

Evaluation, propagation and study of life cycle in vitro of *Morchella esculanta*

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Abstract: Among the edible mushrooms, *Morchella esculenta* (Pers.) is a highly prized mushroom which consists of a short stalk and a conical, pitted, hollow pileus. It is commonly known as amarel or sponge mushroom, or guchhi, etc. It belongs to the class Ascomycetes, order Pezizales and family Morchellaceae. It is found throughout the world. In Jammu and Kashmir, it is found in various forest regions under pine vegetation. It is therapeutic in nature; therefore its medicinal and biochemical properties are under investigation throughout the world, especially in regard to treating the hazardous diseases such as cancer, tumors, etc. The present in vitro study was conducted for the first time. The three media soil and soil extracts Agar and Luria Agar were used. Mycelial growth was obtained on all these media, but the LA medium showed better growth. Microscopic studies had shown pigmentation is most likely due to the secondary mycelium. Among these mycelium ascogenous hyphae are produced that may lead to the formation of ascogonium and antheridium with the dilated tips. The compact mass of prosenchymatous and pseudoparenchyma tissues was observed.

Keywords: microscopic study, mushroom, culturing, Jammu and Kashmir.



Morchella spp. (guchhi)

I. Introduction:

They are well represented in India as well as various regions of Jammu and Kashmir, Himachal Pradesh and in Shivalik, Kumaon and Hairshi hills of Uttar Pradesh (Waraitch, 1976 and Kaul, 1997). World over 215 taxa belong to the genus *Morchella* have been reported (Index Fungorum, 2008). In India ten species viz., *Morchella esculenta*, *M. conica*, *M. deliciosa*, *M. angusticipes*, *M. crassipes*, *M. vulgaris*, *M. rotunda*, *M. hybrida* (*M. semilibera*) and *M. gigaspora* are known to

exist (Waraitch, 1976; Jamaluddin et.al., 2004). While in Jammu and Kashmir six species of this genus have been recorded (Abraham, 1991). They are highly prized edible mushrooms which have been used traditionally for centuries (Pegler, 2003). As more and more mushrooms are being investigated for their role as nutritional foods, morels (*Morchella* spp.) still occupies the pinnacle position amongst them. Morels have a long-standing history in Indian cuisines and culture and their collection from the wild dates back centuries Scenario of Morels in Jammu and Kashmir In Jammu and Kashmir, of all the edible mushrooms collected from the wild, morels (commonly called as 'Guchchi') are prime edibles with immense commercial importance and their strong demand makes them an important non-timber forest produce (NTFP). The sale of morels provides substantial monetary benefits to rural livelihoods and local traders. Morel collection, in this northern state of India, is undertaken twice in a year between March-May and August-September. Due to lucrative returns, in many villages, almost all the families are actively involved in morel collection.

plants by connecting mycelium with roots. This symbiotic association is called the mycorrhizal association. *Morchella esculenta* is an example. It is considered a saprotroph (Kuo, 2008; Frank et al., 2010). *Morchella* are heterotrophs (Pandey, . Some species of *Morchella* form symbiotic associations with *Morchella esculenta* is a proteinacious edible mushroom also known as guchchi in kashmiri . It has great importance as it is used as a vegetable and is equivalent to or better than meat. It is delicious. Medically, it is very important, containing interferon which are anticancerous. The generic name *Morchella* is derived from the German word "Morel" meaning "Mushroom," while the specific epithet *esculenta* is Latin meaning "edible". So it is considered an edible fungus (Reilly, 2011). Morphologically, the *Morchella* species is commonly known as the common morel, true morel, sponge morel, Black morel, yellow morel, *M. esculenta* morel mushroom, haystack, dry land fish, etc. (Dörfelt, 2001; Roody, 2003). It is also known as *Phallus esculentus* (Fries, 1753). It is also known as a sponge mushroom because its fruiting body (cap) has honey-comb like depressions. *Morchella* vary in color, shape and size. Its fruit bodies are polymorphic (Masaphy et al., 2010). It is classified in the kingdom fungi, division ascomycota, class pezizomycetes, order pezizales, family Morchellaceae, genus *Morchella* and species *esculenta* (Persoon, 1801). *Morchella* are commonly found under trees. They grow in temperate forests, and appear as the frost melts. They are also found on burnt soil and trees burnt by fire (Wurtz et al., 2005). The *Morchella* is native to India and India also exports it. In India, it is found in Himalayan regions. It is found in the Northern areas starting in forests of Kashmir, as well as Azad Kashmir (Kaul, 1975). It is also found in Britain, Ireland, Europe (Reilly, 2011), Brazil (Cortez et al., 2004), Turkey (Taskina et al., 2010), Israel (Masaphy et al., 2009) and the Himalayas (Kanwal et al., 2010). *Morchella* are also found in Asian countries and from sites throughout most of North America. This edible mushroom is very rarely found in Australia, where several other members of the genus *Morchella* are known to occur. It is found in the same season in different countries. The fruit body of *Morchella esculenta* has several medicinal properties such as anti-tumor effects, immuno-regulatory properties (Duncan et al., 2002), Fatigue resistance and antiviral effects (Wasser, 2002; Rotzoll I 2005; Nitha and Janardhanan, 2008). Extracts from the fruit body have antioxidant properties, i.e. polysaccharides (Mau, 2004; Elmastas et al., 2006; Gursoy et al., 2009). Christian Handrik Persoon (1801) named it *Morchella esculenta*, while other synonyms of *Morchella esculenta* include *Helvella esculenta* (L), *Phallus esculentus* L and *Morchella rotunda* (Reilly, 2011). Mycelium is always found in the form of masses of hyphae. It consists of inconspicuous, branched, filamentous and septate hyphae that grows in the humus soil. This septate hypha contains many nuclei. Apothecia (fruiting body) consist of asci containing ascospores. The ascocarp consists of Stipe, Pileus, Hymenium and Sub-hymenium. Stipe consists of a fleshy stalk-like structure. Its height is around 3 inches and its diameter is one inch. This stalk-like structure bears a sponge-like cap which is also termed as pileus (Ower, 1982; Vashista et al., 1939). Pileus is about 7-5cm long and 4-5cm wide. The presence of pits and ridges is because of the growing structure of hymenium. Basically hymenial layer grows unequally. The depression and ridges are brown in colour (Negi, 2006). This hymenial layer later consists of elongated, cylindrical cells called asci. Asci contain ascospores; a single ascus contains 8 ascospores, which later are released from it (Ammirati, 1985). The asci are surrounded by protective structures, called paraphysis (Sharma, 1988). The sub-hymenium layer consists of pseudoparenchymatous tissue that is present beneath the hymenium layer. These tissues are present in the depression region of the pileus, termed the hypothecium (Vashista et al., 1939; Pandey, 2000). There are different views about the germination of ascospores. After the dispersal of ascospores, they fall in soil. In the presence of the required condition, e.g. moist humus soil, they germinate and produce mycelium (Harvey et al., 1978). They germinate at a low temperature (2°C), and after one year they do not remain viable in this soil surface (Schmidt, 1983). Sexual reproduction is reduced in *Morchella*; it consists of plasmogamy and karyogamy (Vashista et al., 1939).

