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Research Article

Ectomycorrhizal habit of *Cedrus deodara* roots in beneficial association with *Inocybe pallidicremea* (Inocybaceae) and *Tricholoma psammopus* (Tricholomataceae) from the Kashmir Himalayas, India

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Abstract

The present article interpret morphological and anatomical detailing of the ectomycorrhizal feeder roots of *Cedrus deodara* symbiotically associated with two lamellate taxa viz. *Inocybe pallidicremea* Grund & D.E. Stuntz, and *Tricholoma psammopus*(Kalchbr.) Quél. weredocumented for the first time from India. Morpho-anatomic investigations were undertakan in the temperate forests of Kashmir Himalayas, India, where *Cedrus deodara* dominantly flourishes. The putative ectomycorrhizal (ECM) association between sporophores and roots of *Cedrus deodara* were carried out with the help of standard methodology. The presently examined colonized ECM roots revealed distinct differences in the shape organisation and differences in colour tone of ectomycorrhizal system, mantle surface texture, emanating elements like cystidial organisation, anatomy of mantle layers and colour reactions of ECM roots with different chemical reagents. The present work is illustrated with microphotographs along with Camera Lucida drawings to evaluate mycorrhizal investigation.

Keywords: *Cedrus deodara; lamellate taxa; Ectomycorrhiza, Kashmir Himalaya.*

Introduction

Cedrus deodara (Roxburgh) G. Don is an essential coniferous tree that belongs to the Kingdom Plantae, Division Pinophyta, Class Pinopsida and Order Pinales. It is commonly called as Himalayan Cedar in English and Devdaar in Hindi and Devdaru in Sanskrit. *Cedrus deodara* is widely distributed inthe Western Himalayas and found profusely in countries like China, Afghanistan, Pakistan, North-West India (Kashmir, Himachal Pradesh, Uttarakhand and Nepal at an altitude of 2,000 - 3,200 m. Besides great lucrative and ramunerative importance of *Cedrusdeodara,* it is an important phytobiont for many ectomycorrhizal agarics (Wang and Qiu. 2006; Vaario. *et al.*, 2006; Hibbett and Matheny, 2009). According to (Le. *et al.*, 1987) ectomycorrhiza are the dominant forms of mycorrhiza found associated with the trees in temperate and boreal forests. The fine roots of more than 90% of vascular plants in terrestrial ecosystems form symbiotic beneficial root-fungal associations known as mycorrhiza (Azul. *et al.*, 2008). Amongst several different types of mycorrhizal associations the ectomycorrhizal fungi (ECM) often are considered an ecological union distinguished by their stable and constant biotrophic alliance with the roots of woody plants and production of sporocarps (Luoma. *et al.*, 1991).The ectomycorrhizal fungi usually cannot survive in the soil for long periods without a host, so hyphae in the form of rhizomorphs are typically attached to feeder roots. Moreover, the formation of the symbiotic association is required for the completion of the fungal life cycle. To investigate the ectomycorrhizal fungal species, the first step is to characterize, distinguish and recog-

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nize the ectomycorrhizal taxa. These ectomycorrhizal species can be identified by their sexual structures such as sporophores or fructifications. But these structures are not always illustrative of all the taxa on root tips in the form of ectomycorrhizae (Visser. 1995; Gardes and Bruns. 1996; Durall. *et al*., 1999; Jonsson. *et al*., 1999).

The *Inocybe*(Fr.) Fr. (Inocybaceae**;** Basidiomycota)is considered as predominant ectomycorrhizal genus, known by 58 species from India (Latha and Manimohan 2016) and (Pradeep. *et al*., 2016). The genus *Tricholoma*(Fr.) Staude, is also a dominant ECM genus represented by 13 species from India (Mohanan. 2011) and (Henry and Rajakumar. 2014). The family *Inocybaceae* occurs worldwide forming ectomycorrhizal (ECM) associations with numerous families of angiosperms and gymnosperms in tropical and temperate regions making it one of most diverse families of Agaricales (Kirk. *et al*., 2008). The members of family *Tricholomataceae* develop putative ectomycorrhizal associations with gymnosperms trees in the Kashmir Himalaya represented it highly diverse family of Agaricomycetes (Sheikh. *et al*., 2019). Forest ecosystems are balanced by the reciprocated symbiosis between tree roots and basidiomycete fungi. A particular mutualistic symbiosis between basidiomycete fungi and host trees, the fungal partner construct a mantle around the short feeder roots of its host, which has been suggested to protect the tree from pathogen attack (Smith and Read. 1997, 2008). Approximately, 70% - 80% of taxa in the family *Inocybaceae* have been documented from the north temperate zone in association mainly with the ectomycorrhizal (ECM) plant families Pinaceae, Fagaceae, and Salicaceae (Alexander and Selosse. 2009; Smith. *et al*., 2011). On the basis of morphotyping, 5800 fungal species belonging to 184 genera form ectomycorrhizal associations (Agerer. 2006). As per (Taylor and Alexander. 2005), 7000-10000 fungal species form ectomycorrhizal association worldwide. Recently, (Rinaldi. *et al*., 2008) documented 343 genera including 11,950 species, of which 252 genera belong to Basidiomycota, 84 to Ascomycota and 5 to Zygomycota that form ectomycorrhizal association. The members of Betulaceae, Pinaceae, Fagaceae, Salicaceae and Dipterocarpaceae families form ectomycorrhizal association with fungal species (Buscot. *et al*., 2000). Earlier, (Agerer. 1987-2002) described mycorrhizal formation of *Tricholoma sulfureum* and *T. vaccinum* with spruce. Putative ectomycorrhizae of *Tricholoma aurantium* with *Pinus wallichiana* and *Abies pindrow* from Pakistan was described by (Niazi. *et al*., 2010). Mycorrhizae of *T. aurantium* with *Abies alba* in Italy was described by (Comandini. *et al*., 1998). (Zeitlmayr and Linus. 1976) reported *Tricholoma pardinum* in ECM association with fir and beech. The ectomycorrhizal fungal taxa namely *Inocybe amicta* and *I. mimica* were collected in the vicinity of *Pinus wallichiana* characterized by morphological methods and nrDNA ITS molecular analysis by (Saba. *et al*., 2015). The two ectomycorrhizal lamellate species of genus *Inocybe* namely *Inocybe geophylla* and *I. mimica* were documented from temperate forests of Kashmir Himalaya (Itoo and Reshi, 2014).

The Kashmir Himalaya situated within the biogeographic zone of the North-Western Himalayas in India (Rodgers and Panwar. 1988). It lies between 33º20′ to 34º54′ N Latitudes and 73º55′ to 75º 35′ E Longitudes, covering an area of 15, 948 sq. km, with forest area about 8,123 sq. Km. During the present investigation the sporophores and colonized ECM roots were excavated in their natural habitat by tracing the organic connection between mycobiont and phytobiont from forests of Kashmir Himalaya. Deciphering the rhizomorphs connections between fructification and host colonized ECM short roots is standstill a most consistent and unique way of evaluate the fungal ECM status in the field conditions (Agerer. 1986, 2006). The lamellate ectomycorrhizal taxa were identified up to species level with the help of classical taxonomy. However, the present work depicts to characterize the ECM details of *Inocybe pallidicremea* and *Tricholoma psammopus* associated with *Cedrus deodara* sampled from Kashmir Himalayas, for the first time from India.

