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Mycelial colony characteristics of some macrofungi collected from Langate forest division of Kashmir

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Abstract
A study was conducted to prepare the mycelial cultures of wild macrofungi from the Langate Forest Division of Kashmir. A total of 10 mycelial cultures of macrofungi were prepared. Most of the cultures had colony colour White and its different shades while few had different coloured colonies *e.g.*, brown in *Morchella esculenta*, pale pinkish in *Hericium collarides*, White with pinkish bands in *Candolleomyces candolleanus* (syn. *Psathyrella spadiceogrisea*) and pale yellowish in *Mycena pura*. The colony form varied from circular to irregular, filamentous to rhizoidal. Four colonies were circular in form, four were of filamentous form and one each was of irregular and rhizoidal forms. The colony elevation varied between flat in four colonies, Raised in two, Crateriform in two and one colony each having umbonate and convex forms. The colony margins were of three types filiform in five colonies, entire in three and lobate in two colonies. The growth rate varied between 2.8 mm to 13 mm per day with the colony of *Pleurotus citrinopileatus* being fastest growing and that of *Entoloma vernum* the slowest.

Keywords: Mycelial colony characteristics, macrofungi, *Morchella esculenta*

Introduction
Due to their unique morphological, ecophysiological and phylogenetic characteristics, fungi form the second largest group of organisms on Earth, only outnumbered by insects. They have a cosmopolitan distribution and have colonized all terrestrial and aquatic ecosystems (Perez-Moreno, 2021) [1]. Fungi are essential components of almost all ecosystems, especially terrestrial environment (Yager *et al.*, 2021) [21]. They contribute to the function of healthy range ecosystems through symbiotic association with plants by decomposing organic matter, contributing to nutrient cycling, and providing food for forest animal (Lori *et al*., 2012; Lee and Robert, 2015) [7, 5]. Ecologically, macrofungi can be grouped as saprobes, parasites and symbiotic species (for example the mycorrhiza). Most terrestrial macrofungi are saprobes or mycorrhiza symbionts, but some are pathogens of plants, while those fruiting on woody substrates are usually either saprobes or plant pathogens (Maria and Tzenka, 2014; Yager *et al*., 2021) [8, 21]. Macrofungi are crucial component of biodiversity essential for decomposition, carbon cycling and nutrient transport in forest ecosystems (Lin *et al*., 2015) [6]. Some are edible and non-edible and are often used as innovative in biotechnological and ecological application (Yager *et al*., 2021) [17]. Fungi also have direct interactions with plant, animal and bacterial community structure through a wide variety of interactions. Therefore, any interference within fungal community structure may also disrupt the balance of other organisms in the environment, for example, many plants are dependent on mycorrhizal fungi for minerals and nutrients in order to grow (Mohammad, 2013) [9]. The symbiotic relationship between plant roots and mycorrhizal fungi enables plants to acquire mineral, nutrients and water while fungi obtain sugars in exchange (Peay *et al*. 2008) [10]. Absence of appropriate fungi can significantly alter plant community structure (Weber *et al*. 2005) [16]. Despite our understanding of fungal roles in decomposition activity, nutrient recycling in ecosystems and associations between fungi and other organisms, there are many things relevant to the spatial and temporal aspects of macrofungal communities that have yet to be discovered (Mohammad, 2013) [9].
Jammu and Kashmir State of India which also lies in the Northwest Himalayas is bordered on the north and east by main Himalayan ranges and to the south by Punjab plains. Due to its varied climatic and topographic conditions, this state provides a congenial environment for the lavish growth of mushrooms (Kumar and Sharma 2011; Wani *et al.* 2013) [4, 15]. The UT of Jammu and Kashmir has a geographic area of 42241sq. Km with 20194 Sq. Km area under forests.

The Kashmir region has 15948 Sq. Km geographical area and 8128 Sq. Km area under forests. Many studies have been conducted in Jammu and Kashmir to study the diversity but many more studies in a systemic and coordinated manner are needed to study, discover and preserve the rich mycoflora of the UT.

A study was conducted to study the macrofungal diversity of the Langate Forest Division in North Kashmir. Mycelial cultures of macrofungi are routinely used for their preservation and for planting the substrate beds for commercial cultivation where it is known as Spawn. The mycelial cultures are also used in mushroom breeding for increasing the yield and quality of the mushroom. It has been seen that the similar ingredients present in the fruiting body of mushrooms are also present in the mycelium of the corresponding macrofungus and hence are used in many mushroom based products. In some commercial products of some highly sought mushrooms like Morels, only mass cultured mycelium is used. Hence, during the present study mycelial cultures of some macrofungi collected from the Langate Forest Division of Kashmir were prepared.

