

In Vitro Propagation of *Morchella esculenta* and Study of its Life Cycle

Nazish Kanwal

Institute of Natural and Management Sciences, Rawalpindi, Pakistan

Kainaat William

Bioresource Research Centre, Islamabad, Pakistan

Kishwar Sultana

Institute of Natural and Management Sciences, Rawalpindi, Pakistan

Follow this and additional works at: <https://corescholar.libraries.wright.edu/jbm>



Part of the [Biodiversity Commons](#), and the [Biology Commons](#)

Recommended Citation

Kanwal, N., William, K., & Sultana, K. (2016). *In Vitro* Propagation of *Morchella esculenta* and Study of its Life Cycle, *Journal of Bioresource Management*, 3 (1).

DOI: <https://doi.org/10.35691/JBM.6102.0044>

ISSN: 2309-3854 online

This Article is brought to you for free and open access by CORE Scholar. It has been accepted for inclusion in *Journal of Bioresource Management* by an authorized editor of CORE Scholar. For more information, please contact library-corescholar@wright.edu.

In Vitro Propagation of *Morchella esculenta* and Study of its Life Cycle

© Copyrights of all the papers published in Journal of Bioresource Management are with its publisher, Center for Bioresource Research (CBR) Islamabad, Pakistan. This permits anyone to copy, redistribute, remix, transmit and adapt the work for non-commercial purposes provided the original work and source is appropriately cited. Journal of Bioresource Management does not grant you any other rights in relation to this website or the material on this website. In other words, all other rights are reserved. For the avoidance of doubt, you must not adapt, edit, change, transform, publish, republish, distribute, redistribute, broadcast, rebroadcast or show or play in public this website or the material on this website (in any form or media) without appropriately and conspicuously citing the original work and source or Journal of Bioresource Management's prior written permission.

***IN VITRO* PROPAGATION OF *Morchella esculenta* AND STUDY OF ITS LIFE CYCLE**

Nazish Kanwal¹, Kainaat William², Kishwar Sultana¹

¹Institute of Natural and Management Sciences (INAM), Rawalpindi, Pakistan.

²Bioresource Research Centre (BRC), Islamabad, Pakistan.

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 a morel or sponge mushroom, or gucchi, etc. It belongs to the class Ascomycetes, order Pezizales and family *Morchellaceae*. It is found throughout the world. In Pakistan, it is found in northern areas from Murree, Patriata, and Gilgit 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 in Pakistan. The three media soil and soil extracts Agar and Luria Agar were used. Mycellial 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, Pakistan

Introduction:

Morchella are heterotrophs (Panday, 2000). Some species of Morchella form symbiotic associations with 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 esculenta is a proteinacious edible mushroom also known as gucchi in Punjab (Repin, 1901; Panday, 2000). It has great importance as it is used as a vegetable and is equivalent to or better than meat (Abel *et al.*, 1993). 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 Pakistan and Pakistan also exports it. In Pakistan, it is found in the Northern areas starting in Murree, Patriata, and the Gilgit pine forest, as well as Azad Jamu 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). *Morchellas* 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 grow 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 asci contains 8 ascospores, which later are released from it (Ammirati, 1985). The asci are surrounded by protected 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).

MATERIALS AND METHODS

The sample was collected from the Kashmir Valley in the spring season of 2012. The dried sample of *Morchella esculenta* was processed for cultivation. To grow *M. esculenta*, soil was also brought from Azad and Jammu 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 polar 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 microscope. The spore size and diameter was taken by a standardized ocular micrometer and stage micrometer.

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 that was 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.

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 (Table 1).

MICROSCOPIC STUDY OF CULTURE

The cultural studies of soil medium showed different hyphal structures like coil form hyphae, rhyziomorph, (fig 1 a,b) compact mass of prosenchyma, (fig1 c) and pseudoparenchyma tissue (fig 1d) 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 (fig 1e and 1f)

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 (fig 1g), healthy mycelium formed on this media as compared to first media (soil). The compact form of hyphae developed, and was termed the plectenchyma (fig 1 h). Among the compact hyphae, differentiated hyphae with curved swollen tips can be seen, and might be representing the ascogonium and antheridium of the culture (fig1 i).