Materials and Methods

Area Surveyed

The area selected for the present study is Kashmir Himalayas being phytogeographically placed at the intersection and falling within the North-Western Himalayas. It is largely dominated by the temperate coniferous forests with gymnosperms tree species of *Cedrus deodara* and *Pinus wallichiana*. The area of investigation is located between 33°20′-34°54′ N Latitudes and 73°55′-75° 35′ E Longitudes, covering an area of 15, 948 sq. km, with forest area about 8,123 sq. Km. The altitude of the main valley itself ranges from 1500 -1800 m (amsl), whereas, the average elevation of its surrounding mountains varies from 3000-4000 m (amsl). Topographically, the Kashmir Himalayas comprises of an deep elliptical bowl-shaped valley bounded by the Pir-panjal range of lesser Himalayas in the south and south-west, and the Zanskar range of the Greater Himalayas in the north and north-east. The forests of Kashmir Himalayas are classified into main three groups along with altitudinal ranges viz. Kashmir temperate forests (17, 00 - 29,00m), Sub-alpine forests (31,00 - 35,00m) and Alpine forests (above 35,00m). The forests of Kashmir Himalayas are dominated with conifers with dominant members as *Cedrus deodara*, *Pinus wallichiana* and *Pinus gerardiana* with pockets of *Picea smithiana, Abiespindrow, Abiesspectabilis*, *Betula utilis*, *Taxus wallichiana*, and *Rhododendron anthopogan*.

Collection, Identification and Characterization of Sporophores

The epigeous sporophores and ECM root tips of ectomycorrhizal genera were sampled from coniferous forests of Kashmir Himalayas. Sporophores growing in the proximity of *Cedrus deodara* dominating forests were collected for collection material at an altitude of (2,500m) 34°14'14"N-74°18'05"E, and (2750 m) 33°59'45 " N-75°21'05 " E, during September-June in 2016 and 2019. The *Inocybe pallidicremea* and *Tricholoma psammopus* sporophores and their colonized ECM root tips were sampled by carefully tracing the hyphal connections between host trees and sporophores. The macroscopic features of the collected sporophores were documented on the field Key (Atri. *et al*., 2005). The major portion of the sporophores were hot air dried between 45-50°C by using a low voltage (500 watt) heater in a drier particularly designed for drying mushroom collections (Atri. *et al*., 2005) The dried material were finally packed in the cellophane sheets along with a few crystals of 1,4 para-dicholorobenzene or Naphthalene balls for preservation in Herbarium. The taxonomic diagnosis of macroscopic and microscopic characters of examined species was worked out as per standard methodology (Singer 1986; Arora 1986) and (Kirk. *et al*., 2008) has been followed.

Ectomycorrhizal Studies

Collection of ECM Material

The fresh, fine, young feeder mycorrhizal roots excavated from underneath the sporophores has been collected by carefully tracing their hyphal connections and delicate rhizomorphs and wrapped in polythene bags and finally carry them to the laboratory for further examination. The ectomycorrhizal root materials were collected from pure conifer forests barely. A tree of *Cedrus deodara* was selected as a host for collection of ectomycorrhizal root tips. Soil blocks of 15 cm3 including roots of the tree were excavated at 15 cm to 1m away from the trunk of each tree. The soil blocks were placed in plastic bags, labelled with collection number, locality and date. The samples were brought to the laboratory and stored at 4oC until washing and morphotyping.

Cleaning of Roots

The soil blocks were soaked in water for overnight to loosen the adhering soil particles then shifted to 2 mm sieve and placed under running water to remove the attached soil and debris for prevent the loss of delicate mycotips. The ectomycorrhizal root tips were carefully sorted into morphotypes under incandescent light according to their morphological features. The attached soil particles were removed with the help of fine brush under Magnus MSZ-TR stereomicroscope. Individual root tips were dissected.

Storage of ECM Roots

The ECM roots were preserved in (5 ml formalin (37%) + 5 ml acetic acid (100%) + 90 ml alcohol (50%) for morpho – anatomical characterisation. The morphotypes were kept in McCartney bottles in distilled water for later morphological examination.

Morphotyping of ECM Roots

Morphological characteristics were noted under the stereomicroscope (Magnus MSZ-TR), digitally photographed and characterized by cautious examination by following methodology given by Agerer (1987-2012) and Agerer and Rambold (2004-2020). The root tips were observed particularly for morphological features like mycorrhizal system, ramification, shape, size, colour, texture, presence/absence of rhizomorphs and extralimitical mycelium (Emanating hyphae, Rhizomorphs, Cystidia, Sclerotia). The ECM roots lack root hairs as described by (Smith and Read. 1997). The ECM roots found were showing monopodial pinnate to dichotomous, branching patterns.

Anatomical Studies of ECM Roots

The ECM roots were preserved in (5 ml formalin (37%) + 5 ml acetic acid (100%) + 90 ml alcohol (50%) for anatomical characterisation. The verification of ectomycorrhizal colonization has been acquire by carefully examining the cross sections of ECM roots (dissected manually) under a compound microscope and microphotography under digital microscope (Leica DM4000 B LED) for the presence of the fungal mantle and Hartig net. The surface views of ectomycorrhiza have been analysed using peeling and squashing techniques. The colour terminology of Kornerup and Wanscher (1978) was used.

Results

DescriptionofEctomycorrhizalRoots

Description of Ectomycorrhizae

***Inocybe pallidicremea* Grund & D.E. Stuntz + *Cedrus deodara*(Roxb. Ex D. Don.) G. Don.** Figure 1-2

Morphological Characters

Ectomycorrhizal system simple to monopodial pinnate with 0-1 order of ramification, upto 9.0 mm long; main axes 0.2-0.5 mm in diameter. Unramified ends straight to bent, smooth, swollen towards tips, 2.5-7.0 mm in length and 0.3-0.6 mm in diameter, tips slightly swollen. Surface of unramified ends smooth, shiny, soil particles adhere to the surface, slightly constricted in the middle; younger ectomycorrhizae redish brown and older grayish brown to dark brown; latex or any other fluid not secreted on injury; mantle surface not transparent; hydrophobicity present; tips dark brown to light brown in colour. Rhizomorphs present, cottony like, white to cream coloured. Emanating hyphae present, not specifically distributed. Cystidia present. Sclerotia not found.