Material and Methods

The study area was fortnightly surveyed for the collection of the macrofungi for studying the diversity. At the collection site important field data required for identification of the macrofungi was recorded and then some young and undamaged sporocarps were bought to the laboratory in paper bags for further studies.

All the glassware used in the study was washed properly with detergent under running tap water, dried and then sterilized in hot air oven for 20 minutes at 180 ºC prior to use. Potato dextrose agar (PDA) medium was used for the isolation, purification and maintenance of macrofungal cultures. 200 grams of fresh peeled potato chips were boiled in sufficient amount of water (<1000 ml) till the texture of chips became soft. After filtering through muslin cloth, the potatoes were discarded and 20 gram of dextrose was added to the boiling filtrate in a two liter capacity graduated beaker followed by addition of 20g agar powder while constantly stirring using a glass rod. The volume was made up to 1000 ml and then dispensed into Erlenmeyer flasks plugged by non-absorbent cotton and wrapped with paper. The media was autoclaved at 15 lb. psi for 15 minutes for sterilization.

The collected macrofungi were attempted for mycelium culture in the laboratory either from spore print or directly from sporocarps by tissue bit transfer method. The healthy undamaged macrofungus was washed under running tap water to remove the dirt and then surface sterilized using 1 per cent sodium hypochlorite and then washed three times with sterile water. A shallow cut was given to the cap in middle and small tissue bits were transferred to the fresh solidified potato dextrose agar (PDA) medium plates under laminar airflow cabinet and incubated at 23±2 ºC. The plates were wrapped with thin transparent food grade poly-film in order to prevent any contamination during incubation and handling. The plates were checked for mycelial growth and the plates with similar mycelial growth were used for purification while the contaminated ones were discarded.

From the non-contaminated plates, the mycelium was aseptically transferred to freshly prepared sterilized media plates for development of colonies using a 5 mm diameter cork-borer. The disks of mycelium were taken at the edge of the growing mycelium. After three to 7 days of incubation, depending upon the growth rate of the colony it was again transferred to fresh media plates and slants for purification using hyphal tip transfer technique. The colonies were then preserved at 4 ºC in refrigerator for storage and further use. The observations like colony colour, colony type (form, elevation and margin) and growth rate were recorded. The growth rate was determined by measuring the colony growth in 60mm petri-dishes at 23±2 ºC after every 24 hours of incubation for seven days.

Results and Discussion

The data on Traditional Chinese Medicine and modern studies have demonstrated the possibility of using fruiting bodies and mycelial cultures as the source of substances that are applied in cosmetology. Due to their high antioxidant potential, fungal extracts have been utilized in antiaging preparations, and due to the presence of anti-inflammatory or antimicrobial compounds, they are more commonly used, in the preparation for problematic skin. In addition, fungal extracts possess whitening, moisturizing, and nutritional effects and have the ability to absorb ultraviolet rays, and so they can be used as a natural sunscreen (Hyde *et al*. 2010) [3]. PSK (protein and polysaccharide complex) isolated from *T. versicolor* is used in Japan as an adjuvant in cancer therapy and is considered as the first confirmed drug of fungal origin (Sakagami *et al*. 1991) [13]. Triterpenes found in Ganoderma sp. are responsible for the anticancer or antioxidant effects (Chairul and Hayashi 1994) [1]. It has been observed that the colonization characteristics of vegetative mycelium developed in solid nutritional environments showed different qualities (Harvey, 1978) [2]. The colony characteristics and growth rate of the ten mycelial cultures are given in table 1 and discussed one by one as follows:

1. *Hericium coralloides*

The mushroom is commonly known by the name ‘Comb Tooth’ due to the formation of tooth like projection on both sides of a branch. The fruiting body is a large hairy or spiny mass with spines less than 1 cm long. The mycelium of the mushroom was white coloured initially developing pinkish hue and leathery mycelium with age. The colony is circular thick with lobate margins. Growth rate is 10.2mm per day (24 hrs), however, sometimes thick mycelium arises directly from the tissue bit which grows very slowly at 3-4.5 mm per day. Plate 1.