TABLE: 1 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 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 the ascogonium and anthredium of the culture (fig i)
Av. Dia. of hyphae	Ranging from 2 to 5 μm	Ranging 2 to 6 μm	Ranging from 4 to 6.3 μm

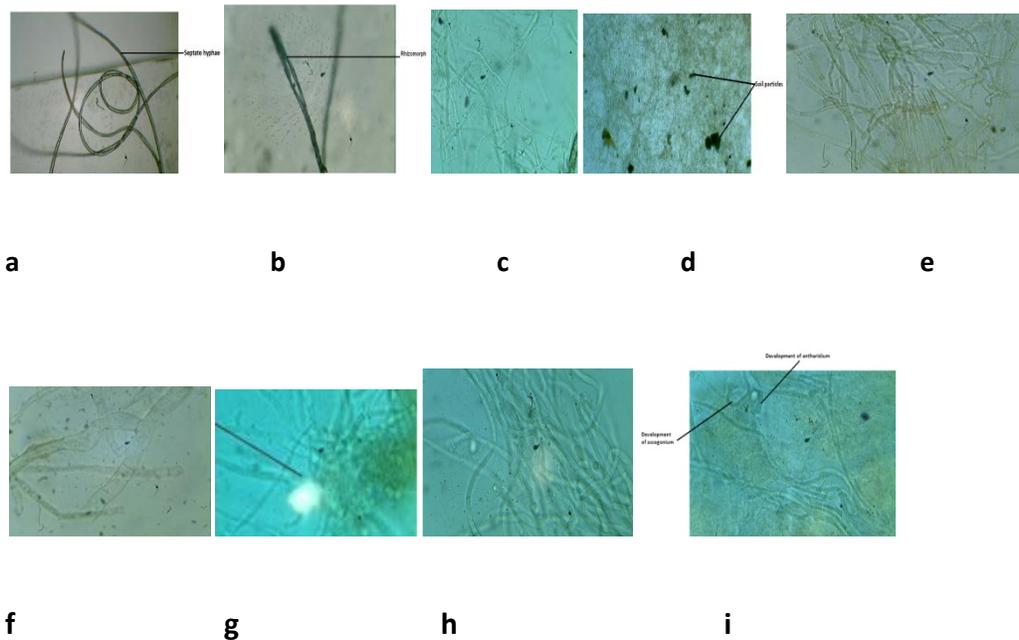


Figure 1: **a-** hyphae grew parallel and other spreading outwards trying to form rhizomorph; **b-** hyphae grew in coil form; **c-** thin mat of mycelium where single hyphae can be observe easily termed as prosenchyma or prosoplectenchyma; **d-** comparatively thick but single hyphae cannot be distinguishd termed as pseudoparenchyma; **e-** pigmented healty mycelium developed was consinting of interwoven hyphae; **f-** highly pigmented thick growth mycelium developed comparatively; **g-** Actively growing hyphae from the grounded inoculums; **h-** Formation of healthy mycelium tending to becoming parallel; **i-** convolution structure of hyphae might be representing ascogonium and antheridium.

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, well mycelial 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).

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

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.

REFERENCES

- Abel D, Horn B, Kay R (1993). A Guide to Kansas Mushrooms. Lawrence: University Press of Kansas. pp.63.
- Amir R, Levanon D, Hadar Y, Chet I. 1993. Morphology and physiology of *Morchella esculenta* during sclerotial formation. Mycol Res. 97 (6), 683-689.
- Ammirati J, Traquair JA, Horgen PA (1985). Poisonous Mushrooms of Canada. Fitzhenry and Whiteside in cooperation with Agriculture Canada. pp. 287-88.
- Arora D (1986). Mushrooms demystified. Ten Speed Press. pp. 23.
- Buscot F (1993). Mycelial differentiation of *Morchella esculenta* in pure culture. Mycol Res. 97 (2), 136-140.
- Buscot F (1993). Synthesis of two types of association between *Morchella esculenta* and *Picea abies* under controlled culture conditions. J Plant Physiol. 141 (1), 12-17.
- Chang S, Phillip GM (1989). Mushrooms: cultivation, nutritional value, medicinal effect and environmental impact. CRC Press. pp. 4-6.
- Cortez VG, Coelho G, Guerrero RT. 2004. *Morchella esculenta* (Ascomycota): A rare species found in Santa Maria, Rio Grande do Sul, Brazil. Biocienc. 12 (1), 51-53.
- David P, Weber NS, Carter MC, Parks CG (2004). Productivity and diversity of morel mushrooms in healthy, burned, and insect-damaged forests of North Eastern Oregon. Forest Ecol Manage. 198(3), 367-386.
- Dorfelt H (2001). *Morchellaceae*. In: Hanelt P. Mansfeld's Encyclopedia of Agricultural and Horticultural Crops: (Except Ornamentals) and Mansfeld's Encyclopedia of Agricultural and Horticultural Crops 1. Springer. p.17.