Anatomical Characters

Mantle thickness 20-35 µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 12.0 -17.0 µm thick, almost plectenchymatous giving rise to a prominent cystidia and emanating hyphae, compactly arranged, representing type D pattern (Agerer 1987 – 2012, Agerer and Rambold, 2004 – 2020); hyphal cells 6.7-15.0 µm tangentially and 5.2-8.2 µm radially, smooth walled, gelatinized, oval, elliptical to cylindrical in shape. Inner mantle layer 13.4-20.0 µm, pseudoparenchymatous posessing angular to mounds of roundish cells representing type K pattern (Agerer 1987 – 2012, Agerer and Rambold, 2004 – 2020); hyphal cells hyaline, thick walled, irregular in shape, measuring 5.0- 12.0 µm tangentially and 3.0-4.5 µm radially, possessing oval, ovoid to rounded shape. Labyrinthine Hartig net hyphae protruding towards endodermis, 2.5-6.2 µm tangentially and 2.0-12.0 µm radially, infrequently lobed type, rounded to cylindrical in shape.

AnatomicalCharactersofEmanatingElements

Rhizomorphs measuring 2.2-3.7 µm in diameter, made up of longitudinally tangled, interwoven, smooth, entangled, clampless, fused, non-anastomosic, septate hyphae; cell wall upto (0.5 µm) thick. Emanating hyphae measuring 3.7-5.2 µm in diameter, smooth, ramified, clamped, slightly inflated near septa, bifurcated, interwoven, broad, septate hyphae. Cystidia measuring 15.0- 25.3 x 3.7 -5.2 µm, present on the outer mantle layer, distinct and often frequent representing type 1 (Agerer

1987 – 2012, Agerer and Rambold, 2004 – 2020), unramified, sub-cylindric to capitate representing type N pattern (Agerer 1987 – 2012, Agerer and Rambold, 2004 – 2020), granulated, septate without clamps.

Color Reactions with Different Reagents

Potassium hydroxide (10%): dull brown; FeSO4: n.r. (no reaction); Cotton blue: blue; Acetic acid: n.r. (no reaction); Sulfovanillin: reddish brown; Melzer's Reagent: brownish.

Description of Ectomycorrhizae: *Tricholoma psammopus* (Kalchbr.) Quél. **+ *Cedrus deodara* (Roxb. ex D. Don.) G. Don.** Figure 3-4

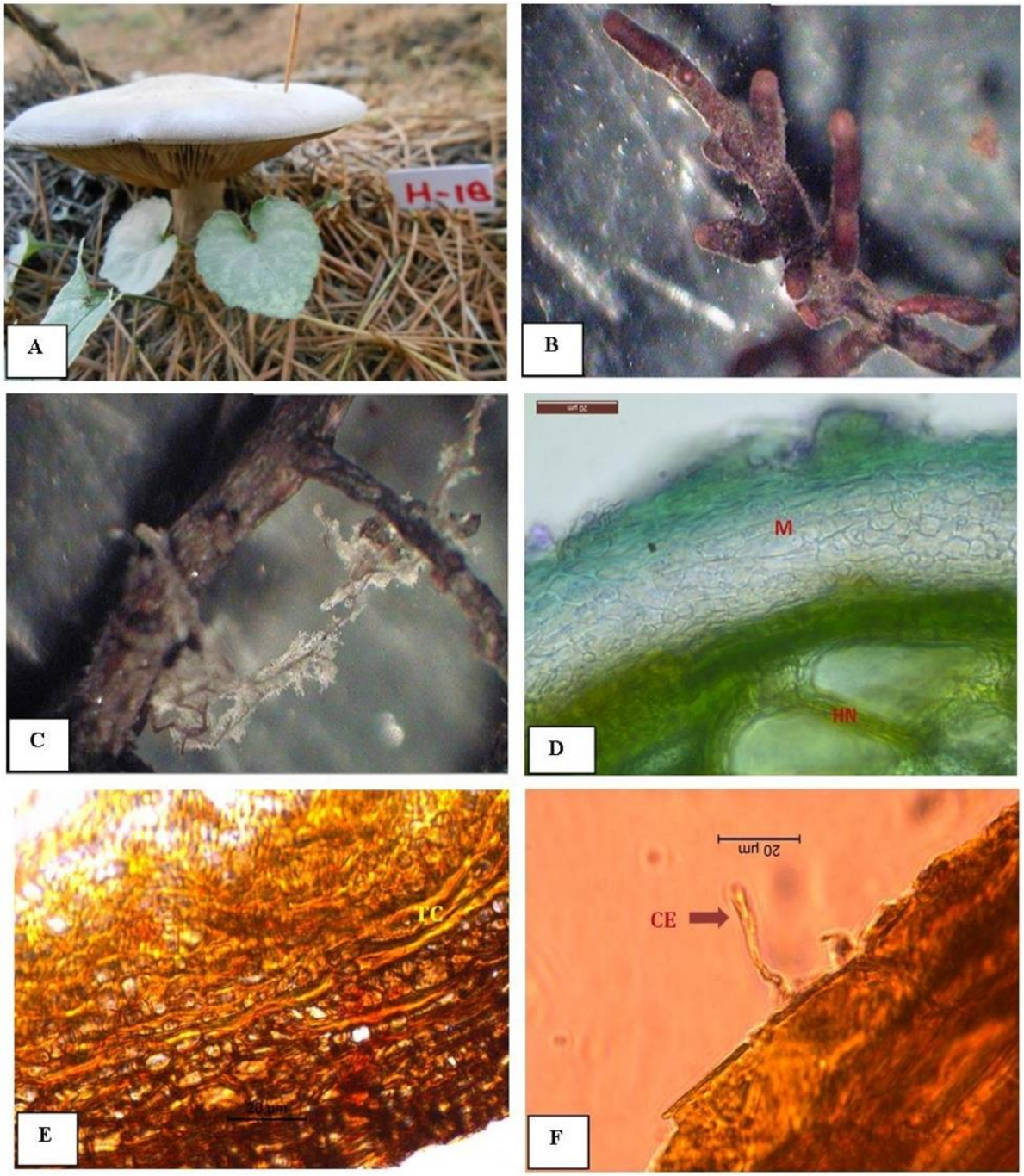

Figure 1: ***Inocybe pallidicremea* + *Cedrus deodara***

(a) Sporophore in association with *Cedrus deodara* root, (b) Mycorrhizal system, (c) Rhizomorphs cottony like, (d) Cross section of ectomycorrhizal root showing mantle (M) and Hartig net (HN), (e) Cross section of root showing elliptic to cylindrical tannin cells (TC), (f)

Cross section of root showing N type cystidial elements (CE), Scale bar a =3cm, b-c =1mm