2. *Pleurotus ostreatus*

The mushroom grows as saprophyte on hardwoods from spring to autumn season and is commonly known as ‘Grey oyster mushroom’. The cap is white to greyish coloured turning brownish with age and measures 4-10 cm. Stipe is usually absent and when present is very small 1- 2cm. Lamellae are white coloured and decurrent. The colony of the mushroom is white coloured, thick and fast growing on PDA with filamentous colony form having raised mycelium and filiform margins. The average growth rate observed was 7 mm per day. Plate 2.

3. *Pleurotus cystidiosus*

Known commonly as ‘Brown Oyster’ this mushroom grows on hardwood logs in bunches of many caps from a single base as saprophyte. The cap is brownish white initially and changes to full brown with age. The pileus has a central depression and entire and arched wavy margin which splits

with age measuring 6-10 cm across at maturity. The stipe of many caps fuses and is 1-4 cm long and white in colour. Gills are decurrent and white in colour. The mycelial colony is white coloured, filamentous, wioth raised mycelial strands and entire margins. The colony is fast growing with 10.6 mm colony growth per day on PDA medium. Plate 3.

4. *Pleurotus citrinopileatus*

Known commonly as 'Golden Oyster' this mushroom grows on hardwood logs during summer season as saprophyte. The cap is golden coloured and grows in bunches of many caps together from a single base. The cap has a deep depression at the point of stipe attachment and has entire and wavy margin. Stipe is rudimentary to well defined measuring 1-3 cm. Gills are white and decurrent. The mycelial colony of the mushroom is white coloured, with irregular colony form, and flat mycelium and lobate margins. The colony has very vigorous growth of 13 mm per day on PDA medium. This was the fastest growing colony of the ten cultures. Plate 4.

5. *Lentinus tigrinus*

Growing on hardwood logs as a saprobe; solitary or gregariously during spring summer and autumn seasons. The cap 4-8cm in diameter, is cream coloured initially turning brownish with age and has patterns of dark brown to black scales and a navel like depression at center and incurved margins. Stipe 3-4cm long has dark brown scales initially which are shed with age giving bald to fibrillose whitish stipe. The culture is mostly white to off white coloured, fast growing and good in thickness on PDA. However, sometimes the colony develops greyish coloration and becomes very slow growing. Usually the colony is white coloured, circular, Crateriform in elevation and has entire margins and with an average growth rate of 5.8 mm per day on PDA medium. Plate 5.

6. *Morchella esculenta*

Known by the common name 'Yellow morel' and locally called 'Gichh' or 'Kann Kechh' or 'Kechh'. The cap measured 4-6 cm in height and 1.5- 2.3 cm in diameter. The cap was cone shaped shape with ochreous yellow colouration and thrown into many irregular fertile furrows/ depressions separated by sterile ridges on the outer surface giving it a honey comb-like appearance. The stipe was cylindrical in shape, slightly swollen towards the base, furrowed, cream coloured, hollow and measured 2.5-5 cm with a diameter of 1.5-2.5 cm. the mycelial colony was initially whitish then turned brown coloured developing minute sclerotia in the mycelial mass. The colony was circular, flat with entire margins. The colony grew at the rate of 7.8 mm per day on PDA. Shiekh 2013 reported the mycelial culture of Morchella esculenta to be white coloured. Plate 6.

7. *Morchella conica*

The mushroom was collected from coniferous forest growing under a broad-leaved shrub. The cap is conical in shape, ochreous brown to gelatinous brown coloured with irregular fertile pits on the surface of fruiting body separated by lighter coloured ridges and measured 5-6 cm in height and 2.5- 3.8 cm wide at the widest point. The stipe was cylindrical, hollow, cream white coloured, measuring 2.5-4 cm in height and 1.5-3 cm wide in size. The colony started as an off white coloured mycelium, developing brownish tinge with age. The colony form was filamentous with convex elevation and filiform margins. The colony showed a growth of 5.4 mm per day on PDA medium. Shiekh 2013, reported that the mycelium of *Morchella conica* was white when young but turned brown with which is in conformity to this study. Plate 7.