II. MATERIALS AND METHODS:

The sample was collected from the Kashmir Valley in the spring season of 2017. The dried sample of *Morchella esculenta* was processed for cultivation. To grow *M. esculenta*, soil was also brought from growing regions of morchella of Jammu and Kashmir.

MEDIA USED:

1. Soil (Schmidt, 1983).
2. Luria agar (L.A) (Sambrook, 2001).
3. Soil extract agar (SEA) (Subbannayya et al., 2011).

COMPOSITION OF FIRST MEDIUM (SOIL)

Soil and water

COMPOSITION OF 2ND MEDIUM (L.A)

Distilled Water 100 mL

Tryptone 1 g

Yeast extract 0.5 g

NaCl 1 g

Agar 1 g

One hundred millilitres of L.A medium was prepared by weighing 1g of tryptone, 0.5g of yeast extract and 1g of NaCl. Distilled water was added up to the mark. These were mixed thoroughly and pH 6.4 was maintained. Agar (1g) was added at the end. Media was then autoclaved at 120 °C and 15 psi.

COMPOSITION OF 3RD MEDIUM (SEA)

Soil 2g

Water 100g

Agar 2%

2 g of soil was weighed and added to 100 ml of water. It was mixed thoroughly and the homogenized solution was filtered. 2% agar was added into this filtrate. The pH was observed before and after filtration by the pH meter. Observed pH was 6.6. Media was then autoclaved at 120 °C and 15 psi.

PREPARATION OF SOIL

The soil had been sterilized in the autoclave at 120 °C and 15 psi. Autoclaved soil was transferred to a new polythene box. Soil was watered according to requirement.

PREPARATION OF INOCULUM

Sample was ground. The prepared material was termed the inoculum.

INOCULATION UNDER ASEPTIC CONDITION

Inoculum was inoculated into the soil box containing the prepared soil. A thermometer was set with it. This medium was then placed in at 4°C. The pouring and inoculation of the 2nd (L.A)

and 3rd media (soil extract) was done in a laminar flow bench. Roxithromycin (antibiotic) was

also added to these media before inoculation. After inoculation in both media (L.A and soil extract

agar), plates were placed at 4 °C. Pileus of *Morchella esculenta* was examined under the

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microscope. The spore size and diameter was taken by a standardized ocular micrometer and stage micrometer.

III. STUDY OF CULTURE

The growth in the first media, i.e. soil media, was observed after 1st, 2nd, 4th and 6th week Of inoculation. The growing material was examined under the microscope. The staining procedure Thatwas followed was: Place a drop of waste water sample on a slide. Add one, or at most, two drops of the Lactophenol cotton blue (Nagamani et al., 2005) and observe the slide under the microscope.

For the 2nd and 3rd medium, some material from the media had been picked and the slide was prepared in water and examined under the microscope. The measurements of hyphae were taken with a stage and ocular micrometer for 1st, 2nd, 4th and 6th week.