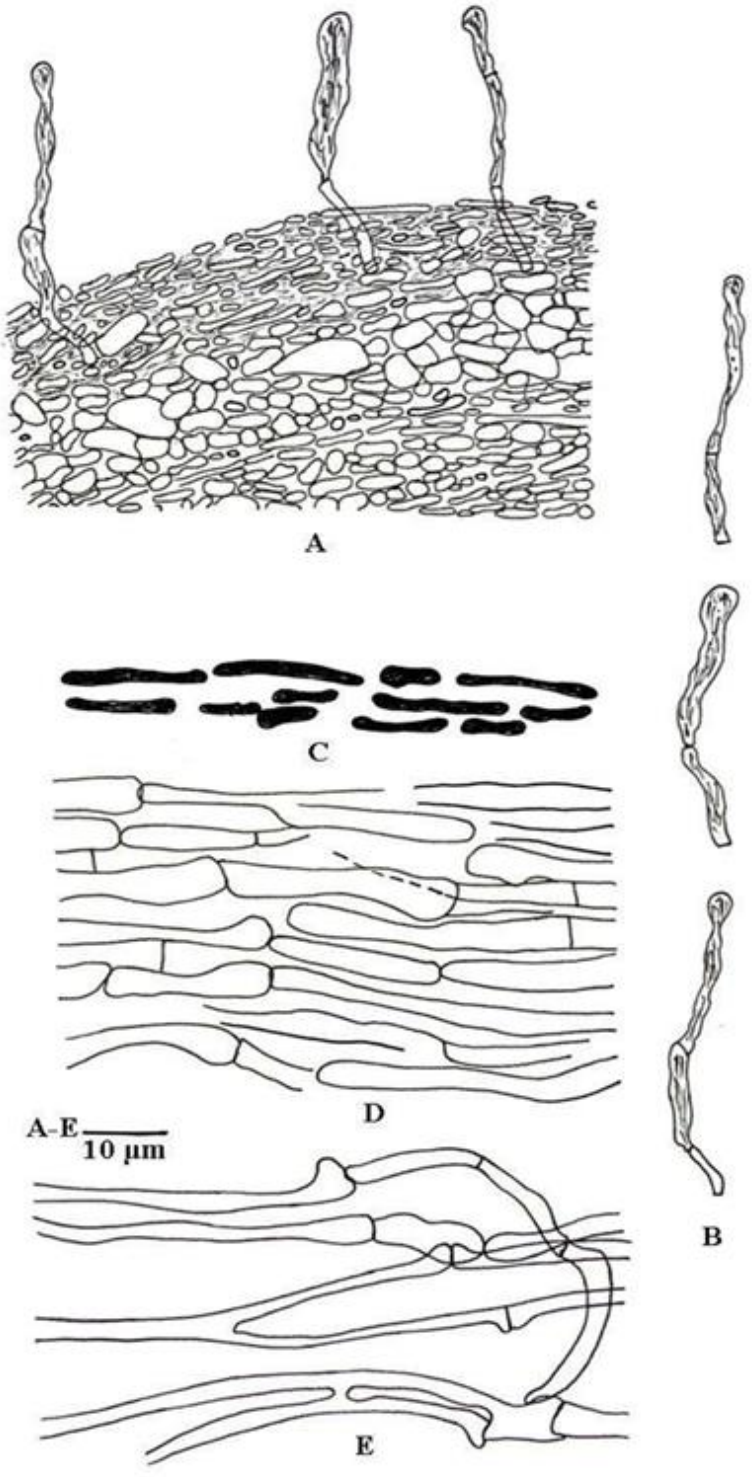

Figure 2: ***Inocybe pallidicremea + Cedrus deodara***
(a) Mantle (b)Cystidial elements (c)Tanin cells (d)Rhizomorphs hyphae (e)Emanating hyphae.

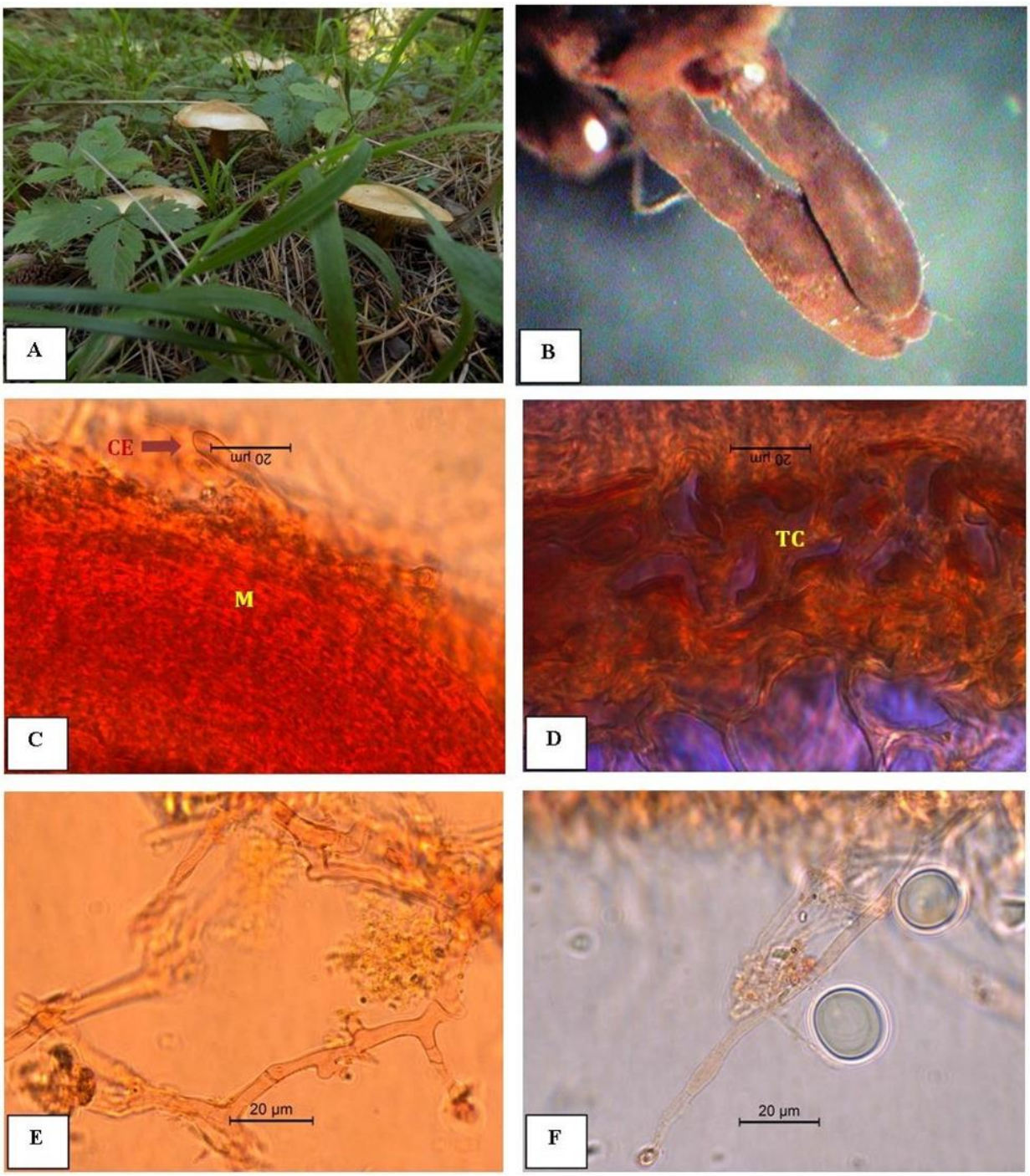

Figure 3: ***Tricholoma psammopus* + *Cedrus deodara***

(a) Sporophores in ectomycorrhizal association with *Cedrus deodara* root, (b) Mycorrhizal system, (c) Cross section of ECM root showing mantle (M) and cystidial elements (CE), (d) Tanin cells (TC) in rows, (e) Rhizomorphs hyphae, (f) Emanating hyphae. Scale bar a= 3cm, b=lmm