- Du XH, Zhao Q, O'Donnell K, Rooney AP, Yang ZL (2012). Multigene molecular phylogenetics reveals true morels (*Morchella*) are especially species-rich in China Original Research Article. *Fungal Genet Biol.* 49(6), 455-469.
- Duncan CJG, Pugh N, Pasco DS, Ross SA (2002). Isolation of a galactomannan that enhances macrophage activation from the edible fungus *Morchella esculenta*. *J Agric Food Chem.* 50 (20), 5683–5685.
- Elmastas M, Turkekul I, Ozturk L, Gulcin I, Isildak O, Aboul-Enein HY (2006). Antioxidant activity of two wild edible mushrooms (*Morchella vulgaris* and *Morchella esculenta*) from North Turkey. *Comb Chem High Throughput Screen.* 9 (6), 443–448.
- Elmer LS (1983). Spore germination and carbohydrate colonization by *Morchella esculenta* at different soil temperature. *Mycologia.* 75(5), 870-875.
- Fu L, Wang Y, Wang J, Yang Y, Hao L (2013). Evaluation of the antioxidant activity of extracellular polysaccharides from *Morchella esculenta*. *Food Funct.* 4(6), 871-9.
- García-Pascual P, Sanjuán N, Melis R, Mulet A (2006). *Morchella esculenta* (morel) rehydration process modelling. *J Food Eng.* 72 (4), 346-353.
- Gilbert F (1960). The submerged culture of *Morchella*. *Mycologia.* 52, 201-209.
- Goldway M, Amir R, Goldberg D, Hadar Y, Levanon D (2000). *Morchella conica* exhibiting a long fruiting season. *Mycol Res.* 104 (8), 1000-1004.
- Greis H (1940). Befruchtungsarten bei *Morchella*. [Kinds of fertilization in *Morchella*]. *Jahrbücher für Wissenschaft Botanik.* 89: 245–253.
- Guler P, Bozcuk S, Mutulu F, Sorku K (2005). Propolis effect on sclerotial formations of *Morchella conica* pers. *Pak J Bot.* 37(4), 1015-1022.
- Gursoy N, Sarikurkcu C, Cengiz M, Solak MH (2009). Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. *Food Chem Toxicol.* 47 (9), 2381-2388.
- Hara R, Kino K (2009). Characterization of novel 2-oxoglutarate dependent dioxygenases converting L-proline to cis-4-hydroxy-L-proline. *Biochem Biophys Res Commun.* 379 (4), 882-886.
- Hatıra T, Büyükalacaa S, Doğanb HH, Stephen AR, O'Donnell K (2010). A multigene molecular phylogenetic assessment of true morels (*Morchella*) in Turkey. *Fungal Genet Biol.* 47 (8), 672–682.
- Jeanne DM, Johann NB, Bonello P (2007). Spatial and temporal patterns of morel fruiting. *Mycol Res.* 111 (3): 339-346.

- Jeanne DM, Bruhn JN, Bonello P (2007). Spatial and temporal patterns of morel fruiting. *Mycol Res.* 111(3), 339-346.
- Kanwal HK, Acharya K, Ramesh G, Reddy MS (2010). Molecular Characterization of *Morchella* species from the Western Himalayan Region of India. *Current Microbiol.* 62 (4), 1245–1252.
- Kaul TN (1975). Studies of the genus *Morchella* in Jammu and Kashmir I. Soil composition in relation to carpophore development. *Bull Bot Soc Bengal.* 29, 127–134.
- Kimbrough JW (1970). Current trends in the classification of discomycetes. *Bot. Rev.*, 36: 91-161.
- Korf RP (1973). Discomycetes and Tuberales. In: *The Fungi*. Eds G. C Ainsworth, F. K. Sparrow and A. S. Sussman. Vol IV A. Academic Press, New York. pp. 249-318.
- Kurbanoglu EB, Algur OF, Zulkadir A (2004). Submerged production of edible mushroom *Agaricus bisporus* mycelium in ram horn hydrolysate. *Ind Crops Prod.* 19 (30): 225-230.
- Masaphy S (2005). External ultrastructure of fruit body initiation in *Morchella*. *Mycol Res.* 109 (4), 508-512.
- Masaphy S, Zabari L (2013). Observations on post-fire black morel ascocarp development in an Israeli burnt forest site and their preferred micro-sites. *Fungal Ecol.* 6(4), 316-318.
- Masaphy S, Zabari L, Goldberg D (2009). New long-season ecotype of *Morchella rufobrunnea* from Northern Israel. *Micol Applicada Int.* 21 (2), 45–55.
- Masaphy S, Zabari L, Goldberg D, Jander-Shagug G (2010). The Complexity of *Morchella* Systematics: A case of the yellow morel from Israel. *Fungi Magazine.* 3 (2), 14–18.
- Mattila P, Suonpää K, Piironen V (2000). Functional properties of edible mushrooms. *Nutrition.* 16 (7–8), 694-696.
- Mau JL, Chang CN, Hunag SJ, Chen CC (2004). Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chem.* 87 (1), 111–18.
- Meng F, Zhou B, Lin R, Jia L, Liu X, Deng P, Fan K, Wang G, Wang L, Zhang J (2010). Extraction optimization and in vivo antioxidant activities of exopolysaccharide by *Morchella esculenta* SO-01. *Bioresour Technol.* 101 (12), 4564-4569.
- Moriguchi M, Sada S, Hatanaka S (1979). Isolation of cis-3-Amino-L-Proline from Cultured Mycelia of *Morchella esculenta* Fr. *Appl Environ Microbiol.* 38(5), 1018-1019.
- Negi CS (2006). Morels (*Morchella* spp) kumaun Himalaya. *Nat Prod Radiance.* 5(4), 306-310.