8. *Mycena pura*

Found under the conifers among grass and mosses growing as a saprophyte. The cap of this mushroom is light purple coloured darker towards the center and lighter towards margins measuring 2-3 cm. Striations run down from the cap center. The stipe is dark purple brown coloured with white granular dots scattered on the length of cap and denser towards the top and lacks annulus. Gills are white with hints of cap colour and attached to the stipe by a notch. The mycelial colony of the fungus was pale white coloured with a sprase rhizoidal growth without any prominent elevation and with filiform margins. The colony was slowest growing with a growth rate of mere 2.8 mm per day. However, the colony sometimes produced airel mycelial growth as well. Shiekh 2013 reported the mycelium of mycena galopus on PDA medium was whitish gray in colour and wooly subfelty to cottony in appearance. Plate 8.

9. *Candolleomyces candolleanus*

Widely distributed in time and space saprophyte grows on rotting wood. Cap is hygrophanous, pale brown to buff coloured, conical to convex in shape and 2-5cm across with remains of universal veil attached at margins. Stipe is white, hollow, 3-6cm long and broadened at base. Gills are adnately attached to the stem, pale brown initially and greyish at maturity. The mycelial colony of the macrofungus on PDA medium was white coloured, filamentous with an umbo shaped elevation at the center of the colony and filiform margins. The colony was slow growing with a growth rate of 3.2 mm per day. Plate 9.

10. *Entoloma vernum*

This mushroom was observed growing as saprophyte in early spring season on pine needle debris. The cap is shiny purple brown to dark brown in colour measuring 3-5cm in diameter with slightly incurved and wavy margin. The cap has small white shiny granules. The stipe was cream to light brown coloured, fragile and lacking annulus, broadened abruptly at base with white mycelial strands attached. Gills were cream coloured with a slight tinge of cap colour and attached to stem by a notch. The spore print was pink coloured and spores were polygonal in shape. The mycelial colony on PDA medium was pale whitish in colour, with sprase rhizoidal mycelium thinner towards the ends and thicker in middle with flat mycelium and filiform margins. However, sometimes the colony produced cottony aerial mycelium. The colony was the slowest growing among the ten colonies with 2.8 mm average colony diameter per day. Plate 10.

The mycelial colonies of the fungi are known to show variation in their characteristics between colonies of same species and between different species due to minute differences in the nutrition and other growth conditions besides the differences in their genetic make-up (Rather *et al.*, 2018) [12].

Table 1: Characteristics of mycelial cultures of macrofungi collected from Langate Forest Division of Kashmir

S. No.	Macrofungi	Colony colour	Colony type			Growth rate*
			Form	Elevation	Margin	
1.	*Hericium collarides*	White Pale pink	Circular	Flat	Lobate	10.2
2.	*Pleurotus ostreatus*	White	Filamentous	Raised	Filiform	7
3.	*Pleurotus cystidiosus*	White	Filamentous	Raised	Entire	10.6
4.	*Pleurotus citrinopileatus*	White	Irregular	Flat	Lobate	13
5.	*Lentinus tigrinis*	Off White	Circular	Crateriform	Entire	5.8
6.	*Morchella esculenta*	Brown	Circular	Flat	Entire	7.6
7.	*Morchella conica*	Off White Brownish tinge	Filamentous	Convex	Filiform	5.4
8.	*Mycena pura*	Pale Yellow	Circular	Crateriform	Filiform	5.2
9.	*Candolleomyces candolleanus*	White	Filamentous	Umbonate	Filiform	3.2
10.	*Entoloma vernum*	Pale white	Rhizoid	Flat	Filiform	2.8

*mm/day (24 hours)

Plates 1-10: Mycelial colonies of some of the macrofungi collected from Langate Forest Division of Kashmir

Conclusion

A total of 10 mycelial cultures of macrofungi were prepared. Most of the cultures had colony colour White and its different shades while few had different coloured colonies *e.g.*, brown in *Morchella esculenta*, pale pinkish in *Hericium collarides*, White and pinkish bands in *Candolleomyces candolleanus* (syn. *Psathyrella spadiceogrisea*) and pale yellowish in *Mycena pura*. The colony form varied from circular to irregular, filamentous to rhizoidal. Four colonies were circular in form, four were of filamentous form and one each was of irregular and rhizoidal forms. The colony elevation varied between flat in four colonies, Raised in two, Crateriform in two and umbonate and convex in one colony each. The colony margins were of three types filiform in five colonies,

entire in three and lobate in two colonies. The growth rate varied between 2.8 mm to 13 mm per day with the colony of *Pleurotus citrinopileatus* being fastest growing and that of *Entoloma vernum* the slowest.

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