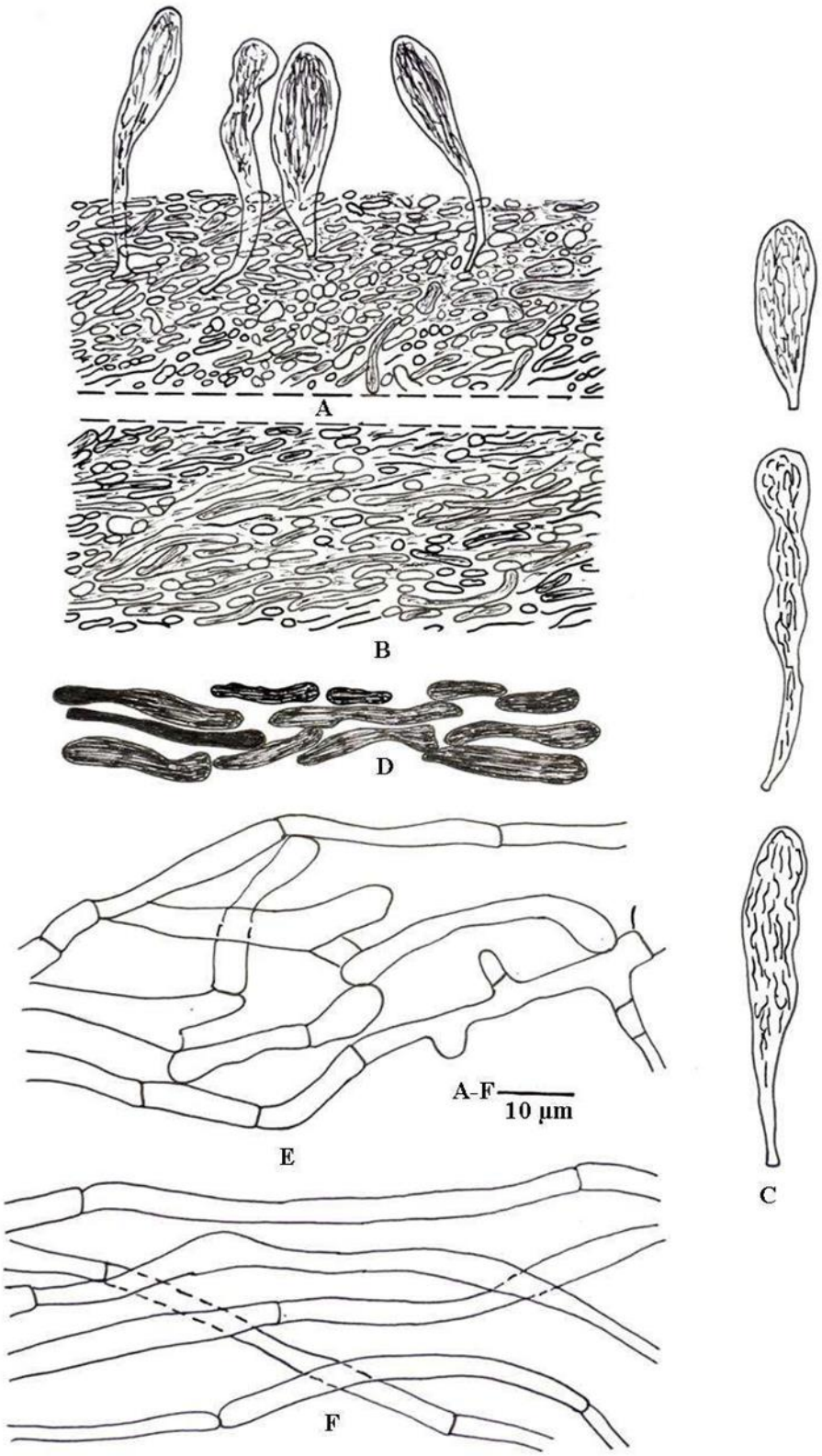

Figure 4. *Tricholoma psammopus* + *Cedrus deodara* : A. Outer mantle, B. Inner mantle, C. Cystidial elements, D. Tanin cells, E. Rhizomorphs hyphae, F. Emanating hyphae.

Morphological Characters

Ectomycorrhizal system dichotomous, with 0-1 order of ramification, 3.0-16.0 mm long; main axes 0.5-0.9 mm in diameter. Unramified

ends straight to slightly bent, occasionally constricted between older parts and younger parts; inflated, club shaped, 2.0-6.0 mm in length and 0.3-0.5 mm in diameter; tips considerably swollen. Surface of unramified ends smooth, occasionally covered with soil particles; younger ectomycorrhizae redish brown and older one dark brown to deep brown in colour; not changing; latex or any other fluid not exudes from root on injury; cortical cells invisible under mantle, transparency of mantle absent, mantle dots absent, carbonization absent, hydrophobicity absent, tips rounded, light brown to dark gray in colour; laticifers invisible. Emanating hyphae offwhite, rope like, infrequently present on the ectomycorrhizal system. Rhizomorphs present in the form of flat fans, usually occurring at the restricted points, white to off white. Cystidia present. Sclerotia absent.

Anatomical Characters

Mantle thickness 38.7-67.0 µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 20.9-32.8 µm, plectenchymatous, not discernable, giving rise prominent cystidia on the outer mantle layer, representing type D (Agerer. 1987 – 2012; Agerer and Rambold. 2004 – 2020); fungal hyphae 2.2-3.7 µm broad, not discernable, gelatinous, loosely arranged, smooth walled, aseptate, clampless, irregular and variable in shape. Inner mantle layer 12.0-23.8 µm, deeply plectenchymatous with an irregularly arranged hyphae without special discernable pattern, representing type B (Agerer. 1987 – 2012; Agerer and Rambold. 2004 – 2020) intermingled with few patches of roundish cells representin type F (Agerer. 1987 – 2012; Agerer and Rambold. 2004-2020); hyphae 3.0-4.5 µm broad, thin walled, tangentially elliptical to cylindrical in shape , aseptate without clamps; anastomosis absent, loosely placed, cell wall upto (0.7 µm) thick, unramified, granular contents present in few hyphae. Hartig net lobed type, protruding towards endodermis, measuring 13.4-31.3 µm tangentially and 12.0-22.3 µm radially, possessing rounded, oval to rectangular cells. Epidermal cells measuring 3.7- 6.7 x 2.3-3.7 µm tangentially and radially, bearing oval to elliptical shape. Tanin cells measuring 16.4-23.8 µm tangentially and 3.0-5.2 µm radially, lying elliptical to cylindrical and oriented parallel to the root axis.