IV. RESULTS:

The aim of in vitro propagation of Morchella esculenta is to proceed for cultivation commercially.

If successful, it could help meet the protein needs of the growing world population, With commercial cultivation, we may able to provide it to people at lower cost than wild harvested Morchella esculenta.

The section of pileus was examined in Lactophenol. It bears the asci and paraphysis in layers. The Paraphysis are septate, and may or may not be swollen at their tips. The 8 spored asci were cylindrical, and measured about 240 -280 x 18 - 21. The ascospores were 16 - 21 x 7.8 - 18

ellipsoidal, slightly grained at the ends, smooth and yellowish.

Macroscopic studies

The mycelia growth on the SEA is light brown and milky white on the L.A and soil media.

MICROSCOPIC STUDY OF CULTURE

The cultural studies of soil medium showed different hyphal structures like coil form hyphae,

rhiziomorph, compact mass of prosenchyma, and pseudoparenchyma tissue after one, four and six week respectively.

The mycelial colony on the soil extract agar showed no growth after the first week, but it showed pigmented healthy mycelium. The highly pigmented mycelial growth increased in thickness after four and six weeks respectively.

On the L.A media, comparatively better growth of mycelium was obtained with slightly compact patches. With the germination of spore and actively spreading hyphae, healthy mycelium formed on this media as compared to first media (soil). The compact form of hyphae developed, and was termed the plectenchyma . Among the compact hyphae, differentiated hyphae with curved swollen tips can be seen, and might be representing the ascogonium and antheridium of the culture.

Cultural characteristics of Morchella esculenta on selected 3 media.

WEEKS SOIL SOIL EXTRACT AGAR LURIA AGAR

Colony

color

Milky white Light brown Milky white

1st Mycelium well developed

ramifying outwards in

some places and tending

to form rhizomorph

There was no growth Mycelial growth better and

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form compact patches. From where hyphae were actively growing outward.

4TH The mycelium forming networking; where the single hyphae can be observed, forming prosenplectenchyma Thin layer of hyphae developed.

The compact mycelium forming the plectenchyma tissues. The hyphae becoming thick walled and pigmented

6TH The colony showed comparatively thick mycelia growth where the single hyphae cannot be distinguished, the tissues named as

pseudoparanchymatous; the different hyphae measured 1 to 6 m dia.

Mycelium growth increased in thickness, showing slow growth.

The pigmented mycelium seems to be the secondary mycelium, among the compact tissues, the differentiated hyphae with curved swollen tips can be seen, might be representing

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the ascogonium and

antheridium of the culture

Av. Dia. of

hyphae

Ranging from 2 to 5 µm Ranging 2 to 6 µm Ranging from 4 to 6.3 µm

A hyphae grew parallel and other spreading outwards trying to form rhizomorph; hyphae grew in coil form; thin mat of mycelium where single hyphae can be observe easily termed as prosenchyma or prosoplectenchyma; comparatively thick but single hyphae cannot be distinguished termed as pseudoparenchyma; pigmented healthy mycelium developed was consisting of interwoven hyphae; highly pigmented thick growth mycelium developed comparatively; Actively growing hyphae from the grounded inoculums; Formation of healthy mycelium tending to becoming parallel; convolution structure of hyphae might be representing ascogonium and antheridium.

V. Discussion:

In the present study, the original soil of the same locality was used, which may have all the Required substances such as sugars, minerals etc., where they were already growing. As in vitro Culturing was carried out, wellmycelia growth was observed on all these media at the temperature 2.2°C (Schmidth, 1983). Powdered form inoculum was used. The growth of mycelium was abundant and thick on soil, soil extract agar and the L.A medium at 2.2°C (Schmidth, 1983; Guler et al., 2005; Guler, Winder, 2006; Gilbert, 1960). However, Gilbert (1960) had used the semi broth media. Microscopic studies showed ascospore germination at 2.2°C (Winder, 2006; Schmidth, 1983). Mycelial formation occurred after the first week on soil media in scattered and rhizomorphic form (Guler et al., 2005). Guler et al (2005) obtained the same structure by using malt extract agar. Thick and Compact mycelium formation developed after 4-6 weeks in three media (Goldway et al., 2000).

Convuluted and compact mycelium were observed in the L.A media. The compact mycelia growth seems to be showing a tendency to the formation of ascogonium and antheridium.

VI. Conclusion:

In the present study, the mycelial growth of *M. esculenta* was obtained on the following media: Soil, Soil extract agar and L.A media. The L.A medium showed better growth than the other two. Temperature and pH parameters were considered. Microscopic studies showed different hyphal structures, like rhizomorph and the compact mass of prosenchyma and pseudoparenchyma tissue, were obtained. The pigmented formation of mycelium observed most are probably the secondary mycelium. Among these, ascogenous hyphae are produced that lead to the formation of ascogonium and antheridium with dilated tips. The media used in the present study could be better if it could be supplemented with other natural substances of rich protein like wheat bran, rice bran etc.

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