and corky, providing structural support to the fruiting body.

Table: 1 Sporophores characters of *P. sapidus*

Pileus size (cm)	Stipe length (cm)	Stipe thickness (cm)
4.2	3.2	0.7
6.4	4.8	0.9
4.6	3.8	0.9
6.8	2.8	1.1
5.3	3.3	0.8

Hymenium

The hymenium featured gills with undulating, straight margins along the back of the cap.



Fig. 2 and 3: Fruiting body and Gills of *Pleurotus sapidus*

Fig. 4: Spore print of *Pleurotus sapidus*

Lamellae (Gills)

The gills were decurrent, running down the stipe, and were white or creamish-white in color with a smooth, lanceolate surface. Initially, the gills were crowded, closed, and lanceolate, remaining attached to the stipe (Fig. 2). In

mature fruiting body, their color changed to light yellow. The lamellae played a key role in spore dispersal.

Spore and Spore Print

The spores were white in mass, smooth, and ranged from cylindrical to oblong in shape. The spore print was white in colour (Fig. 4).

Fruiting Body

Several fruiting bodies were joined at their base to form a large common cluster, while some fruiting bodies grew individually also. Fresh samples had a soft texture, fleshy consistency and mild odor.

Table 2, 3 and 4; Morphological characteristics of *Pleurotus sapidus* Pileus

Color	Whitish to creamish-white and grey
Shape	Fan-shaped or shell-shaped
Depression	Central depression at mature stage
Margin	Margins were curled inward towards top during the early stages, later becoming wavy
Surface	Smooth, velvety, soft upper surface
Odor	Mild slight similar to anise-like odor
Lamellae	
Color	White or creamish-white
Spacing	Crowded
Margin	Smooth
Lamellulae	Absent
Latex	Absent
Stipe	
Color	Slightly creamish white-colored
Attachment to the pileus	Lateral
Surface	Smooth
Consistency	Solid
Annulus	Absent
Volva	Absent
Fruiting body attachment	Stipitate

Life Cycle of *Pleurotus sapidus*

The life cycle of *Pleurotus sapidus* consists of different stages

A. Mycelial Growth

In *Pleurotus sapidus* cultivation, spores are not used due to their small size, making them difficult to handle, furthermore, mushroom

spores take longer to germinate, whereas competing organisms like green molds and other contaminants can germinate and spread more rapidly. To cultivate commercially the *P. sapidus* mushroom, vegetative part of the fruiting body introduced in the contamination-free MEA medium to get pure culture. Pure culture consists of white colored, thread-like hyphae that colonizes complete MEA medium plate in 8- 10 days.

B. Spawn

Pure culture was inoculated onto sterile wheat grains. This inoculated material when covered completely with mycelium is referred to as spawn. Spawn covered with white colored mycelium ensures the cultivated mushroom colonizes the substrate quickly, then other fungi or bacteria.

Substrate Colonization

Spawn introduced in paddy straw filled in polythene bag and left it for spawn run. During the spawn run, the mycelium spreads throughout the substrate filled in bag, which utilized available nutrients. This stage is optimal at a temperature of about $24\pm 2^{\circ}\text{C}$ for *Pleurotus sapidus*. High carbon dioxide (CO_2) concentrations also favor mycelial growth during this phase, but it is not necessary for fruiting. Once the substrate is fully colonized, the mycelium transitions to the reproductive phase, producing fruiting bodies. So many changes are introduced only after the mycelium has completely colonized the substrate so that conditions can encourage fruiting.

C. Primordia Formation:

Environmental changes such as temperature, humidity, and light trigger the formation of tiny pin head like structures called primordial or pin head stage, which are the precursors to fruiting bodies. As primordia developed into fruiting bodies, nutrients are transported from the mycelium through a steady flow of moisture. Over-watering or excessively high relative humidity can disrupt this process, potentially spoiling the crop.

D. Fruiting Body Development

Primordia mature into full-sized fruiting bodies in 3-4 days after pin head formation, containing structures like pileus, gills, stipe and it is ready for reproduction. These structures contain the spore-producing basidia.

E. Spore Release and Dispersal

Basidia within the gills, producing basidiospores that are dispersed into the environment, for regeneration naturally and completing the life cycle of *Pleurotus sapidus*. Morphological features of *Pleurotus sapidus* such as cap expansion, gill color, and stipe firmness indicate developmental stages and overall health. It also helps in determining the right time for harvest.

CONCLUSION

The morphology, cultural characteristics and life cycle of *Pleurotus sapidus*, is a cornerstone of mycological research. It provides critical insights for identification of species, cultivation optimization, ecological sustainability and commercial exploitation. Knowledge of morphological and cultural characteristics supports the development of biotechnological products and understanding the life cycle facilitates the genetic improvement of mushroom strains for enhanced productivity. Studying the life cycle stages of mushrooms also helps to identify critical points vulnerable to pests, diseases, and environmental stress, enabling the implementation of effective control measures. This knowledge serves as an educational resource for training in mycology and sustainable agricultural practices.

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