AnatomicalCharactersofEmanatingElements

Rhizomorphs hyphae measuring 3.7-6.0 µm broad, made up of loosely arranged, branched, joined to entangled hyphae, septate without clamps, septa enlarged in few hyphae; anastomosis absent, few hyphal cells inflated at their ends near septa, hyaline. Emanating hyphae measuring 3.7-5.2 µm broad, made up of straight to slightly bent, interwoven, unramified, septate hyphae without clamp connections, few hyphae slightly inflated at septa with normal ends, unbranched, elongated to cylindrical in shape. Cystidia measuring 22.3-47.7 x 6.0-8.2 µm, possessing clavate to capitate shape, representin type N/O (Agerer. 1987 – 2012; Agerer and Rambold. 2004-2020), needle like contents present in cystidia, hetergenous knobless, unramified, thick walled, aseptate, clampless.

Color Reactions with Different Reagents

Potassium hydroxide (10%): brownish green;FeSO4: n. r. (no reaction); Ethanol (70%): brownish orange; Melzer reagent: brownish gray, Cotton blue: bluish green; Acetic acid: n. r. (no reaction).

Discussion

The ECM Sporophores of *Inocybe pallidicremea* has been reported to grow solitary, scattered or in groups on soil in the western states and provinces like Alaska, British Columbia, Washington, Oregon, Wyoming, Colorado and Arizona, eastward to Michigan, New York, New England, and the eastern provinces of New Foundland, Labrador and Nova Scotia, associated with conifers including *Pseudotsuga*, *Picea*, *Tsuga* and *Pinus*, occurring in the months of August to December (Matheny and Swenie. 2018). Presently, this species was found growing in groups in ECM association with *Cedrus deodara* in the pure coniferous forest in the month of September from Kashmir Himalayas, India.

The sporophores of *Tricholoma psammopus* have been commonly found in the United

Kingdom usually under *Larix* but also rarely found under *pinus* and *Picea* on calcareous soils (Kibby. 2010). It is usually widespread in Europe in association with *Larix*, particularly in mountainous regions and on rich calcareous soils, but this species also found in association with *Pinus* (Christensen and Noordeloos, 1999). This species also shows association with *Abies* and *Picea* (Bon, 1984a). Presently, this species has been found amongst grasses on outskirts of forest under the proximity of *Cedrus deodara* in pure coniferous forest at an altitude of 2750m from Kashmir Himalayas, India.

The ECM roots of *Inocybe pallidicremea* are diagnostic by the presence of simple to monopodial pinnate ectomycorrhizal system with 0-1 order of ramification, smooth to shiny, reddish brown, grayish brown to dark brown surface. Cystidia distinct and often frequent representing type 1 pattern, unramified, subcylindric to capitate representing type N pattern, granulated, septate without clamps. The *Tricholoma psammopus* is characterized by possessing dichotomous ectomycorrhizal systemwith 0-1 order of ramification, smooth, reddish brown, dark brown to deep brown surface. Cystidia distinct and often frequent presenting type 1 pattern, possessing clavate to capitate shape, representing type N/O pattern, needle like contents present in cystidia, hetergenous, knobless, unramified, thick walled, aseptate, clampless.

The *Inocybaceae* is a species-rich family shows tremendous diversity in temperate forest ecosystems with ca. 700 species worldwide (Matheny. *et al.*, 2009). The family *Inocybaceae* occur worldwide developing ectomycorrhizal (ECM) associations with various families of angiosperms and gymnosperms in tropical and temperate ecosystems making it one of most diverse families of Agaricales (Kirk. *et al.*, 2008). Approximately 70% - 80% of taxa in this ectomycorrhizal family already have been documented from the north temperate zones in putative association mainly with the ectomycorrhizal (ECM) plant families *Pinaceae*, *Fagaceae*, and *Salicaceae*. However, normal studies on fungal ecology and mycofloristic diversity have determined focus on species outside the tropical ecosystems, where ECM fungal taxa require more attention (Alexander and Selosse. 2009; Smith. *et al.*, 2011).

The morphological and molecular identification of ECM species of genus *Inocybe* have been recorded from the temperate forests of Kashmir Himalayas, India (Itoo and Reshi. 2014). The *Cedrus deodara* is obligatory excellent photobiont makes ectomycorrhizal association with these lamellate basidiomycetes (Itoo and Reshi. 2014). Earlier, (Hanif. *et al.*, 2012) characterized four ectomycorrhizal fungi associated with *Cedrus deodara* using morpho-anatomic methods described for the first time from Pakistan. A small number of species have been recorded form ectomycorrhizae with *Cedrus deodara* only from India (Singh and Lakhanpal. 2000; Deepika. *et al.*, 2011; Saini and Singh, 2011). The *Inocybaceae* all together is inadequately acknowledged in tropical regions of Asia. In spite of this, India appears to be phylogenetically diverse in *Inocybaceae* containing lineages from six of seven major clades in the family (Matheny. *et al.*, 2009, 2012b). Previously, (Sheikh. *et al.*, 2019) investigated to document an inventory of ectomycorrhiza prevailing in Zabarvan forest range of Western Himalayas of Kashmir and evaluate their diversity index and species richness and described status of 76 potential ECM taxa. The inventory decipher the habitat and altitude of numerous *Tricholoma* and *Inocybe* species associated with *Cedrus deodara* viz. *Tricholoma terreum*, *T. album*, *T. malvacereum*, *T. portentosum*, *T. scalpturatum* and *T.* sejenctum and *Inocybe appendiculata*, *I. fastigata*, *I. geophylla* and *I. maculata*.

The technique of tracing hyphal connections or organic connections between sporophores and ECM roots is quite useful for deciphering ectomycorrhizal diversity and a clear picture of ECM fungi symbiotically associated with roots of host trees. Our observations and findings are in complete agreement with the ectomycorrhizal study proposed by Iotti and (Zambonelli. 2006) and (Hanif. et al., 2012). These workers used the same technique to characterize mycorrhizae associated with

Cedrus deodara. The *Cedrus deodara* now added another mycobiants namely *Inocybe pallidicremea* and *Tricholoma psammopus* were found in ectomycorrhizal association with its roots. The morpho-anatomical details of ectomycorrhizal roots of *Cedrus deodara* associated with two ECM fungal species were documented for the first time from India.