- Nitha B, Janardhanan KK (2008). Aqueous-ethanolic extract of morel mushroom mycelium *Morchella esculenta*, protects cisplatin and gentamicin induced nephrotoxicity in mice. *Food Chem Toxicol.*, 46 (9), 3193–3199.
- Nitha B, Strayo De, Adhikari SK, Devasagayam TPA, Janardhanan KK (2010). Evaluation of free radical scavenging activity of morel mushroom, *Morchella esculenta* mycelia: A potential source of therapeutically useful antioxidants. *Pharm Biol.* 48(4), 453-460.
- Overholts LO (1934). The morels of Pennsylvania. *Proc Penn Acad Sci.* 8, 108-114.
- Ower R (1982). Notes on the development of the morel ascocarp: *Morchella esculenta*. *Mycologia.* 74(1), 142-168.
- Perihan G, Arkan O (2000). Cultural characteristics of *Morchella esculenta* mycelium in same nutrients. *Turk J Biol.* 24, 783-794.
- Person CH (1801). *Synopsis Methodica Fungorum.* pp. 618.
- Rachel A, Steudle E, Levanon D, Hadar Y, Chet I (1995). Tugor changes in *Morchella esculenta* during translocation and sclerotial formation. *Exp Mycol.* 19 (2), 129-136.
- Reilly P (2011). *Fascinated by Fungi.* pp 1-450.
- Repin C (1901). Sur la culture de le Morille. *Revue Générale des Sciences Pures et Appliquées.* 12, 595–96.
- Richard SW (2006). Cultural studies of *Morchella elata*. *Mycol Res.* 110 (5), 612-623.
- Roody WC (2003). *Mushrooms of West Virginia and the Central Appalachians* Lexington, Kentucky. University press of Kentucky. pp. 485.
- Rotzoll N, Dunkel A, Hofmann T (2005). Activity-guided identification of (S)-malic acid 1-O-D-glucopyranoside (morelid) and gamma-aminobutyric acid as contributors to umami taste and mouth-drying oral sensation of morel mushrooms (*Morchella deliciosa* Fr.). *J Agric Food Chem.* 53 (10), 4149-4156.
- Sambrook J, Russell DW (2001). *Molecular Cloning, a Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sandrina AH, Stojković D, Barros L, Glamočlija J, Soković M, Martins A, João M, Queiroz RP, Ferreira ICFR (2013). A comparative study of chemical composition, antioxidant and antimicrobial properties of *Morchella esculenta* (L.) Pers. from Portugal and Serbia. *Food Res Int.* 51 (1), 236-243.
- Segula M, Zabari L, Amir R, Levanon D, Hadar Y, Chet I (1993). Morphology and physiology of *Morchella esculenta* during sclerotial formation. *Mycol Res.* 97 (6), 683-689.
- Sharma OP (1988). *Textbook of Fungi.* Boston: McGraw Hill Higher Education. pp. 193-96.

- Subbannayya K, Rao VA, Raghunath P (2011). Fishmeal Extract Dextrose Agar-A New Mycological Medium-A Preliminary Report. *Am-Euras J Sci Res.* 6 (3), 146-148.
- Tsai S, Weng C, Huang S, Chen C, Mau J (2006). Nonvolatile taste components of *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *LWT-Food Sci Technol.* 39 (10), 1066–1071.
- Volk T, Leonard TJ (1990). Cytology of the lifecycle of *Morchella*. *Mycol Res.* 94(3), 399-406.
- Wasser SP (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl Microbiol Biotechnol.* 60 (3), 258-274.
- Weber NS (1988). In *A Morel Hunter's Companion*. Two Peninsula Press: Lansing. pp. 111-67.
- Winder RS (2006). Cultural studies of *Morchella elata*. *Mycol Res.* 110(5), 612-623.
- Wojciech K, Malinowska E, Suchocki P, Kleps J, Olejnik M, Herold F (2009). Isolation and quantitative determination of ergosterol peroxide in various edible mushroom species. *Food Chem.* 113(1), 351-355.
- Wurtz TL, Wiita L, Amy S, Weber, Nancy SS, David P (2005). Harvesting morels after wildfire in Alaska. Research Note RN-PNW-546. Portland, OR: U.S. Forest Service Pacific Northwest Research Station. pp 31.