The morpho-anatomical description and ectomycorrhizal pattern were confirmed by directly examining the rhizomorphs or hyphal connections between host roots and lamellate mushrooms. Previously, (Dar. *et al*., 2009; Beig. *et al*., 2008) have also documented some ECM species associated with various conifer species from different localities of Kashmir Himalayas. (Sharma and Singh. 1990) recorded species of lamellate ectomycorrhizal genera from Himachal Pradesh, India. (Abraham and Kaul. 1985) documented 175 species from Kashmir Himalayas of which 53 taxa were found to form ECM associations with different coniferous species. Later on, (Watling and Abraham. 1992) reported 77 ectomycorrhizal fungal species from Kashmir Himalayas. (Dar. *et al*., 2009) proposed that conifer forests in the Kashmir Himalayas harbours 260 taxa of macrofungi. (Pande. *et al*., 2004) reported that out of a total of 98 ectomycorrhizal species from Western Himalayas, 55 were associated with *Cedrus deodara* and *Pinus wallichiana*. Earlier, (Kumari. *et al*., 2010) documented *Cantharellis pseudoformosus*, a new species associated with *Cedrus deodara* from Himachal Pradesh, India. The present results shows a complete match with the results given by (Hanif. *et al*., 2012) in terms of morpho-anatomic details of ECM roots of *Cedrus deodara* for confirmation of symbiotic association of *Inocybe pallidicremea* and *Tricholoma psammopus* with *Cedrus deodara* under their natural habitat. The morpho-anatomical details of ectomycorrhizal roots of *Cedrus deodara* associated with two lamellate fungal taxa of genera *Inocybe* and *Tricholoma* were investigated for the first time from India.

Conclusion

The present work will establish a baseline data for mycorrhizal research in temperate coniferous forests dominated by *Cedrus deodara* and forest management projects in Kashmir Himalayas. Hence, the presently examined ECM taxa were found in direct organic contact with the colonized ECM roots of *Cedrus deodara* help in improve the information regarding Cedar ectomycorrhizal biology and help in replenishment of forest ecosystems regarding ectomycorrhizal diversity in the Kashmir Himalayas.

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References

1. Agerer, R. "Colour Atlas of Ectomycorrhizae." 1st–15th ed. *Einhorn Verlag, Schwäbisch Gmünd, Germany* (1987–2002).
2. Agerer, R. "Fungal relationships and structural identity of their ectomycorrhizae." *Mycological Progress* 5 (2006): 67-107.
3. Agerer, R. "Studies on ectomycorrhizae III. Mycorrhizae formed by four fungi in the genera *Lactarius* and *Russula* on spruce." *Mycotaxon* 27 (1986): 1-59.
4. Agerer, R. "Colour atlas of ectomycorrhizae." 1st– 15th ed. *Einhorn Verlag, Schwäbisch Gmünd, Germany* (1987-2012).
5. Agerer, R. and G. Rambold. "DEEMY –A DELTA based Information System for Characterization and Determination of Ectomycorrhizae." *Lud- wig-Maximilians-Universität München*Dept. Biologie I–Systematische Mykologie, München, *Germany*. http://www.deemy.de, 2004-2020.
6. Atri, N. S., A. Kaur and H. Kour. "Wild Mushrooms-Collection and Identification." In: Rai, R. D., Upadhyay, R. C. and Sharma, S. R. eds., *Frontiers in*

Mushroom Biotechnology NRCM Chambaghat, Solan, India (2005): 9-26.

7. Abraham, S. P. and Kaul, T. N., "Larger fungi from Kashmir-III." *Kavaka* 13 (1985): 77-81.
8. Arora, D. "Mushrooms Demystified: A comprehensive guide to the fleshy fungi." *Ten Speed Press* (1986).
9. Azul, A. M., Vitor, R., Anabela, P., Matilde, A. and Helena, F. "Mycorrhizal types in the Mediterranean Basin: safety teaching and training." *Journal of Biological Education* 42.3 (2008): 130-137
10. Alexander, I. and Marc, A. S. "Mycorrhizas in tropical forest: a neglected research imperative." *New Phytologist* 182.1 (2009): 14–16.
11. Bon, M. "Les Tricholomes de France et d'Europe occidentale." *Encyclopedie Mycologique* Paris 36 (1984).
12. Buscot, F., Jean, C. M., Jean, Y. C., Monique, G., Uwe, N. and Rüdiger, H. "Recent advances in exploring physiology and biodiversity of ectomycorrhizas highlight the functioning of these symbioses in ecosystems." *FEMS microbiology reviews* 24.5 (2000): 601-614.
13. Beig, M. A., Dar, G. H., Nadeem, A. G. and Nisar, A. K. "Mycorrhizal biodiversity in Kashmir forests and some new records of macrofungi from J&K state." *Applied Biological Research* 10 (2008): 26-30.
14. Comandini, O., Giovanni. P. and Andrea, C. R. "Fungi in ectomycorrhizal associations of silver fir (*Abies alba* Miller) in Central Italy." *Mycorrhiza* 7.6 (1998): 323–328.
15. Christensen, M. and Machiel, E. N. "Notulae ad floram agaricinam neerlandicam Tricholoma." *Persoonia* 17(1999): 295-317.
16. Gardes, M. and Thomas, D. B. "ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae rusts." *Molecular Ecology* 2 (1993): 113–118.
17. Durall, D. M., Melanie, D. J., Elaine, F. W., Paul, K., and Coates, K. D. "Species richness of ectomycorrhizal fungi in cutblocks of different sizes in the Interior Cedar-Hemlock forests of northwestern British Columbia: sporocarps and ectomycorrhizae." *Canadian Journal of Forest Research* 29.13(1999): 22–1332.
18. Dar, G. H., Beig, M. A. and Ganai, N. A. "Biodiversity of macrofungi in Kashmir forests -1." *Indian Journal of Forestry* 32 (2009):119-121.
19. Deepika, K. C., M. Ramesh., Ramesh, C. U. and S. Reddy. "*Cantharellus pseudoformosus*, a new species associated with *Cedrus deodara* from India." *Mycoscienc* 52 (2011): 147–151.
20. Hibbett, D. S. and Matheny, P. B. "The relative ages of ectomycorrhizal mushrooms their plant hosts estimated using Bayesian relaxed molecular clock analyses." *BMC Biology* (2009): 7-13.
21. Hanif, M., Abdul, N. K. and Samina, S. "Additions to the ectomycorrhizae associated with Himalayan cedar (*Cedrus deodara*) using rDNA-ITS." *International Journal of Agriculture Biology* 13 (2012):1062–1067.
22. Henry, L. D. C. and Rajakumar, R. S. "Nutritional status of certain wild edible mushrooms from the eastern ghats of Tamil Nadu." *Mysore Journal of Agricultural Sciences* 48.1 (2014): 50-55.
23. Iotti, M. and lessandra, Z. "A quick and precise technique for identifying ectomycorrhizas by PCR." *Mycological Research* 110 (2006): 60–65.
24. Itoo, Z. A. and Zafar, A. R. "Ectomycorrhizal diversity associated with *Cedrusdeodara* and *Pinus wallichiana* in the Kashmir Himalaya, India." *Pakistan Journal of Biological Sciences* 17.1(2014): 32–40.
25. Jonsson, L., Anders, D., Marie, C. N., Olle, Z. and Ola, K. "Ectomycorrhizal fungal

communities in late-successional Swedish boreal forests, their composition following wild fire." *Molecular Ecology* 8 (1999): 205–215.

26. Kornerup, A. and Wanscher, J. A. "Methuen Hand- book of Colour. 3rd ed." *Eyre Methuen*, London, 1978.
27. Kirk, P. F., Cannon, P. F., Minter, D. W. and Stalpers, J. A. "Ainsworth and Bisby's 'Dictionary of Fungi'."(10th Ed.) *CABI Bioscience* U.K (2008).
28. Kibby, G. "The genus *Tricholoma* in Britain." *Field Mycology* 11.4 (2010): 113-40.
29. Latha, K. P. D. and Manimohan, P. "Five new species of *Inocybe* (Agaricales) from tropical India." *Mycologia* 108.1 (2016): 110–122.
30. Le, T. F., Jean, G. and Geoff, C. "The use of mycorrhizas in temperate and tropical forests." *Symbiosis* 3 (1987): 179-206.
31. Luoma, D. L., Robert, E. F. and James, M. T. "Fruiting of hypogeous fungi in Oregon Douglas-fir forest: Seasonal and habitat variation." *Mycologia* 83 (1991): 335-353.
32. Matheny, P. B., Pradeep, C. K., Vrinda, K. B., P. Shibu, P. V. "*Auritella foveata*, a new species of *Inocybaceae* (Agaricales) from tropical India." *Kew Bulletin* 67 (2012): 119 – 125.
33. Matheny, P. B., Aime, M. C., Neale, L. B., Bart, B., Dennis, E. D., Egon, H., Bradley, R. K., Lodge, D. L., Trappe, J. M. and Hibbett, D. S. "Out of the palaeotropics? Historical biogeography and diversification of the cosmopolitan mushroom family *Inocybaceae*." *Journal of Biogeography* 36 (2009): 577- 592.
34. Mohanan, C. "Biodiversity of Terricolous and Lignicolous Macrofungi of the Western Ghats." Final Technical Report No. F. No. 23/15/2006-RE. MoEF, Government of India, New Delhi, 2011.
35. Matheny, P. B and Rachel, A. S. "The *Inocybe geophylla* group in North America: a revision of the lilac species surrounding *I. lilacina*." *Mycologia* 110 (2018): 618–634.
36. Niazi, A. R., Khalid, A. N. and Iqbal, S. H. "New records of ectomycorrhizae from Pakistan." *Pakistan Journal of Botany* 24 (2010): 4335–4343.
37. Pande V., Uma,T. P. and Singh, S. P. Singh. "Species diversity of ectomycorrhizal fungi associated with temperate forest of Western Himalaya: A preliminary assessment." *Current Sci*ence 86 (2004): 1619-1623.
38. Pradeep, C. K., Vrinda, K. B., Shibu. P. V., Hailee, B. K. and Matheny, P. B. "New and noteworthy species of *Inocybe* (Agaricales) from tropical India." *Mycological Progress* (2016): 15-24.
39. Rodgers, W. A. and Panwar, H. S. "Planning a Wildlife Protected Area Network in India." Vol. 1 and 2. A report prepared for the Department of Environment, Forests and Wildlife, Government of India at the Wildlife Institute of India, Dehradun (1988): 608.
40. Rinaldi, A. C., Ornella, C., Kuyper, T. W. "Ecto- mycorrhizal fungal diversity: Separating the wheat from chaff." *Fungal Diversity* 33 (2008): 1-45.
41. Smith, S. E. and David, J. R. "Mycorrhizal symbiosis." *Academic Press*. London, UK (1997).
42. Smith, S. E. and David, J. R. "Mycorrhizal Symbiosis, 3rd edn." *Academic Press*, London, UK (2008).
43. Smith, M. E., Terry, W. H., Aime, M. C., Alex, K. F. and Rytas, V. "Ectomycorrhizal fungal diversity and community structure on three co- occurring leguminous canopy tree species in a Neotropical rainforest." *New Phytologist* 192 (2011): 699-712.
44. Saba, M., Ahmad, I. and Khalid, A. N. "New reports of *Inocybe* from pine forests

in Pakistan." *Mycotaxon* 130 (2015): 671–681.

45. Sheikh, P. A., S. Baby., Dar, G. H., Sajad, U.N. and Khurshid, A. M. "Ectomycorrhizal Diversity in Zabarvan Forest Range of North Western Himalaya." *International Journal of Current Microbiology and Applied Sciences* 8.1 (2019): 2312-2323.
46. Singer, R. "The Agaricales in Modern Taxonomy 4th ed." *Koenigstein*, Federal Republic of Germany (1986).
47. Singh, L. and Lakhanpal, T. N. "Growth and ectomycorrhiza development in *Cedrusdeodara* seedlings inoculated with different vegetative inoculums formulations." *Journal of Mycology and Plant Pathology* 30 (2000): 64–67.
48. Sharma, B. M and Singh, B. M. "Ectomycorrhizal fungi associated with different forest trees of Himachal Pradesh." *Proceedings of the National Conference on Mycorrhiza*, February 14-16, Haryana Agricultural University, Hisar, India (1990): 13-15.
49. Saini, M. K. and Yadwinder, S. "Systematic Studies on Genus *Amanita* Section *Validae* from India, (Abstract)." *Proceedings of the Ninety Eighth Session of the Indian Science Congress*, Chennai, India (2011).
50. Taylor, A. F. S. and Alexander, I. "The ectomycorrhizal symbiosis: life in the real world." *Mycologist* 19.3 (2005): 102–112.
51. Vaario, L. M., Shu,T. X., Zong, Q. X., Zhi, M. L., Xue, S. and Yu, H. L. "In situ in vitro colonization of *Cathaya argyrophylla* (*Pinaceae*) by ectomycorrhizal fungi." *Mycorrhiza* 16 (2006): 137–142.
52. Visser, S. "Ectomycorrhizal fungal succession in jack pine stands following wild fire." *Newphytologist* 129 (1995): 389–401.
53. Watling, R. and Abraham, S. P. "Ectomycorrhizal fungi of Kashmir forests." *Mycorrhiza* 2 (1992): 81-87.
54. Wang, B. and Qiu, Y. L. "Phylogenetic distribution evolution of mycorrhizas in plants." *Mycorrhiza* 16 (2006):299–363.
55. Zeitlmayr and Linus. "Wild Mushrooms: An Illustrated Handbook." *Hertfordshire*, UK: Garden City Press (1976):72-73.

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